Norwegian University of Life Sciences
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PhD Supervisors

Professor John Morken (main supervisor)
Faculty of Science and Technology
Norwegian University of Life Sciences
P.O. Box 5003 NMBU, No-1432, Ås
Norway

Associate professor Odd Ivar Lekang (co-supervisor)
Faculty of Science and Technology
Norwegian University of Life Sciences
P.O. Box 5003 NMBU, No-1432, Ås
Norway

Assistant professor Zerihun Demrew Yigezu (co-supervisor)
College of Agricultural
Hawassa University
P.O. Box 05, Hawassa
Ethiopia

Associate professor Volha Shapaval (co-supervisor)
Faculty of Science and Technology
Norwegian University of Life Sciences
P.O. Box 5003 NMBU, No-1432, Ås
Norway

PhD evaluation Committee

Professor Kestutis Navickas

Vytautas Magnus University Agriculture Academy

Institute of Energy and Biotechnology Engineering

Studentų g. 15, 53362, Akademija, Kauno r.

E-mail: kestutis.navickas@vdu.lt

Lithuania

Associate Professor Zeynep Çetecioğlu-Gürol,

Royal Institute of Technology-KTH

School of Engineering Sciences in Chemistry, Biotechnology and Health

Dept. of Chemical Engineering

Resource Recovery

Teknikringen 42, Floor 6

SE-100 44 Stockholm

E-mail: zeynepcg@kth.se

Sweden

Associate professor Lars Kåre Grimsby

Faculty of Landscape and Society

Department of International Environment and Development Studies

Norwegian University of Life Sciences

P.O. Box 5003 NMBU, NO-1432, Ås

Norway

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Gebresilassie Asnake Ewunie
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Summary

Increasing global fuel prices, growing energy consumption, and profound environmental concerns are the key factors that motivated the search for alternative energy sources. Biofuels such as bioethanol, biodiesel, and biogas have been identified as promising fuels that potentially substitute the consumption of diesel, gasoline, and natural gas, respectively. Biodiesel is gaining popularity among mentioned biofuels due to its easiest applicability in all diesel engines without major modifications, while biogas is the alternative domestic energy source in the rural area of developing countries and could be also upgraded into biomethane for increasing its energy content and applicability. As a result, many efforts have been made to produce biodiesel and biogas from various feedstocks. However, more than 95% of biodiesel is produced from edible oil crops, which brought scathing criticism due to unsustainability, higher edible oil cost, and fuel versus food conflicts. The higher price of edible oil increases the overall biodiesel production cost; thus, the biodiesel production would not be economically viable as compared to petrol diesel. Therefore, researchers and industries are strongly motivated to search for low cost and non-edible feedstocks to secure sustainable biodiesel production. *Jatropha curcas* L (*J. curcas*) has been identified as a promising tropical, and subtropical plant for biodiesel production and the application of *J. curcas* biodiesel as engine fuel has been tested successfully.

However, sustainable biodiesel production from *J. curcas* is not achieved yet due to various factors such as low seed production, higher biodiesel production cost, and limited technologies in seed harvesting and biodiesel production. Likewise, fruit processing for seed production, oil extraction, and biodiesel production generates large volumes of *J. curcas* residues such as *J. curcas* fruit shell (JCFS), *J. curcas* press cake (JCPC), and crude glycerol (CG). JCPC and JCFS share above 80% of the dry fruit weight, while transesterification of 100 kg oil generates 10 kg of CG. These residues can neither be used as animal feed nor as organic fertilizer because of toxic chemicals such as crucin and phorbol ester. As a result, an open disposal of these residues may adversely affect the environment unless adequately managed. The migration of gas and leachate from the wastes into the surrounding environment could result in severe environmental concerns such as groundwater pollution and climate change through methane gas emission. Interestingly, these residues could be considered as additional valuable resources in the *J. curcas* value chain since they have a large potential for biogas production. The economic return of biofuel production from *J. curcas* could be maximized through improving the biofuel conversion process and utilizing the full potential of *J. curcas*, i.e., the oil and various residues. However, biogas production from the residues could be affected by higher lignocellulosic constituents of JCFS and JCPC.

These phenomena motivated for characterizing the potential of *J. curcas* for biofuel production before the establishment of large-scale biodiesel and biogas plants. Therefore, the main objective of the present PhD thesis was to analyze the biofuel production potential of Ethiopian variety *J. curcas* by explicitly focusing on examining the potential and suitability of *J. curcas* oil and various residues for biodiesel and biogas production, respectively. For this purpose, this study attempted to investigate the oil yield, composition, and physicochemical properties of *J. curcas* seed grown in different areas of Ethiopia using various analytical, instrumental, and empirical methods. Furthermore, various pretreatment methods that could improve the methane yield of *J. curcas* residues were investigated, and the optimum pretreatment conditions that resulted in higher methane yield were determined and modeled.

The present PhD thesis contains five scientific papers that are geared together towards achieving evaluation of the biofuel production potential of *J. curcas* oil and various residues. *J. curcas* is a tropical and subtropical species growing for multiple applications such as soil conservation and soap production, and numerous studies across the world have been conducted to study the biodiesel production potential of *J. curcas* oil. The first review paper (**Paper I**) focused mainly on the identification of factors affecting the sustainability of biodiesel production from *J. curcas* grown across tropical and subtropical regions. The result from **Paper I** reveals that the biodiesel production potential of *J. curcas* oil is affected by various ecological, social, economic, policy, and technological barriers. The type and degree of these individual impacts on the cultivation, oil extraction, and biodiesel process varied across various *J. curcas* growing countries. Therefore, it was indispensable to narrow the study into a specific location (Ethiopia) to identify the fundamental factors affecting the potential and suitability of *J. curcas* for sustainable biodiesel production. Therefore, the second paper (**Paper II**) explicitly focused on investigating the oil yield, composition, and physicochemical properties of *J. curcas* grown in different areas of Ethiopia. Furthermore, various biodiesel fuel properties were predicted from the fatty acid composition of *J. curcas* oils. The experimental result from **Paper II** showed that the oil yield of *J. curcas* kernel ranged between 47.10 to 59.32%, depending on the biophysical condition of growing areas. *J. curcas* grown at both higher and lower altitudes showed significantly lower oil yield as compared to these growing at the middle altitudes. The fatty acid composition of the oil was dominated by oleic (34.20-42.20%) and linoleic (34.80-41.80%) acids, while fuel properties such as kinematic viscosity, density, cold filter plugging point, and cetane numbers predicted from the fatty acid composition of oils were agreed with EN-14214 standards. Thus, considering the higher kernel oil content and suitable physicochemical properties of the oil, Ethiopian variety *J. curcas* is confirmed as a promising feedstock for biodiesel production.

In the biodiesel production chain, various *J. curcas* biowastes such as JCFS, JCPC, and CG were generated in massive volume, and the biochemical methane potential (BMP) of these biowastes was enhanced through applying proper pretreatment methods (**Paper III-V**). The methane yield of JCPC was significantly improved using alkaline pretreatment and co-digestion with CG (**Paper III**). The effect of NaOH concentration, incubation temperature and retention time on the methane yield of JCPC was investigated and modeled using response surface methodology coupled with a central composite design (RSM-CCD). JCPC was further co-digested with CG by varying the organic loading (OL) and CG levels. The alkaline pretreatment process was significantly affected by the linear and quadratic effect of NaOH concentration, incubation temperature, and retention time ($p < 0.05$); however, the interactive impact between two process variables was not significant. Thus, soaking the JCPC with 7.32% NaOH at 36 °C for 54 hrs has been predicted for maximum methane yield of 353.90 ml g^{-1} VS, whereas the co-digestion experiment employed at 2 g L^{-1} OL that contained 2% CG was identified as an optimum co-digestion process for higher methane production of 325.47 ml g^{-1} VS.

The promising result from alkaline pretreatment has motivated the authors to search for other environmentally benign and low-cost lignocellulosic pretreatment options. Thus, non-catalyzed steam explosion (SE) pretreatment was selected as the best option, and the effect of temperature and retention time on the methane yield of JCPC was investigated and modeled using RSM-CCD (**Paper IV**). A series of SE pretreatments were employed at different temperature-time combinations. This study found that all the linear, interactive, and quadratic effects of explosion temperature and retention time were significantly correlated with the methane yield of JCPC ($p \leq 0.05$). Lower methane yield was obtained from JCPC pretreated at lower and higher severity factor. The prediction from the RSM-CCD model revealed that heating the biomass at 202 °C for 9.39 mins would result in a maximum methane yield of 330.14 ml g^{-1} VS. However, the maximum methane yield obtained at this optimum condition exceeded by only 1.70% as compared to the co-digestion process. Thus, considering the environmental and economic advantage of JCPC and CG, the co-digesting process was suggested as a promising approach for enhancing the methane yield of the mixture over SE pretreatment.

The other potential biowaste generates during seed processing for oil extraction is lignocellulosic rich JCFS. Mechanical, alkaline, and SE pretreatments at various process conditions were employed for enhancing the methane yield of JCFS (**Paper V**). The effect of mechanical pretreatments on the methane yield of JCFS was examined by grinding its particle size into ≤ 1 mm. The particle size distribution, median diameter (d50), and mean diameter of ground JCFS was investigated using laser diffraction. The effect of alkaline pretreatment on the chemical composition and methane yield of JCFS was examined using the optimum condition defined in **Paper III**, i.e., 7.32% NaOH, 36 °C incubation temperature, and 54 hrs retention time. Likewise, the SE experiments were carried out at different explosion temperature (160-220 °C) and retention time (5-20 mins). The methane yield obtained from milled JCFS was estimated to be 349.56 ml g^{-1} VS, which is higher by 74.23% as compared to untreated biomass. Similarly, the alkaline pretreatment employed at specified conditions has resulted in 44.05% (288.6 ml g^{-1} VS) more methane yield than untreated JCFS. The alkaline pretreatment was found to be more efficient in lignin removal. The SE pretreatment was effective in hemicellulose dissolution; however, the methane yield has linearly decreased from 179.49 to 310.32 ml g^{-1} VS as severity factor increases from 2.47 to 4.83. Therefore, mechanical pretreatment was found more effective for enhancing the methane yield of JCFS as compared to alkaline and SE pretreatment methods.

Sammendrag

Økende globale drivstoffpriser, økende energiforbruk og miljøbekymringer er nøkkkelfaktorer som har motivert til søken etter alternative energikilder. Biodrivstoff som bioetanol, biodiesel og biogass er identifisert som lovende fornybare energibærer som erstatter henholdsvis diesel, bensin og naturgass. Biodiesel har blitt populært på grunn av den kan brukes uten større motormodifikasjoner. Biogass er den beste alternative innenlandsk energikilden på landsbygda i utviklingsland og kan dessuten bli ytterligere oppgradert til biometan som kan brukes som drivstoff. Som et resultat har det blitt gjort mange bestrebelser på å produsere og utnytte biodieselen og biogassen til forskjellige bruksområder. Imidlertid, produseres på verdensbasis mer enn 95 % av biodiesel fra vegetabilske oljer som kunne vært brukt til mat, noe som har ført til mye kritikk om bærekraftighet, produksjonskostnader og konflikter mellom drivstoff og mat. Høyere pris på oljer som kan brukes til mat fører til økte produksjonskostnader av biodiesel. Dermed vil ikke biodieselproduksjonen være mer økonomisk levedyktig enn fossil diesel.

Derfor er forskere og næringer sterkt motivert av å finne billige og ikke-spiselige råvarer for å sikre bærekraftig produksjon av biodiesel uten å påvirke matforsyningskjedene. *Jatropha curcas* L (*J. curcas*) er identifisert som en av de lovende tropiske og subtropiske plantene for produksjon av biodiesel, og bruken av *J. curcas* til biodiesel som drivstoff er blitt forsøkt. Imidlertid oppnås ikke økonomisk bærekraftig biodieselproduksjon fra denne energiveksten ennå på grunn av flere årsaker slik som lav avkastning, høye produksjonskostnader på biodiesel og begrenset teknologi for effektiv høsting av Jatropha-frøene. Samtidig danner avfall fra framstillingen fra fruktprosessering for frøproduksjon, oljeutvinning og biodieselproduksjon store mengder *J. curcas*-rester som *J. curcas* nøtteskall (JCFS), *J. curcas* presskake (JCPC) og rå-glyserol (CG). JCPC og JCFS utgjør over 80% av tørrstoffet, mens transesterifisering av 100 kg olje generer 10 kg CG. Disse restene kan verken brukes som dyrefôr eller som organisk gjødsel på grunn av giftige kjemikalier som crucin og forbolester. Med mindre håndtering av avfallet blir tilstrekkelig håndtert vil en avhending uten restriksjoner påvirke miljøet negativt. Utlekking av gass og sigevann fra avfallet til det omgivende miljøet kan føre til alvorlige miljøproblemer som grunnvannsforurensning og klimaendringer gjennom metangassutslipp.

Det er imidlertid interessant at disse bioavfallsstoffene kan betraktes som ekstra verdifulle ressurser i *J. curcas* verdikjeden, siden de har et stort potensial for biogassproduksjon. Den økonomiske avkastningen av biodrivstoffproduksjon fra *J. curcas* kan maksimeres ved å forbedre effektiviteten for konvertering av biodrivstoff og utnytte bedre de potensielle *J. curcas*-

ressursene. Imidlertid er biogassproduksjon fra JCFS og JCPC utfordrende på grunn av innholdet av lignocellulose komponenter.

Disse utfordringene har ført til et behov for å karakterisere *J. curcas* potensial og egnethet for produksjon av biodrivstoff, noe som er viktig før etableringen av store biodieselanlegg. Derfor var hovedmålet med denne doktorgradsavhandlingen å analysere biodrivstoffsproduksjonspotensialet til etiopiske sorter av *J. curcas* ved å karakterisere potensialet og undersøke egnetheten til henholdsvis *J. curcas* olje for produksjon av biodiesel og ulike rester fra produksjon til biogass. For dette formålet har man i denne avhandlingen ved hjelp av ulike analytiske, instrumentelle og empiriske metoder undersøkt oljeutbyttet, sammensetningen og de fysisk-kjemiske egenskapene til *J. curcas* frø dyrket i forskjellige områder av Etiopia. Videre ble forskjellige forbehandlingsmetoder som mulig forbedrer det biokjemiske metanpotensialet til *J. curcas*-restene undersøkt, og i tilegg ble forbehandlingsbetingelsene som kunne resultere i høyere metanutbytte analysert og modellert.

Doktorgradsavhandlingen inneholder fem vitenskapelige artikler som er satt sammen for å oppnå evaluering av potensialet for produksjon av biodrivstoff til *J. curcas* olje og forskjellige rester. *J. curcas* er en tropisk og subtropisk art som har forskjellige bruksområder, og det er utført mange studier over hele verden på biodieselproduksjonspotensialet til *J. curcas*. Den første artikkelen som er en litteraturstudie, (**Paper I**) fokuserer hovedsakelig på identifisering av faktorer som påvirker bærekraftig produksjon av biodiesel fra *J. curcas* dyrket over flere tropiske og subtropiske regioner. Resultatet fra artikkelen (**Paper I**) avslører at biodieselproduksjonspotensialet til *J. curcas* olje påvirkes av ulike økologiske, sosiale, økonomiske, politiske og teknologiske barrierer.

Resultatet indikerer at faktorer som påvirker dyrking, oljeutvinning og biodieselprosess, varierte i de ulike regionene. Derfor var det ikke mulig å begrense studien til et bestemt sted (land) for å identifisere de grunnleggende faktorene som påvirker potensialet og egnetheten til *J. curcas* for produksjon av biodiesel. Typen og graden av hvordan hver av disse faktorene påvirket dyrking, oljeutvinning og biodieselprosess varierte i forskjellige land. Derfor var det nødvendig å begrense studien til et bestemt sted (Etiopia) for å identifisere de grunnleggende faktorene som påvirker potensialet og egnetheten til *J. curcas* for bærekraftig produksjon av biodiesel. I den andre artikkelen (**Paper II**) var målet å undersøke av oljeutbyttet, sammensetningen og de fysisk-kjemiske egenskapene til *J. curcas* som vokste under forskjellige betingelser i Etiopia. Man undersøkte om *J. curcas* hadde potensial og egnethet av olje for produksjon av biodiesel. Resultatet fra forsøket viste at oljeutbyttet av *J. curcas*-kjernen varierte mellom 47.10 og 59.32%, avhengig av voksestedets biofysiske betingelser. *J. curcas* dyrket i både høyere og lavere høyder over havet viste betydelig lavere oljeutbytte sammenlignet med de som ble dyret i mellomhøyde.

Fettsyresammensetningen i oljen var dominert av oljesyre (34.20-42.20%) og linolsyre (34.80-41.80%) syrer, mens de forskjellige biodieselens drivstoffegenskaper slik som fettsyresammensetningen av oljer var i samsvar i EN-14214-standardene. Konklusjonen, med tanke på høyt oljeutbytte og gode fysikalsk-kjemiske egenskaper til oljen, ble den etiopiske sorten av *J. curcas* vurdert som et lovende råstoff for produksjon av biodiesel.

I biodiesel-produksjonskjeden ble de ulike restproduktene som JCFS, JCPC og CG samlet opp, og det biokjemiske metanpotensialet (BMP) for disse restproduktene ble forbedret ved å anvende riktige forbehandlingsmetoder (**Paper III-V**). Metanutbyttet av JCPC ble betydelig forbedret ved bruk av alkalisk forbehandling og samutråtnet med CG (**Paper III**). Effekten av NaOH-konsentrasjon, inkubasjonstemperatur og behandlingstid på metanutbyttet av JCPC ble undersøkt og modellert ved hjelp av en responsoverflatemetodikk kombinert med sentralt sammensatt design (RSM-CCD). JCPC ble samutråtnet med CG ved å variere organisk belastning (OL) og ulike mengde tilsatt CG. Den alkaliske forbehandlingsprosessen ble sterkt påvirket av NaOH-konsentrasjon og behandlingstid (p <0.05). Imidlertid var effekten av inkubasjonstemperaturen svak. Effekten av alkalisk forbehandling på metanutbyttet av JCPC ble variert avhengig av de ulike prosessbetingelsene. Oppfukting av JCPC med 7.32% NaOH ved 36 °C i 54 timer ble identifisert som optimal forbehandling ut fra maksimalt forventet metanutbytte på 353.90 ml g^{-1} VS, mens samutråtningsforsøket som ble utført ved organisk belastning på 2 g L^{-1} hvor det var tilsatt 2% CG gav en metanproduksjon på 325.47 ml g^{-1} VS.

Det lovende resultatet fra alkalisk forbehandling motiverte til å søke etter andre miljøvennlige og billige forbehandlingsalternativer for lignocellulose-materialer. Dermed ble ikke-katalysert SE-forbehandling valgt som et alternativ, og effekten av temperatur og behandlingstid på metanutbyttet av JCPC ble undersøkt og modellert ved bruk av RSM-CCD (**Paper IV**). En serie med forbehandlinger med dampeksplosjon (SE) ble utført ved forskjellige temperatur-tid-kombinasjoner. Fra denne studien ble det funnet at eksplosjonstemperaturen og behandlingstiden påvirket eksplosjonsprosessen ($p \leq 0.05$) betydelig. Analysene fra RSM-CCD-modellen viste at oppvarming av biomassen til 202 °C i 9.39 minutter gav et maksimalt metanutbytte på 330.14 ml g^{-1} VS. Imidlertid var økningen bare 1.7% sammenlignet med maksimalt metanutbyttet fra samtrenings prosessen. Dermed, med tanke på den miljømessige og økonomiske fordelen med JCPC og CG, ble samutråtningsprosessen vurdert til den mest lovende for å forbedre blandingens metanutbytte framfor SE-forbehandlingen.

Det andre mulige restavfallet som skapes under prosessering av frø for oljeutvinning, er lignocelluloserikt JCFS. Mekanisk, alkalisk og SE-forbehandling ved forskjellige prosessbetingelser ble undersøkt for å forbedre metanutbyttet av JCFS (papir V). Effekten av mekaniske forbehandlinger (malinger) på metanutbyttet av JCFS ble undersøkt ved å sortere ut partikkelstørrelser på under 1 mm. Partikkelstørrelsesfordelingen, median diameter (d50) og gjennomsnittlig diameter fra JCFS ble undersøkt ved bruk av laserdiffraksjon. Effekten av alkalisk forbehandling på den kjemiske sammensetningen og metanutbyttet av JCFS ble undersøkt ved å bruke den optimale tilstanden definert i **Paper III**, dvs. 7.32% NaOH, 36 °C inkubasjonstemperatur og 54 timers behandlingstid. Likeledes ble SE-eksperimentene utført ved forskjellig eksplosjonstemperatur (160-220 °C) og behandlingstid (5-20 minutter). Metanutbyttet oppnådd fra malt biomasse ble estimert til å være 349.56 ml g^{-1} VS, som var 74.23% høyere sammenlignet med ubehandlet biomasse. På den annen side resulterte den alkaliske forbehandlingen i 44.05% (288.6 ml g^{-1} VS) mer metanutbytte enn ubehandlet JCFS. Den alkaliske forbehandlingen ble funnet å være mest effektiv for ligninoppløsning. SE-forbehandlingen var effektiv ved hemicelluloseoppløsning; imidlertid ble metanutbyttet redusert lineært fra 179.49-310.32 ml g^{-1} VS avhengig av økning av produktet av faktorene temperatur og tid (severity factor) fra 2.47-4.83. Derfor ble mekanisk forbehandling vurdert som mer effektiv for å forbedre metanutbyttet av JCFS sammenlignet med de alkaliske og SE-forbehandlingsmetodene.

List of articles

The PhD thesis is based on the papers listed below and referenced by their roman number (**I-V**).

Paper I

Ewunie, G.A., Morken, J., Lekang, O.I., Yigezu, Z.D. Factors affecting the potential of *Jatropha curcas* for sustainable biodiesel production: a critical review. *Renewable and Sustainable Energy Reviews*. 2021; 137:110500. https://doi.org/10.1016/j.rser.2020.110500.

Paper II

Ewunie, G.A., Lekang, O.I., Morken, J., Yigezu, Z.D. Characterizing the potential and suitability of Ethiopian variety *Jatropha curcas* for biodiesel production: variation in yield and physicochemical properties of oil across various growing areas (accepted for publication under *Energy Report, 2021).*

Paper III

Ewunie, G.A., Morken, J., Yigezu, Z.D. Alkaline and co-digestion pretreatments: process optimization for enhancing the methane yield of *Jatropha press cake* (in press under *Biomass Conversion and Biorefinery*, 2020). https://doi.org/10.1007/s13399-020-00732-y.

Paper IV

Ewunie, G.A., Yigezu, Z.D., Morken, J. Biochemical methane potential of Jatropha press cake: effect of steam explosion pretreatment and co-digestion with crude glycerol. *Journal of Renewable and Sustainable Energy*, 2020, 12: 063102-3. https://doi.org/10.1063/5.0005935.

Paper V

Ewunie, G.A., Yigezu, Z.D., Morken, J. Biochemical methane potential of *Jatropha curcas* fruit shell: comparative effect of mechanical, steam explosion and alkaline pretreatments (in press under *Biomass Conversion and Biorefinery, 2021*). http://dio.org/10.1007/s13399-020-01159-1.

List of abbreviations

AD	Anaerobic digestion
ANOVA	Analysis of variance
AOCS	American Oil Chemists' Society
AV	Acid value
BMP	Biochemical methane potential
CCD	Central composite design
BCR	benefit-cost ratio
CFPP	Cold filter plugging point
CG	Crude glycerol
DU	Degree of unsaturation
FFA	Free fatty acid
GC-MS	Gas chromatography mass spectrometry
GHG	Greenhouse gas
HHV	Higher heating value
IL	Recently ionic liquid
ISR	Inoculum-to-substrate ratio
IV	Iodine value
JCFS	*Jatropha curcas* fruit shell
JCPC	*Jatropha curcas* press cake
KV	kinematic viscosity
LCSF	Long-chain-saturated factor
NGOs	Nongovernmental organizations
PV	Peroxide value
RSM	Response surface methodology
sCOD	Soluble chemical oxygen demand
SE	Steam explosion
SV	Saponification value
TCD	Thermal conductivity detector
OL	Organic loading
VFA	Volatile fatty acid
VOP	Volatile organic products

Contents

1.Introduction

1.1. Background information

The continuous global population growth, emerging economy, and fast urbanization have requested the production and utilization of sustainable and environmentally sound energy sources. However, more than 1-1.50 billion people do not still have access to electricity [1], and future energy demands will increase by 1.30% each year until 2040 [2]. A study conducted in 2014 indicates that the average electricity coverage in the sub-Saharan countries was 38% [3], and most household energy demands are mainly relayed on fossil fuel and traditional biomasses. Fossil fuel covers 85-87% of world energy requirements [4, 5], while 10% of the energy demand is accessed from traditional biomass [6]. More than 2.50-3 billion people in the world used traditional solid fuels as a primary energy source [1, 7], while the daily petroleum oil and natural gas consumption stretched to more than 85.40 million barrels and 261 billion cubic feet, respectively [8]. The transportation fuel is entirely relayed on fossil fuel oil, in which only 4% of the total energy requirement is derived from biofuels [6]. The transport sector alone consumed 30% of global energy production and above 60% of the worldwide petroleum oil product. The average world's transportation fuel consumption has expected to increase by 60% by 2030 [9]. In contrast, studies have shown that the global fossil-based oil, coal, and natural gas reserve will be exhausted in less than ten decades if the rate of oil consumption continues as usual [8]. Besides its scarcity, fossil-based energy resources are identified as a primary source for greenhouse gas (GHG) emissions [10-12]; thus, the energy-related emissions hit another historic high in 2018 [2]. As a result, the accumulation of CO_2 in the atmosphere increases by over 100 ppm as compared with pre-industrial accumulations (280 ppm) [13].

Like other developing countries, the energy requirement of Ethiopia is highly dependent on traditional biomass and imported petroleum oils. The country is the number one energy-poor country in Africa [14] and ranked at the bottom of the global energy poverty index despite the existence of high energy potential resources. More than 46 million people in Ethiopia live without access to the national electricity grid, and 93% of residential energy requirements have relied on the burning of traditional biomasses [15]. The rapid economic growth of the country further fueled the rapid increase in energy demand across all sectors. For instance, studies done in Sub-Saharan countries like Ethiopia showed that increasing the gross domestic product by 1% requires 0.53% more energy demand [16]; thus, energy poverty could significantly affect the poverty reduction efforts. The energy poverty of Ethiopia is further manifested by full dependence on traditional biomasses such as firewood and charcoal [17], which have resulted in massive deforestation and emission of GHGs [18]. As a result, firewood becomes a scarce resource due to increasing population pressure and deforestation [18]. Thus, the rural communities have shifted from wood-based energy consumption to animal dung and crop residues that triggered land degradation and soil nutrients loss. Therefore, food security becomes the main problem for rural households whose livelihood depends on small-scale subsistence farming systems [19].

Millions of women and children have traveled long hours to collect firewood, and urban dwellers spend large proportions of their income on their minimum daily fuel needs [20]. In developing countries, respiratory disease caused by indoor air pollutant account for 3.7-6.6% disease burden, which is the 3rd risky factor next to malnutrition, HIV/AIDS, and malaria [16, 17, 21]. For instance, the annual number of deaths caused by acute respiratory infections due to the direct burn of biomass was estimated to be 1.30-4.30 million [18, 21], and Ethiopia shared about 50,000 deaths per year [19].

Ethiopia is also a net oil importing country among other sub-Saharan regions. The Ethiopian transportation sector entirely depends on imported fossil oils, and this sector is consuming more than 52% of imported oil [22], while kerosene is mainly used as a source of light and cooking in the rural part of Ethiopia [21]. Although the share of petroleum oil from the total energy consumption is estimated to be 7% [23], it is imported with the expense of more than 50% of the total export income [16]. For instance, in 2018/2019 alone, 3.99 million metric tons of petroleum products were imported, and the expense of these imports was more than 2.40 billion US dollars [24]. The cost associated with port rent, long-distance transportation, and petroleum oil price fluctuation has severely affected Ethiopia's trade balance. Energy insecurity is another challenge in the Ethiopian economy since most oils are importing from countries where the political situation is usually unstable [20]. As a result, substituting the conventional fossil fuel and traditional biomass energy resources with value-added and locally available bioresources would be the appropriate measures to mitigate all the above challenges. Moreover, the production and utilization of biofuels like biodiesel, bioethanol, and biomethane in the transportation and household energy matrix are viewed as a means of GHGs emission reduction and energy source diversification [25]. Ethiopia has also realized biofuel as a means of rural development, job creation, and foreign exchange thrift [25]. Therefore, biofuels such as biodiesel, bioethanol, and biogas have been identified as potential alternative energy resources that potentially substitute petrol diesel, gasoline, and traditional biomass utilization, respectively.

Liquid biofuels have attracted the interest of governments and policymakers because of their immediate usability in the existing transport sector and the easiest blendability with fossil fuel oils [26]. Biodiesel is gaining popularity over diesel due to its potential to be used as vehicle fuel without major engine modification [27]. Biodiesel is a monoalkyl ester of long-chain fatty acids and is produced by the reaction of fat/oil with monohydric alcohol in the presence of a catalyst. The fuel can be used in its pure form (B100) or by blending with petrol diesel at any proportion. Biodiesel is biodegradable, almost zero life cycle CO_2 emission, and free from sulfur emission. It has improved cetane number and oxygen and thus giving improved combustion over petrol diesel [23]. For the last several decades, however, over 95% of biodiesel has been produced from edible oilseeds [28, 29], which resulted in severe competition with food and feed production [30]. The other challenge associated with biodiesel is its inherent higher cost and limited availability of low-cost biodiesel feedstock since 70-80% of the overall biodiesel production price is associated with feedstock growing [10, 31]. Thus, feedstock-growing cost and the associated impact should be considered critically while planning large-scale biodiesel plants, which helps to assure the economic competitiveness of biodiesel with conventional diesel fuels [10]. As a result, various alternative non-edible oil crops that can grow in non-arable land have been explored and identified for substituting edible oils targeted for biodiesel production.

The availability of ample land for energy crops cultivation combined with the dramatic impact of imported oil on the national economy, had inspired the Ethiopian Government to launch an ambitious biofuels expansion strategy in 2007 that aimed to promote biodiesel production from various non-edible energy crops [14]. *Jatropha curcas* (*J. curcas),* castor bean, moringa, pongamia, palm, and croton have been identified as promising non-edible energy feedstocks for biodiesel production [16]. The former two feedstocks have been acknowledged as a primary biodiesel feedstock since they can grow in less fertile land and wider environmental conditions [32].

J. curcas has been identified as a promising energy crop with good quality and lower cost than other feedstocks [33]. It is a small tree or large perennial shrub up to 5-7 m in height, which belongs to Euphorbiaceae's family [34, 35]. It has a life expectancy of 50 years [36]. The plant is native to Central America and Mexico, then widely distributed into Africa and Asia. *J. curcas* oil is non-edible, toxic, and contains a higher level of oleic and linoleic fatty acid, which are suitable for fatty acid alkyl ester (biodiesel) production [14]. The biodiesel produced from *J. curcas* is successfully utilized in a diesel engine without major modification [36]. The application of *J. curcas's* oil and biodiesel could be further extended for heat, light, and electric production [20], which is good news for rural communities who are not connected to the national electricity grid.

Despite many advantages in the utilization of *J. curcas*, there is no successful *J. curcas* cultivation for sustainable biodiesel production in Ethiopia. Its economic importance and potential for biodiesel production are not realized due to the absence of proper evaluation and promotion of the local variety. More specifically, sustainable, and cheap feedstock supply are major uncertain factors that are significantly affecting the success of *J. curcas* biodiesel projects [10, 31]. Studies showed that *J. curcas's* seed yield and its oil content and composition are significantly varied with growing ecologies [37, 38]. In other words, the physiochemical properties of *J. curcas* are affected by various biotic and abiotic factors. The effect of temperature, rainfall distribution, soil moisture, land slope, and soil quality are categorized under abiotic factors [16]. In contrast, the impact of genetic variation, pests, and diseases are considered as biotic factors. As a result, characterizing the potential and suitability of *J. curcas* seed and oil for biodiesel production would be indispensable for successful biodiesel plant establishment since the biodiesel production process, reaction parameters, reactor design, feedstock handling, and preprocessing are strongly affected by physicochemical properties of *J. curcas oil*.

Seed processing, oil extraction, and biodiesel production usually generate massive volumes of organic wastes such as *J. curcas* fruit shell (JCFS), *J. curcas* press cake (JCPC), crude glycerol (CG), and wastewater [39]. These residues can neither be used as animal feed nor as organic fertilizer because of toxic chemicals such as crucin and phorbol ester [40, 41]. As a result, open disposal of these residues may adversely affect the environment unless adequately managed. The migration of gas and leachate from the wastes into the surrounding environment could result in severe environmental concerns such as groundwater pollution and climate change through methane gas emission. Interestingly, these agro-industrial wastes are economical, abundant, renewable, and provides a unique natural resource for large-scale and cost-effective biogas production. Thus, exploring and exploiting the value-added products from *J. curcas* residue could maintain a competitive advantage in the world market and ensure a sustainable economic return in biodiesel production from *J. curcas* oil. However, JCPC and JCFS are enriched with lignin, cellulose, and hemicellulose [42, 43], while CG significantly lacks nitrogen content [44].

Lignocellulosic materials are recalcitrant for the anaerobic digestion (AD) process. The carbon to nitrogen ratio (C/N) of JCPC was also lower than the optimum level required for the stable AD process [45]. Thus, these *J. curcas* residues should be either pretreated and/or co-digested with other substrates for maximizing their methane production. Therefore, this thesis aimed to investigate the biodiesel production potential of *J. curcas* by characterizing the yield, composition, and physicochemical properties of the seed oils grown at different study sites of Ethiopia. Furthermore, the biochemical methane potential (BMP) of various residues generated during seed processing (JCFS), oil extraction (JCPC), and biodiesel production (CG) was examined, and their methane yields were enhanced using various pretreatment methods.

1.2. Research objectives

Although *J. curcas* has been identified as a promising feedstock for biodiesel production, sustainable biodiesel production from this plant is not achieved yet in Ethiopia. The economy and biodiesel production potential of Ethiopian variety *J. curcas* seed is not realized, which invites us to examine the potential and suitability of *J. curcas* for sustainable biodiesel production. On the other hand, different *J. curcas* residues such as JCFS, JCPC, and CG are generated with massive volumes during conversion of *J. curcas* oil into biodiesel (Fig. 1). These biowastes are neither used as animal feed nor as organic fertilizer due to various toxic constituents. Within this aspect, biomethane production from *J. curcas* biowastes could be an alternative solution as the waste to energy conversion could minimize the higher cost of biodiesel production and waste stream treatments. Therefore, the main objective of this thesis was to examine the biofuel production potential of *J. curcas* oil and various residues with the following five specific objectives:

1. Identification of factors affecting the potential of *J. curcas* for sustainable biodiesel production (**Paper I**).
2. Characterizing the potential and suitability of Ethiopian variety *J. curcas* for biodiesel production (**Paper II**).
3. Examining and modeling the impact of alkaline and co-digestion pretreatments on the methane yield of JCPC (**Paper III**).
4. Exploring the optimum conditions in the steam explosion pretreatment and co-digestion process for higher methane yield of JCPC (**Paper IV**)
5. Investigating the effect of mechanical, steam explosion and alkaline pretreatments on the methane yield of JCFS (**Paper V**).

1.3. Scope and structure of the thesis

The accomplishment of the present thesis objective was relayed on five papers, while Fig. 1 shows the conceptual framework for investigating the biofuel production potential of *J. curcas* oil and various residues. The description and the contents of each paper are outlined below.

In Paper I, a state-of-the-art review has been conducted on the latest publication related to factors affecting the potential of *J. curcas* for sustainable biodiesel production, and all factors that affected *J. curcas* cultivation for sustainable biodiesel production have been categorized as ecological, economic, social, policy and technological barriers. Furthermore, the impact of various biotic and abiotic factors on the physicochemical properties of *J. curcas* grown across tropical and subtropical regions were identified and discussed. Different techniques employed for oil extraction and biodiesel production were identified and evaluated. The performance, combustion, and emission characteristic of various engines fueled with *J. curcas* biodiesel were examined and compared with that of the engines working with petroleum diesel.

The results obtained from **Paper I** showed that various physicochemical properties of *J. curcas* were highly dependent on the biotic and abiotic factors of the growing regions. Therefore, **in Paper II**, *J. curcas* seeds grown across different study areas of Ethiopia were characterized for their oil yield, oil fatty acid composition, and various physicochemical properties. Thus, all potential areas growing *J. curcas* have been identified and then clustered as lowland and midland based on their altitudes. Representative study areas were then randomly selected, and sample *J. curcas* seeds were collected for oil yield, oil fatty acid composition, and various physicochemical characterization. The study areas were then grouped into three classes based on their oil yield using a dendrogram. Then top ten study areas that shown higher oil yield were selected for detailed physicochemical and fuel property analysis. The fuel properties of biodiesel predicated from the fatty acid composition of *J. curcas* oil were examined and evaluated against international standards. Finally, the various residues generated during seed processing and oil extraction for biodiesel production were characterized for biogas production.

In Paper III, the effect of alkaline pretreatment on the methane yield of JCPC was investigated by varying NaOH concentration, incubation temperature, and retention time. The linear, interactive, and quadratic effects of these process variables on the soluble chemical oxygen demand (sCOD) and methane yield of JCPC were investigated and modeled using RSM-CCD. Then the optimum conditions that resulted in higher methane yield were explored and defined. Besides, JCPC was co-digested with CG, and the impact of organic loading (OL) and CG levels on the methane yield of the mixture were evaluated by running a series of batch AD. Finally, a preliminary energy balance and economic viability assessments were done for the optimum pretreatment conditions defined for higher methane yield.

In Paper IV, the methane yield of JCPC was enhanced through steam explosion (SE) and co-digestion with CG. The effect of different temperature-time combinations on sCOD and methane yield of JCPC was investigated and modeled using RSM-CCD. While the impact of co-digesting JCPC with CG on the mixture's methane yield was examined by employing a series of batch anaerobic digestion run with different OL and CG levels. The methane yield after the steam explosion and co-digestion process was utilized as a response variable for selecting the optimum process conditions. The variations in an optimum condition defined for higher methane and sCOD was used as a quick indicator of process impediment during the pretreatment process. For investigating the application of SE and co-digestion process at a large scale, a preliminary energy balance and economic viability assessments were employed for conditions identified for higher methane yields.

Taking the lesson learned from **Paper III** and **IV**, JCFS generate during seed processing for oil extraction was pretreated using mechanical, alkaline, and SE methods since each technique act differently on different parts of the material (**Paper V**). **Paper V** aimed to investigate and compare the impacts of mechanical, alkaline, and SE on the degradation, methane yield, and physicochemical composition of JCFS. The alkaline pretreatment was carried out using the optimum conditions defined in **Paper III**. The effect of particle size on the rate of degradation and methane yield of JCPC was examined after grinding the biomass into smaller particle sizes. The SE pretreatment was conducted at various temperature-time combinations, and the optimum conditions for higher methane yield were identified. Besides, detailed compositional change analyses were performed to understand the exact effect of alkaline and SE pretreatments on cellulose, hemicellulose, and lignin content of JCFS.

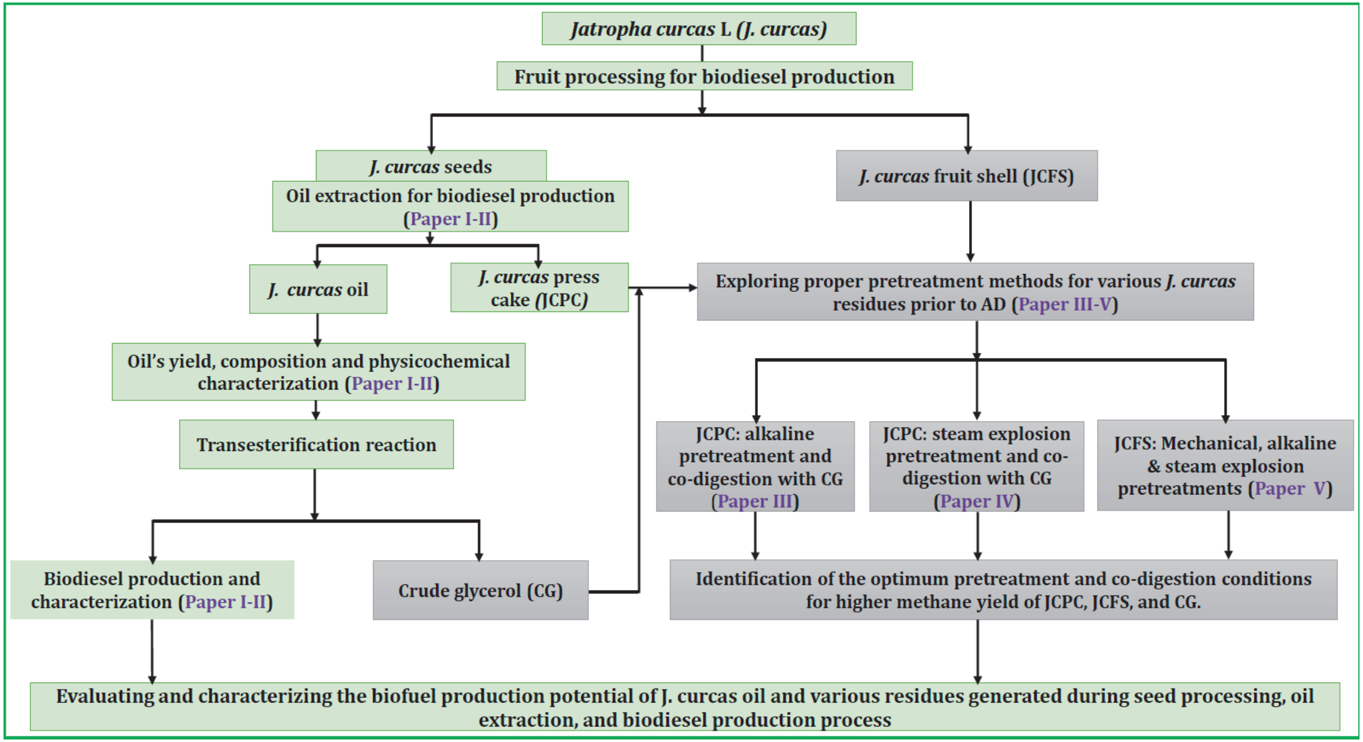

Fig. 1. The conceptual framework for investigating the biofuel production potential of *J. curcas.*

2. Research backgrounds and Justification

2.1. Overview of Ethiopian biofuel status

Increasing global fuel prices, growing energy consumption, and deeper environmental concerns are the key factors in Ethiopia that motivated the search for alternative energy sources and biofuel in particular [46]. Modern energy access is very crucial for economic development and livelihood improvement. However, the energy sector of Ethiopia has faced a dual challenge, i.e., inadequate access to modern energy and heavy dependence on traditional biomass energy sources [47]. The energy produced from conventional biomass resources such as firewood, crop residues, charcoal, and animal dung cover 80-91% of Ethiopian primary energy consumption [15, 17, 47], from which 99% is consumed in the residential sector [48]. A specific case study conducted in Wondo Genet District, Ethiopia, indicates that firewood covers more than 46% of traditional biomass consumption [49]; however, in the northern part of Ethiopia (Tigray and Amhara regions), animal dung is the primary energy resource due to firewood scarcity [50]. Direct biomass burning has been identified as the primary source of GHGs emission and the main driver of deforestation and land degradation [18]. For instance, the emission of CO_2 due to biomass combustion increased from 5.10 to 6.50 million tons between 2005-2010 [47]. Firewood consumption in the 1980s decimated 0.10-0.20 million ha of forest per year [51]. In 2009, the traditional firewood consumption in Ethiopia for domestic cooking and lighting was estimated to be 77 million m^3 $year^{-1}$; however, the sustainable fuelwood supply was limited to 9.3 million m^3 $year^{-1}$ [52]. A recent study conducted in Ethiopia indicates that the forest stock of Ethiopia will be exhausted by 2050 if the firewood consumption continues as usual [53]. Besides, cooking food with firewood on open fires causes respiratory diseases and sometimes resulted in death in the rural part of Ethiopia [54].

Although the share of petroleum oil to the total energy profile of Ethiopia accounts for only 7% [23], it is imported with the expense of more than 2.4 billion US dollars per year [24]. The rapid economic growth of the country, coupled with the expansion of infrastructure, has raised the volume of imported petroleum oils starting from 2009 to 2020. For instance, the consumption of petroleum oil increased by 17.90 % in 2019 as compared to 2018 [55]. The annual imported petroleum products in 2019 were estimated to be 140 million metric tons [24], which embraces 16.40% of total imports of goods and services [56]. From the total imported petroleum, 82% of the oil was used for transportation, while the rest was used in residential and industrial sectors [56]. The cost associated with port rent coupled with long-distance transport of oil from the port to different parts of Ethiopia triggers the fuel costs, which severely affect the trade balances of

Ethiopia. The price hike and unstable supplies of petroleum oils in the global market are also further affecting the national economies of Ethiopia.

Ethiopia has a huge potential for electric production from hydropower, wind, geothermal, and solar [24]. A study indicates that 95.50% of electric power usage in Ethiopia is generated by hydropower, while the remaining 4.50% is generated from wind and biomass sources [24]. The amount of electric power generated in 2020 was estimated to be 13.80 billion kWh [55], which covers only 2% of the primary energy consumptions of Ethiopia [24]. A study conducted in 2018 indicates that 38.70% and 33.70% of generated electricity was consumed in residential and industrial sectors, respectively, while the remaining 26.90% of electricity was granted for commercial and public services [48]. However, according to the Sustainable Energy for Africa Report published in 2016, only 26.50% and 2.50% of the total rural household have access to electricity and clean fuels for cooking, respectively. The per capita energy consumption of Ethiopia is estimated to be 70 kWh in 2014 [57], which is far from the average per capita (500 kWh) calculated for other African countries.

The adverse impact of traditional biomass and petroleum oil consumption on the national economy and environment, coupled with limited modern energy access in the rural area of Ethiopia, have inspired searching for alternative domestic energy sources. As a result, the Ethiopian government launched two strategies, i.e., Biofuel Development and Utilization and National Biogas Program, in 2007/2008 to promote and support the production and utilization of biofuels from various feedstocks [14, 58]. Therefore, tremendous effort has been made to solve the energy crisis and shortage in more isolated and poorly integrated areas by providing biofuels such as bioethanol, biodiesel, and biogas.

2.1.1. Bioethanol production in Ethiopia

The primary feedstock identified for ethanol production in Ethiopia is sugarcane bagasse left over after sugar production. Ethiopia started the blending of ethanol with gasoline with a 5:95% ratio between one sugar factory (Fincha) and Nile petroleum in 2009. The blending is growing by 10% starting 2011 between two sugar factories (Fincha and Metehara) and three blender companies (Nile petroleum, Oil Libya and NOC). Studies indicated that Ethiopia saved more than 30.90 million US dollars between 2008-2014 by blending 38.20 million liters of ethanol with gasoline [59]. The annual ethanol production from two sugar factor was estimated to be 11 million liters, which covers only 2.7% needed for blending the total consumed gasoline.

2.1.2. Biodiesel production status in Ethiopia

The other promising alternative engine fuel targeted for substituting the higher demand for petrol diesel in Ethiopia is biodiesel. In pursuit of an ideal energy crop for sustainable biodiesel production, the Ethiopian Government has intensively promoted biodiesel production from various non-edible oil crops starting in 2007. *J. curcas*, Castor bean, Moringa, Pongamia, Palm, and Croton have been identified as promising non-edible energy plants for biodiesel production [16]. Among them, *J. curcas* and castor bean have been identified as primary feedstocks since they can grow in moisture stress areas [32] despite the seed production under this type of area is seriously criticized [26, 41]. Thus, the country has identified 23.30 million hectares of land for *J. curcas* and castor bean cultivation [14, 26]. However, this estimation seems exaggerated due to many uncertainties during the assessment [16, 20, 26]. Other remote sensing-based studies indicated that the availability of 16.61 million ha of land, which is described as 'highly suitable' for *J. curcas* cultivation [60]. Likewise, World Bank [61] reported that the availability of 7 million ha of no cultivated and non-protected lands, which is suitable to grow energy crops. Other studies downsized the land available for *J. curcas* and castor bean cultivation to 3.5 million ha [16, 61]. Although variability is observed among reported land sizes, all studies indicated the possibility of cultivating non-edible oil crops for sustainable biodiesel production.

Accordingly, Ethiopia had advocated a massive ad hoc investment promotion to cultivate the above two biodiesel crops. The Government was provided supports for ensuring the economic attractiveness of biodiesel production and the expansion of investments in this sector by incentivizing investors with tax holidays, cheap land leases cost, and long-term credit facilities [16]. As a result, more than 14 foreign, local, and joint companies were registered in 2012 and got an investment license. From the licensed companies, however, only five of them were started the cultivation of *J. curcas* and castor bean for biodiesel production [26]. A survey conducted in 2016 indicates that 0.05 million ha of land was leased for private companies, and 47% of the leased area was planted with *J. curcas* and castor bean [14]. Another study reported that the availability of 20000 ha of *J. curcas* that planted as hedge, life fence, and soil conservation purposes [16]. However, biofuel development in Ethiopia has threatened natural forest and wildlife areas. For instance, the National (Sun) Biofuel project has cleared 50 ha of natural forest for *J. curcas* cultivation. While the land leased for Flora Eco Power was a natural wildlife habitat and plowing for castor bean cultivation has severely affected the wildlife species [20]. Moreover, most biodiesel investment deals were executed in a nontransparent, piecemeal, fragmented manner, as well as the involvement of the local community was minimal [16].

The adverse impact of biofuel development in Ethiopia raised opposition from international and local communities, environmentalists, and NGOs. As a result, the lease of land for biodiesel investment has been critically criticized, and the opposition from many stockholders forced the Government to reconsider the lands leased for biofuel investment [16]. Therefore, the policy that promotes biofuel development in Ethiopia was revised, and then the incentives provided for biodiesel investment were significantly down-sized after a few years of passion (2010-2015) [16]. The key reason behind this biofuel policy shift was the increasing concern over competition between food and biofuel crops and the leasing of improper lands for energy crop cultivation [26]. The absence of clear biofuel police, lack of biofuel impact assessment, and allocation of pastoral, arable, forest, and wildlife habitats for *J. curcas* and castor bean cultivation were the major concerns of local and international experts [62].

The revised policy encouraged the cultivation of biodiesel energy crops on the land called 'Marginal land'. The marginal land was defined in two perspectives, i.e., land that could not grow food crops due to moisture stress and low soil quality, and lands underutilized [26]. However, the latter type of land is confusing and not widely used in the marginal land definition. The question is 'is it possible to produce socioeconomically viable *J. curcas* seed on the marginal land for sustainable biodiesel production?'. As a result, some studies were carried out to investigate factors that affect biodiesel production in Ethiopia. The seedling survival rate, vegetative growth, and seed yields were used as primary indicators for evaluating the agronomic performance of *J. curcas* plantation for large-scale biodiesel production. Moisture stress, sowing of wild germplasms, poor soil quality, resource conflict, and poor agronomic performance of *J. curcas* seed were the major identified factors that contributed to the termination of most *J. curcas* projects [63]. All leased areas were planted with seeds collected at the various locality of Ethiopia despite *J. curcas* being strongly heterozygosity, and high-quality genotype seeds require clonal or tissue culture techniques [26]. On the other hand, the lower agronomic performance of the plant was directly associated with moisture stress since the average annual rainfall of all areas leased for *J. curcas* cultivation was less than 750 mm.

Behera *et al.* [64] showed that selecting suitable planting varieties is crucial for cultivating feedstocks for sustainable biodiesel production. Trees that can yield above 2 kg seed per tree per year with a minimum of 30% oil content could be considered as a good source of planting material. However, breeding and agronomic research on Ethiopian *J. curcas* variety are extremely low. Only few research centers have recently started the germplasm selection trials. However, no improved *J. curcas* variety is adopted and distributed to cultivators yet. Studies have also indicated that the absence of feasibility studies such as soil tests and adaptation trials before large scale plantation, coupled with insufficient experience and knowledge on the agronomic performance of *J. curcas* under different agro-ecological zones of Ethiopia, caused the failure of most *J. curcas* projects [26].

2.1.3. Biogas technologies in Ethiopia

Biogas production is a stepwise process that comprises four essential stages, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis [65]. The type and abundance of microbes and archaea involved in each stage of AD are significantly varied. Biogas is a clean and renewable energy source that mainly contains methane (60%–70%), carbon dioxide (30%-40%), hydrogen (1-5%) [49], and traces amount of nitrogen, hydrogen sulfide, oxygen, and water vapors [54, 66]. Biogas is produced by AD of organic substrates, and the produced biogas could be converted into various form energies such as heat, light, and electricity [67].

Biogas technology was introduced in Ethiopia as early as 1979 [58], and the number of installed biogas digester throughout the country were not exceeded 1000 plants until 2013 [50]. The size of the digester varies between 2.50-200 m^3 [50]. The five dominant types of biogas reactors observed in Ethiopia are Indian floating drum, Chinese fixed dome, Camar tech, Deenbandhu, and Polyethylene. The primary feedstock source for these installed biogas digesters is animal manure. Previous studies have reported that the availability of 35.40 million cattle in Ethiopia, which a provides higher volume of manure with an estimated biogas production potential of 10.60-14.20 million m^3 [68]. Such huge biogas production potential of animal manure motivated the establishment of a National Biogas Program in 2008 to assist and monitor the establishment of biogas technologies across various regions of Ethiopia [58]. The national biogas program was planned to build thousands of small-scale biogas digesters in two phases, i.e., phase-I (2009-2013) and phase-II (2013-2017). More than 8063 biogas digesters have been built in the first phase, while in the second phase, 10109 installed domestic biogas plants have been reported [50]. A recent study conducted in the Amhara region, Ethiopia, reported that the availability of 4500 active small-scale household biogas digesters and the annual biogas production from all reactors was estimated to be 0.13 petajoule per year [17]. The fixed dome is the most preferred digester in the region due to its economic feasibility and needs a small installation area as compared to other digester types [69].

The Green Economy Strategy set forth by the Ethiopian Government has prioritized the production and utilization of biofuels to foster sustainable growth and reduce demand for fuelwood [58]. In Ethiopia, biogas plants are establishing by targeting multiple benefits. Some of the motives are the potentiality for improving the local community's energy access and reducing reliance on traditional biomass (fuelwood) that cases GHGs emissions, deforestation, and forest degradation [54, 70]. There are also many arguments among scholars that biogas production from various types of organic waste can improve households' health through indoor air pollution reduction and save firewood and charcoal consumption [49, 58]. Some studies have proved that biogas technology significantly saved time and energy at the household level [54]. Biogas has also been reported to relieve health risks while providing environmental, agricultural, economic, and social benefits [71]. The digestate generated after AD is also identified as an essential nutrient for plant growth [21].

Although numerous advantages are realized from biogas technology, the small-scale household biogas digesters installed in the various region of Ethiopia are adversely affected by multiple factors. Studies indicated that at least two caws per capita are needed to obtain enough cattle manure for household biogas digester [70]. Similarly, another study reports that a minimum of 20 kg of manure is needed for feeding the biogas digester daily to produce enough biogas for a single-family (ca. 1 m^3) [54]. However, the number of livestock owned by rural households has decreased due to drought and grazing land shortage [50]. Thus, sustainable biogas production from animal manure becomes impossible, and many built digestors were closed. An assessment employed in 2007 indicates that 40% of installed biogas digesters wore not functional due to poor management, lack of follow-up, technical problems, evacuation of ownership, and feedstock and water shortage [58]. Likewise, Eshete *et al.* [72] raised the number of unfunctional biodigester reactors to 60% due to similar factors mentioned above. The installed biogas digesters are also entirely relayed on animal manure [17, 54, 58, 68]. However, the methane production potential of animal manure is lower as compared to most other substrates [73].

2.2. Characteristics, biology, and ecology of *J. curcas*

J. curcas has been identified as a promising energy crop with good quality and lower cost than other feedstocks [33]. *J. curcas* belongs to the family of Euphorbiaceae that contains more than 175 different species [34, 35]. Although there is no common agreement among studies, *J. curcas* is native to Central America and Mexico, then widely introduced into Africa and Asia from the Caribbean region by Portuguese seafarers [41, 74]. *J. curcas* is a small tree or large perennial shrub up to 5-7 m in height. Under favorable conditions, the height can reach up to 8-10 meters [74]. The plant is propagating either through seed sowing or stem cutting [41]. Growth is fast, and germination occurs after ten days of sowing. After 12 months of planting, the plant bears seeds, but the higher seed yield starts after five years of planting [38, 75]. It has a life expectancy of 30-50 years [36, 41]. *J. curcas* is monoecious, meaning it contains separate male and female flowers on the same plant [41], and flowering mostly occurs during the rainy seasons [76, 77]. In the permanent humid environment, however, flowering occurs throughout the year [78].

Due to its higher adaptability potential, *J. curcas* can grow in wider environmental ranges, i.e., from semiarid (300 mm) to humid (3000 mm) climatic conditions [79]. However, areas with higher precipitation are likely to cause a fungal attack and restrict root growth, while flowering and fruiting are highly limited if the annual rainfall of the growing area is less than 600 mm [80]. Thus, 1000-1500 mm average annual rainfall was noted as an optimum condition for *J. curcas* cultivation [81]. *J. curcas* is a thermophyte plant that grows better at a higher temperature (18-40 °C) and zero tolerance to below 0 °C [82, 83]. The optimum temperature identified for successful *J. curcas* cultivation ranges between 20-28 °C [41]. The plant can also grow in various soil types, including dry, stony, shallow, and soils with low nutrient content. However, for economically viable seed production, *J. curcas* needs soil with enough nutrients and moisture contents [41]. Well-drained sandy or gravelly soils with proper aeration and at least 45 cm depth are identified as suitable conditions for adequate *J. curcas* seed production [36]. *J. curcas* should not be planted on heavy clay soil, particularly where drainage is impaired, as *J. curcas* is less tolerant for waterlogged environments. Likewise, *J. curcas* can also strive in a wider altitudinal range (500-2150 m.a.s.l) [41, 80]. However, the optimum altitudes identified for higher seed production have been reported between sea level to 1500 m.a.s.l [60], while altitudes from 1500 to 2150 m.a.s.l are moderately suitable for *J. curcas* plantation [60, 80]. Although *J. curcas* is believed to has a higher resistance to pest and disease attacks [83], monoculture plantation is susceptible to pest and disease attacks [41, 84].

2.3. Factors affecting *J. curcas* cultivation for biodiesel production

Despite many advantages in *J. curcas* utilization, there are no successful cultivations and biodiesel production across many tropical and subtropical regions. Many companies invested in *J. curcas* cultivation have either ceased or suspended their investments after a few years of farming. The primary factor that adversely affects biodiesel production from *J. curcas* is the lower economic return of biodiesel production as compared to conventional petrol diesel [85]. The economic viability of biodiesel production depends on ensuring cheap and sufficient feedstock with acceptable quality and physicochemical compositions [86, 87]. More specifically, sustainable and cheap feedstock supply are major uncertain factors that are significantly affecting the success of *J. curcas* biodiesel projects since feedstock price accounts for about 70-80% of overall biodiesel production costs [10, 31]. Therefore, minimizing the cost of biodiesel production and increasing the yield of feedstocks has been the main agenda of many researchers, governments, and industries [10].

Segerstedt and Bobert [24, 26] noted that high seed yields are crucial for the economic feasibility of biodiesel production, which can only be achieved on good soil quality with sufficient nutrients and moisture. Study also indicates that for economic cultivation of *J. curcas* for biodiesel production, the average dry seed production should be at least 2 tones ha^{-1} with 30% seed oil content [41]. In contrast, several studies claim that *J. curcas* is the best candidate feedstock for biodiesel production as it can grow on moisture stress and degraded land [88, 89]. However, lands possessing moisture stress and low fertility level have impaired the commercial cultivation of *J. curcas* for large-scale biodiesel production [26, 83].

Studies showed that *the* seed yield, seed oil content, and physicochemical properties of oil are significantly varied between different growing agro-ecological zones [37, 38]. In other words, the physiochemical properties of *J. curcas* oils are affected by various biotic and abiotic factors. Growing temperature, rainfall amount and distribution, soil nutrient status, soil moisture content, and slope of the land are the critical factors categorized under abiotic factors [16]. The effect of genetic variation, pests, and diseases on the quality and composition of *J. curcas* is considered as biotic barriers. In Ecuador, the seed production of *J. curcas* has been estimated between 1-10 tons per hectare, depending on the age of the plants, soil conditions, and use of irrigation [90]. Tiwari [91] indicates that the oil content of *J. curcas* seeds ranged between 40-60% in weight due to variation in growing site conditions, while according to Agyemang *et al.* [92], *J. curcas*' seed oil content was significantly affected by the dryness and wetness of the growing area. The oil content of *J. curcas* growing in dry climatic conditions was considerably higher than these grown in wet climates [92]. Another study also noted that significant variations of kernels grown in 18 different provenances in its crude protein, crude fat, neutral detergent fiber, and ash contents [93].

A study conducted on a large-scale *J. curcas* plantation in Ethiopia revealed that moisture stress, sowing of wild varieties, and land use conflict were the major identified factors that contributed to the termination of most large-scale *J. curcas* cultivation projects [26]. Jingura *et al.* [83] argued that the availability and suitability of land are not granted for cultivation of *J. curcas* for sustainable biodiesel production as feedstock production could affect access to food and ecology of the natural environment. According to this study, areas categorized as lowland (< 900 m.a.s.l) were recommended for *J. curcas* cultivation since these sites lack soil nutrient and moisture content for economically viable crop production. A study conducted in India showed a substantial effect of environmental variation on the *J. curcas'* genetic constituents such as seed size, seed weight, and oil contents [39]. Variation in ecotypes, provenances, and seed sources of *J. curcas* plants were the reported factors that affected the various component of *J. curcas*.

The other environmental factors that potentially affected *J. curcas's* seed yield and its oil content are average annual rainfall, soil type, and growing altitude [83]. For instance, under semi-arid conditions, areas with a slope of 15-30°, 250-3000 mm annual rainfall, and less than 500 m elevations were identified as ideal environmental for *J. curcas* cultivation [36, 82, 83]. In a semi-arid environment with low soil quality, the achievable seed yield has been estimated between 2-3 tones ha^{-1} year^{-1}, while areas with suitable soil type and moderate rainfall (900-1200 ml) more than 5 tones ha^{-1} year^{-1} seed production was achieved [94].

Soil quality includes its type, texture, depth, organic content, macronutrients, and pH. The impact of soil type and quality varies across *J. curcas* growing regions [82]. Most studies showed that well-drained sandy or gravelly soils with proper aeration are suitable for *J. curcas* growth [36]. Other studies indicate that *J. curcas* could grow under saline soil [83], but its seed production was not reported. In contrast, *J. curcas* planted in ephemeral waterlogging soils such as vertisols or heavy clay soils showed lower growth performance. Jingura [83] has characterized the effect of various soil types on *J. curcas* cultivation. According to this study, vertisol and siallitic soil are suitable for both crop and *J. curcas* cultivation. However, to avoid the fuel-food conflict, these soil types were not recommended for *J. curcas* cultivation. On the other hand, some soil types such as sodic and regosol soil types are the highly recommended soils for *J. curcas* cultivation, despite the amount of seed that could be harvested on these soil types has not been reported. Likewise, *J. curcas* can grow at different pH, but the optimum soil pH should be between 6.00-8.50 [41].

Moreover, the seed and oil yield of *J. curcas* was significantly affected by several agronomic practices. Pruning, thinning, and weeding are the primary identified agronomic practices that alter the plant's morphological and physiological characteristics. For instance, pruning, thinning, and weeding results in the production of more branches and simulating the abundance and healthy inflorescence; thus, eventually enhancing proper fruit setting and seed yields. Studies also showed that propagation methods such as seed sowing, vegetative cutting, and tissue culture practices affected the survival rate, growth, and seed production potential of *J. curcas*. The detailed *J. curcas* propagation methods and the advantages and disadvantages of each technique are well described in Brittaine [41]. Among the various propagation methods, vegetative cutting and directly planted in the field were identified as the best option for fast growth and higher seed production as compared to seed sowing.

Fruit maturity and planting space are other growing conditions that significantly affected the yield, composition, and physicochemical properties of *J. curcas* seed oil [38]. *J. curcas* fruit with yellow-brown color gives higher oil yield with desirable physicochemical properties [36]. On average, *J. curcas* fruit is matured after 90 days of flowering; however, the number of days needed for maturity significantly varies with the growing agro-ecological conditions.

2.4. *J. curcas* oil as biodiesel feedstock

J. curcas is cultivated for numerous benefits. Some of the multiple advantages of the plant include the use of its oil for biodiesel production, reclaim degraded lands and promotion of rural entrepreneurships [41]. The plant is ranked in the third place after palm and coconut species in terms of its oil yield [95]. The oil content of *J. curcas* seed varies between 18-42% [41], but under-optimized extraction method, the estimated oil yield can reach 35-48% [83, 92]. Studies have also shown that the oil content of *J. curcas* seed varies across different provenance and growing ecologies [37, 96]. In the rural part of tropical and subtropical regions, *J. curcas* oil is directly utilized for lighting and cooking. *J. curcas* oil is also well known to produce soap, medicines, and pesticides. In China, *J. curcas* oil is used as furniture varnish after boiling with iron oxide [41].

For the last two/three decades, however, *J. curcas* has received great attention from researchers and governmental organizations for utilizing it as a source of biodiesel production [97]. *J. curcas* oil can potentially substitute the edible oil and reduce the opposition between food and fuel that potentially occurred during edible oils usage for biodiesel production. Biodiesel production from *J. curcas* can also reduce the growth of energy crops on arable land as the plant can be cultivated on less fertile and moisture stress soil. Thus, the suitability and potential of *J. curcas* for biodiesel production have been characterized in some tropical and subtropical regions [75]. The application of *J. curcas* oil as a diesel engine fuel was started in the Second World War in Madagascar, Benin, and Cape Verde [41]. However, the direct utilization of *J. curcas* oil as engine fuel shows numerous poor performances, combustion, and emission characteristics. In contrast, the biodiesel produced from *J. curcas* oil has been successfully utilized in a diesel engine without major modification [36, 41]. *J. curcas* oil has physical and chemical properties that make it highly suitable for processing into biodiesel. The oil is non-edible, toxic, and contains a high level of unsaturated fatty acids [14]. However, oil physicochemical characteristics appear to be affected by the environment and genetic interaction [38, 41]. This indicates that *J. curcas* grows across various tropical and subtropical regions showed different composition and physicochemical properties.

J. curcas oil is highly viscous, and its viscosity varies between 28.40-33.50 $mm^2 s^{-1}$ [35, 98]. The higher viscosity of oil leads to incomplete fuel combustion because it is inadequately mixed with air inside the engine cylinder [99]. The low combustion is manifested by higher exhaust emission [100]. The high viscosity is related to the large molecular mass and chemical structure of vegetable oils. Interestingly, transesterification reaction can significantly reduce the higher viscosity of *J. curcas* oil. For instance, Ong *et al.* [35] reported a reduction of oil viscosity from 28.35 mm^2 s^{-1} to less than 3.1 mm^2 s^{-1} after two-stage acid-base catalyzed transesterification reactions. The other parameter that motivates the conversion of *J. curcas* oil into alkyl ester is density. The higher density of oil leads to less compressibility, which significantly affects the fuel injection system of the engine [35]. The reported density of *J. curcas* oil varies between 913-940 kg m^{-3} [96, 101]. Likewise, biodiesel produced from *J. curcas* oils was characterized for its density, and the result indicates that the transesterification reaction can lower the density of *J. curcas* oil. A study conducted in Malesia showed that the density of *J. curcas* oil decreased from 940 to 880 kg m^{-3} after the conversion of *J. curcas* oil into biodiesel [101]. However, biodiesel is still denser and less compressible than petrol diesel [35, 102, 103], but it satisfied the EN 14214 standards.

The drawbacks associated with the utilization of *J. curcas* oil as biodiesel feedstock is its inherent higher acid value [104]. The reported free fatty acid (FFA) value of *J. curcas* oil ranges between 6.39-15% [35, 105], which is far from the acceptable limit (≤ 1%) for alkaline catalyzed transesterification reaction [106]. Higher FFA content resulted in soap and water formation during conventional transesterification reaction and caused incomplete ester conversion. The soap formed due to higher FFA may result in the gelling of biodiesel [103]. Thus, *J. curcas* oil requires a pretreatment process before the transesterification reaction. The acid value, viscosity, density, and other fuel properties such as cetane number, oxidative stability, lubricity, and cold flow properties of *J. curcas* biodiesel are directly affected by the fatty acid composition of the oil [107, 108]. The fatty acid composition of *J. curcas* oil varies across many growing regions. *J. curcas* oil is dominated by unsaturated fatty acids [38]. Oleic and linoleic acids account for more than 70% of the fatty acid composition of *J. curcas* oil [109], while among various saturated fatty acids, palmitic acid is determined to be the main constituent [110]. However, the quantitative experimental studies employed on each fatty acid composition showed that the fatty acid composition of *J. curcas* oil varies due to various factors [105, 111].

For instance, as it is shown in Fig. 2, the fatty acid composition of *J. curcas* oil has significantly varied due to changing in growing agro-ecological conditions (Thamaga, Maun, Shashe, and Mmadinare) and seed maturity stages. Linoleic and oleic acids were affected considerably by seed maturity stages and growing agro-ecological conditions, while the other fatty acids showed a similar trend. *J. curcas* oil is dominated by oleic and linoleic acids, which are categorized under unsaturated fatty acids (Fig. 2). However, unsaturated fatty acids are chemically unstable as compared to saturated fatty acids [38]. Thus, biodiesel produced from unsaturated *J. curcas* oil could be susceptible to oxidation and degradation unless care is taken during storage and utilization.

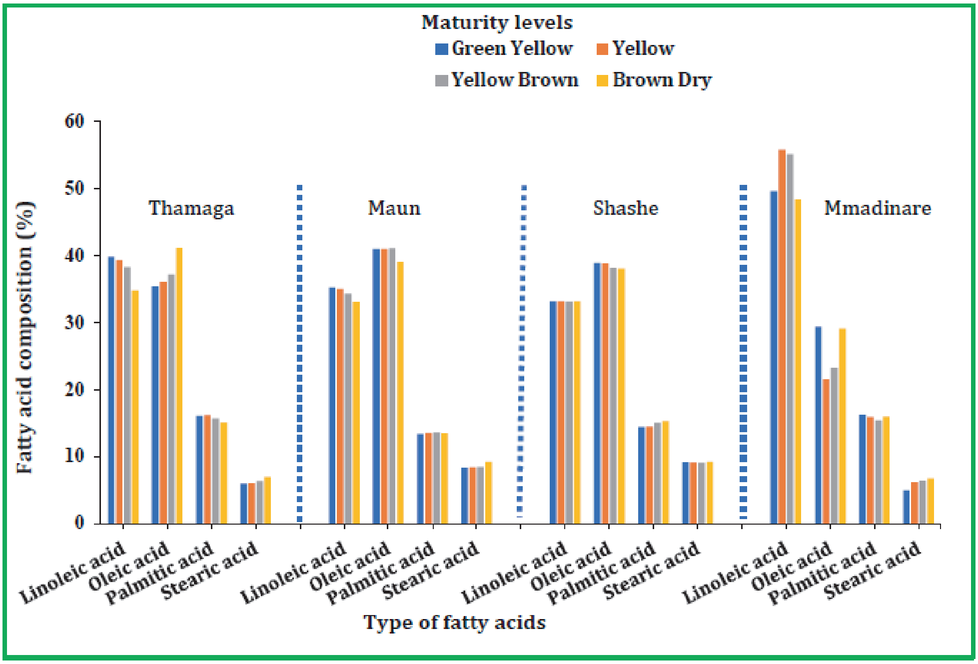

Fig. 2. Fatty acid profile of J. curcas seed oil at different fruit maturity stages harvested from areas with different agro-ecological conditions [38].

2.5. Biogas production potential of *J. curcas* residue

The seed processing, oil extraction, and biodiesel production processes usually generate a higher volume of *J. curcas* residues such as JCFS, JCPC, CG, and wastewater (Fig 3). The former two residues share above 80% of the dry fruit weight, while transesterification of 100 kg oil generates 10 kg of CG [112]. On average, 70-liter wastewater is generated from the purification of 100 L biodiesel [113]. Briefly, one hectare of land produces around 3500 kg of fruits; 1000 kg (29%) of the gross weight is estimated as fruit shell, while the remaining 2500 kg (71%) is the seeds [114]. The seed by itself has two components, i.e., 1025 kg (41%) seed coat and 1475 kg (59%) kernels. Other studies have shown that JCFS alone accounts for about 34-40% of the dry fruit weight [36], while JCPC weighs 60-70% of the dry seed weight [115].

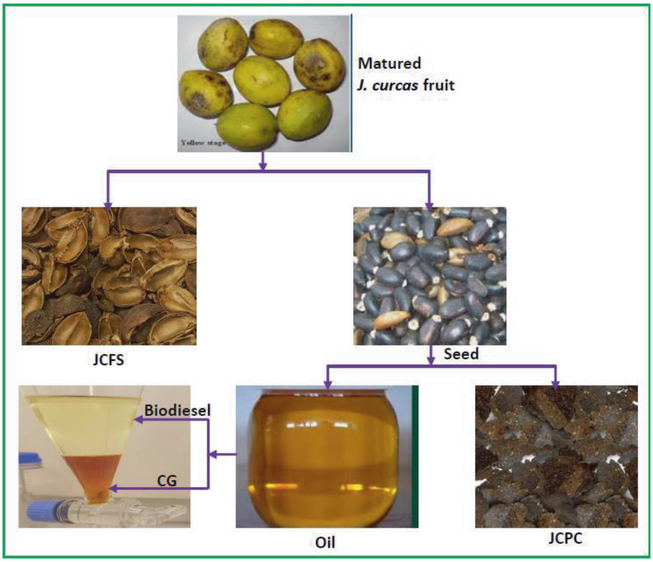

Fig. 3. Components of *J. curcas* fruits and wastes generated during oil extraction and biodiesel production process.

The raw glycerin (CG) contains methanol, catalyst, oil/biodiesel, and other contaminants [41]. The CG can be easily purified into high-grade, and it can be used in various applications such as in food, cosmetics, and pharmaceutical industries. However, massive CG production from large scale biodiesel plant and higher purification cost forced the CG to be considered as organic waste. JCPC contains higher organic and protein content for various applications [36]. The average crude protein and oil content of JCPC were estimated to be 58.10% and 9-12%, respectively [36]. However, due to the presence of different toxic compounds such as saponins, phorbol ester, and phytates, JCPC can neither be used as animal feed nor as organic fertilizer [36, 40, 41]. Heller [78] has also noted a phytotoxicity effect due to the direct application of JCPC as organic fertilizer. Studies have also tried to investigate the potential of the JCFS as an energy source through direct combustion [116], gasification [117], bio-briquettes production [118], and pyrolysis processes [119]. However, all trials were not successful due to substantial environmental and technical challenges associated with their physicochemical properties. Thus, open disposal of these residues may adversely affect the environment unless adequately managed. Interestingly, all *J. curcas* biowastes could be used as a potential feedstock for biogas production [120-122], and the toxicity levels could be reduced after the AD process [123].

JCFS, JCPC, and CG are economical, abundant, renewable, and provides a unique natural resource for large-scale and cost-effective biogas production [36, 120, 124, 125]. The cost associated with biogas production from *J. curcas* residues is lower due to the simple process and cheaper feedstock availability. Utilization of *J. curcas* biowastes as an energy source could also minimize the cost associated with waste management. The revenue obtained from the biowaste could further reduce the overall biodiesel production cost as biodiesel production alone is not economically feasible [36, 126, 127]. Gunaseelan [122] pointed out that the economic return of biodiesel production from *J. curcas* is significantly influenced by how the byproducts are used. Biogas produced from these residues could be converted to heat or electricity for utilizing as a source of energy during the biodiesel production and purification process. Recently, new technologies have been proven to synthesize methanol from biogas [11, 13]; thus, the methanol produced from *J. curcas* residue can also be utilized as green alcohol during the esterification or transesterification process. Therefore, exploring and exploiting the value-added products from *J. curcas* residues could maintain a competitive advantage in the world market and ensure sustainable economic returns in biodiesel production from *J. curcas* oil.

In Ethiopia, utilizing the *J. curcas* residue for biogas production is very important since all household digesters are running using animal manure [17]. However, studies have shown that 40-60% of domestic biogas plants built in the country become unfunctional due to lack of water and animal manure [21, 58, 72]. Thus, substituting or co-digesting the cow manure with *J. curcas* residue could be considered as the best solution to solve the shortage of feedstock for biogas production. Besides, water shortage during biogas production will not be a question since the biodiesel purification process generates a higher amount of wastewater. The wastewater mainly contains oil, glycerol, and alcohol that are highly biodegradable under anaerobic conditions [128], which could boost the BMP of *J. curcas* residues and cow manure. However, JCPC and JCFS are enriched with lignin, cellulose, and hemicellulose [42, 43], while CG significantly lacks nitrogen. The respective carbon to nitrogen (C/N) ratio of JCPC and CG is also considerably lower and higher as compared to the optimum levels required for a stable AD process [45].

The lower C/N content of JCPC may inhibit the methanogenic activities [129] and lead to process collapse due to ammonia accumulation [125]. In contrast, the higher C/N ratio from CG causes nitrogen deficiency for maintaining microbial biomass growths [4]. Thus, these *J. curcas* residues should be either pretreated and/or co-digested for improving their methane yields. The possible pretreatment methods that could be applied for reducing the recalcitrant nature of lignocellulosic materials are mechanical [130, 131], thermochemical [132-134], biological [129, 135], and SE [136, 137]; and the merit and demerits of each pretreatment method are discussed below.

2.6. Lignocellulosic pretreatments

Lignocellulose is a ridged part of the plant cell, and it is composed of cellulose, hemicellulose, and lignin. The two significant polysaccharides, i.e., cellulose and hemicellulose, are strongly linked with lignin and make complex lignocellulosic networks. These networks are highly stable and resistant to degradation [138]. Cellulose is a linear polymer of β-1,4-glucan, and its structure aid in having a tightly packed polymer chain, which is resistant to depolymerization and highly crystalline due to the strong interchain hydrogen bonds [126, 139]. Hemicellulose is another polysaccharide component with an amorphous, branched, and irregular structure that contains five to six-carbon sugars and uronic acids. Hemicellulose is connected to lignin by covalent links and enclose the cellulose [139]. Since hemicellulose and cellulose are polymers of sugars, they can be considered as potential sources to produce biodegradable monomeric sugars [32]. Lignin is the third principal component of lignocellulosic biomass, and it has a stable three-dimensional cross-linked structure that acts as a "glue" for cellulose and hemicellulose polymerization [138]. Thus, the hydrolysis stage in AD is always affected by the recalcitrant nature of lignocellulosic biomasses that protect the accessibility of carbohydrates for microbial degradation [126, 138, 140]. Moreover, the hydrolysis stage in AD could be affected by the crystallinity and bioavailability of cellulose.

Studies have indicated that only 20-30% of lignocellulosic biomass is converted to biogas during AD due to the impact of lignocellulosic structure [140]. In contrast, for economic viability, more than 75% of available carbohydrates should be converted to the monosaccharide and then to biofuel [141]. Therefore, lignocellulosic biomass should be pretreated before feeding into the biogas digester. Thus, AD of lignocellulosic biomass is a stepwise process like pretreatment, hydrolysis, and AD. The pretreatment step is the costliest and limiting stage in the biogas production process [142].

Lignocellulosic pretreatment aimed to reduce the recalcitrance nature of biomass to increase its biodegradability [143]. Pretreatments can also reduce the crystallinity and particle size of cellulose and dissolve the hemicellulose and lignin constituents [138]. However, each pretreatment method has its merit and demerit depending on the physicochemical components of lignocellulosic biomass and applied pretreatment conditions. The choice of pretreatment methods depends on the physicochemical properties of the material [126, 143-145], their efficiency [146], environmental soundness [147], and economic gain [148]. Furthermore, each pretreatment should be optimized to increase its economic and environmental viability by avoiding loss of carbohydrates, inhibitor formation, excessive energy consumption, and harmful and expensive chemical utilization [143].

2.6.1. Alkaline pretreatment

Pretreatments carried out using acid or alkaline chemicals facilitate the hydrolysis and decomposition of polysaccharides and lignin. However, after chemical pretreatment, concerns are raised due to the production of toxic degraded products and feedstock with too lower or higher pH values [140]. Thus, it required a neutralization process before the AD process that would complicate and increase the overall biogas production cost. Briefly, alkaline pretreatment is identified as a reliable method due to its intense effect and simple process. Alkaline is highly effective in lignocellulosic biomass pretreatment by cleaving the lignin-carbohydrate linkages [5, 149, 150] and widely applied on various lignocellulosic biomasses such as rice straw, softwood pine, sugarcane bagasse, and wheat straw [150, 151]. Among different alkaline, sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), lime ($Ca(OH)_2$), and alkaline hydrogen peroxide (H_2O_2) solutions are the most effective and widely used chemicals [138]. Strong alkalines (NaOH, KOH, and $Ca(HO)_2$) were more efficient than weak bases [152]. Mainly, NaOH is the most effective alkaline in delignification and able to work at various process conditions as compared to other chemicals [5, 153].

The alkaline pretreatment is further characterized by selective removal of lignin without significant loss of carbohydrates. It can also enhance the porosity and surface area of lignocellulosic biomass [138, 154]. The lignin content is removed either due to dissolution in intact form or degradation into its simple monomeric forms [143]. Alkaline pretreatment is also very beneficial in removal of hemicellulose. The distraction of complex networks in the lignin-cellulose-hemicellulose chain by alkaline pretreatment could be explained by the fact that the hydroxyl group (OH^-) from alkaline chemicals is capable of breaking the ester and ether bonds found between lignin and polysaccharides and weaken the hydrogen bond that connects cellulose with hemicellulose [126]. This process resulted in the separation of cellulose, hemicellulose, and lignin components of pretreated lignocellulosic biomass. At the same time, the acidic fractions (e.g., carboxylic or phenolic groups) ionized by the alkaline solution can trigger the solubility of individual components and swelling of the cell wall that promotes the solubilization of hemicellulose and lignin.

The alkaline pretreatment may increase the feedstock's pH due to the residual chemical left after the pretreatment process [155]. However, the chemical is useful since the next step (AD) requires an alkaline addition for controlling the pH drop due to volatile fatty acid (VFA) accumulation [155]. Thus, alkaline pretreatment is found to be better than acid pretreatments. The drawback associated with alkaline pretreatment is the requirement of relatively longer reaction times (several hours up to one day) at mild conditions as compared to other pretreatment methods

[156]. Moreover, the efficiency of alkaline pretreatments is mainly affected by chemical concentration, incubation temperature, retention time, and type of pretreated biomass [150, 154, 157, 158]. For instance, alkaline pretreatments employed at lower temperatures significantly affected the carbohydrate contents of hardwood, while the softwood pine's lignin content was reduced considerably after increasing the incubation temperature [159, 160]. Moreover, the alkaline pretreatments running at higher NaOH concentration and lower incubation temperature were advantageous for breaking the intramolecular hydrogen bonds between cellulose chains that significantly reduce the cellulose crystallinity [161].

In conclusion, alkaline pretreatment resulted in lower lignin and hemicellulose contents, which increases cellulose availability for microbial degradation and then increases the methane potential of pretreated biomass. However, unoptimized process conditions during the alkaline pretreatment could lead to incomplete hydrolysis of lignocellulosic material or cause degradation of polysaccharides and lignin that have an inhibitory effect on anaerobic microorganisms. For instance, Gu *et al.* [162] indicate that higher concentrated $Ca(OH)_2$ results in the generation of Ca^{2+}, followed by calcium salt precipitation that inhibited methanogenic archaea's activity. Alkaline H_2O_2 performed at higher temperatures could lead to lignin degradation and phenolic compound production, which is harmful to methanogens [151]. Thus, investigation and defining the optimum pretreatment conditions before performing the actual pretreatment process could be the sound measures to increase the economic gain and mitigate the adverse effects of the alkaline pretreatments.

2.6.2. Acid pretreatments

Phosphoric acid, acetic acid, hydrochloric acid, and sulfuric acid are the most common types of chemicals utilized in the acid pretreatment process [163]. Acid pretreatment can increase the recovery of major monomeric sugars for bioethanol and biogas production by breaking all bonds that link the cellulose, hemicellulose, and lignin components [153]. Sulfuric acid is the most used chemical due to its higher hemicellulose removal efficiency [164]. Moreover, sulfuric acid pretreatment can remove the surface layer of lignocellulosic biomasses and result in pore size increment and crystallinity index reduction [126]. The acid could also cleave the glycosidic bond found between xylose and arabinose units. As a result, hemicellulose hydrolysis is significantly higher during acid pretreatments than alkaline pretreatments [153].

The wealth of acid pretreatment method is attributed to the disruption of all lignocellulosic matrix and the amorphous cellulose. However, the higher cost of acid recovery, coupled with the formation of toxic and inhibitor compounds following the acid pretreatment, hindered its application in the biofuel production process [153]. Acid pretreatment also needs a higher temperature (>100 °C) over the alkaline pretreatment. The higher energy requirement could increase the over biogas production cost, and the degradation of carbohydrates increases with the rising of incubation temperature due to the auto-hydrolysis reaction [126]. Thermo-acidic pretreatments are corrosive and require high corrosion resistance reactor and piping materials [165]. Sulfuric acid pretreatment followed by biogas production is not suggested since remain sulfur could inhibit the methanogens [126, 143]. In acidic situations, precipitation of degraded lignin is higher, and then coagulate and condense into the biomass surface. It has been noted that acid pretreatment is not also suggested for utilizing the digestate as biofertilizer due to the risk of chemical residue in the slurry. The effect of acid pretreatment is highly dependent on solids loading, acid concentration, incubation temperature, and residence time [153]. Diluted acid (1–5%) pretreatment needs higher heat energy, which could increase the cost of biofuel production; thus, concentrated acid could solve the problem, but it would also significantly reduce the optimum pH levels required during the AD process.

2.6.3. Thermal and steam explosion pretreatments

Thermal pretreatment is useful for enhancing the biomass's surface area by degrading the lignin and hemicellulose, disorganizing the hydrogen bonds in the polymeric structure, and swelling the biomass [166]. SE is a more energy-efficient and inexpensive method than thermal pretreatment. It has been successfully applied in various lignocellulosic biomasses at different temperatures, pressure, and retention times [136, 137, 140].

Lee and Park [140] investigated the effect of SE pretreatments on the biodegradability and methane yield of sunflower biomass. The result revealed that SE pretreatment was highly effective in increasing biodegradability and methane yield of sunflower biomass. The non-catalyzed SE pretreatment is recognized as a low-cost option [149, 167] for feedstock pretreatment with significantly lower environmental impacts since the addition of external chemicals is not needed [168]. For instance, studies have shown that SE pretreatment needs only 30% of the mechanical pretreatment energy requirement to achieve the same size reduction [169, 170]. SE is also called auto-hydrolysis since carbohydrate depolymerization occurs during the process following three consecutive steps, as described in Fig. 4. During the SE process, the thermal expansion opens the cell wall of pretreated biomass, and then the auto-hydrolysis reaction starts soon. The acetic acid produced from the released acetyl group triggers the hydrolysis reaction and generate two simple sugars: glucose and xylose [126].

Studies also indicate that SE needs only 1.50 kg of steam to treat 1 kg of biomass, which is significantly lower than 5-10 kg of water required during hot water pretreatment [139]. Heating the lignocellulosic material with high-pressure saturated steam allows an explosive decompression of the lignocellulosic biomass due to the sudden pressure release [171]. The impacts of SE on the subsequent AD process varies according to the pretreatment condition (temperature and residence time), biomass type, and moisture contents [137, 170]. Under the optimized method, SE pretreatment can increase the methane yield of lignocellulosic biomass by disintegrating the bond found between cellulose-hemicellulose-lignin networks. SE pretreatment is mostly manifested by increasing the biomass's surface area and dissolution of hemicellulose with lower degradation products. However, unoptimized SE pretreatment may produce inhibiter and toxic compounds such as soluble organic acids, phenolic compounds, and furan derivatives [140].

Studies have also shown that condensation and re-polymerization reaction between degraded compounds (e.g., furfurals) increases the acid-insoluble lignin fraction called pseudo-lignin [139, 172]. After pretreatment, the increased lignin content could also be associated with the transformation of hemicellulose and partly cellulose to water-soluble or volatile compounds [126] since lignin is estimated relative to the mass of these carbohydrates [149]. Hemicellulose degradation is associated with the removal of thermally labile acetyl groups during SE pretreatment employed at higher temperatures [126]. However, in the literature, little information is available on the systematic design and optimization of SE process variables such as explosion temperature and residence time for preventing the occurrence of inhibitor compounds. Thus, a systematic study to determine the optimum SE pretreatment conditions for lignocellulosic biomass requires to compare the bioconversion efficiency of pretreated biomasses under the optimum conditions with that of a none exploded sample.

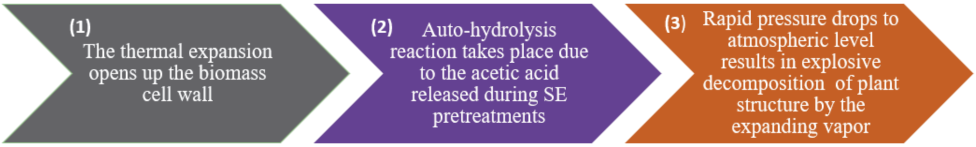

Fig. 4. Processes in biomass disintegration during SE pretreatment (adopted from Millati *et al.* [126]).

2.6.4. Mechanical pretreatments

The digestibility of lignocellulosic material in the AD process is significantly influenced by the cellulose crystallinity index, porosity, particle size, and lignin and hemicelluloses distribution [173]. Several studies have utilized mechanical size reduction (grinding) before carrying out other lignocellulosic biomass pretreatment methods. Size reduction is highly efficient in biofuel production when combined with different pretreatment methods [138]. For instance, the dried sunflower biomass was chopped into a particle size of 10 mm before the hydrothermal pretreatment [140]. The size of various energy crops such as maize, barley, sunflower, and sorghum were chopped into a particle size of 0.50-10 cm using a crop chopper before SE pretreatments [174]. According to Wang *et al.* [31], the size of the bulrush harvested from the sea was reduced to 3-4 cm particle size prior to adding into the SE units. These milling processes could increase the efficiency of proceeding pretreatment techniques. The most common applied milling methods are hammer milling, disk milling, ball milling, and vibratory milling [138]. Size reduction before any other pretreatment methods accounts for 33% of the energy required to bring the process to the end product [175].

In addition to combining it with other pretreatment methods, grinding pretreatment alone has been also used as a potential pretreatment method and is has been tested on various lignocellulosic biomasses such as Pennisetum hybrid [176], meadow grass [177], and switchgrass [178]. Grinding could alter the inherent ultrastructure of the biomass, increase the accessible surface area, reduce the degree of cellulose crystallinity, and decrease cellulose polymerization [126, 138, 179]. It could also alter both the external surface (size and shape of the particle) and the internal surface (capillary structure of cellulosic fibers) [143]. Mechanical pretreatment is also a preferable method to scale up for broader and large-scale applications [126]. Like SE, mechanical pretreatment is environmentally sound since external chemicals are not necessary [149, 167].

The effect of mechanical pretreatment is highly dependent on the characteristic of the biomass and achieved particle size reduction [138]. Moisture content, time of grinding, and grinder type are the primary parameters that significantly affected the performance of mechanical pretreatments [126, 176]. For instance, grinding performed on the dry and wet biomass of *Pennisetum hybrid* using ball grinder achieved particle size reduction of 47-234, and 149-290 µm, respectively [176]. The particle size reduction was positively correlated with grinding time. However, the crystallinity index (CI) increases with increasing of grinding time due to removal of amorphous cellulose. In contrast, particle size, surface area, and pore volume after mechanical pretreatment affected by the type of grinder and intensity of the method. For instance, mechanical pretreatments carried out using shear or compression forces resulted in 10-30 mm size reduction [180], while air classifier mill, continuous ball mill, and high-speed mill generate fine particles with 100-230 μm sizes [181].

Smaller particle size reduction brought higher cellulose availability for microbial degradation [138]. Thus, the methane yield increases with increasing surface area and pore volumes [126, 176]. However, too higher surface areas and smaller particle sizes could cause acidification during the AD process due to the fast accumulation of VFAs [176]. The higher power consumption combined with lower lignin and hemicellulose removal efficiency hamper the broader application of mechanical pretreatments in biofuel production and utilization process [138].

2.6.5. Biological Pretreatments

Biological pretreatments are mainly performed using fungi, microbial consortium, and enzymes that can use or degrade lignin and hemicelluloses components of lignocellulosic biomasses [182]. For instance, white-rot fungi were prevalent in the lignin removal over brown and soft-rot fungi [126]. A recent study shows that *Pleurotus ostreatus*, *Phanerochaete chrysosposrium*, and *Ganoderma lucidum* were amongst the white-rot fungi that effectively disintegrate the lignin content of lignocellulosic materials [183]. The fungi pretreatment process is significantly affected by cellulose to lignin ratio and processing temperature [183]. Studies have also indicated that cellulose degradation might occur during the biological pretreatment, which results in lower recovery of monomeric sugar for biofuel production [126]. Thus, lignocellulosic biomass pretreatments using various pure ligninolytic enzymes such as manganese peroxidase (MnP), lignin peroxidase (LiP), versatile peroxidase (VP), and laccase were proposed as alternative methods than fungi pretreatments [126].

For instance, corns trover was enzymatically pretreated using laccase (LA, 2 U/g biomass) and peroxidases, i.e., a combination of manganese peroxidase (MnP, 5 U/g biomass) and versatile peroxidase (VP, 1.5 U/g biomass) for 0-24 hrs at 30 ^{0}C [184]. Compared to the untreated corn stover, LA enzyme pretreatment resulted in 25% more methane after 24 hrs of incubation, while peroxidase enzymes (MnP+VP) increased the BMP of corn stover by 17% after six hrs of incubation. In the same scenario, summer harvested mulched switchgrasses was pretreated using lignin peroxidase (LiP, 1U/ml) and MnP (2 U/ml) at 22 and 37 ^{0}C, respectively, for 8 hrs [178]. The methane yield of LiP and MnP pretreated switchgrasses increased by 29 and 42%, respectively, compared to the mulched sample.

The other microbial communities utilized in biological pretreatment are microbial consortiums [126], and they are mainly screened from nature in decayed-lignocellulosic biomasses. Microbial consortium contains several hydrolytic microbes that can degrade the cellulose and hemicellulose components of lignocellulosic biomass [185]. However, this type of pretreatment results in loss of carbohydrates. The loss of cellulose and hemicellulose is mainly associated with the loss of sCOD and volatile organic products (VOP) during the pretreatment process. Although biological pretreatments do not directly impact the microbes involved in the AD process, this pretreatment technique is expensive and time-consuming due to slower processes, as well as finding a specific enzyme for removing a particular component of lignocellulosic biomass is complicated [5, 135, 186, 187].

2.6.6. Ionic liquid and organic solvent pretreatment methods

Recently ionic liquid pretreatments are gaining popularity over other pretreatment methods due to their higher biomass dissolution potential, and release of high carbohydrates yield for biofuel production [31, 174]. Ionic liquid and organosolv pretreatments are potent in removing pure lignin with small lignin structure defection [126]. Commonly used ionic liquids are imidazolium-based ($[(C_3N_2)Xn]^+$), pyridinium-based ($[(C_5N)Xn]^+$), pyrrolidinium-based $[(C_4N)Xn]^+$), ammonium-based ($[NX_4]^+$, phosphonium-based ($[PX_4]^+$ and sulfonium-based ($[SX_3]^+$) [126].

Ionic liquid pretreatment can selectively dissolve either one or more components of lignocellulosic biomasses, while acidic ionic liquid pretreatment significantly distracts the ether bond and has a remarkable effect on lignin de-polymerization [138]. As a result, ionic liquid pretreatment has been applied on various lignocellulosic biomasses such as barley straw, bamboo, rice straw, and sugarcane bagasse [188]. Like other pretreatment methods, temperature, residence time, biomass loading, and moisture contents were the primary process conditions that significantly affect the hydrolysis rate of ionic liquid pretreatments [189]. However, this pretreatment technique is manifested by higher energy consumption to recycle pure ionic liquid, and higher waste is generating during the pretreatment process.

Organic solvent pretreatment has also been widely utilized to treat lignocellulosic biomasses due to various positive characteristics like extracting pure cellulose, hemicellulose, and lignin separately and quickly recover and reuse of solvents as compared to ionic liquid pretreatment [138]. Organosolv is a type of organic pretreatment method that utilizes organic or aqueous-organic solvent at temperatures ranges between 100 to 250 °C. Diverse types of organic solvents such as alcohols, phenols, esters, propionic acids, acetones, formaldehyde dioxanes, and amines have been utilized with and without catalyst [143, 190]. Due to their lower cost and boiling point, ethanol and methanol are the preferred chemicals used during organosolv pretreatments [191].

The two advantages of this method are the separation of high purity cellulose with minor degradation and higher efficiency of hemicellulose fractionation. Acid-catalyzed organosolv pretreatment allows the removal of hemicellulose and lignin contents within a short time and at a lower temperature [192]. Adding the catalyst during organosolv pretreatment allows the breakdown of β-aryl ether bond, which accounts for about 40 to 65% of the lignins' total linkages. In contrast, organosolv pretreatment without catalyst addition was advantageous for breaking α-aryl ether linkages in a lignin structural unit that contain free phenolic hydroxyl groups in the para position [126].

Studies indicated that organosolv pretreatment had been successfully applied on wheat straw [193], cotton stalks [194], and sorghum bagasse pretreatments [195]; thus, significantly higher lignin and hemicellulose reduction were observed among these studied biomasses. The intact lining obtained after the organosolv pretreatment can be used to generate electricity, heat, and lignin-based adhesives [143], which can reduce the cost of organosolv pretreatments, while carbohydrates are a valuable substrate for biogas production. However, it should be noted that excess sulfate left after organosolv pretreatment can activate the sulfate-reducing bacteria, which adversely influence methane production due to nutrient competitions with anaerobic methanogens [126]. Moreover, organosolv pretreatment is predominantly affected by catalyst concentration, temperature, retention times, and solvent type [126, 192].

3. Material and methods

3.1. Characterizing *J. curcas* for biodiesel production

3.1.1. Study site

For **Paper II**, thirty-two different sites (Site:1, Site:2, … Site:32) distributed throughout Ethiopia (Fig. 5) were selected at potential areas that are growing *J. curcas* abundantly. The brief procedures we followed in the selection of these representative sites are described as follows: (1) all *J. curcas* growing areas are selected and mapped using previous studies [196], stakeholder interviews, and field observations; (2) the selected study areas were then grouped as lowland and midland study areas based on their relative altitude; (3) 16 study sites from each altitudinal range were selected randomly, and sample *J. curcas* fruits were collected from all study areas.

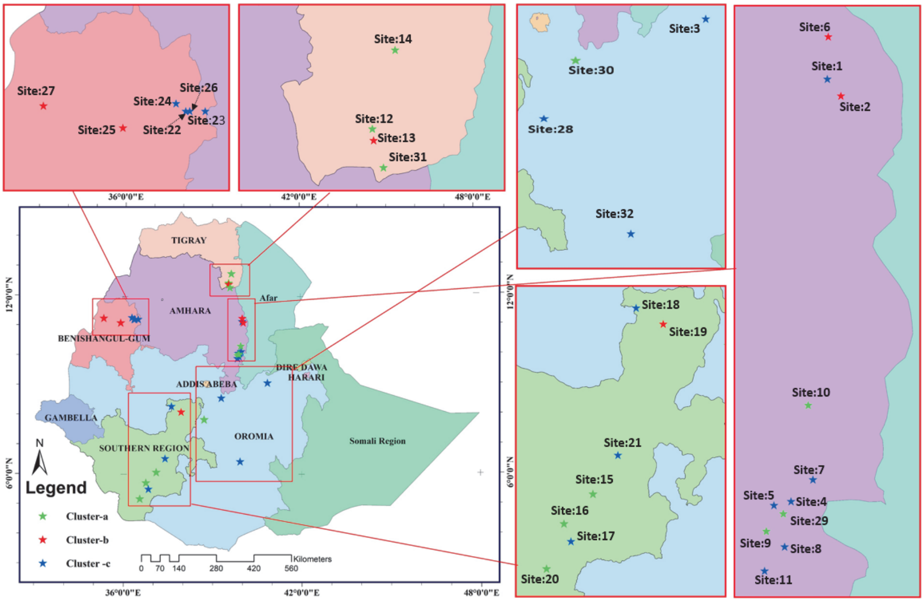

Fig. 5. Map of the study sites (star points on the map with green, blue, and red colors indicates areas showing relatively higher, moderate, and lower oil yields, respectively).

3.1.2. *J. curcas* seed collection and oil extraction processes

Sample fruits with yellowish color were collected from randomly selected *J. curcas* trees/shrubs [38] and immediately transported to Wondo Genet College of Forestry, Wondo Genet, Ethiopia. The fruits were then dried under open-air until their moisture contents were reduced below 50%. Forty fruits from each sample site were randomly picked, and the seeds from these fruits were then manually removed. The shells and seeds were further dried at room temperature until constant weights were recorded for three consecutive measurements. For ensuring removal of all moisture content, the shell and seeds were dried in an oven drier for 16 hrs at 105 °C. Finally, the dry weight of 100 randomly selected seeds were measured, and then the seed coat from 100 dried seeds was carefully removed using a sharp metal stick to estimate the weight proportion of seed coat, kernel, and oil retained in the kernels [197]. The weight of listed components was measured carefully using Shimadzu AW320 analytical balance with a precision of ± 0.0001 g.

Before oil content determination, the required amount of kernel was further dried overnight at 80 °C. The dried kernels were then ground and sieved using ISO stainless steel sieve with a mesh size of 1 mm. Finally, the kernel's oil content was determined following the conventional Soxhlet extraction process at the following conditions: 50g kernel weight, 6:1 n-hexane to solid ration (v/m), 70 °C extraction temperature, and 8 hrs extraction time. After extraction, the n-hexane was removed from the oil using a rotary vacuum evaporator at lower pressure, and the oil content of the kernel was estimated using Eq. 1.

$$\text{Oil yield} = \left[\frac{(W_2 - W_1)}{W_3}\right] X100 \qquad (1)$$

Where; W_1, W_2, and W_3 are weights of the boiling flask, boiling flask contained the oil, and kernel powder dipped into the thimble, respectively.

3.1.3. Oil physicochemical characterization

After determining the oil content of 32 different sites, all study sites were ranked chronologically based on their oil yield; then top 10 best sites that showed the highest oil yields were chosen for further compositional and physicochemical property characterization. The sampled oils were characterized for kinematic viscosity (KV), acid value (AV), FFA, iodine value (IV), saponification value (SV), and peroxide value (PV) contents, and the value of each chemical component was estimated using the equation shown in Table 1. The AV of crude *J. curcas* oil was determined by titration with potassium hydroxide following the method described in Asmare [198], then the FFA % was estimated by multiplying the AV with 0.501 [199]. The IV was determined using the Wijs method [200], while the SV was determined using the AOCS Cd 3-25 specification [201, 202]. The PV of crude *J. curcas* oil was estimated using AOCS Cd 8-53 method [202], whereas the oil's viscosity was measured using Cannon 9721-R56 Viscometer at room temperature [203].

Table 1. Basic equations utilized to determine the various physicochemical properties of *J. curcas* oil.

Properties	**Used equations**	**Eq.**	**Remarks** [a]
Acid value (mg KOH g^{-1} oil)	$\frac{56.1 \text{ x N x V}}{\text{sample oil } (g)}$	(2)	N is normality of alcoholic KOH and V is the volume (ml) of alcoholic KOH used in the titration
Free fatty acid (%)	AV x 0.501	(3)	-
Iodine value (g I_2 100^{-1} g oil)	$\frac{(V_b - V_s)\ x\ N\ X\ 12.69}{sample\ oil\ (g)}$	(4)	N is normality of $Na_2S_2O_3$, V_b and Vs are volumes (ml) of $Na_2S_2O_3$ solution utilized for blank and oil titration, respectively
Saponification value (mg KOH g^{-1} oil)	$\frac{(V_b - V_S)\text{x N x } 56.1}{\text{sample oil (g)}}$	(5)	N is normality of alcoholic KOH, V_b and Vs are volumes (ml) of HCl solution utilized for blank and oil titration, respectively
Peroxide value (meq kg^{-1} oil)	$\frac{(V_b - V_S)\ x\ M\ X\ 1000}{sample\ oil\ (g)}$	(6)	M is morality of $Na_2S_2O_3$, V_b and Vs are volumes (ml) of $Na_2S_2O_3$ solution utilized for blank and oil titration, respectively

[a] $Na_2S_2O_3$ is sodium thiosulphate; 12.69 is the equivalent weight of iodine, 56. 10 is the molecular weight of KOH, and 1000 is a constant number.

3.1.4. Fatty acid composition of *J. curcas*

The fatty acid composition of 10 sampled oils was determined using GC-MS following the methods described in Tsegay *et al.* [204]. Prior to fatty acid determination, *J. curcas* oil was transesterified using 1% KOH, 5:1 methanol to oil molar ratio at 50 °C for 50 mins. More specifically, 25 g of preheated *J. curcas* oil was taken and transferred into a boiling flask that contained 0.25 grams of KOH dissolved in 5.50 ml of methanol. The transesterification reaction was then performed at 50 °C for 50 mins by gently stirring with a magnetic stirrer. The produced mixture (methanol, catalyst, and fatty acid methyl ester) was then dissolved with the required amount of n-hexane and then gently shacked and centrifuged at 6000 rpm for 15 mins.

The supernatant was carefully taken with a syringe, and samples for GC-MS analysis were prepared by mixing 9 µg ml^{-1} of supernatant with 5µg ml^{-1} of standard decanoic acid methyl ester. Chromatographic separations were carried out using a DB-1701 column with 30 m length, 0.25 mm internal diameter, and 0.25µm column phase thickness. Injection mode was splitless while helium was used as a carrier gas, and 1µl volume of the sample was injected into the inlet heated to 275 °C. The oven temperature condition was programmed to be 60 °C for initial and hold for 2 mins and reached up to 280 °C. The program was separated into the rate of 20 °C min^{-1} until it reaches 200 °C, and the rate of 3 °C min^{-1} until it reaches 240 °C with zero hold time. Conditions used for the MS were a source temperature of 230 °C, 40-650 m/z scanning range, and operated in positive electron impact mode with ionization energy of 70 eV. The chromatogram and mass spectral data were processed using the instrument installed software (MS-Chem Station; Agilent Technologies, USA). Wiley's and Nist's libraries were used for identification purposes, and its quantification was calculated by the internal standard with the relationship of relative response factors.

3.1.5. Prediction of biodiesel fuel properties

The fuel properties of biodiesel have been predicted from the fatty acid composition of *J. curcas* oil. The detailed empirical equations utilized for predicting various fuel properties such as degree of unsaturation (DU), long-chain-saturated factor (LCSF), cetane number, KV, density, higher heating value (HHV), cold filter plugging point (CFPP), and surface tension of biodiesel are described in **Paper II**. More specifically, the DU and LCSF of the methyl ester were determined using the empirical model developed by [87]. At the same time, the empirical models developed by Ramírez-Verduzco *et al.* [205] were utilized to determine the cetane number, KV, density, and HHV values of *J. curcas* biodiesel. The surface tension of biodiesel synthesized from *J. curcas* oil was determined from its fatty acid composition using a simplified regression model developed by Allen *et al.* [206].

3.2. Biogas production potential of *J. curcas* residues

3.2.1. Experimental design for optimizing the alkaline and SE pretreatments (Paper III-IV)

In **Paper III and IV**, the alkaline and SE pretreatments were employed at various levels of NaOH concentration (X_{1A}), temperatures (X_{2A} and X_{1B}), and retention times (X_{3A} and X_{2B}). The coded and real values of each process variables calculated by RSM-CCD are presented in Table 2, and both the alkaline and SE pretreatments were employed for each combination of all variables as shown in Table 6 and 2 of **Paper III and IV**, respectively. A second ordered polynomial model was used to relate the effect of X_{1A}, X_{2A}, X_{3A}, X_{1B}, and X_{2B} on the sCOD and methane yields of JCPC. Furthermore, the second-order polynomial equation from RSM-CCD was used to define the optimum condition for each process variable that could maximize the methane and sCOD yields. As a result, the linear (X_{1A}, X_{2A}, X_{3A}, X_{1B}, and X_{2B}), quadratic (X_{1A}^2, X_{2A}^2, X_{3A}^2, X_{1B}^2, and X_{2B}^2), and interactive ($X_{1A}X_{2A}$, $X_{1A}X_{3A}$, $X_{2A}X_{3A}$, and $X_{1B}X_{2B}$) effects of these process variables on the sCOD and methane yield of JCPC were evaluated using multivariate analysis of variance (ANOVA) developed from the experimental value of methane and sCOD yields.

Table 2. Levels of X_{1A}, X_{2A}, X_{3A}, X_{1B}, and X_{2B} utilized during the alkaline and SE pretreatment processes.

	Variable	**Symbol**	**Levels** [a]				
			-α (-1.68)	-1	0	+1	+α (1.68)
Paper III	NaOH concentration (%)	X_{1A}	2.64	4	6	8	9.36
	Incubation temperature (°C)	X_{2A}	26.60	30	35	40	43.40
	Retention time (hrs)	X_{3A}	7.68	24	48	72	88.32
			-α (-1.41)	-1	0	+1	+α (1.41)
Paper IV	Explosion temperature (°C)	X_{1B}	186	190	200	210	214
	Retention time (mins)	X_{2B}	4.40	6	10	14	15.70

[a] α is the distance from the axial point to the center point calculated by $2^{k\,(1/4)}$ (k is the number of independent factors being used in the alkaline and SE pretreatments).

3.2.1.1. Mechanical pretreatment (Paper V)

The effect of mechanical pretreatments on the BMP of JCFS was investigated by grinding the dry biomass into a particle size of ≤ 1 mm using a coffee grinder (DeLonghi-KG 40) for 3 mins as described in **Paper V**. The milled biomass was then transferred into ISO stainless steel sieve with a mesh size of 1 mm and forced to pass through this sieve using a shaker (Edmund Buhler GmbH). Furthermore, the actual biomass distribution, median diameter (d50), d10, d90, and the average particle sizes of the grounded biomass were determined using a Beckman Coulter Laser Diffraction (LS 13 320). The average particle diameter estimated by the laser diffraction was used for assessing the effect of grinding (particle size reduction) on the methane yield of JCFS [207].

3.2.1.2. Alkaline pretreatment (Paper III and V).

JCFS and JCPC generated after seed processing (**Paper V**) and oil extraction (**Paper III**) were pretreated using alkaline pretreatments. **In Paper III**, JCPC was pretreated by varying NaOH concentration (2.64-9.36%), incubation temperature (26.60-43.40 °C), and retention time (7.68-88.32 hrs) as described in Table 2, while in **Paper V**, JCFS was pretreated using the optimum alkaline pretreatment conditions defined for higher methane yield of JCPC. Briefly, prior to alkaline pretreatments, JCPC was ground into a particle size of ≤ 1 mm (**Paper III**). Then, 100 g of sun-dried JCPC (with 8% moisture content) was transferred into one litter beaker containing 2.64%, 4%, 6%, 8%, and 9.36 % of NaOH, based on the dry weight of JCPC. The samples were then adjusted into a moisture content of 82% by adding the required amount of tap water. After adding the water, all samples were gently stirred with a glass rod to homogenize the mixture, then all beakers were covered with plastic films and sealed with plastic rings and incubated at 26.60 °C, 30, 35, 40 for 43.40 °C for 7.68, 24, 48, 72, and 88.82 hrs. Following the same procedure described for **Paper III**, the chipped JCFS was soaked with 32% NaOH at 35.86 °C for 54.05 hrs (**Paper V**).

3.2.1.3. Steam explosion pretreatment (Paper IV and V)

The JCPC and JCFS were pretreated using the SE unit designed by Cambi Aas (Asker, Norway) and situated at the Norwegian University of Life Science, Aas, Norway, as described previously [208]. Before adding the samples into the SE unit, the reactor was preheated to the desired temperature for 10 min [209]. Then without any size-reduction, 0.40 kg of dry JCPC or JCFS was added into a 20-liter pressure vessel tank and pretreated by supplying steam from the electric steam boiler (Parat, Flekkefjord, Norway). In **Paper IV**, JCPC with a moisture content of 10% was added into the reactor and subjected to 186, 190, 200, 210, and 214 °C for 4.40, 6, 10, 14, and 15.70 mins of retention time (Table 2). Likewise, JCFS with the same moisture content was exploded by varying the temperatures between 160 to 220 °C, using intervals of 20 °C, and each temperature was maintained for 5, 10, 15, and 20 mins (**Paper V**). During the SE pretreatment, the supplied temperature was indirectly controlled by regulating the reactor's pressure using a manometer connected to the automatic valve. Finally, the exploded biomasses were collected from the removable bucket, cooled down to room temperature, and then stored in airtight plastic bags at 4 °C until further processing.

3.2.1.4. Co-digestion experiments (Paper III and IV)

JCPC contains a high level of nitrogen content, while CG is significantly lacking nitrogen for utilizing as a potential substrate for biogas production. Therefore, co-digesting both substrates could enhance the carbon-nitrogen ratio required for stable AD processes. Thus, JCPC was co-digested with CG at different OL (2-7 g VS L^{-1}) and CG levels (0-4%) following a completely randomized design approaches [4].

3.2.2. Biochemical methane potential assay (Paper III-V)

The BMP of untreated and pretreated JCPC and JCFS samples were tested in batch serum bottles with different total and working volumes (Table 3). Before adding the batch reactor, the manure-based inoculum was further anaerobically incubated at 37±0.5 °C for one week to reduce the endogenous biogas production. The required amount of inoculum was then measured and poured into the batch reactors. Then, excluding the bottles reserved for the control test (inoculum), a required amount of untreated or pretreated JCPC and JCFS was added to all bottles. The inoculum-to-substrate ratio (ISR) for all experiments was maintained to be 1.50 (based on the VS bases), as suggested in the previous study [136]. The control batch bottles that contained inoculum alone were used to correct the endogenous biogas production. All batch reactors were then closed with a rubber stopper and aluminum crimps and flashed with pure nitrogen using a syringe for 5 mins to make an anaerobic environment [210]. Finally, the batch reactors were incubated inside the shaker (Multitron Standard, Infors HT, Switzerland) at 37 °C by continuously centrifuging at 90 rpm for required days until the daily biogas yield reduced bellow 3-5% of the total biogas production.

Table 3. The various protocols established for the batch AD of JCPC and JCFS.

AD protocols	**Paper III**		**Paper IV**		**Paper V**		
	Alkaline Prt.	Co-digestion process	SE Prt.	Co-digestion process	Alkaline Prt.	SE Prt.	Mechanical Prt.
OL (g VS L^{-1})	3.34	3.34	2.00	3.34	3.34	3.34	3.34
ISR	1.50	1.50	1.50	1.50	1.50	1.50	1.50
In. temp (°C)	37	37	37	37	37	37	37
TBR's vol. (ml)	122	530	122	530	530	530	530
WBR's vol. (ml)	70	350	60	350	300	300	300
Sk. S (rpm)	90	90	90	90	90	90	90
DD (days)	61	56	58	56	64	64	64

Prt. is pretreatment; SE is steam explosion; OL is organic loading; ISR is inoculum to substrate ratio in VS bases; In. temp is incubation temperature; TBR's vol is total batch reactor's volume; WBR's vol. is working batch reactor's volume; Sk. S is shaking speed; and DD is digestion duration.

3.2.3. Analysis of biogas composition and volume (Paper III-V)

The biogas produced from the individual batch reactor were regularly monitored by measuring the reactor's headspace gas pressure using a digital manometer (GMH 3161 Reisinger Electronic, Germany). Following the subsequent pressure measurement, biogas composition (CH_4 and CO_2) was determined using gas chromatography (3000 Micro GC, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) [211]. More specifically, the gases were separated using two parallel capillary columns (MolSieve 5 Å PLOT, 10m × 0.32 mm ×12 μm, and PLOT Q, 10m × 0.32 mm × 10 μm) connected to the TCD by using helium as a carrier gas. The injector and column temperatures for MolSieve 5 Å PLOT capillary were maintained at 90 and 70 °C, respectively, while the PLOT Q column was operated at 50 and 45 °C. The measured overpressure, reactor's headspace volume, and normalized methane concentration were used as input variables during methane volume calculation [209]. All measured gas volumes were reported at standard temperature (273 K) and pressure (101.3 kPa) using **Eq. 7**. The endogenous methane produced from the control (inoculum) was deducted from the total methane yield.

$$V_b = \frac{\mathrm{n\ x\ R\ x\ 273}}{\mathrm{P_o}} = \left(\frac{\mathrm{dp\ x\ V}}{\mathrm{R\ x\ T}}\right) \mathrm{x} \left(\frac{\mathrm{R\ x\ 273}}{\mathrm{P_o}}\right) = \left(\frac{\mathrm{dp\ x\ V\ x\ 273}}{\mathrm{T\ x\ P_0}}\right) \tag{7}$$

Where; V_b: volume of biogas (L) at the standard condition of 273 K (0 °C) and 1 atm total pressure; Po: 1 atm; n: the number of moles; R: the ideal gas constant; V: the volume of head-space (L); T: the temperature in the incubator room (310 k); dp: the overpressure measured in the bottle (atm) (1013.25 mbar = 1 atmosphere).

3.3. Statistical analysis

In **Paper II**, ANOVA was carried out using R software (version 3.6.2) to investigate the mean difference among treatments. Likewise, a post hoc Tukey test was performed to determine which specific group's mean difference was statistically significant, and the means differences were considered statistically significant if the p-value is ≤ 0.05. Pearson correlation matrix analysis was performed to depict the relationship between seed, seed coat, kernel, and kernel oil contents. Furthermore, a hierarchical Euclidian cluster analysis was performed to group similar study sites that showed the same average oil yields. In the co-digestion process, the interactive effects between OL and CG levels on the biogas and methane yield of the mixture were tested using a two-way ANOVA (**Paper III-IV**). In **Paper III**, the linear, interactive, and quadratic effect of NaOH concentration (X_{1A}), incubation temperature (X_{2A}), and retention time (X_{3A}) on the methane and sCOD yield of JCPC were analyzed using Design-Expert software (version 12). Multivariate ANOVA analyses were performed using the same software, and then a Tukey's test was employed to investigate the actual impact of all process variables on the methane and sCOD yield of JCPC. The linear (X_{1A}, X_{2A}, and X_{3A}), interactive ($X_{1A}X_{2A}$, $X_{1A}X_{3A}$, and $X_{2A}X_{3A}$), and quadrative (X_{1A}^2, X_{2A}^2, X_{3A}^2) effects were reported as statistically significant if the p-value for the average methane and sCOD mean difference is ≤ 0.05. The same statistical analysis applied in **Paper III** was also followed in **Paper IV**, although SE pretreatments were employed at different explosion temperatures (X_{1B}) and retention time (X_{2B}). In **Paper V**, the effect of various pretreatment techniques such as a mechanical, steam explosion, and alkaline pretreatments employed at different process conditions on the composition and cumulative methane yield of JCFS was tested using either linear regression or one-way ANOVA. All ANOVA was carried out using a Tukey's test, and the mean differences were considered statistically significant if the p-value is ≤ 0.05.

4. Main result and discussion

The present thesis focused on investigating and characterizing the biofuel production potential of *J. curcas* oil and various residues. The biofuel in the present study encompasses the biodiesel produced from *J. curcas* oil and biogas produced from various biowastes generated during seed processing, oil extraction, and biodiesel production processes. As a result, this thesis is organized as part-I and part-II. In the first part of this thesis, global experiences about factors affecting the potential of *J. curcas* for sustainable biodiesel production have been assessed through the art of critical review (**Paper I**). Factors affecting the cultivation of *J. curcas* for sustainable biodiesel production, oil yield, oil's physicochemical properties, and various oil extraction and biodiesel production methods were identified and critically discussed. Besides, the performance, combustion, and emission characteristics of various diesel engines fuelled with *J. curcas* biodiesel were examined and compared with petrol diesel. Taking the global experiences obtained from **Paper I**, a detailed and more specific study was conducted to investigate the potential and suitability of Ethiopian variety *J. curcas* for biodiesel production (**Paper II**). The yield and physicochemical properties of *J. curcas* oil grown in various study areas of Ethiopia were characterized, and their potential and suitability for biodiesel production were examined. In part II of this thesis, major *J. curcas* residues have been identified, and their potential for biogas production has been characterized. Various pretreatment techniques like mechanical, SE and alkaline pretreatment methods were designed and employed to enhance the degradation and methane production potential of different *J. curcas* residues (**Paper III-V**). The effect of various pretreatment process variables such as incubation temperature, retention time, alkaline concentration, organic loading, and particle size on the methane yield of *J. curcas* residues was investigated and modeled using either RSM-CCD or completely randomized design experimental approach.

4.1. Potential and suitability Ethiopian variety *J. curcas* oil for biodiesel production (Paper II)

In this paper, the potential and suitability of Ethiopian variety *J. curcas* for biodiesel production has been assessed by collecting seeds grown in 32 different study areas. Briefly, all potential areas that grown *J. curcas* have been identified across five regions of Ethiopia. Then the identified areas were stratified as lowland (800-1320 m.a.s.l) and midland (1320-2089 m.a.s.l) based on their altitude. Finally, 16 sample areas from each altitude were randomly selected and mapped, and sample *J. curcas* fruits were collected from all sampled study sites. The collected fruits were characterized for their dry weight proportion of seeds, seed coats, and kernels. Afterward, the oil content of all sampled kernels was determined using the conventional Soxhlet method under optimized extraction temperature and time. Then ten study sites that showed higher oil content was selected for further composition and physicochemical analysis. Likewise, various fuel properties such as cetane number, kinematic viscosity, density, HHV, CFPP, and surface tension of biodiesel were predicted from *J. curcas* oil, and each parameter was evaluated against the EN 14214 standards.

The dried seed weight per 100 randomly selected seeds ranged between 50.53-68.97 grams, while the dry weight proportion of seed coats and kernels relative to the dry seed weight varied between 33.26-66.74% and 47.10-59.32%, respectively. The other parameter used to assess the potential of a given feedstock for biodiesel production is the seed/kernel oil content [87]. As a result, the oil content of *J. curcas* kernels determined from the optimized Soxhlet extraction process ranged between 47.10-59.32%. The data presented in Table 4 showed that the dry weight proportion of seeds, seed coats, kernels, and kernel's oil content were significantly varied across *J. curcas* growing sites. Although the overall effect of altitudinal variation was not statically significant, the oil content had declined when the altitudes were getting too low and high (Fig. 3 of **Paper II**). For instance, the estimated oil yield varied between 47.16-51.61% for kernels harvested from study sites having lower altitudes (875-1038 m.a.s.l). Similarly, *J. curcas* seeds growing at higher altitudes (1767-2082 m.a s. l) contained lower oil content (47.10-50.85%) as compared to the oil content of kernels sampled from middle altitudes. However, the k-means clustering reveals that kernels harvested from different study sites were grouped in the same cluster, which suggests that altitudinal variations did not significantly affect the oil content of *J. curcas* kernels (Fig. 2 of **Paper II**). This could be due to the impact of other biotic and abiotic factors since all study areas are distributed in a different part of Ethiopia, and most sampled *J. curcas*es kernels are wild [212, 213]. The reported biotic factors were genotype, pests, and diseases. In contrast, the probable abiotic factor includes soil type and its nutrient content, rainfall pattern and amount, soil moisture content, average temperature, and agronomic practices such as planting density, pruning, thinning, and weeding.

Table 4. One-way ANOVA on the influence of study site variation on different components of *J. curcas* fruit (n=32).

Parameters	**Mean square**	**F-test**	**Significance (P-values)**
Seed weight/100 seeds (g)	Between groups (31.90) Within groups (5.55)	14.58	$2.2\text{x}10^{-16}$
Kernel (% in dry seeds)	Between groups (37.03) Within groups (4.13)	8.98	$1.2\text{x}10^{-13}$
Oil content (% in dry kernels)	Between groups (31.90) Within groups (5.51)	5.79	$1.1\text{x}10^{-9}$
Seed coat (% in dry seeds)	Between groups (37.03) Within groups (4.13)	8.98	$1.2\text{x}10^{-13}$

In addition to weight proportion analysis, *J. curcas* oils sampled across ten different study sites were characterized for their fatty acid composition, viscosity, AV, SV, IV, PV, and KV that substantially affect its suitability for biodiesel production. The ANOVA model from Table 5 shows that the AV, IV, SP, and PV were significantly varied due to variation in growing site conditions. In contrast, no significant variation in oil's KV was observed among sampled kernel oils. The IV and SV ranged between 98.80-112.20 mg I_2 and 180.90-202 mg KOH per gram of oil, respectively. Thus, the IV of J. curcas oil was below 120 mg I_2 g^{-1} oil restricted by the EN1424 standard, which explicitly motivates its application for biodiesel production. The higher IV could help in the storage and utilization of *J. curcas* biodiesel in its liquid form; however, oxidation reaction could be the other challenging issue during the storage of oils or methyl esters. The IV could also affect biodiesel polymerization, and thus, it leads to the formation of deposits in diesel engine injectors. At the same time, the higher SV indicates that the availability of triglycerides in *J. curcas* oil, which is the ideal properties of vegetable oils suggested for biodiesel production. However, excessive saponification of triglyceride and dissolution of ester by glycerol resulted in loss of ester yields [214]. Likewise, the peroxide value estimated among treatments varied between 2.60-9.00 meq kg^{-1} oil, which is relatively higher as compared to Nigerian and Indian varieties [215]. The higher peroxide value indicates the deterioration of lipids due to autoxidation at the double bond of unsaturated fatty acids.

Table 5. One-way ANOVA on the influence of growing site variation on different physicochemical properties of *J. curcas* kernel oil (N= 10).

Properties	Mean square	F-test	Significance (P-value)
Acid value (mg KOH g^{-1} oil)	Between groups (3.07) Within groups (0.04)	76.95	4.8×10^{-8}
Iodine value (mg I_2 g^{-1} oil)	Between groups (74.5) Within groups (19.76)	3.77	2.5×10^{-2}
Saponification value (mg KOH g^{-1} oil)	Between groups (61.29) Within groups (8.75)	7.00	2.7×10^{-3}
Peroxide value (meq kg^{-1} oil)	Between groups (6.38) Within groups (0.73)	8.71	1.1×10^{-3}
Kinematic viscosity at 25 °C (mm^2 s^{-1})	Between groups (6.24) Within groups (3.89)	1.60	8.1×10^{-1}

As shown in Table 3 of **Paper II**, the AV and FFA content of *J. curcas* oil were in between 0.70-5.30 mg KOH g^{-1} oil and 0.40-2.70%, respectively. From the total sampled oils, 50% of oils showed above 1% FFA, which is not suitable for alkaline catalyzed transesterification reaction [97, 216]. The higher FFA content of vegetable oil can affect biodiesel quality and determine the type and amount of catalysts used in the biodiesel production process [217]. Moreover, higher FFA content in the oil could result in soap formation and, therefore, incomplete reaction during the alkaline catalyzed transesterification process. Besides, the KV of Ethiopian variety *J. curcas* oil was estimated to be 34.60-39.30 mm^2 s^{-1} (Table 3 of **Paper II**), which is far from the value standardized by EN 14214. High viscosity and density could reduce fuel atomization and lead to a weak spray of the fuel and less accurate fuel injectors [218]. High viscose oil is also manifested by poor cold engine startup, high ignition delay, incomplete fuel combustion, gelling of lubricants, filter clogging, and breakage of certain types of injection pumps [219, 220].

The other parameter frequently used to assess the quality of *J. curcas* oil for biodiesel production or quality of biodiesel is fatty acid composition. On the other hand, the fatty acid composition can be used to predict various biodiesel's physicochemical properties such as viscosity, density, cetane number, IV, calorific value, lubricity, oxidation stability, and cold flow properties [75, 87]. In the present study, the fatty acid composition of the oil was assumed to be the same as biodiesel since transesterification does not significantly alter the fatty acid profile of the raw materials [10]. Ethiopian variety *J. curcas* oil contains 75.80-80.30% of unsaturated and 19.70-24.20% of saturated fatty acids (Table 4 of **Paper II**). Oleic (34.22- 42.18%) and Linoleic (34.78 - 41.83%) acids dominated the fatty acid composition of *J. curcas* oil, which are a promising characteristic of feedstock ascribed for biodiesel production. At the same time, palmitic (13.10-15.20%), stearic (4.80-10.30%), palmitoleic (0.10-0.90%), and eicosanoic (0.10-0.20%) acids were detected as minor fatty acid component of *J. curcas* oil (Fig. 6).

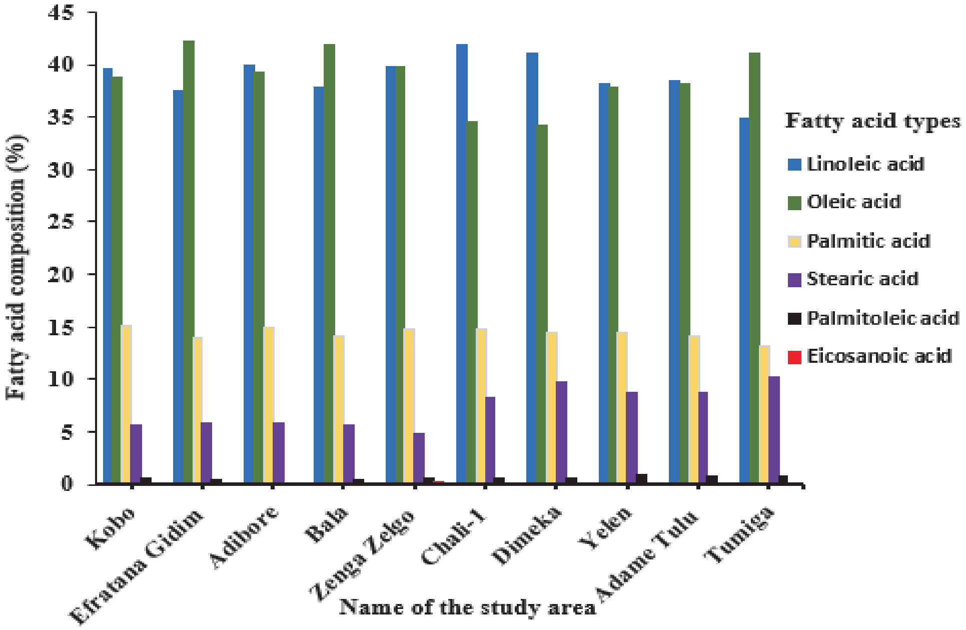

Fig. 6. Fatty acid profile of *J. curcas* oil harvested from different study areas of Ethiopia.

Higher saturated fatty acid content could potentially deteriorate the cold flow properties of biodiesel, but they could also improve the cetane number and oxidative stability [87]. Vegetable oils with a higher level of mono and di-unsaturated (Cn:1, Cn:2), lower polyunsaturated (Cn ≥3), and controlled saturated (Cn: 0) fatty acids are good characteristics of feedstocks for biodiesel production [109, 221]. Furthermore, the fuel properties of fatty acid methyl esters (biodiesels) produced from ten different J. curcas oil samples were predicted using oil's fatty acid compositions (Fig. 7). Thus, the predicted density and KV varied between 0.86-0.88 g cm^{-3} and 4.29-5.16 mm^{2} s^{-1}, respectively. Likewise, the estimated CFPPs and the cetane number of biodiesel syntheses from ten different oil collection ranged between -13.40 to -13.47 °C and 56.33 to 61.89, respectively. These results indicate that all predicted fuel properties are agreed with the EN 14214 standards. Furthermore, the predicted surface tensions and higher heating value values of biodiesel produced from Ethiopian variety *J. curcas* oil ranges between 28.85 to 28.92 mN m^{-1} and 39.85 to 39.94 MJ kg^{-1}, respectively. In conclusion, Ethiopian variety *J. curcas* oil showed suitable composition and physicochemical properties for biodiesel production. However, the yield, composition, and physicochemical property of Ethiopian variety *J. curcas* oil was significantly affected by growing site conditions.

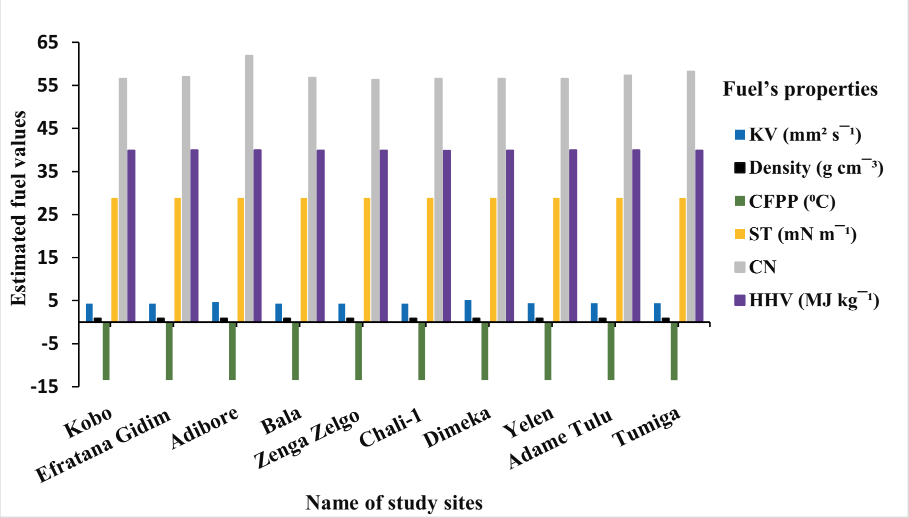

Fig. 7. Fuel property of *J. curcas* biodiesel synthesized from oils different study sites.

4.2. Effect of alkaline pretreatment and co-digestion with CG on the methane yield of JCPC (Paper III)

JCPC is one of the major biowastes generated during the oil extraction process. The press cake contains higher organic content that could be considered as a potential feedstock for biogas production. However, JCPC is enriched with cellulose, hemicellulose, and lignin contents that potentially affect the hydrolysis stage of the AD process. Similarly, the carbon to nitrogen ratio (C/N) of the press cake was estimated to be 12:1, which is significantly lower than the optimum ratio (20:1-30:1) suggested for a stable AD process. Substrates with higher nitrogen content may inhibit the methanogen activities through ammonia accumulation [130] and subsequently lead to process collapse [125]. On the other hand, CG generated during the biodiesel production process contains too low nitrogen content, i.e., 0.008-0.037% [44]. Thus, pretreatment could solve the problems associated with the recalcitrant nature of lignocellulosic material, while co-digesting the JCPC with CG can optimize the lower C/N ratio. Therefore, **Paper III** aimed to enhance the methane yield of JCPC through alkaline pretreatment and co-digesting with CG.

The effect of alkaline pretreatment on the methane and soluble chemical oxygen demand (sCOD) was investigated by varying NaOH concentration (X_{1A}), incubation temperature (X_{2A}), and retention time (X_{3A}). The linear, interactive, and quadratic effects of these process variables on the methane and sCOD were examined and modeled using RSM-CCD. The independent variables (X_{1A}, X_{2A}, and X_{3A}) were defined in five levels of the following ranges: X_{1A} varies between 2.64-9.36%, X_{2A} varies between 26.60 to 43.40 °C, and X_{3A} varies between 7.68-88.32 mins.

Simultaneously, the effect of the co-digestion process on the methane yield of the mixture (JCPC and CG) was investigated by varying the OL (2-7 g VS L^{-1}) and CG levels (0-4%).

4.2.1. Co-digesting JCPC with CG

The co-digestion process has significantly affected the rate of degradation and cumulative methane yield of JCPC as compared to treatments without CG. The methane yields from the co-digestion process ranged between 165.86-325.47 ml g^{-1} VS. The ANOVA module with a small p-value of < 0.001 indicates a significant methane yield variation among treatments (Table 4 of **Paper III**). All batch rectors worked at 2 g VS L^{-1} OL showed relatively higher cumulative methane yields as compared to the rest treatments (Fig. 8). However, the cumulative methane yield has declined with increasing of both OL and CG levels. More specifically, co-digesting 2% of CG with JCPC and maintaining the OL into 2g VS L^{-1} was identified as optimum conditions that provided a maximum methane yield of 325.47 ml g^{-1} VS. However, further increasing the level of CG and OL into 4% and 7 g VS L^{-1}, respectively, the methane yield was significantly declining into 165.86 ml g^{-1} VS.

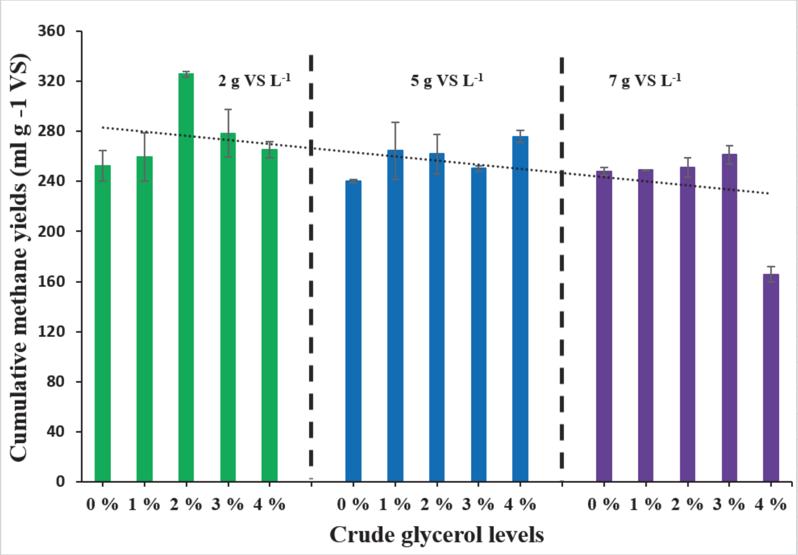

Fig. 8. Cumulative methane yield of JCPC when co-digested with various levels of CG.

The effect of CG on the mixture's methane yield was also investigated by varying the level of added CG levels at constant OL. The batch reactors that worked at lower (2 g VS L^{-1}) and higher (7 g VS L^{-1}) showed a significant cumulative methane yield variation, while the batch anaerobic digester operated at 5 g VS L^{-1} OL showed relatively similar cumulative methane yields. Similar result was reported previously [4], in which the biogas and methane yield of laying hen waste was strongly dependent on the amount of added glycerol.

4.2.2. Impact of alkaline pretreatment on methane and sCOD yields

Besides the co-digestion process, detail experimental works were conducted to enhance the methane yield of JCPC through alkaline pretreatments. The effect of alkaline pretreatment on the methane and sCOD yield of JCPC was investigated by varying the X_{1A}-X_{2A}-X_{3A} combination. Briefly, different regression models such as linear, quadratic, cubic, and two-factor interaction (2FI) models were fitted using experimental sCOD and methane yields. The second-order polynomial model was selected as the best-fitted model to depict the correlation between predictors (X_{1A}, X_{2A}, and X_{3A}) and response variables (sCOD and methane yields) (Eq. 8 and 9).

The cumulative methane yield of untreated JCPC was estimated to be 252.41 ml g^{-1} VS. Compared to the untreated sample, the methane yield increments due to the alkaline pretreatments were varied between 16.90% (295.10 ml g^{-1} VS) to 40.20% (353.90 ml g^{-1} VS) depending on the intensity of supplied X_{1A}, X_{2A}, and X_{3A}. As shown in Eq. (8), all coded linear and two interaction terms ($X_{1A}X_{2A}$ and $X_{1A}X_{3A}$) have positively affected the sCOD values, while all quadratic terms and the interaction between X_{2A} and X_{3A} were negatively correlated with the degree of solubilization. Likewise, the linear effect of X_{1A}, X_{2A}, X_{3A} and two interaction terms of $X_{1A}X_{2A}$ and $X_{1A}X_{3A}$ have a positive effect on the methane yield of JCPC, whereas all quadratic terms (X_{1A}^2, X_{2A}^2, and X_{3A}^2) and the interaction between X_{2A} and X_{3A} inversely correlated with the predicted methane yield of JCPC (Eq. 9). The absolute value of β_j from the two models indicates that X_{3A} was the most important variable that significantly affected the sCOD and methane yield of JCPC as compared to other variables.

$$
\begin{aligned}
&sCOD\ yields\ (coded) \\
&\qquad = 145.96 + 12.25X_{1A} + 6.26X_{2A} + 20.13X_{3A} + 3X_{1A}X_{2A} + 0.25X_{1A}X_{3A} \\
&\qquad - 4.5X_{2A}X_{3A} - 7.67X_{A1}^2 - 2.19X_{2A}^2 - 8.38X_{3A}^2
\end{aligned}
\tag{8}
$$

$$
\begin{aligned}
&Methane\ yields\ (coded) \\
&\qquad = 348.85 + 9.80X_{1A} + 3.64X_{2A} + 5.83X_{3A} + 1.02X_{1A}X_{2A} + 2.62X_{1A}X_{3A} \\
&\qquad - 1.48X_{2A}X_{3A} - 8.047X_{A1}^2 - 11.45X_{2A}^2 - 14.52X_{3A}^2
\end{aligned}
\tag{9}
$$

Moreover, a multivariate ANOVA was carried out to test the strength and significant effect of linear, interactive, and quadratic terms of X_{1A}, X_{2A}, and X_{3A} on the sCOD and methane yield of JCPC. The data presented in Table 6 shows that the linear impact of X_{1A} and X_{3A}, and quadratic effects from the same variables (X_{1A}^2 and X_{3A}^2) have significantly affected the sCOD yield of JCPC. Likewise, the linear and quadratic effects of X_{1A}, X_{2A}, and X_{3A} on the methane yield of JCPC were found to be statistically significant. However, the interaction effects between the three process variables ($X_{1A}X_{2A}$, $X_{1A}X_{3A}$, and $X_{2A}X_{3A}$) were weak and inadequately correlated with the sCOD and methane yield of JCPC.

Table 6. ANOVA for the model regression representing the sCOD and methane yields.

		sCOD		Methane yield	
Source	**df**	**F-value**	**p-value**	**F-value**	**p-value**
Model	9	10.14	0.001*	34.10	< 0.0001*
X_{1A}	1	18.61	0.002*	57.50	< 0.0001*
X_{2A}	1	4.86	0.052	7.92	0.018*
X_{3A}	1	50.23	< 0.0001*	20.34	0.001*
$X_{1A}X_{2A}$	1	0.65	0.438	0.37	0.558
$X_{1A}X_{3A}$	1	0.006	0.948	2.41	0.152
$X_{2A}X_{3A}$	1	1.47	0.253	0.77	0.401
X_{1A}^2	1	7.70	0.019*	40.78	< 0.0001*
X_{2A}^2	1	0.63	0.446	82.69	< 0.0001*
X_{3A}^2	1	9.19	0.013*	132.98	< 0.0001*
Residual	10				
Lack of Fit	5	2.15	0.211	0.37	0.851
Pure Error	5				

4.2.3. Effect of independent parameters on the methane and sCOD yields

The effect of individual variables on the methane yield of *J. curcas* has been investigated by keeping the other two variables into their center values (Fig. 9). As shown in Fig. 9, the methane yields were increased with increasing all process variables (X_{1A}, X_{2A}, and X_{3A}) from their lower value (-α) to the center values (0), then started to decline progressively as the intensity of each process value raised into their higher ranges (+α). The actual values calculated for the axial (±α) and center points are depicted in Table 2 of **Paper III**. Increasing the applied X_{1A}, X_{2A}, and X_{3A} from their lower value to the center values resulted in a methane yield increment of 14%, 13%, and 18%, respectively, as compared to the corresponding methane yield obtained at the lower ranges (Fig 9). However, further increase of X_{1A}, X_{2A}, and X_{3A} from the center value into axial values (+α), the methane yields have declined by 1.30%, 8%, and 9.80%, respectively, as compared to the higher methane yield obtained from JCPC pretreated at the center values. In contrast, the sCOD yields were continuously increased with increasing X_{1A}, X_{2A}, and X_{3A} values (Fig. 3b, d and f of **Paper III**).

The probable reason for lower methane yield at severe pretreatment conditions could be the degradation of lignin and cellulose, and hemicellulose [222, 223]. Degradation of carbohydrates mainly results in generation of inhibitor compounds such as furans and 5-hydroxymethylfurfural, while the production of various phenolic compounds is manifested by lignin degradation. Other toxic and inhibitor compounds such as saccharinic, lactic, formic, and different dihydroxy and dicarboxylic acids could also be developed due to peeling reactions at severe alkaline pretreatment conditions [222].

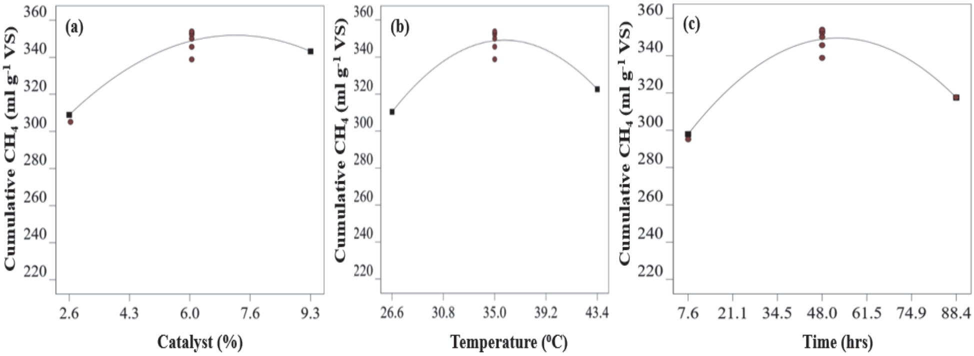

Fig. 9. Effect of individual process variable: X_{1A} (a), X_{2A} (b), and X_{3A} (c) on the methane yield of JCPC; one parameter was varied while the rests were maintained to their center value.

4.2.4. Effect of interactive factors on the methane and SCOD yields

Variation in the impact of each process variable on the methane and sCOD yield of JCPC and reduction of methane yield at severe pretreatment conditions have motivated for searching of the optimum pretreatment condition that results in higher methane yield without carbohydrate and lignin degradation. The two-way interactive effect between $X_{1A}X_{2A}$, $X_{1A}X_{3A}$, and $X_{2A}X_{3A}$ on the sCOD and methane yield of JCPC was examined by keeping the third variable into its center value. The sCOD yield has increased progressively with the simultaneous increment of two process variables at all levels while keeping the third variable into its center value (Fig. 4b, d, and f of **Paper III**). However, when the interaction between two process variables was getting more severe, the solubilization rate was decreased. In contrast, the methane yield of JCPC increased with the increasing of two process variables from their lower range (-α) to center values (Fig, 4a, c and e of **Paper III**). However, the methane yields of JCPC have been significantly reduced when two process variables interacted at more severe pretreatment conditions. Therefore, the present study defined the optimum alkaline pretreatment conditions that would result in higher methane yield without carbohydrate and lignin degradation using a second-order polynomial equation (Eq. 10) developed from the experimental value of methane yields. Thus, soaking the JCPC using 7.32% NaOH at 36 °C for 54.05 hrs was identified as optimum conditions predicted for maximum methane yield increment of 40.23% (353.90 ml g^{-1} VS) as compared to untreated JCPC.

$$\begin{aligned} &Methane\ yields\ (actual) \\ &\qquad = -392 + 22.83X_{1A} + 32.77X_{2A} + 2.77X_{3A} + 0.10X_{1A}X_{2A} + 0.05X_{1A}X_{3A} \\ &\qquad - 0.01X_{2A}X_{3A} - 20.01X_{A1}^2 - 0.46X_{2A}^2 - 0.03X_{3A}^2 \qquad (10) \end{aligned}$$

Finally, energy balance and benefit-cost ratio (BCR) analyses were performed to investigate the environmental soundness and economic viability of the alkaline pretreatment. The BCR analysis was performed based on two assumptions, i.e., the average market price of NaOH is assumed to be 412 €/ton [224], and the estimated cost of methane sale is 0.57 € m^{-3} [225]. Simultaneously, the energy required for alkaline (NaOH) production was considered as input energy, while the energy gained from the extra methane production was considered output energy. The extra methane obtained due to alkaline pretreatment at the optimum conditions was estimated to be 101.50 m^3 $tone^{-1}$ VS, while the alkaline required for pretreating one tone VS of JCPC was estimated to be 0.078 tone. From the energy balance calculation, the energy produced from the extra methane yield was estimated as 3270.30 KJ kg^{-1} VS. In contrast, 548.80 KJ of energy was needed to produce 0.078 kg of NaOH consumed during alkaline pretreatment of one kg VS of JCPC. Thus, the extra energy obtained after alkaline pretreatment was 5.96 times higher as compared to the energy consumed for NaOH production. Likewise, the income obtained from sales of extra methane yields due to the alkaline pretreatment (101.50 m^3 ton^{-1} VS) was estimated to be 57.86 €, while 0.078 tons of NaOH production needs 32.30 €. Therefore, the BCR obtained by dividing the total methane selling price with NaOH market cost was estimated to be 1.79, which was positive and encourage the application of alkaline pretreatment at a larger scale.

4.3. Effect of steam explosion pretreatment and co-digestion with CG on the methane yield of JCPC (Paper IV)

In this paper, the effect of SE and co-digestion with CG on the methane yield of JCPC was investigated by employing a series of pretreatment processes employed at various conditions. The SE pretreatments were performed at multiple temperatures (X_{1B}) and retention time (X_{2B}) combinations, and the linear (X_{1B} and X_{2B}), interactive ($X_{1B}X_{2B}$), and quadratic (X_{1B}^2 and X_{2B}^2) effects of each process variables on the sCOD and methane yield of JCPC were investigated and modeled using RSM-CCD. Simultaneously, the effect of co-digestion process on the methane yield of the mixture (JCPC and CG) was investigated by varying the OL and CG levels. For exploring the optimum SE pretreatment condition that results in a higher sCOD and methane yields, the X_{1B} and X_{2B} were defined in five levels of the following ranges: X_{1B} ranges between 186 to 214 °C, and X_{2B} ranges between 4.4 -15.7 mins (Table 1 of **Paper IV**). The values for R^2, adj. R^2 and pred. R^2 were used to evaluate the strength of models that best explained the correlation between predictors (X_{1B} and X_{2B}) and response (methane and sCOD yields) variables. Furthermore, JCPC was also co-digested with CG, and its impact on the methane yield of the mixture was investigated by varying the OL (2-7 g VS L^{-1}) and CG levels (0-4%), as described in Table 1 of **Paper III**. Afterward, the application of SE pretreatment and co-digestion processes at a larger scale was further evaluated against their energy balance and BCR.

4.3.1. Effect of co-digestion on the methane yield of JCPC

The co-digestion process employed at various OL and CG levels has significantly affected the methane yield of JCPC and CG. The cumulative methane yield obtained in the co-digestion process ranges between 165.87-325.47 ml g^{-1} VS. The impact of OL and CG levels on the rate of anaerobic degradation and cumulative methane yield of the mixture was highly significant on the batch reactors operated at lower (2 g VS L^{-1}) and higher (7 g VS L^{-1}) OLs. In contrast, the AD performed at the middle (5 VS L^{-1}) OL showed a small variation in cumulative methane yields. As shown in Fig. 10, the anaerobic batch reactor worked at 2g VS L^{-1} that contained 2% CG seems the optimum condition that resulted in higher cumulative methane yield (325.47 ml g^{-1} VS), while co-digesting 4% CG with JCPC and maintaining the OL into 7 g VS L^{-1} resulted in significantly a lower cumulative methane yield of 165.87 ml g^{-1} VS. Oliveira *et al.* [226] reported a similar result, which stated that co-digesting 2% CG with sargassum resulted in 18% more methane than the control treatments. In contrast, Veroneze *et al. [227]* noted that the biogas production rates from swine manure were impaired when the added glycerol raised to more than 5%.

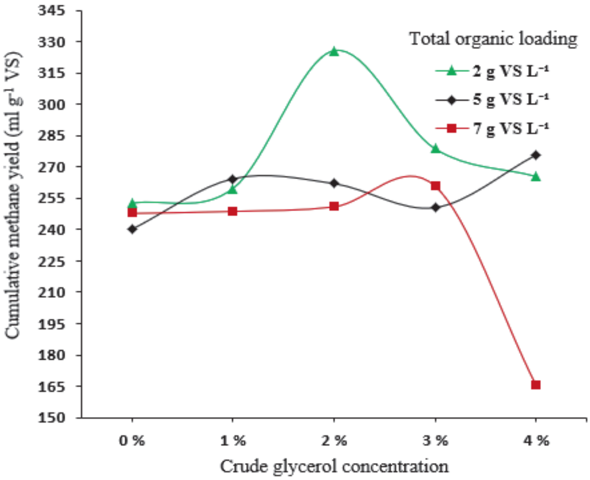

Fig. 10. The interactive effect between OL and CG levels on the cumulative methane yield of JCPC.

4.3.2. Modeling the effect of steam explosion on the methane and sCOD yields

The effects of X_{1B} and X_{2B} on the sCOD and methane yield of JCPC was examined by developing a second-order polynomial model (Eq. 11 and 12). The data displayed in Table 7 shows the significant linear, interactive, and quadratic effects of X_{1B} and X_{2B} on the methane yield of JCPC. More specifically, the linear effect of X_{1B} and the interactive effect between X_{1B} and X_{2B} were positively associated with the predicted methane yields. In contrast, the linear effect X_{2B} and quadratic effect of both variables (X_{1B}^2 and X_{2B}^2) were negatively associated with the methane yield of JCPC. Similarly, the linear and interactive effect of X_{1B} and X_{2B} on the sCOD values was positive and statistically significant at a 95% confidence interval, while the quadratic impact of X_{2B}^2 was negative and statistically significant (Table 7). The higher F-value and smaller p-values presented in Table 7 indicate that the linear and quadratic effect of X_{2B} was very important on the methane yield of JCPC as compared to other variables.

Table 7. ANOVA for the model regression representing the sCOD and methane yields.

		sCOD		Methane yield	
Source	**df**	**F-value**	**p-value**	**F-value**	**p-value**
Model	5	92.49	< 0.0001*	79.87	< 0.0001*
X_{1B}	1	152.15	< 0.0001*	20.91	<0.0026*
X_{2B}	1	254.98	< 0.0001*	41.99	0.0003*
$X_{1B}X_{2B}$	1	15.06	0.0061*	50.80	0.0002*
X_{1B}^2	1	0.56	0.4784	89.46	< 0.0001*
X_{2B}^2	1	40.25	0.0004*	228.66	< 0.0001*
Residual	7				
Lack of Fit	3	5.38	0.0689	4.82	0.0814
Pure Error	4				

$$sCOD\ yield\ (Coded) = 69.21 + 12.91X_{1B} + 16.72X_{2B} + 5.75X_{1B}X_{2B} + 0.84X_{1B}^2 - 7.12X_{2B}^2 \quad (11)$$

$$Methane\ yield\ (Coded) = 329.07 + 5.84X_{1B} - 8.27X_{2B} + 12.87X_{1B}X_{2B} - 12.95X_{1B}^2 - 20.71X_{2B}^2 \quad (12)$$

4.3.3. Effect of independent parameters on the methane and sCOD yields

The effect of individual process variables on the sCOD and methane yield of JCPC was investigated by varying the value of one variable at a time while keeping the other into its center value. As shown in Fig. 11a and b, the sCOD yield increased with increasing of X_{1B} at all levels, i.e., from 186 to 214 °C. The sCOD yield from exploded JCPC increased by 49.68% when the value of X_{1B} raised from 186 to 200 °C. However, with a further increase of X_{1B} from 200 to 214 °C, the sCOD yield increment was reduced to 28.32%. Likewise, the sCOD yield was increased by 122.46% when the value of X_{2B} increased from 4.34 to10 mins at a constant X_{1B} (200 °C), but the increment was reduced to 15.71% when X_{2B} further increases from 10 to 15.70 mins. The higher sCOD yield of JCPC after SE pretreatment may be associated with carbohydrates and lignin solubilization [228]. Unlike the sCOD yields, the increment of X_{1B} and X_{2B} from their center value (0) to higher axial points (+α) resulted in lower methane yields. Briefly, the methane yield of JCPC was increased by 13.35% when the supplied X_{1B} increases from 186 to 200 °C at a constant X_{2B}; however, with a further increment of X_{1B} from its center value to axial points (214 °C), the methane yield has significantly declined by 5.83% (Fig. 11C). Similarly, the methane yield of JCPC was increased by 11.21% when X_{2B} increases from 4.3 to 10 mins. However, the methane yields significantly declined by 19.95% when X_{2B} was further increased from 10 to 15.60 mins. Reduction of methane yields at higher X_{1B} and X_{2B} could be due to the accumulation of inhibitor compounds at severe pretreatment conditions [136]. Sever pretreatment condition caused degradation of cellulose and hemicellulose constituents of the biomass [228].

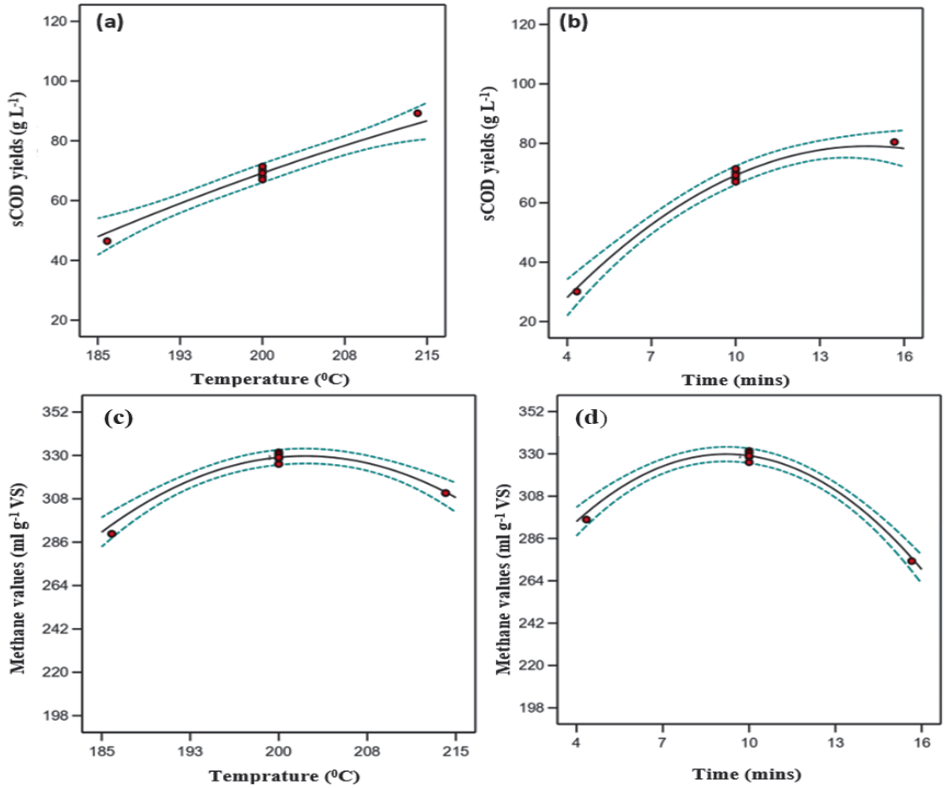

Fig. 11. Effect of individual process variable: explosion temperature (a, c) and retention time (c, d) on the sCOD (a, b) and methane (c, d) yields. One parameter was varied while another maintained to its center value.

4.3.4. Effect of interactive factor on the methane and sCOD yields

Investigating the impact of one variable on the sCOD and methane yield of JCPC by keeping the other variable into a constant value has its own limitation since the impact of one variable could be significantly modified by the other factor [228]. Therefore, it was indispensable to investigate the interaction effect between X_{1B} and X_{2B} on the methane and sCOD yield of JCPC, as shown in Fig. 5b and 7b**,** respectively**,** of **Paper IV**. The sCOD values were increased with increasing of both X_{1B} and X_{2B}. At the initial increment of X_{1B} and X_{2B}, the sCOD yield increased rapidly and reached a maximum value at the midpoint of X_{1B} and X_{2B} (approximately 200 °C and 10 mins). However, the sCOD yields started slightly decline when X_{2B} further increases at higher X_{1B}. In contrast, the effect of X_{1B} was constant in which the sCOD yield was continuously increased with increasing of X_{1B} at all level of X_{2B}.

Likewise, as shown in Fig. 5b of **Paper IV**, the methane yield of JCPC was increased with the increment of both applied X_{1B} and X_{2B} from their lower range (186 °C, 4.40 mins) to their center values (200 °C, 10 mins), and then again declined when the severity of X_{1B} and X_{2B} exceeding above the center values. The reduction of methane yields due to higher X_{2B} looked higher as compared to methane reduction due to extremes X_{1B} values (Fig. 11d). The possible reason for higher methane yield at the middle range of pretreatment conditions (200 °C, 10 mins) might be higher solubilization of hemicellulose and lignin dissolution over pretreatments carried out at lower and severe pretreatment conditions. Harsher pretreatment condition caused degradation of cellulose, hemicellulose, and lignin that manifested by the accumulation of aliphatic acids, furan aldehydes, and phenolic compounds, which are highly inhibitor and toxic for microbes involved in the AD process [136, 137, 229]. Finally, the optimum SE pretreatment conditions that would provide higher sCOD and methane yield was defined using the RSM-CCD model developed from the actual experimental values (Eq. 13 and 14). Accordingly, exploding the JCPC at 209 °C for 13.68 mins would result in a maximum sCOD yield of 94.48 g L^{-1}, while the SE pretreatment performed at 202 °C for 9.39 mins was predicted as optimum conditions for maximum methane yield of 330.14 ml g^{-1} VS.

$$\begin{aligned} sCOD\ yield(actual) &= -324.39 + 3.22X_{1B} - 15.64X_{2B} + 0.14X_{1B}X_{2B} - 0.01X_{1B}^2 \\ &\quad - 0.45X_{2B}^2 \qquad (13) \end{aligned}$$

$$\begin{aligned} Methane\ yield\ (actual) &= -4434 + 49.18X_{1B} - 40.45X_{2B} + 0.32X_{1B}X_{2B} - 0.13X_{1B}^2 \\ &\quad - 0.29X_{2B}^2 \qquad (14) \end{aligned}$$

After defining the optimum SE and co-digestion process that resulted in higher methane yield, the economic viability, and energy balance analysis was performed considering the various input and output components involved during SE pretreatment and AD processes (Table 7 of **Paper IV**). The result from the analysis revealed that the total energy required for pretreating one tone of JCPC was estimated to be 733.63 MJ, which costs 5.30 €, while one-ton dry JCPC produced 77.50 m^3 extra methane over untreated JCPC, which prices 44.18 €. The energy content of 77.50 m^3 methane was estimated to be 2497.05 MJ. Thus, the BCR obtained by dividing the revenue gained from the sales of extra methane yield by the sum of energy cost during the pretreatment process was estimated as 8.33. Besides, the energy produced from additional methane yield exceeded by 3.4 times as compared to the sum of energy consumed during the SE pretreatment process.

4.4. Effect of mechanical, steam explosion and alkaline pretreatments on the methane yield of JCFS (Paper V)

The other biowaste substantially generated during the seed process for oil extraction is JCFS. More specifically, JCFS alone shares 34-40% of the dry fruit weight [117]. However, this residue can be either be used for animal feed or organic fertilizer because of poisonous chemicals such as crucin and phorbol ester [40]. As a result, numerous studies have been conducted to convert this potential biomass into a different form of bioenergies through direct combustion [116], gasification [117], bio-briquettes production [118], and pyrolysis processes [119]. However, none of them was successful due to various environmental and technical challenges incurred during the conversion of JCFS to the mentioned energy forms. Likewise, some studies have been employed to produce biogas from the shell [120, 230], and these studies reported a lower methane yield. The lower methane yields of JCFS could be due to the recalcitrant nature of lignocelluloses materials during the AD process [43]. Therefore, the present study sought to maximize the methane yield of JCFS by applying different pretreatment methods. The effect of mechanical, alkaline, and SE pretreatment on the methane yield of JCFS was investigated and compared since each method acts differently on different parts of the material.

4.4.1.Effect of SE and Alkaline pretreatment on chemical composition of JCFS

The effect of alkaline and SE pretreatment on the compositional and methane yield of JCFS was investigated. The cellulose, hemicellulose, and lignin content of untreated JCFS were estimated to be 29.83, 13.95, and 37.21%, respectively, which motivated pretreatment actions before the anaerobic degradation process. Thus, all SE pretreatments employed at various temperatures-time combination had significantly reduced the hemicellulose content of JCFS (Fig. 12a). The estimated hemicellulose content of exploded JCFS ranged between 3.79-12.75%, and the values were negatively correlated ($R^2 = 0.85$) with the severity factor. The steam generated from the boiler combined with acetic acid produced from the acetyl groups of the biomass can accelerate the hydrolysis of hemicelluloses. In contrast, the cellulose and lignin content of exploded JCFS were linearly increased with increasing severity factors. The increased cellulose content at severe pretreatment intensity was mainly associated with the dissolution and degradation of hemicellulose [149]. Simultaneously, the condensation and repolymerization reaction between degraded compounds (e.g., furans) at higher severity factors resulted in the generation of acid-insoluble lignin fraction called pseudo-lignin [172].

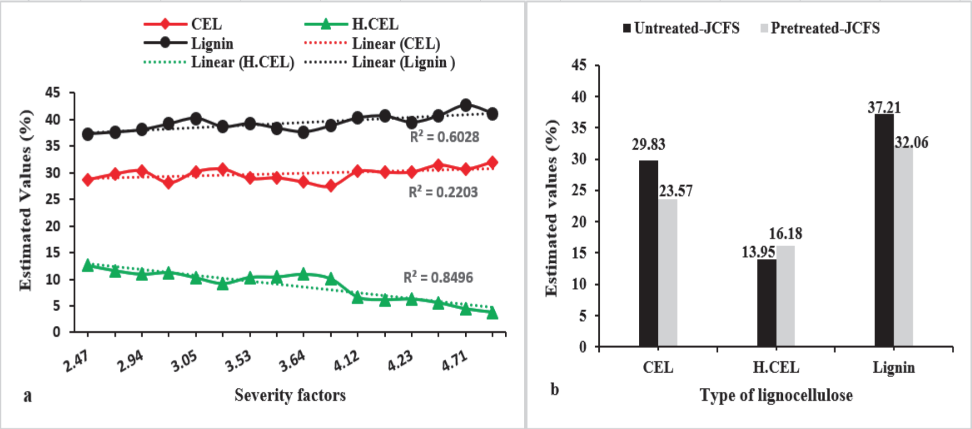

Fig. 12. Effect of SE (a) and alkaline (b) pretreatment on lignocellulosic components of JCFS (CEL is cellulose and H.CEL is hemicellulose).

The JCFS pretreated with NaOH showed significantly lower cellulose (23.57%) and higher hemicellulose (16.18%) contents as compared to the untreated and steam-exploded JCFS (Fig 12b). In contrast, the lignin content of alkaline catalyzed JCFS significantly declined by 16% compared to 37.33% estimated from untreated JCFS. The reduction of cellulose could be associated with its dissolution by the thermochemical pretreatment processes [149]. It should also be noted that cellulose could be degraded at higher NaOH concentrations and result in significant loss of carbon. It has also been reported that the cellulose content of biomass deteriorates when the alkaline concentration exceeds a specific dosage, which leads to lower methane yields.

4.4.2. Effect of mechanical pretreatment on Methane yield of JCFS

The effect of mechanical pretreatment (grinding) on the rate of anaerobic degradation and methane yield of JCFS was investigated by varying the particle size, i.e., ≤1 mm, 1-1.5 mm, and 1.5-2.0 mm. However, the optimum particle size (≤ 1mm) that showed significantly higher methane yield was reported in the present study. The methane yield of untreated JCFS was estimated to be 200.53 ml g^{-1} VS after 64 days of AD time. Interestingly, mechanical pretreatment (grinding) substantially enhanced the methane yield of JCFS by 74.23% (349.56 ml g^{-1} VS) as compared to untreated-JCFS (Fig. 13). The mechanical pretreatments can increase the pore size and surface area of lignocellulosic biomass [231]. The crystallinity and degree of cellulose polymerization could also be significantly reduced after grinding the biomass into smaller particle sizes [232]. Thus, the availability of carbohydrates for microbial degradation significantly increases. Besides methane yield enhancement, grinding also considerably reduced the time needed to obtain 80% methane potential of JCFS. Accordingly, 25 days were needed to attain more than 80% of the theoretical methane potential of JCFS, whereas the untreated-JCFS required a longer digestion time (> 64 days) to achieve the same level of theoretical methane potential. These results confirmed that mechanical pretreatment improved the rate of AD as shown in Table 3 of **Paper V**. The maximum rate of methane production from ground biomass was estimated to be 19.25 ml g^{-1} VS day^{-1}, which is significantly higher than a value calculated for untreated JCFS (8.67 ml g^{-1} VS day^{-1}).

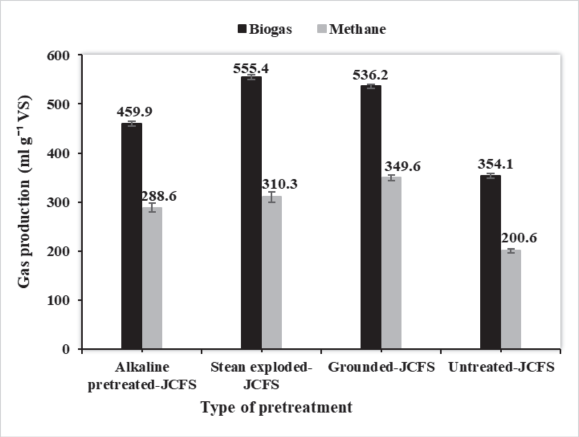

Fig. 13. Effect of various pretreatments on the biogas and methane yield of *J. curcas* residues

4.4.3. Effect of SE and Alkaline pretreatment on the methane yield of JCFS

Variation is lignocellulosic biomasses due to the steam explosion, and alkaline pretreatment has significantly affected the methane yield of JCFS. Soaking the JCFS with 7.32% NaOH solution at 36 °C for 54 hrs resulted in 288.61 ml g^{-1} VS methane yields, while the methane yields obtained from steam-exploded JCFS varied between 179.49-310.32 ml g^{-1} VS depending on the severity factors. The higher methane yield (310.32 ml g^{-1} VS) was obtained when JCFS heated at 160 ^{0}C for 5 mins (Fig. 13). However, the methane yields were significantly declined when the severity factors were getting more severe. The alkaline pretreatment caused a significant dissolution of lignin (Fig. 12b), which indicates the breakdown of complex lignocellulosic networks [149]; thus, higher methane yield was obtained after the pretreatment. The lower methane yield at severe SE conditions might be due to cellulose, hemicellulose, and lignin degradation. At severe pretreatment conditions, the hydrolytic reaction may also cause exothermic degradation of monomeric sugars. Degradation of carbohydrates is mainly manifested by the formation of furfural and 5-hydroxymethylfurfural [171]. Besides, the degradation of hemicellulose could be explained by the production of various organic acids, which could significantly reduce the pH of the digester [211]. At the severe pretreatment conditions, re-polymerization and condensation of degraded products could increase the pseudo-lignin contents (Fig. 12a), which negatively affects the overall biogas production process [146]. Therefore, all degraded compounds and secondary lignin could inhibit methane production by adversely affecting the activity of anaerobic micro-organisms.

5. Conclusions

The increased cost and severe competition with food/feed production due to the utilization of edible oil for biodiesel production have motivated researchers, governments, and industries to find new low-cost and non-edible energy oil crops. *J. curcas* is identified as a promising feedstock for biodiesel production due to its proper physicochemical composition and higher seed oil content. However, ensuring economically viable and adequate *J. curcas* oil supply for sustainable biodiesel production is not achieved yet. Biodiesel production from *J. curcas* oil is also not economically competitive with petrol diesel. The seed yield, oil content, and oil physicochemical properties of J. curcas are significantly varied across various growing areas. Seed processing and oil extraction for biodiesel production generate different residues, which could be used as a potential substrate for biogas production. Biogas production from resides could enhance the economic competitiveness of *J. curcas*. Thus, all potential components of *J. curcas* can be utilized for maintaining the economic viability of biofuel production from *J. curcas*. The present thesis investigated the biofuel production potential of *J. curcas* by explicitly focusing on the oil and various residues generated during seed processing, oil extraction, and biodiesel production process.

In **Paper I**, factors affecting the potential of *J. curcas* for sustainable biodiesel production has been critically examined using previous works. The identified factors were grouped as ecological, technological, economic, and legislative/policy barriers. Lower market opportunity, inadequate agronomic practices, small incentives from the government, absence of clear policy and legislation, ownership problem, shortage of land, and limited technology in seed collection, seed processing, and biodiesel production were the main constraints that affected the potential of J. curcas for sustainable biodiesel production. However, the type of factor and their degree of impact varies across different *J. curcas* growing regions/countries. Therefore, it was necessary to narrow the study into a specific region. Thus, **Paper II** entirely focused on investigating the suitability and potential *J. curcas* grown in various areas of Ethiopia for biodiesel production, and different biodiesel fuel properties were predicted from the fatty acid composition of the *J. curcas* oil. Ethiopian variety *J. curcas* kernel showed a higher oil content, but the oil yields have significantly varied across different growing areas. The iodine and saponification values were in the range recommended for biodiesel production. However, most sampled oils showed more than 1% FFA contents, which is not recommended for alkaline catalyzed transesterification reactions. Oleic and linoleic acids shared 76-80% of the fatty acid compassion of *J. curcas oil*, and all biodiesel fuel properties predicted from the fatty acid composition were agreed with EN 14214 standards.

The biodiesel production process from *J. curcas* generates a massive volume of JCPC, JCFS, and CG in which disposal of these residues could adversely affect the environment unless adequately managed. Interestingly, these biowastes could be used as a valuable resource for biogas production. However, JCPC and JCFS contained considerable lignocellulosic material, which acts as a barrier to biological degradation. Thus, various pretreatment techniques such as mechanical (grinding), alkaline, and SE pretreatment methods were designed and implemented to minimize the recalcitrant nature of lignocellulosic biomasses (**Paper III-V**). JCPC was pretreated using alkaline and SE pretreatments at a different incubation/explosion temperature, alkaline concentration, and retention time (**Paper III-IV**). Likewise, the effect of mechanical, SE, and alkaline pretreatments on the composition, rate of degradation, and methane yield of JCFS was examined and compared (**Paper V**).

In **Paper III**, Alkaline pretreatment was very effective in enhancing the methane yield of JCPC, and the methane yields varied depending on the intensity of supplied incubation temperature, alkaline concentration, and retention time. JCPC pretreated at lower and higher pretreatment severity resulted in lower methane yields. Thus, the pretreatment conditions that could result in higher methane yield was examined and modeled using RSM-CCD. The ANOVA model from RSM-CCD indicates that the linear and quadratic effect of NaOH concentration, incubation temperature, and retention time have significantly affected the alkaline pretreatment processes. The model indicates that pretreating the JCPC using 7.32% NaOH at 35.86 °C for 54.05 hrs would result in maximum methane production of 353.90 ml g^{-1} VS. The lower methane yield of JCPC pretreated at both lower and higher severity could be associated with lower hydrolysis and accumulation of various inhibitor compounds, respectively.

Paper IV discussed the potential of SE and the co-digestion process on the methane yield of JCPC. The SE pretreatment was employed at various explosion temperature and retention time, while the JCPC was co-digested with CG. The effect of explosion temperature and retention time on the methane and sCOD yield of JCPC was examined and modeled using RSM. Likewise, the impact of the co-digestion process on the methane yield of the mixture was assessed by varying the OL and CG levels. The result of this study showed that the linear, interactive, and quadratic effect of explosion temperature and retention time brought a significant change ($P \leq 0.05$) on the methane yield of JCPC. Increasing the explosion temperature and retention time from their lower range to the optimum levels resulted in higher methane yields. However, a further increment of severity factors caused a reduction of methane yields. Therefore, it was indispensable to define the optimum SE pretreatment that could provide higher methane yield without process inhabitation. Thus, exploding the JCPC at 202 °C for 9.39 mins was predicted as optimum conditions for maximum methane yield of 330.14 ml g^{-1} VS, which exceeded by 33.56% as compared to the methane yield obtained from untreated JCPC. Likewise, in the co-digestion processes, the methane yields of the mixture were significantly varied depending on OL and CG levels, and co-digesting 2% CG with JPC at 2g VS L^{-1} OL was the optimum condition to obtain a maximum methane yield of 325.25 ml g^{-1} VS. Thus, considering the economic advantages and environmental soundness, co-digesting JCPC with CG was suggested as the best option for improving the mixture's methane yield as compared to SE pretreatments.

Paper III and IV clearly indicate that the methane yield of JCPC was significantly affected by the type of pretreatment and pretreatment process conditions since each method acted differently on different parts of the material. Thus, **Paper V** investigated the comparative effect of mechanical, SE, and alkaline pretreatments on the composition, rate of degradation, and methane yield of JCFS. All pretreatment techniques have significantly improved the rate of anaerobic degradation and methane yield of JCFS. Considering the optimum conditions obtained in the present study, mechanical, SE, and alkaline pretreatment increased the methane yield of JCFS by 74.23%, 54.75%, and 43.92%, respectively, as compared to the untreated sample (200.55 ml g^{-1} VS). The methane yield and compositional change of JCFS were significantly affected by the severity of the supplied explosion temperature and retention time. SE pretreatment was powerful in hemicellulose dissolution; however, the methane yields have been progressively declined with increased severity factors. In contrast, alkaline pretreatment was effective in lignin removal, but the methane yield obtained after alkaline pretreatment was lower as compared to mechanical and SE pretreatments.

6. Futures perspectives

The numerous research papers reviewed under **Paper I** calmed that *J. curcas* is a drought and pest resistance tropical and subtropical species that can grow on degraded and moisture stress areas. However, there is no concurrent evidence that showed the actual nutrient and water requirements of *J. curcas* for securing economically viable seed production for sustainable biodiesel production. The seed production of *J. curcas* grown in degraded and moisture stress areas was lower than the expected yields. Therefore, for commercial and economical cultivation of *J. curcas* for sustainable biodiesel production, its agronomic performance, nutrients, and water requirement, as well as its pest and disease vulnerability, should be investigated rather than calming as '*J. curcas* is drought and disease-resistant plants'. The results presented in **Paper II** showed that the oil yield of *J. curcas* kernel oil and its various physicochemical properties varied across studied areas despite the effect of altitudinal variation was not significant. However, this study did not examine the actual factors that contributed to oil yield and its physicochemical variation. Considering this gap, future studies should focus on the identification and examination of main factors affecting the potential of *J. curcas* for sustainable biodiesel production. The *J. curcas* examined in **Paper II** of this thesis is wild variety with high heterozygosity. Thus, J. curcas should be domesticated to select proper varieties for higher seed yielding with better oil contents. Intensive experimental works are also needed on the molecular and genetic improvement for securing adequate and quality feedstock for sustainable biodiesel production.

In **Paper III**, alkaline pretreatment was identified as the best option to improve the methane yield of JCPC as compared to the co-digestion process. To reduce the downstream processing cost and effective utilization of the catalyst, future research work in this area should focus on the recovery of NaOH from the hydrolysate using different techniques. **In Paper IV**, the preliminary energy balance and BCR analysis motivated the application of steam explosion pretreatment for enhancing the methane yield of JCPC. However, the investigation was not considered energies consumed for heating the air and reactor itself, as well as potential energy losses during rapid decomposition of steam, air, liquid water, and dry biomasses. Thus, future study should include all input and output energy components while analyzing the energy balance and BCR of steam explosion pretreatment process. In **Paper V**, mechanical (grinding) pretreatment was effective for enhancing the methane yield of JCFS over steam explosion and alkaline pretreatments. However, the energy balance and economic viability of the mechanical pretreatment were not discussed. Therefore, future studies should assess the energy consumption and economic viability of the mechanical pretreatment prior to applying for large scale application.

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Errata list

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18 February 2021

List of errata:			
Page	Line	To be changed from	To be changed to
v	33	PCFS	JCFS
vi	1	JCPC	JCFS
13	10	… and the produced biogas could be used converted …	… and the produced biogas could be converted …
33	8	groped	grouped
36	23	…temperature (X_{2A} and X_{2A})	…temperature (X_{2A} and X_{1B})
36	31	$X_{1A}X_{2A}$	$X_{1A}X_{3A}$
62	24	… various explosion pretreatment and retention time…	… various explosion temperature and retention time…

Paper I

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Factors affecting the potential of *Jatropha curcas* for sustainable biodiesel production: A critical review

Gebresilassie Asnake Ewunie [a,*], John Morken [a], Odd Ivar Lekang [a], Zerihun Demrew Yigezu [b]

[a] *Department of Science and Technology, Norwegian University of Life Science (NMBU), P. O. Box, As, N-1432, Norway*
[b] *College of Agriculture, Hawassa University, P. O. Box, 05, Hawassa, Ethiopia*

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ABSTRACT

Scarcity, insecurity, and severe environmental impact of fossil fuel-based energy consumption have enthused the production and utilization of alternative energy resources. Biodiesel is identified as promising renewable energy that can substitute the petrol diesel consumption with numerous advantages. However, more than 95% of biodiesel is produced from edible oil crops, which jeopardizes the food supplies. As a result, exploring inexpensive and non-edible oil-bearing energy crops such as *Jatropha curcas* (Jatropha) has been the target of governments, researchers, industries, and policymakers. However, sustainable biodiesel production from this plant is not achieved yet due to various ecological, socioeconomic, legislative, and technological factors. Previous reports showed that the individual impact of those factors; however, all factors are strongly correlated, and the impact of one factor is significantly affected by the situation of other factors. Therefore, the present review is devoted to critically examine and discuss the sole and interactive effect of various factors affecting the cultivation of Jatropha for sustainable biodiesel production by reviewing more than 185 published articles. Various oil extraction and biodiesel production technologies and factors affecting the physicochemical properties of Jatropha oil and biodiesel were profoundly investigated. Moreover, the performance, combustion, and emission characteristic of diesel engines fuelled with Jatropha biodiesel were carefully reviewed and compared with petrol diesel. In conclusion, factors affecting the sustainable biodiesel production potential of Jatropha vary across growing regions due to variation in determinants, and the performance and emission characteristic of diesel engines fuelled with Jatropha biodiesel slightly differed from petrol diesel.

1. Introduction

In recent years, energy demand is steadily increasing due to modernization and industrial expansion. The world energy consumption was doubled between 1973 and 2013 [1]. It is also predicted that the global energy demand will increase by 49% in 2030 [2]. However, 86.4% of energy demand is still relayed on fossil fuel and nuclear power [1]. The transportation sector has utilized 60–65% of the total oil production, and oil consumption is continuously increasing [3]. On the other hand, petroleum reserves are depleting at an alarming rate [4,5], and the reservation could be drained within the coming 45 years [6]. In addition to its scarcity, relying on fossil fuel-based energies resulted in the release of exhaust gases such as oxides of nitrogen (NOx), carbon monoxide (CO), unburned hydrocarbons (UBHC), carbon dioxide (CO_2), and particulate matter (PM). Demirbas and Karslioglu [7] estimated that 4.1 million metrics tons of CO_2 were emitted into the atmosphere within 2007–2020 alone. The transportation sector alone accounts for 98% of CO_2 emission. The emission of CO_2 is further projected to increase by 8.6 million metric tons between 2020 and 2035 [8]. Therefore, developing greenhouse gases (GHGs) emission reduction and mitigation measures become global priorities since they cause global warming by trapping the infrared radiation. In conclusion, energy insecurity, depletion of fossil fuel reserves, and the environmental impact of fossil-based energy sources have motivated for searching of alternative energy resources that are renewable and environmentally benign [8,9].

Despite the fact that many efforts are made to expand alternative energy resources utilization, renewable energy sources contribute only 13.5–17.9% of the global energy consumption [1,10]. The share of renewable energy in Africa is reported to be 50%, which is significantly higher than other continents. However, renewable energy estimation in developing countries consider the traditional biomass energy sources such as firewood, crop residue, animal dung and charcoals that utilized

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[*] Corresponding author.
E-mail address: asnake.ewunie.gebresilassie@nmbu.no (G.A. Ewunie).

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by more than 2 billion inhabitants [11]. Unfortunately, energy production form these traditional biosources are neither sustainable nor environmentally benign. The various indoor air pollutants released due to direct burn of solid biomass accounts for 3.7–6.6% disease burden, which is the 4th risk factor next to malnutrition, HIV/AIDS, and malaria [12–14]. For instance, the annual number of deaths caused by acute respiratory infections due to indoor air pollutants was estimated to be 1.3–4.3 million [14,15]. Moreover, the use of firewood and charcoal for domestic energy production has resulted in massive deforestation and emission of greenhouse gases, while direct burning of animal dung and crop residues triggered land degradation and soil nutrient depletion [14–16].

For maintaining the economic, social, and environmental sustainability of biobased energy production, the traditional solid fuels should be converted into value-added and modern biofuels such as bioethanol, biodiesel, and biogas rather than direct combustion. Moreover, biofuel production and utilization from locally available biomass offer energy security, agriculture development, and job opportunities [17]. Among biofuel, biodiesel has been identified as versatile fuel for diesel engines applications [5]. Biodiesel refers to the mono-alkyl esters of long fatty acids (C14–C24) with desirable renewability, biodegradability, low sulfur content, and excellent lubricity [18–20]. Biodiesel has an improved cetane number (CN) and flashpoint, and relatively lower calorific values and cold flow property over petrol diesel [21]. The higher CN in biodiesel allows safe storage and transport of the fuel. Utilizing the biodiesel as a source of energy is also reported to reduce CO_2 emissions by 78% as compared to fossil diesel [22]. Furthermore, biodiesel can reduce particulate UNHC and PM emissions due to its higher oxygen content [8].

The global biodiesel production was around 36 billion liters in 2017, while the annual biodiesel production of the European Union (EU) and USA is estimated to be 13.5 and 6.9 billion liters, respectively. For the last two-three decades, 350 oil-bearing crops have been identified for biodiesel production [23]. However, securing adequate feedstock for sustainable biodiesel production has been the most challenging task [20]. Studies indicate that more than 95% of biodiesel is produced from edible oils such as rapeseed, sunflower, maize, soybean, palm tree, coconut, and linseed oils [9,24–26]. Biodiesel production from edible crops is expensive and brought food shortage as compared to non-edible vegetable oils [5]. Large scale biodiesel production requires the cultivation of these energy crops on arable and forest lands, which results in food crises, deforestation, loss of vital soil resources, and loss of biodiversity [27]. As a result, inexpensive, non-edible, and sustainable alternative oil-bearing energy crops and by-products have been assessed and identified in different parts of the world [2,28]. *Jatropha curcas* L (Jatropha hereafter), *Madhuca indica*, castor bean, sea mango, algae, *Sterculia foetida, Ceiba pentandra,* waste cooking oils, low-quality animal fats, and side-streams from oil refinery were identified as a non-edible low-cost feedstock for sustainable biodiesel production [4,29–32].

Jatropha has been identified as a promising energy crop with good quality oil and lower processing cost than other feedstocks [33]. The plant has higher adaptability in moistures stress and degraded land with higher seed oil content. The higher percentage of mono and di-unsaturation fatty acid composition promotes the utilization of Jatropha oil for biodiesel production [34]. Jatropha can also improve the structure and quality of the ecosystem when planted on degraded lands [35]. The biodiesel produced from Jatropha is successfully utilized in a diesel engine without major modification [36]. The application of J. curcas oil and biodiesel could be further extended for heat, light, and electric production [37], which is good news for rural communities who are not connected to the national electricity grid.

Despite many advantages in the cultivation and utilization of Jatropha for various applications, many Jatropha cultivars and biodiesel producer companies across the world have terminated their investment [38]. More specifically, sufficient and cheap feedstock supply is the major uncertain factors that is affecting the success of Jatropha biodiesel projects since feedstock price account for about 70–80% of overall biodiesel production cost [39,40]. Various ecological [41,42], environmental [43,44], social [45–48], economical [41,45,49], legislative [43,44], and technological [45] factors are reported as the main factors that potentially contributed to the failure of many Jatropha projects established across various counters. As a result, various studies have been employed to investigate the sole effect of these individual factors. However, factors that are affecting the cultivation, oil extraction and biodiesel production process are strongly inter-correlated [50], and the impact of one agent is significantly modified by the other factor/s [51]. For example, if low or no income is mentioned as a barrier for continued cultivation, this lack of income may be due to low yields caused by nutrients and water scarcity of the area, while the low oil and biodiesel quality and yields might be due to improper usage of extraction and production technologies. However, as the authors' information, there are no adequate reports that showed both the sole and interactive effect of all potential factors affecting the potential of Jatropha for sustainable biodiesel production rather than focusing on a single factor. Moreover, there is no comprehensive review on the level of variation in yield, composition, and physicochemical property of oil and biodiesel produced from Jatropha grown in various tropical and subtropical regions. Some of the motives and initiatives to write this review are shown in Table 1.

This review is, therefore, all-inclusive and critically examined and discussed all factors that are affecting the sustainable biodiesel production potential of Jatropha; thus, the suitability and potential of Jatropha for large-scale biodiesel production were evaluated against the ecological, economic, social, technological, and legislative aspects of growing regions. Moreover, this review examined and compared the performance, combustion and emission characteristic of diesel engines fuelled with Jatropha biodiesel and its blends. Relevant information was collected from published and unpublished documents, and the lesson gleaned from this review could be used as sources of information for sustainable biodiesel production from Jatropha.

2. Challenges and opportunities in Jatropha cultivation

Jatropha is a fast-growing small tree or large perennial shrub of up to 3–7 m height, and it belongs to the family of Euphorbiaceae [31,59]. The plant is native to tropical Mexico and Central America, and it is widely distributed in Asia and Africa [2,24,25,60]. Many claims have been made about Jatropha as a potential biodiesel crop, and it has been hailed as a 'miracle crop' that can resist drought and grow well on 'marginal land' [61]. The deep taproot and shallow lateral roots of Jatropha improve soil stabilization against landslides and soil erosion caused by wind and water [36]. Growing the plant on marginal land also eliminates competition with arable land utilized for food crop cultivation. It has also been described as a 'miracle tree' that can alleviate energy crisis and generate income in rural areas of developing countries [62]. The species is also called 'green gold', which implies its superiority in terms of environmental friendliness and the economic gain over any other conventional biofuel crops grown in tropical climates [63]. It is sometimes described as a 'new magic bullet' that can quickly treat the complex prevailing problems of energy security, climate change, and rural development [64]. As a result, Jatropha has been considered as a promising tropical and subtropical plant for sustainable biodiesel production due to its multiple advantages and many favorable physicochemical properties.

The plant has a life expectancy of over 49 years [31,33], and active for seed production for 30–40 years [65]. Seed sowing and stem cutting are the best methods for Jatropha propagation [36]. The average time duration for first flowering since plating is 12 months, and seeds are ready for harvesting within 90 days after flowering [66]. The water and fertilizer requirements of Jatropha are lower as compared to other feedstocks. Jatropha needs relatively lower agronomic practices such as weeding, pest/disease control, and pruning/thinning. It can grow and

Table 1
Factors affecting the sustainable biodiesel production from Jatropha and identified research gaps.

Factor category	Main targets of the previous review (focused points)	Identified gaps for assessing the potential of Jatropha for sustainable biodiesel production.
Policies and institutional aspects	Barriers related to planning, institutional setups, police barriers, community participation, compensation practices, and involvement of civil society and chiefs [51].	Ecological, technological and economic aspects, and the seed yield and its oil content are not assessed.
Biology of Jatropha	Variation in seed and oil content, genetic diversity, ecological, and the interaction between genotype and environment [52].	Technologies in oil extraction and biodiesel production, policies in place that promote biodiesel production and consumption, and the economy of Jatropha biodiesel are not intensively discussed
Technical, ecological and performance characteristics	Jatropha cultivation, land requirement, the productivity of Jatropha plantation, methods employed for utilizing Jatropha oil as fuel, advantage and disadvantage of biodiesel, emissions from biodiesel and diesel [53].	Variation in yield, composition, and physicochemical properties of Jatropha oil and biodiesel, the social, economic, and policy-related issues in the cultivation of Jatropha are not addressed.
Economical, technological and environmental aspects	Properties of Jatropha oil and biodiesel, biodiesel production technologies, economic and, environmental aspect of biodiesel production and utilization [54].	The impact of institutions, social, legislatives, and physicochemical properties of biodiesel produced from Jatropha oil are missed.
Uses and ecological characteristics of Jatropha	Taxonomy and botany, ecological requirements, propagation method, the toxicity of seed, and multiple uses of Jatropha [55].	Technologies in oil extraction and biodiesel production, the economy of Jatropha biodiesel, factors affecting the cultivation and biodiesel production, and the various physicochemical properties of oil and biodiesel are not widely reviewed.
Biology of Jatropha	Biology, genetic,seed yield variability, and factors affection these components [56].	Technologies in oil extraction and biodiesel production, characteristic of Jatropha oil and biodiesel, techno-economic, and socio-economic factors are not addressed.
Techno-economic and ecology of Jatropha	Cultivation, land and soil requirement, techno-economic viability and multiple-use of Jatropha and its residues [57].	Alternative technologies in oil extraction and biodiesel production, physicochemical properties of Jatropha oil and biodiesel, potential social and environmental impacts during oil extraction and biodiesel production process are not examined.
Technological, ecological and technical aspects	Physicochemical properties, ecology and distribution, biodiesel production process, fuel properties, performance and GHG emissions characteristics [4].	The various technologies in oil extraction and biodiesel production, factors affecting sustainable biodiesel production, and the ecological requirement of Jatropha are not intensely discussed.
Technological aspects	Botanical description, technologies for Jatropha seed processing and pretreatment, use of Jatropha byproducts and seed storage [58].	This study focused only on the available technologies for seed processing for biodiesel production; the cost effectiveness and accessibility of these technologies are not widely discussed.

thrive under broader environmental conditions such as temperature (18–40 °C), rainfall (250–3000 mm), soil type (saline, sandy, and gravelly), soil nutrients (nitrogen, phosphorus, and potassium), soil depth (not less than 45 cm) and altitude (500-2150 m.a.s.l) [31,59,67]. However, Jatropha is highly susceptible to frost and very low temperature [68]. Five years old Jatropha can give five tones ha^{-1} year^{-1} seeds [33] with acceptable oil content [69]. Jatropha is pests and disease resistance plant [67], but many studies also claimed that Jatropha is highly vulnerable to disease and pest attacks [70–72]. Jatropha is ranked third place after palm and coconut species in terms of oil yield (2.236 L oil tree^{-1}) [73]. Jatropha oil is non-edible, toxic, and contains a higher level of oleic and linoleic fatty acids suitable for fatty acid alkyl ester (biodiesel) production [74].

The cost of Jatropha seed is relatively lower than that of edible oils, although it accounts for 75% of the total operating price in biodiesel production [42]. Although the seed yield is lower than the anticipated, Jatropha can grow on marginal land without competing with arable land for food crop production [75]. The cost associated with the land lease and farming equipment is relatively lower over most biodiesel feedstock. Planting of Jatropha is easy since the propagation could be employed by seed sowing or stem cuttings. Overall, the above characteristics have inspired the cultivation of Jatropha for biodiesel production and other applications. As a result, numerous Jatropha projects have been implemented during the last few decades in Africa, Asia, and Latin America by local and foreign companies [56].

However, the experience of several countries shows that there is no adequate and successful cultivation and biodiesel production from Jatropha. Many companies that invested in Jatropha cultivation and biodiesel production have ceased or suspended their investments after a few years of operation. Most investment deals have been carried out in a non-transparent, piecemeal, and fragmented manner and, stakeholder's involvement was minimal [12]. Furthermore, studies indicate that lower seed yield than the anticipated production contributed to the termination of many Jatropha projects established in various regions [56]. The agricultural characteristic of Jatropha is overrated, and the lower seed yield is mostly associated with growing Jatropha in areas that possessed poor and infertile soil quality. A study conducted on a large-scale Jatropha plantation in Ethiopia revealed that moisture stress, poor soil quality, and poor agronomic performance of the area were the major identified factors for the termination of many Jatropha projects [76]. In turn, the lower productivity of the area was directly associated with moisture stress since the average annual rainfall of the area was less than 750 mm. In a semi-arid environment with poor soil quality, the achievable seed yields were estimated to be 2–3 tones ha^{-1} year^{-1} lower than the expected yield [33]. In contrast, areas with suitable soil type and moderate rainfall (900–1200 ml) can produce more than 5 tons ha^{-1} year^{-1} [77]. However, there are no universal standards for evaluating the influence of soil type and quality on the yield, quality, and composition of Jatropha seeds and its oil content. The impact of soil type and quality varies across Jatropha growing regions [78]. Most studies showed that well-drained sandy or gravelly soils with proper aeration are suitable for Jatropha production [36]. In contrast, Jatropha planted in ephemeral waterlogged soils such as vertisols or heavy clays oils showed lower seed production performance [79].

Despite the fact that various technologies are adopted for converting Jatropha oil to biodiesel [80], most techniques are successful only in the laboratory and had many limitations at a large-scale oil extraction and biodiesel production process. The seed collection and processing for oil extraction are still carried out manually, which are labor-intensive and increase the overall biodiesel production cost. A study conducted in southern India indicates that among various factors affecting Jatropha cultivation, 24% was related to the economical issues [50].

Moreover, limited markets for seed and valorization of by-products from Jatropha' value chain contributed to the failure of many Jatropha projects [71]. Many project developers and researchers underestimated the labor and field maintenance costs during project development phases over the actual expense required for establishing large-scale biodiesel plants [71]. Therefore, currently, profitability and the financial viability of Jatropha cultivation are under questions. Thus, the implementation of new Jatropha projects has been declined, and several ongoing initiative projects have been terminated [81]. In fact, numerous policy directives have been put in place for the last two/three decades that favored biofuels as a rural development strategy [51]; theire implementation on the ground is very week. The main barriers affecting the potential of Jatropha for sustainable biodiesel production across 17 growing regions are discussed and summarized in Table 2.

2.1. Factors affecting Jatropha seed yield and its oil content

To consider any feedstock for sustainable biodiesel production, sufficient seed yield from cultivated land with acceptable oil content is the main parameter [23]. Even though numerous oil extraction methods were used, only techniques that have been frequently used were selected for evaluating the oil content of Jatropha seed growing in different tropical and subtropical regions. Therefore, this review assumed that all mentioned oil extraction methods were performed in their optimum condition, and higher oil yield was obtained after each extraction. However, in reality, the oil yield of Jatropha seeds obtained after extraction was lower than the expected [56], and variation in oil extraction method could significantly affects the oil yield of Jatropha seeds. As a result, it was indispensable to evaluate the oil content of Jatropha seed either through blocking variation in extraction methods or extraction parameters. For instance, the oil content of Jatropha collected across nine regions was estimated using a conventional oil extraction method [80]. The kernel powder was heated to an average of 65 °C for 6 h without any interruption, and the estimated oil content of Jatropha kernel was significantly varied between 40.0–48.4% (Fig. 1).

Vaknin *et al.* [59] evaluated the oil content of Jatropha seeds collected from different geographical locations using near-infrared spectroscopy. The study has shown that a slight variation in seeds oil content was noted upon the growing agroecological conditions (Fig. 2). Ethiopia's origin Jatropha seeds showed relatively lower oil content as compared to other origins.

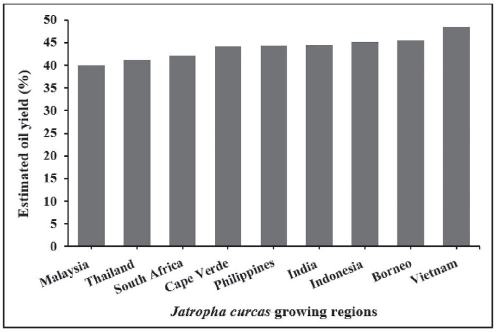

Fig. 1. Oil content (%) of Jatropha seed kernels grown in different origins [80].

Table 2
The main constraints in sustainable biodiesel production from Jatropha across tropical and subtropical regions.

Country	Identified major barriers in cultivation and biodiesel production from Jatropha	Refs.
Nicaragua	Absence of interest from the farmers, inadequate promotion of the project, deficiency of good technical advisor, lack of market for oil and byproduct selling.	[45]
Brazil	Poor agronomic practices like inappropriate management of seedling (excessive weed growth, no trimming).	[42]
Costa Rican	Very low seed yield, financial and organizational problems.	[45]
Guatemala	Limited genetic improvement, poor seed estimation, and market problem for seed and oil selling	[45]
El Salvador	Inadequate plantation management, lower seed yield, absence of risk share between farmer and investor, poor technical advisor for Jatropha farming, no information about estimated oil and seed yields.	[45]
Bolivia	Shifting of the Jatropha cultivator to other areas, absence of local biodiesel producer for a commercial purpose that could use locally harvested seeds.	[82]
Ghana	Limited cultivation and management experience, poor business planning, institutional barriers, limited community participation, unfair compensation practices, obstacles posed by civil society, and unconstructive involvement of chiefs.	[33,51]
Kenya	Monoculture and intercropping were not sustainable due to conflict with food supply, but Jatropha planted as a hedge was found to be successful.	
Senegal	Technical and economic factors, inadequate information on the management and growing of Jatropha, a limited marketing opportunity for Jatropha products.	[41]
South Africa	Intensive labor to harvest (consequently cultivation of Jatropha was not profitable), rainfall was the primary determinant for Jatropha seed yield, lack of knowledge about the plant among stockholders…	[41]
Tanzania	Absence of governmental promotion; structural, infrastructural and logistic problems, lower seed and oil price, technical skill and knowledge gaps, limited local research, cultural barriers…	[83]
Togo	Political pressure, poor microeconomic environment, lower price of seed and oil, poor agronomic practices…	[49]
China	High volume seed requirements, high capital needs, market risks, limited learning level, and inadequate knowledge sharing between Jatropha actors, weak public support and development, and reduced access to technical and managerial information…	[75]
India	The conflict between cultivator and farmers, big expectations from farmers than the actual truth, landholding issues, limited water accessibility for irrigation, and lower seed yield in the selected wasteland…	[46–48]
Ethiopia	Land resource conflict, lower seed production on moisture stressed and degraded land, technical and knowledge gaps, limited follow-up and support from the government, absence of clear policy on Jatropha investment, little participation of stockholders, ambitious biodiesel production plan, limited research on the performance and genetic improvement of Jatropha varieties, poor agronomic practices, weak management system, allocation of improper land for Jatropha cultivation, computation between food and fuel …	[43,44]
Rwanda	Lack of improved seeds, land shortage and poor soil quality, poor seed yields, high seed production cost, lower seed selling price due to lack of reliable markets, low level of sensitization among stockholders…	[65,66]
Mexico	Deterioration in the perception of Jatropha profitability, on-payment of expected subsidies and the wealth position of the household, and pest and disease damage played a significant role for abandoning of Jatropha cultivation.	[71]

Jatropha seed's oil content was changed considerably between different agroecological zones of single growing regions. Kumar and Das [84] and Tiwari *et al.* [85] showed that the oil content of Jatropha seed varied between 13.74%-54.37% and 40–60%, respectively. A study

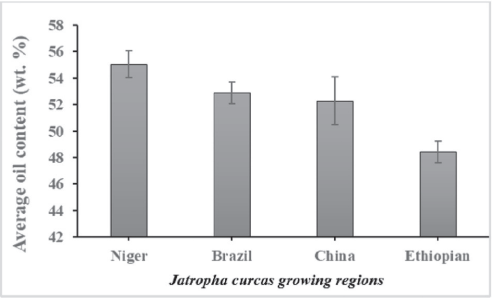

Fig. 2. Oil content of Jatropha oil detected by near-infrared spectroscopy content.

employed in Ghana has also shown variation in oil content across the various agroecological zones [2]. Jatropha grown in dry climatic conditions possessed higher oil content than those grown in a wet climates. Jatropha oil content grown in Brazil, Nigeria, China, and Congo-Brazzaville showed a significant variation [86–89]. Jingura *et al.* [67] tried to characterize the effect of land use types and agroecological conditions on the oil yield of Jatropha and found that the impact of land use was quite intense as compared to the agroecological conditions.

The seed yield and oil content of Jatropha are highly dependent on various abiotic and biotic factors [12,41,71]. The biotic factor includes genotypes, human intervention, pests, and diseases. In contrast, abiotic factors are soil properties (type, texture, depth, organic content, and pH), temperature, rainfall, and agronomic practices such as planting density, pruning, thinning, and weeding [41,71]. Jatropha is a thermophyte plant that grows better at a higher temperature and has zero tolerance to a temperature below 0 °C [68]. Furthermore, other studies have shown that Jatropha grown in temperature between 15 and 17 °C produced lower seed and oil [36]. On the other hand, the growing area's moisture content is positively correlated with the seed yield of Jatropha. However, areas that are too arid and moist compromise the growth of Jatropha [78]. Under semi-arid conditions, areas with temperature of 15–30 °C and an average annual rainfall ranging between 250 and 3000 mm and elevation bellow 500 m.a.s.l were identified ideal condition for growing production [36,67,78]. Although previous studies reported that Jatropha is highly resistant to pest and disease, many studies also claimed that there are various diseases and pests such as stem/capsule borer, sapsuckers root rot, fruit webbers, and bark eaters that could potentially affect the Jatropha plantation [70]. While *Pachycoris klugii*, *Agonosoma trilineatum*, *Scutellera nobilis*, *Pempelia morosalis*, *Stomphastis thraustica, Achaea janata* and *Oxycetonia versicolor* are the dominant pests infecting Jatropha plantation [72]. In Mexico and Central America, true bugs of the genera *Pachycoris* and *Leptoglossus* have been identified as dominant pest that affects the fruits and seed of Jatropha [71].

Pruning, thinning, and weeding are the primary perceived agronomic practices that could alter the plant's morphological and physiological properties. Furthermore, those agronomic practices result in more branches and stimulating the abundance and healthy inflorescence, thus enhancing proper fruit setting and seed yields. The genotype also another factor that affects the oil yield of Jatropha seed. Different genotypes were investigated under a controlled environment (greenhouse), and the result revealed that the oil yield and composition of Jatropha were strongly correlated with germplasms [90]. The maturity level and age of the plant at the time of harvesting could also affect the seed yield and oil content of Jatropha. The oil content of Jatropha seed increased as the seed's maturity level increased for a certain level [91]. Jatropha fruit with yellow-brown color gives higher oil yield with desirable physicochemical properties [36]. However, it should also be noted that the oil extraction methods and the applied process condition during the extraction process could also affect the estimated oil yield. For instance, Farahani *et al.* [92] (2016), the oil yield of Jatropha grown in Iran varied between 10.38% and 59.38% depending on the extraction time, extraction temperature, and solvent to solid ratios. The amount of oil estimated from Jatropha seed using different methods and process conditions are investigated and summarized in Table 3. The higher oil yields were achieved through supercritical and ultrasound-assisted solvent extraction methods, while significantly lower oil yields were obtained after mechanical oil extraction.

Table 3
Jatropha seeds oil content originated from different growing regions.

Growing regions	Estimated oil yield (wt%)	Method of oil extraction	Reference
Ghana	43.2–48.7.	Solvent extraction	[2]
Mexico	57.4	Solvent extraction	[94]
Brazil	31.6	Solvent extraction	[87]
Nigeria	47.25	Solvent extraction	[86]
China	38.90	Solvent extraction	[89]
Congo-Brazzaville	47–50	Solvent extraction	[88]
India	13.7–54.4	Solvent extraction	[84]
India	25–30	Mechanical extraction	[95]
Tanzania	26.15	Mechanical extraction	[1]
Tanzania	22.02	Traditional extraction	[1]
China	40.28	Supercritical CO_2 extraction	[96]
Indonesia	43.51	Supercritical CO_2 extraction	[97]
Spain	32.2	Supercritical CO_2 extraction	[98]
India	60	Supercritical CO_2 extraction	[93]
Iranian	10.38.59.38	Ultra-sound assisted solvent extraction	[92]
India	33.44	Aqueous enzymatic extractions after ultrasonication as a pretreatment	[99]

3. Methods for Jatropha oil extraction

Vegetable oil (triglyceride) is a promising renewable feedstock for biodiesel production. Oils from Jatropha seed has been extracted following different preprocessing methods such as shelling, drying, crushing, and grinding. The yield and purity of crude oil used for biodiesel production depend on the extraction method and process conditions (Table 3). Solvent/chemical extraction, mechanical pressing, enzymatic extraction, and supercritical fluid extraction methods have been the dominant methods for expelling the oils from Jatropha seeds/kernels [1,93]. Moreover, microwave-assisted extraction accelerated solvent extraction, and supercritical CO_2 extraction systems have been developed as new oil extraction methods [93]. The process, merit/demerit, and factors affecting each extraction technique are critically investigated and discussed in the subsequent sections.

3.1. Traditional and mechanical oil extraction

Traditional and mechanical oil extraction are dated back to several years, and these methods are not efficient in oil recovery as compared to all other extraction methods. The traditional approach for extracting the oil from the paste uses boiling water as a solvent. In traditional extraction, the seeds should be removed from the shell, dried, and then finally roasted. The roasted seeds will be grounded to appropriate size and soaked in boiling water and heated for a certain time with mechanical stirring. Then the oil is separated from the paste and filtered using different filtration techniques. It has a minimal operation cost and can be carried out by a semi-skilled operator; however, the oil recovery from traditional extraction is lower with a higher water content and volatile components [1]. According to Aboubakar *et al.* [1], the traditional extraction method recovered 67% of the available oil, which was significantly lower than the mechanical extraction methods (75%).

The mechanical method is classified as a manual ram press and engine screw press that could recover 60–65% and 75–80% of available oil, respectively [100]. Mechanical extractions are the preferred method used for commercial oil production [93]. The oil yield efficiency of mechanical extraction was found better than the traditional methods; however, it is less efficient as compared to solvent and supercritical methods. However, both traditional and mechanical extraction methods possess lower oil recovery (Table 3), and the oil contains various impurities like water, metal, and dusts that all compromise the quality of oil for biodiesel production [1,23]. Low-quality oils needed further purification before transesterification reaction, which increase the overall biodiesel production and waste treatment costs.

The effectiveness of mechanical processing is significantly influenced by specific mechanical energy and operating conditions such as screw configuration, screw rotation speed, compression speed, pressing temperature, applied pressure, pressing time, particle size, and moisture content of the seed/kernel [101,102]. Evon *et al.* [101] reported that a twin-screw extruder results in highest oil yield (70.6%) with good press cake quality over mechanical screw press. According to this study, the optimum operating conditions for this specific twin-screw extruder were 153 rpm screw rotation speed, 5.16 kg h^{-1} inlet flow rate of Jatropha seeds, 80 °C pressing temperature, and 4% seed moisture contents. The oil recovery has increased with both increasing processing temperature and pressing time [101].

3.2. Solvent extraction methods

In the standardized solvent extraction technique, the fresh solvent frequently contacts the samples, wash-out the oil from the seed/kernel powder and then drains it into the boiling flask [23]. It is expected to recover all oils in the seeds using standardized and optimized solvent extraction methods [103]. The solvent extraction method is mostly feed with kernel powder and widely applied in laboratory analysis for determining the actual oil content of studied biomass (Table 3), and its application in industrial scale is a nascent stage. A study conducted in India indicates that solvent extraction employed using the kernel was more efficient over the other methods employed on the whole seed [55]. According to this study, the estimated oil content of the kernel was 55.2%, which was significantly higher than the oil extracted from seeds with coat intact (25.5%). On average, solvent extraction process can recover 90–98% of the available oil [104]. These techniques resulted in a higher yield with less turbid oil and less water content than traditional and mechanical extraction methods [105]. However, the efficiency of solvent extraction method is significantly affected by different process variables such as solvent types and amount, extraction time and temperature, solvent to solid ratio, and particle size of the biomass [105–107].

Therefore, several studies have been conducted to define and model the optimum condition used to obtain higher yield and good quality oil from Jatropha seeds [39,88,105,107,108]. For instance, Sepidar et al. [107] has defined 8 h reaction time, 68 °C extraction temperature, 0.5–0.75 mm coarse particle size, and 6:1 v m^{-1} hexane to solid ratio as optimum conditions for getting higher oil yields. The most common solvents used for Jatropha oil extraction were n-hexane, petroleum ether, ethyl ether, pentane, isopropanol, toluene, ethyl acetate, cyclohexane, acetone, chloroform, ethanol, and methanol. N-hexane is a widely used solvent due to its low boiling temperature and low corrosiveness. However, these classical organic solvents are harmful and non-selective, which has led to searching for other oil extraction techniques [106].

3.3. Supercritical oil extraction

Recently, a new technology called supercritical CO_2 extraction has been developed for extracting oil from Jatropha seed. This technique uses supercritical fluid as a solvent. Both the temperature and pressure of supercritical fluids are higher as compared to the critical point [23]. In the supercritical extraction method, supercritical CO_2 is used as a solvent to remove oils from the seed. Higher pure CO_2 could be obtained at low costs, and it can be easily removed from the final product. Supercritical extraction techniques may need relatively higher pressure (2209 bar) and temperature (220 °C) with a specified CO_2 flow rate per unit time [109]. Thus, pressure, temperature, solvent to solid ratio, and particle size are the main parameters affecting the oil recovery efficiency of the supercritical extraction methods, and the optimum conditions were defined by numerous studies [93,96–98]. The performance of supercritical fluid extraction applied to Jatropha seed was compared to reflux extraction, microwave extraction, ultrasonic extraction, and subcritical fluid extraction [96]. The result showed that higher oil yield (40.28%) with good appearances was obtained using the supercritical fluid extraction method at a temperature and pressure of 90 °C and 0.5 MPa over other extraction methods.

According to Chen *et al.* [97], 350 bar, 60 °C temperature, and 125:1 solvent to solid ratio were found to be the optimum condition in the production of higher oil yield (43.51%) as compared to the rest treatments. In supercritical CO_2 extraction, it is not necessary to carry out a degumming and dehydration process. It can also significantly reduce the time and energy needed as compared to solvent extraction methods. Meanwhile, supercritical CO_2 extraction is also environmentally benign since zero utilization of solvent and minimal oil purification process. Supercritical fluids are sometimes considered as “super solvents,” but this is not true when their solvating power is compared to liquids. The data displayed in Table 3 showed that the oil obtained after supercritical extraction is comparable with solvent extraction but higher over mechanical and traditional methods. Supercritical extraction is fast and completed within 10–60 min, whereas liquid solvent extraction needs several hours to days [109]. However, the lower molecular affinity between solvent and solute, coupled with the higher cost for solvent and equipment, causes the supercritical extraction methods to be less efficient than the ultrasound-assisted extraction methods [110].

3.4. Ultrasound-assisted solvent extraction

The weakness of solvent and supercritical critical oil extraction methods due to improper solvent selection and insufficient agitation has been solved by developing an ultrasound-assisted extraction methods [110]. High-intensity ultrasound is a novel technology to enhance the extraction process of lipophilic/hydrophobic compounds from plant/animal materials [92]. Ultrasonication is the application of high-intensity and high-frequency sound waves that interact with the materials. High-intensity ultrasonication can accelerate heat and mass transfer, and it has been successfully tested in the oil extraction process. The ultrasound-assisted extraction can benefit biodiesel producers in multiple ways. It can enhance extraction yields and increase the aqueous extraction processes without using any solvent [85]. Moreover, the ultrasound-assisted extraction method provides the opportunity to use alternative clean and/or green solvents for improving their extraction performance. For instance, Farahani *et al.* [92] demonstrated the application of ethanol as a potential green solvent for extracting oil from Jatropha seed, and the oil yield obtained in ultrasound-assisted ethanol extraction was super higher as compared to the oil yield obtained using chloroform, acetone, n-hexane, and methanol solvents. The result showed that 59.38% of oil (relative to seed weight) was achieved through ultrasound-assisted ethanol extraction performed at 35 °C for 30 mints.

This method also enhances the extraction of heat-sensitive compounds under normal conditions that otherwise have low or unacceptable yields. An integrated technique has recently been proposed for extraction and biodiesel production from Jatropha seeds [111]. This technique used a single-stage called ultrasonic-assisted reactive extraction. It is also called as an in-situ method of transesterification of oilseeds. The ultrasonic reactor reduces processing time, catalyst amount, and alcohol consumption due to cavitation. Ultrasonication is a unique way of putting energy into chemical reactions. A combination of high-speed liquid jets, high pressure (>1013 bar) and high temperatures (>4727 °C), enormous heating and cooling rates ($>10^9$ Ks^{-1}) occur locally concentrated during the implosive compression of cavitation bubbles [112]. Kumar [111] has investigated the effectiveness of the integrated ultrasound reactive extraction/transesterification method on Jatropha oilseed with methanol in the presence of KOH as a catalyst, and the result revealed that 92% conversion was achieved under the condition of 1:100 seed/solvent (w/w) molar ratio, 1–2 mm crushed seed size, 1.5 wt% of KOH concentration, 50% ultrasonic amplitude, and 0.3 seconds cycle.

3.5. *Aqueous enzymatic extractions*

The advantage of aqueous enzymatic extraction (AEE) over solvent extraction is the use of water rather than organic solvent, which is safe, environmentally benign, and economically viable [103]. Aqueous enzymatic extraction technique can simultaneously extract the phospholipids; thus, minimal overall oil purification is needed. The oil produced by AEE has lower free fatty acid (FFA) and better oxidative stability than what is produced by other extraction methods. These techniques play an essential role in rupturing kernel cell wall, which could facilitate the oil extraction process. However, the amount of oil yield obtained after AEE is relatively lower than products estimated after solvent extraction [103]. The other disadvantage associated with AEE is the long process time. The longer processing time during AEE method has been solved using ultrasonication pretreatment before aqueous oil extraction. The maximum oil yield (33.44%) was achieved by ultrasonication of Jatropha seed for 5 min, then followed by aqueous enzymatic oil extraction using an alkaline protease at pH 9.0.

Moreover, the efficiency of enzyme assisted three-phase partitioning (TPP) extraction of oil from Jatropha kernels was investigated by Shah *et al.* [99]. Jatropha kernel was pretreated using fungal proteases at pH 9 before the TPP. The TPP extraction was then performed by mixing slurry of Jatropha kernel (5g ml^{-1}) with different amount of t-butanol and ammonium sulfate. The mixture of Jatropha slurry, t-butanol, and ammonium sulfate was then incubated at 25 °C for 1 h for the three-phase formation. The authors reported a recovery of 97% of available oil using 39% (w/v) ammonium sulfate and 1:1 (v/v) t-butanol. The Jatropha kernel pretreated with fungal proteases was produced significantly higher oil yield over the untreated sample. However, in terms of oil yield, aqueous enzymatic extraction methods were a sub-optimal, and the cost associated with enzyme is higher compared to the classical solvent extraction methods [103]. Particle size, amount of enzyme and water, pH of the mixture, incubation temperature, residence time, and shaking speed are the main process variables that mostly affected the performance of AEE.

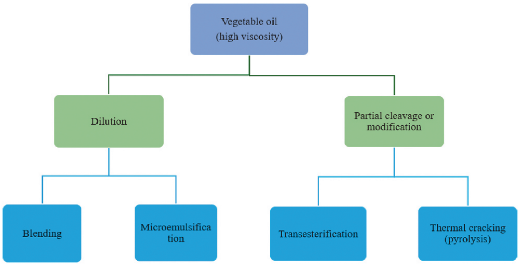

Fig. 3. Techniques employed for converting Jatropha oil to fuels for various applications.

4. Methods for utilizing Jatropha oil as engine fuel

The non-edible oil extracted from Jatropha could be used as a potential domestic energy source for cooking, lighting, and export commodity for foreign markets [1,12]. Jatropha oil has also been used as a fuel source in combustion engines without converting into other forms of fuels; however, it brought many technical, performance, and environmental problems due to its higher viscosity [7,31,113,114]. Therefore, different techniques such as blending, microemulsion, thermal cracking, and transesterification processes have been employed for utilizing Jatropha oil as a fuel for various applications (Fig. 3). The principal objective of these processes is to reduce the higher viscosity of the oil and enhance its volatility by modifying the inherent physicochemical properties of Jatropha oil [20]. Thus, in the following subsequent sections, the various techniques employed to convert Jatropha oil into engine fuels are critically examined and discussed.

4.1. *Direct use and blending of Jatropha oil with diesel*

It is well known that vegetable oils could be used as alternative fuels for diesel engines as they have high heat content. The use of virgin vegetable oil in the combustion engine dates back to 1900 by Dr. Rudolf Diesel using peanut oil as a test oil [115,116]. Likewise, numerous studies have tested the direct application of Jatropha oil in a diesel engine with or without diluting it with petrol diesel. However, the direct use of Jatropha oil in the engine was not satisfactory due to its higher viscosity, lower volatility, and lower heating value as compared to petrol diesel [111]. The viscosity of vegetable oil is 10 times higher than that of grade No. 2D diesel fuel [54,117]. High viscosity could reduce fuel atomization and leads to a weak spray of the fuel and less accurate operation of the fuel injectors [7]. Ong et al. [31] showed that higher viscosity leads to poor cold engine startup and ignition delay (ID). Wang [114] indicates that the high viscosity of Jatropha oil leads to incomplete fuel combustion due to inefficient mixing with air, which contributes to carbon deposits in the engine that further reduce the engine lifespan. Furthermore, higher viscosity of oil can cause the gelling of lubricants, filter clogging, and breakage of certain types of injection pumps [118,119]. The technical and performance problem of direct utilization of Jatropha oil in the compression engine is associated with its large molecular mass, the chemical structure of the oil, and higher viscosity [117].

The blending of Jatropha oil with diesel potentially reduces the mixture's viscosity, lower specific fuel consumption and exhaust gas emissions [18,120]. Studies indicate that 40–50% of Jatropha oil could be mixed with diesel without engine modification and preheating of the blends [121]. Tainaka et al. [113] investigated the soot formation characteristics of Jatropha oil blended with heavy fuel oil at 50/50% in a 550 kW class combustion test furnace equipped with an-industrial-scale steam atomizing burner using an optical measurement systems. The soot volume formation around the fuel nozzle was reduced when Jatropha oil was blended with heavy petrol oil, while according to Hashimoto *et al.* [122], the radiation intensity was declined when Jatropha oil was mixed with C-heavy oil. Reduction in flame radiation intensity resulted in a decrease in heat absorption near the burner. The probable reason for these results may be due to few polycyclic aromatic hydrocarbons available in Jatropha oil [113]. In contrast, Aransiola *et al.* [34] concluded that the blending of vegetable oil with diesel is neither satisfactory nor practical due to the higher viscosity, acid composition, FFA content, and gum formation due to oxidation and re-polymerization during storage and combustion. The detailed disadvantage of blending and direct utilization of vegetable oil in the unmodified diesel engine was critically examined in a previous study [20]. Therefore, various methods, such as microemulsion, pyrolysis, and transesterification processes, could solve the weakness of blended and direct utilization of Jatropha oil [123].

4.2. Microemulsification process (micro-emulsified fuel)

Among various measures developed, emulsification with alcohol is identified as a promising technique to solve the problems associated with higher viscosity of vegetable oils [7]. The microemulsion process is free from any waste generation, simple process, and employed with minimum energy consumption. NOx emission from the combustion of emulsified oil was found to be lower as compared to other fuels [20]. Oil microemulsification is a process to blend the entire oil with another liquid fuel such as diesel or biodiesel or their mixtures with or without surfactants [124]. This process, however, mostly needs surfactant as an emulsifier to prevent phase separation among diesel, Jatropha oil, and solvents such as ethanol, methanol, 1-butanol, 2-octanol, butanol, and hexanol. The suitability of emulsified Jatropha oil in diesel engines and the level of exhaust emissions were investigated by making stably emulsifiable by mixing Jatropha oil with diesel, ethanol, and surfactant (Dehydol LSI) with different ratios. The result revealed that micro-emulsified fuel has suitable properties for utilizing in a diesel engine without any problem. The kinematic viscosity of microemulsified fuel was estimated to be 4.31 to 4.55 cSt at 40 °C, which was significantly lower than that of Jatropha oil (36.62 cSt). The calorific value of the modified oil was increased, while the water content was drastically declined as compare with Jatropha oil. The emulsified oil showed comparative emission of CO and CO_2, but significantly lower smoke emission than petrol diesel [124]. No significant difference was observed in high heating value (HHV) and kinematic viscosity across different levels of the mixture during the emulsification process. However, the fuel's water content increased when the percentage of ethanol during the emulsification process increased, while the exhaust emissions were reduced as the surfactants increased. In contrast, another study showed that the application of microemulsified oils in the combustion engine causes carbon deposit, incomplete combustion, thickening of lubricating oils, and injector needle sticking [20].

4.3. Thermal cracking (pyrolysis)

Pyrolysis is defined as the cleavage of vegetable oils to a smaller molecule by adding thermal energy. The thermal cracking process was frequently used to crack the complex hydrocarbon of triglyceride into a less complicated structure. This process could be carried out with or without a catalyst at a relatively higher temperature and pressure than the conventional transesterification process [125]. Catalytic cracking is a favorable process since it could be employed at much lower temperatures than the conventional pyrolysis process. The most common catalyst used in thermal cracking of vegetable oil are Y zeolite, X zeolite [126], Na_2CO_3 [127], ZrO_2 [128], Co_3O_4, KOH, MoO_3, NiO, V_2O_5, and ZnO [129]. In the thermal cracking process, a batch reactor will be packed with raw oil and measured amount of catalyst. When the heat is supplied, the reaction proceeds faster, and then the complex structure of the triglyceride is split into a simpler structure. The yield and quality of the product are nearly identical to petrol diesel or gasoline [20], but better than the biodiesel produced using the conventional transesterification process.

Catalytic cracking resulted in high-octane gasoline, lowers the yield of heavy fuel oils and light gases [31]. However, an unoptimized cracking process would lead to the degradation of oil before converting into the targeted biofuel. Zheng et al. [130] produced a diesel/gasoline like transportation fuel by cracking Jatropha oil using ultra-stable Y zeolites catalysis at various temperatures and catalyst to-oil ratio. The result showed that higher diesel/gasoline yield was obtained at a larger mesopore volume (88 m^2 g^{-1}) of a catalyst with lower reaction temperature and catalyst to oil ratio. Biswas and Sharma [125] attempted to study the non-isothermal kinetics of cracked Jatropha oil using thermogravimetric analysis. The experiments were carried out at different heating rates of 5, 10, 15, and 20 K min^{-1} in pure nitrogen atmosphere from room temperature to 1073 K. The optimum degradations occurred in the temperature, which ranges between 623 and 753 K, and the degradations were substantially completed in the specified temperature regions. If the amount of catalyst and cracking temperature in the cracking process is out of its optimum condition, it will lead to secondary cracking reactions that form smaller hydrocarbons in the gaseous phase [131]. The disadvantage of biofuel production through pyrolysis is its higher cost, sophisticated technology requirement, and the produced biodiesel has not oxygenated value [20].

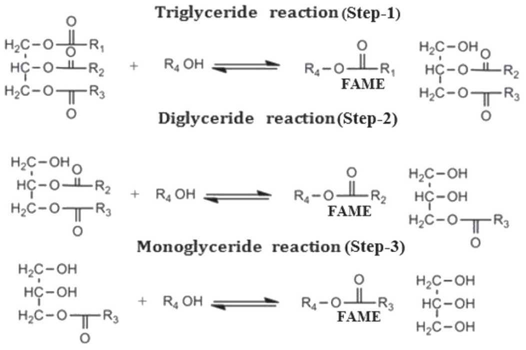

Fig. 4. The three stepwise process of transesterification reaction (adopted from Su *et al.* [133]).

4.4. Transesterification (mono alkyl ester)

In compression to all techniques, transesterification is the easiest and most cost-effective way to produce biodiesel since it results good quality fuel having nearly same physicochemical property of petrol diesel [9, 123]. Transesterification is a three-phase reversible reaction of triglyceride with primary or secondary monohydric aliphatic alcohols in the presence of a catalyst, and each stepwise reaction provided 1 mol of fatty acid methyl ester (FAME) (Fig. 4). Transesterification is the most common method to convert the triglyceride into methyl or ethyl ester with one-third molecular weight and 12% triglyceride's viscosity [7]. Ong *et al.* [31] showed a reduction of Jatropha oil's viscosity from 28.35 mm^2 s^{-1} to less than 3.1 mm^2 s^{-1} after two-step acid-base catalyzed transesterification reaction. Dubey et al. [132] demonstrated that transesterification could minimize the viscosity of Jatropha oil by 74.8% as compared to unprocessed oil. However, the reported viscosities of transesterified oils were slightly higher over petrol diesel (2.26 mm^2 s^{-1}).

Studies indicated that feedstock quality (FFA and water content), molar ratio, reaction temperature and time, catalyst type and amount, and agitation rate were the main process parameters that significantly affected the biodiesel production process [134]. As a result, numerous studies have been conducted to define the optimum condition for higher yield and good quality biodiesel from Jatropha oil [134–137]. The catalysts utilized in the biodiesel production process are categorized as homogenous catalyst (acid and base), heterogeneous solid catalysis (solid alkaline and solid acid), and enzymes. The various catalysts utilized for biodiesel production from Jatropha oil and factors affecting the transesterification reaction are examined, and the optimum conditions that resulted in higher biodiesel yield with acceptable quality are summarized in Table 4.

The alkaline catalyzed transesterification reactions are preferable as it has many advantages such as short reaction time and high biodiesel yield [134]. The most common homogenous alkaline catalysts are strong mineral bases such as sodium hydroxide (NaOH), potassium hydroxide (KOH), and the derivation of their methoxide, i.e., CH_3KO and CH_3ONa [4,20]. However, alkaline catalyzed transesterification reaction becomes the most challenging process since Jatropha oil contains higher

Table 4
The types of catalysts used in biodiesel production from *Jatropha* oil for the last two decades and defended optimum conditions.

Type of catalyst	Temperature (^{0}C)	Time (min.)	Catalyst[a] (wt %)	Molar ratio[b]	Ester (%)	Refs.
NaOH	65	2880	0.004	6:1	95	[138]
NaOH	65	180	1	9:1	99.9	[136]
KOH	65	0.17	1	6:1	90	[139]
NaOH	60	60	0.6	6:1	100	[134]
CaO	70	180	6	9:1	96.1	[140]
Na_2ZrO_3	65	180	3	16:1	99.9	[136]
Bi_2O_3–La_2O_3	150	240	2	15:1	93	[141]
CaO–La_2O_3	65	360	4	24:1	86.5	[142]
Zn_8@Fe–C_{400}	160	240	7	40:1	100	[143]
Sulphated Zr–Al_2O_3	110	180	2.5	12:1	96	[144]
Na_2ZrO_3 (in situ transesterification)	65	480	5	–	99.9	[136]
Na_2SiO_3@Ni/JRC	65	80	7	9:1	96.7	[135]
Metal salt Co $(NO_3)_2$ (cracking)	250	240	3	–	89	[145]
$CaSO_4$/Fe_2O_3–SiO_2	120	240	12	9:1	94	[137]
Sr^{2+}-CaO/MgO	65	120	5	9:1	99.6	[146]
CaMgO & CaZnO	65	360	4	15:1	>80	[147]
CaO–NiO and CaO–Nd_2O_3	65	360	5	15:1	>80	[148]
Fe–Co–Ni–MgO	55	60	15	15:1	97.9	[19]
Enterobacter aerogenes	55	2880	–	4:1	68	[149]
Lipozyme$^{@}$ IM	40	180	–	4:1	100	[150]
Thermomyces lanuginose	35	1440	0.75	15:1	81	[151]
Immobilized Candida antarctica	40	1440	14	3:1	67	[151]

[a] The density of *Jatropha* was estimated to be 926 g l^{-1} [114] for calculating the catalysis concentration in weight percent of oil.
[b] The molar ratio is determined by dividing mole of alcohol to mole of oil.

FFA contents (Table 8). The transesterification reaction catalyzed by homogeneous catalysts would form a soap that results in severe separation problems during the biodiesel purification process, which leads to the reduction of biodiesel yield and increases biodiesel purification steps; thus, the overall biodiesel production cost could be expensive. The alkaline catalyzed reaction could also generate wide waste streams during the biodiesel purification process, and the recovery and recycling of alkaline are almost impossible [20]. Thus, the costs for downstream treatment is also prohibitive. They are not also successfully used in large scale industrial applications due to various technical challenges such as equipment corrosion [141] and applied only in a batch-type reactor.

The problem associated with higher FFA in the alkaline catalyzed reaction could be solved using homogeneous acid catalysts since the acid-catalyzed esterification/transesterification is not significantly affected by higher FFA contents. The most common homogeneous acid catalysts are sulfuric acid, phosphoric acid, hydrochloric acid, and sulfonic acid. However, the acid-catalyzed rates of reaction are lower process, and the conversion of oil to ester is significantly lower than that of the homogenous catalyst. The reaction rate in liquid acid was slower by 4000 times than those catalyzed by the same amount of alkaline catalyst [4]. Therefore, various studies have conducted to substitute the conventional homogenous acid/base catalysts with less FFA-sensitive heterogeneous and enzyme catalysts (Table 4).

Heterogeneous solid base catalysts are desirable due to easy recovery, regeneration, reusing, and potential facilitation of continuous processing [19]. Heterogeneous catalysts are green and economical. Developing solid base/acid catalysts has been the interest of many researchers and industries; thus, dramatic process improvements have been observed for the last two decades for converting lower quality feedstocks into biodiesel using various heterogeneous catalysts [152]. Compared to the homogeneous catalyst, the transesterification reaction catalyzed with heterogeneous catalyst gives higher quality biodiesel with minimal side saponification process [20]. However, the higher catalyst preparation cost, along with higher reaction temperature and more extended time requirement, affects the application of heterogeneous catalysts at the industrial scales [4,20]. Heterogeneous catalysts are also manifested with lesser contact between reactants during the transesterification process; thus, a longer time is needed than the time required by homogenous catalysts. A recent study showed that the mass transfer limitation could be solved using ultrasound technologies [5]. As a result, high frequency ultrasonic ultrasound-assisted transesterification reactions have been developed to shorten the time required for mechanically stirred transesterification reaction from hour/s to minutes with minimal energy consumption [9].

Enzymatic catalysts are green, and the is no need of removal of the catalyst after the reaction. Low-quality feedstock could be converted into biodiesel using various types of enzymatic catalysts. The reaction could occur with mild temperatures and minimal energy consumption (Table 4). Therefore, enzymatic transesterification has drawn much attention from researchers since the product after biological catalysts are high pure over other catalysts. However, enzymes are expensive for industrial applications, and biodiesel production via enzymatic catalyst consumes days to complete the transesterification reaction [4,20]. The slow process during enzymatic catalyzed transesterification reaction is mainly associated with mass transfer limitation [150].

5. Physicochemical properties of Jatropha oil and biodiesel

5.1. Viscosity

The drawback of using vegetable oil as an engine fuel is associated with its higher viscosity. Viscosity is measure of liquid flow resistance due to the internal friction when one part of the fluid moving over the other. Jatropha oil is highly viscose, and the estimated viscosity ranged between 17 and 46.3 $mm^2\ s^{-1}$ depending on the measurement temperature and growing agroecological conditions (Table 5). According to Tables 5 and 6, the viscosity of Jatropha oil and biodiesel decreased as measurement temperature increases. Thus, the measurement temperature should be considered while evaluating the viscosity of Jatropha oil sampled across various growing regions. The common argument across all researchers is that the estimated viscosities were too far from the viscosity of petrol diesel (2.26 $mm^2\ s^{-1}$). The high viscosity is related to the large molecular mass and chemical structure of vegetable oils. Moreover, the freezing point and viscosity of oil are also associated with the fatty acid profile of the feedstock. Canakci and Sanli [153] noticed that the biodiesel freezing point and viscosity increase with the increase of carbon chain length and degree of saturation. Utilizing raw vegetable oil as an engine fuel has brought numerous technical, environmental, and performance problems (Section 4.1). Thus, the viscosity of oil could be reduced using various techniques such as blending with petrol-diesel,

Table 5
Physiochemical property of Jatropha oil originated from different regions.

Properties	Malaysia [154]	China [31]	India [132]	Cape-Verde [114]	Nicaragua [132]	Nigeria [154]	Ghana [2]	Borneo [80]	South-Africa [80]	Philippines [80]	Thailand [80]
Density (kg m^{-3})	940^a	915.1^a	926.0^b	926.0^b	–	–	–	914.0^d	918.0^d	913.0^d	918.0^d
Viscosity (mm^2 s^{-1})	24.5*	28.4^d	33.5^d	46.3^b	39.0^c	–	17.0^c	–	–	–	–
Flash point (°C)	225	190.5	228	240	–	–	–	–	–	–	–
Pour point (°C)	4.0	–	–	1.0	–	–	–	–	–	–	–
Cloud point (°C)	–	–	−9.0	6.0	–	–	–	–	–	–	–
Acid value (mg g^{-1})	28.0	12.8	16.8	2.2	–	–	3.5	4.6	4.6	0.9	1.0
Calorific value (MJ g^{-1})	38.7	38.9	37.6	38.9	–	–	–	40.0	39.4	38.9	39.8
IV (g I_2 100^{-1}g)	–	94.8	100.9	–	95.0	107.0	105.0	84.0	138.1	87.3	115.0
SV (mg g^{-1} oil)	–	–	213.1	–	192.0	190.0	199.0	192.0	193.4	189.5	192.9
Cetane number	–	–	56.1	–	–	–	–	56.0	42.5	33.0	47.7

a, b, c and d indicate the measurements employed at 15, 20, 30, and 40 °C, respectively; IV is iodine value; SV is saponification value; * not indicated.

Table 6
Physiochemical property of Jatropha biodiesel originated from different geographical locations.

Properties	Biodiesel produced from Jatropha						Biodiesel produced from other non-edible oils				EN 14214-standard
	Malaysia [154]	China [31]	India [89]	India [156]	India [157]	Nigeria [19]	*Sterculia foetida* [31]	*Ceiba pentandra* [31]	*Castor* [32]	*Madhuca indica* [157]	
Density (kg m^{-3})	880.0^a	838.8^a	880.2^b	875.0^b	883.0^b	867.3^a	875.0^a	856.9^a	946.1^a	870.7^a	$860–900^a$
Viscosity (mm^2 s^{-1})	4.8^d	3.91^d	7.32^b	13.88^b	4.84^d	2.31^d	6.0^d	4.15^d	15.4^d	4.2^d	$3.5–5.0^d$
Acid value (mg g^{-1})	0.40	0.24	0.04	0.37	0.45	0.59	0.14	0.83	0.63	0.54	0.5
Pour point (°C)	2.0	2.0	–	−6.0	−3.0	−10	−3.0	1.7	−30	2.67	–
cloud point (°C)	–	3	1	1	2.3	2	1.2	2.5	−18	7.67	–
Ash content (%)	0.01	–	–	0.03	–	–	0.003	0.009	–	–	<0.02
Carbon residue (%)	0.2	–	0.1	–	–	–	–	–	–	–	0.3
Flash point (°C)	135.0	161.8	170.0	168.0	162.3	142.0	160.5	163.5	194.0	195.3	>101.
Calorific value (MJ g^{-1})	39.2	40.4	40.0	39.9	38.4	–	40.2	40.5	38.3	–	–
IV (g I_2 100^{-1} g)	–	105.0	–	–	–	–	103.0	107.0	–	65	120.0
Cetane number	–	50.0	50.6	–	–	–	56.5	57.2	43.7	–	–

a, b, c and d indicate the measurements employed at 15, 20, 30, and 40 °C, respectively; EN denotes the European Committee for Standardization.

pyrolysis, micro-emulsification, and transesterification process (Fig. 3). While the data displayed in Table 6 showed that the viscosity of Jatropha oil had been significantly reduced after the transesterification reaction. Thus, the viscosity of biodiesel produced from Jatropha is comparable with *Sterculia foetida, Ceiba pentandra*, and *Madhuca indica* biodiesels, but substantially lower than biodiesel synthesized from the castor bean (Table 6).

5.2. The density of Jatropha oil and biodiesel

Studies showed that vegetable oil and biodiesel are denser and less compressible than petrol diesel [27,31,153]. The reported density of Jatropha oil ranges between 913 and 940 kg m^{-3} depending on measurement temperature and growing regions (Table 5). The density of Jatropha oil is relatively higher than the corresponding biodiesel obtained after the transesterification reaction (Table 6). For instance, the density of Chin's origin Jatropha has declined from 915.1 to 838.8 kg m^{-3} after a transesterification reaction [31]. Studies further showed that the density of oil and biodiesel are affected by the fatty acid composition of the feedstock [31,153]. Biodiesel produced from saturated fatty acids has a comparatively lower density than biodiesel produced form unsaturated fatty acids [31]. The estimated densities of biodiesel reported across various tropical and subtropical regions are presented in Table 6, and the estimated values are significantly varied across growing regions. Jatropha biodiesel possessed similar densities as biodiesel produced from various non-edible oil, but lower than the biodiesel produced from the castor bean (Table 6). The biodiesel density affects the breakup of fuel during injecting into a cylinder [31]. The higher density and less compressibility of biodiesel influence the engine's fuel injection system. Excess fuel may be injected by mass as the fuel got denser [153]. On the other hand, the amount of injected fuel, time of injection, and the injection spray pattern are adversely affected by the density and viscosity of the biodiesel [153,155]. As a result, standards like ASTM D 6750 and EN 14214 have limited the upper range of biodiesel density to be less than 900 kg m^{-3} at 15 °C (Table 6). Thus, referring to the EN 14214 standard, Jatropha biodiesel density could not significantly affect affect the performance, combustion, and emission characteristic of a conventional diesel engine.

5.3. Fatty acid profile of Jatropha oil and biodiesel

The main component of vegetable oil is triglyceride, which consists of different types of fatty acids attached to the glycerin; thus, the physicochemical property fat/oils is highly dependent on the nature of these fatty acids [158]. The fatty acid composition analysis helps to assess the quality of Jatropha biodiesel since various fuel properties such

Table 7
The fatty acid composition of Jatropha oil/biodiesel grown in different climatic conditions and provenances.

Origin	Lauric acid (C12:0)	Myristic acid (C14:0)	Palmitic acid (16:0)	Palmitoleic acid (16:1)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Other	Refs.
Nigeria	–	–	20.3	–	7.4	41.8	32.6	–	–	[86]
Brazil	–	–	0.14	–	6.1	21.8	46.4	–	–	[87]
Indonesia	1.02	0	7.01	–	1.48	45.84	42.64	–	–	[161]
India	–	–	11.88	–	7.8	42.72	30.08	0.28	–	[26]
India	–	–	11.3	–	17	12.8	46.3	–	4.7	[85]
Congo-Brazzaville	–	–	15.6	1.27	5.8	40.1	37.6	0	–	[55]
Indonesian	–	0–0.1	14.1–15.3	0–1.3	3-7-9.8	34.3–44.8	29.0–43.2	0–0.3	0–0.5	[69]
Thailand	–	–	15.2	0.7	6.8	43.6	32.2	0.2	0.3	[108]
Malaysia	0.1	0.1	13	0.7	5.8	43.5	35.4	0.3	0.2	[31]
Cape Verde	–	0.1	15.1	0.9	7.1	43.7	31.4	0.2	0.5	[162]
Nicaragua	–	0.1	13.6	0.8	7.4	34.6	42.2	0.2	0.6	[28]
China	–	–	13.23	0.85	5.4	40.62	36.99	0.22	–	[89]
Nigeria	–	–	12	–	6.87	78.28	–	2.72	–	[19]

as CN, oxidative stability, viscosity, lubricity, and the cold flow properties are directly related the fatty acid composition of vegetable oils [18]. Furthermore, a recent study showed that the performance, combustion, and emission characteristics of a diesel engine are significantly affected by the physicochemical properties of utilized biodiesels [19]. On the other word, the qualities of oil and biodiesel are directly related to degree of saturation/unsaturation. Since esterification and transesterification do not significantly alter the fatty acid composition of the parent material [39], this review is assumed that the fatty acid composition of Jatropha oil is the same as the corresponding biodiesel as shown in Table 7. Mostly the degree of saturation in fatty acid could be classified as saturated fatty acid (SFA, Cn:0), monounsaturated fatty acid (MUFA, Cn:1), and polyunsaturated fatty acid (PUFA, Cn: $\geq$2).

Knothe [159] noted that higher saturated fatty acid (C16:0 and C18:0) or polyunsaturated fatty acid (C18:2 and C18:3) adversely affect the various physicochemical properties of methyl ester. Thus, oils contain high levels of MUFA [34], lower amounts of PUFA and controlled SFA acids [89] are suggested as ideal feedstock for biodiesel production. Biodiesel made from oilseeds with high contents of SFA showed higher oxidation stability and higher heating value; however, it possessed poor cold flow properties due to solidification at higher temperatures as compared to biodiesels produced from oils having higher MUFA and PUFAs [26,160].

Jatropha oil is dominated by unsaturated fatty acids (75%) [26,59]. Islam *et al.* [80] showed that oleic and linoleic acids accounted for about 76–78% of the fatty acid composition. The primary detected fatty acids in the Jatropha oil were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C 18:1), linoleic acid (C18:2), and linolenic acid (C18:3). Moreover, myristic acid (C14:0), palmitoleic acid (C16:1), arachidic acid (C 20:0), and erucic acid (C22:1) were detected as minor components [97]. Moreover, the data displayed in Table 7 showed that the fatty acid composition of Jatropha oil/biodiesel is significantly varied across different growing regions [18]. More specifically, the fatty acid profiles of Jatropha oil are predominated by unsaturated oleic acid (12.8–48%) and linoleic acid (28.7–46.7%), and relatively less amount of saturated palmitic (0.14–20%) and stearic (1.48–17%) acids were reported. The fatty acid composition of Jatropha oil is mostly affected by the biophysical condition of growing regions and the maturity stage of the fruits at the time of harvesting [97]. Sinha [91] clearly showed that the oil yield and content of oleic and linoleic acids were significantly increased when the seeds are getting more mature. In contrast, the palmitic and linolenic acids were decreased as the seeds are getting more mature. Moreover, variation in eco-geography, agronomic practices, genotype, climate, and local conditions of the area could affect the fatty acid composition of Jatropha oils [18,163]. For instance, Kumar and Das [18] showed a significant fatty acid composition variations of Jatropha oil grown across 19 different tropical regions.

5.4. *The free fatty acid content of Jatropha oil and biodiesel*

The free fatty acid is the number of grams of fatty acid in 100 ml of oil [31], while acid value is defined as the amount of alkaline (KOH or NaOH) in milligram required to neutralize the FFA present in 1 g of oil. The FFA content of vegetable oil can affect quality of the biodiesel and determine the type and amount of catalysts used during the transesterification process [164]. Higher FFA content resulted in soap and water formation during transesterification reaction and caused incomplete ester conversion (Equ. 1). Moreover, the soap formed due to higher FFA may result in a gel that increases the viscosity of biodiesel [153]. This process could emulsify the ester with glycerin; thus, biodiesel purification becomes a challenging task. Therefore, pretreatment before the transesterification reaction is indispensable for feedstocks that contain a higher level of FFA [121].

$$\boldsymbol{R_1 - COOH(FFA) + NaHO(alkaline\ catalyst) \rightarrow R_1COONa(soap) + H_2O(water)} \quad (1)$$

Studies conducted in different parts of tropical and subtropical regions reported various FFA contents (Table 8 and Fig. 5). The FFA contents of crude Jatropha oil are far beyond the acceptable limit for the alkaline catalyzed transesterification reaction. The alkaline catalyzed transesterification reaction needs oil with less than 1% FFA content [54]. If the FFA content of the feedstock exceeds the lower limit, a two-step process (esterification and transesterification) is required [2,22,153]. For example, two-stage acid-catalyzed esterification and alkali-catalyzed transesterification reaction were performed for Jatropha oil that contained 14% [80], 15% [69], and 9.2% [89] of FFAs.

Islam [80] investigated the FFA content of Jatropha oils grown in seven tropical regions using the same processes and procedures (Fig. 5). In this study, the extraction process conditions that could affect the FFA content of the oil were kept constant, and the acid values of the oils as oleic was determined. The estimated FFA contents were significantly varied between 0.4% (in South Africa) to 2.3% (in Borneo). The possible reason for FFA variability might be associated with genotypes and growing agroecological conditions. The FFA content of oil also affected by the purity of the oil. Crude oils contain phospholipids, and gums are

Table 8
FFA content of Jatropha oil grown in different regions.

Origin	FFA content (%)	Oil grade	Reference
Indonesia	15	Crude	[69]
India	14	Crude	[85]
India	1.12–1.44	Crude	[26]
Borneo	6.39	Crude	[31]
China	8.8	Crude	[89]
Nigeria	9.2	Crude	[86]

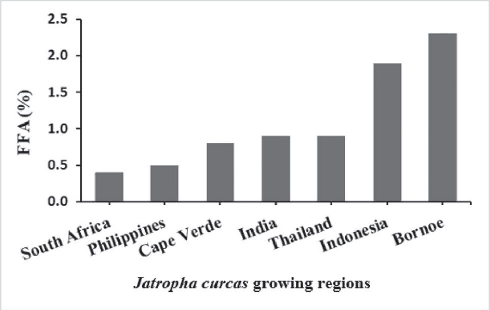

Fig. 5. Estimated FFA percentage of crud Jatropha oil across various regions [80].

susceptible to hydrolysis; thus, the FFA content would increase at the time of storage [165]. High FFA content could also be due to improper handling and storage of the seeds and oil before and after oil extraction [2]. Studies indicate that exposing the oil to open air and sunlight for a prolonged time would significantly increase FFA concentration [69].

5.5. *The saponification value of Jatropha oil and biodiesel*

Saponification value (SV) represents the base required to saponify 1 g of oil under specified conditions. SV also indicates the nature of fatty acid present in the triglyceride. The longer hydrocarbon chain in the fat/oil, hydrolyzed lesser acid liberated per gram, and hence low SV. Moreover, SV shows the average chain length of all the fatty acids present in the oil [166]. The higher SV indicates the lower fatty acid's average length and the lighter mean molecular weight of triglycerides and vice-versa. Studies showed that the average SV of Jatropha oil grown in different regions varied between 189.5 and 213.14 mg KOH g^{-1} of oil (Table 5). Higher SV caused the loss of ester yield during the transesterification reaction. For instance, Vicente *et al.* [167] showed that the saponification of triglyceride and dissolution of the ester by glycerol resulted in the loss of biodiesel yields.

5.6. *The iodine value of Jatropha oil and biodiesel*

Another important criterion for selecting methyl esters as biodiesel is its degree of unsaturation, which is measured as iodine value (IV). On the other word, IV is a measure of the unsaturated fatty acid content of oil and biodiesel that influence the oxidative stability of biodiesel when exposed to oxygen [168]. The reported IV of Jatropha oil ranges between 84 and 107 I_2 100^{-1} g of oil (Table 5). According to Ong *et al.* [31], the IV of methyl ester synthesized from Jatropha oil was estimated to be 105 g I_2 100^{-1} g, which agreed with the maximum values (120 g 100^{-1} g) of EN 14214 standards. Biodiesel produced from vegetable oil with higher level of unsaturated fatty acids (higher IV) is ideal engine fuel and could be utilized in its liquid form without solidification. However, it should also be noted that oxidation reaction could be the other challenging issue during oil/biodiesel storage as oxidation stability is inversely related to the IV. Moreover, high unsaturated fatty acid methyl ester would result in the polymerization of glycerides during the heating process, which leads to the formation of deposits and the lubricant's deterioration.

6. Fuel properties of Jatropha oil and biodiesel

6.1. *Flashpoint*

Flashpoint is the temperature at which the fuel (biodiesel) will start to burn when it comes in contact with fire. It is one of the essential fuel properties to characterize biodiesel safety in storage and transportation. Ong *et al.* [31] estimated 161.8 °C flashpoint for Jatropha biodiesel, while Dubey *et al.* [132] reported the flashpoint of crude Jatropha oil, degummed oil, and biodiesel to be 228 °C, 216 °C, and 168 °C, respectively. This implies that transesterification reaction and purification process reduced the flashpoint of Jatropha oil. As shown in Tables 5 and 6, the flashpoint of Jatropha oil and biodiesel ranges between 190.5-240 °C and 135–170 °C, respectively. The flashpoint of Jatropha oil and biodiesel were significantly higher than that of the petrol-diesel (71.5 °C). The higher flashpoint of vegetable oil and biodiesel is mostly associated with a higher content of MUFA and the non-volatility nature of oil-based fuels [153]. Thus, Jatropha biodiesel is safe in transportation and storage as compared to petrol diesel since they significantly differ in estimated flashpoints. However, as shown in Tables 5 and 6, the flashpoint value of Jatropha oil and biodiesel varied significantly across growing regions.

6.2. *Calorific value*

Calorific value (also named heating value) is a crucial indicator of the fuel energy content. Biodiesel from Jatropha oil has nearly similar calorific value as petrol diesel [114]. Ong et al. [31] investigated the calorific value of Jatropha oil and biodiesel, and the values were estimated to be 38.96 MJ kg^{-1} and 40.42 MJ kg^{-1}, respectively. Moreover, the calorific value of Jatropha oil grown in Brazil was determined to be 40.31 MJ kg^{-1} [87], while the data displayed in Table 5 showed the calorific value of Jatropha oil harvested across different tropical and tropical regions ranged between 37.6 and 40.0 MJ kg^{-1}. In contrast, Aransiola et al. [34] showed that the calorific value of biodiesel was less than by 9–12% as compared to petrol diesel. The lower heating value of Jatropha oil and biodiesel leads to higher fuel consumption than petrol diesel for similar works (Section 6.6).

6.3. *Cetane number*

Biodiesel, in general, has a higher CN than petrol diesel [117]. The CN determines biodiesel's ignition quality and is a valuable parameter for evaluating the quality of biodiesel [22]. The CN of biodiesel affects its ID [169]. The time gap between the start of injection and combustion is called ID. The higher the CN, the shorter the ID and vice versa. Biodiesel from Jatropha oil has higher CN due to its oxygen content [170]; thus, its ID time is shorter than that of the petrol-diesel [153]. A high CN results in an easier cold temperature start, lower noise, lower exhaust emissions, and minimal white smoke because of the complete combustion [171]. A study conducted in China showed that the CN of biodiesel from Jatropha oil was 50 [114]. Moreover, the CN of Jatropha oil and biodiesel reported in various studies are presented in Tables 5 and 6 and significantly varied across growing regions. The increase in carbon chain length and decrease in carbon chain branches is the higher CN values, thus shorter ID, whereas more branching and shorter carbon chains reduce the CN value [7,26]. For example, palmitate and stearate ester had a CN of >70, whereas linoleate methyl ester contains 33 CN. Studies indicated that transesterification reaction increases the CN of vegetable oil [171]. According to this study, the CN number of transesterified oils has been increased by 60–78% over the unprocessed crude oils.

6.4. *Cloud and pour point properties*

The main parameters that determine the application of biodiesel at a lower temperature are cloud point, pour point, and filter plugging point (CFPP). Cloud point refers to the minimum temperature at which the cloud of wax first becomes visible in the fuel when cooling down, while pour point is the temperature at which the wax can gel the fuel or the minimum temperature at which the fuel can freely flow [7,153]. The CFPP is described as the temperature at which the test filter starts to plug due to fuel components that begin to gel. The crystals in the fuel at a lower temperature may plug fuel lines and filters. The degree of saturation affects the biodiesel cold flow property. More saturated biodiesel is characterized by higher numbers associated with individual cold flow properties. Biodiesel dominated by unsaturated fatty acids is a good engine operating fuel at a lower temperature than fuels mainly composed of saturated fatty acids. However, the fatty acid with higher unsaturation content has lower CN, which in-turn compromise the ignition quality. de Oliveira *et al.* [87] investigated the cold flow property of Jatropha biodiesel produced in Brazil. The result revealed that the biodiesel had a pour point of −5 °C, while, according to Kartika [161], the cloud and pour point of Jatropha biodiesel were estimated to be 4 and 3 °C, respectively.

The average cold flow properties of Jatropha biodiesel reported across various geographical regions are depicted in Table 6. However, all reported cold flow properties of Jatropha biodiesel are slightly higher than petrol diesel [31,114]. The pour and cloud point of Jatropha biodiesel agrees with other biodiesels produced from non-edible oils, but significantly lower than the biodiesel synthesized from the castor bean (Table 6). The limited cold flow properties of biodiesel could be solved using branched-chain alcohols like isopropanol and 2-butanol during the transesterification reaction [169]. However, the lower reactivity of those branched alcohols results in higher impurities such as monoglycerides, diglycerides, and triglycerides in the biodiesel due to incomplete transesterification reaction [169,172,173]. Furthermore, blending biodiesel with petrol diesel and kerosene, as well as winterization, have significantly reduced the cloud and pour points of Jatropha biodiesel. For example, Nainwal *et al.* [174] reported that the cloud and pour points reduced to −1 and −2.2 °C, respectively, after the blending of biodiesel with 20% of kerosene.

6.5. *Oxidation stability*

Oxidative stability refers to the fuel's resistance to oxidation and is an important parameter that affects storage time and condition. Unfortunately, biodiesel is susceptible to oxidation degradation due to its higher oxygen content [175]. The oxidative stability of biodiesel is affected by air, light, trace metal, peroxide, and the oil's fatty acid composition [22,89]. The degree of oxidation varies among biodiesels depending on their fatty acid composition [176]. Sarin *et al.* [162] reported that the oxidative stability of linolenic acid is less than linoleic and oleic acids, while oleic acid shows improved stability against oxidation as compared to linoleic and linolenic acids. Moreover, Ong *et al.* [31] noticed that polyunsaturated fatty acids are less stable against oxidation than SAFA and MUFAs, which indicates that the position and the number of double bonds in unsaturated fatty acids affect the oxidation rate of biodiesel. High level of methyl oleate (monounsaturated), but lower saturated and polyunsaturated fatty acid content in feedstock may improve the oxidative stability, ignition quality (CN), and cold flow property of biodiesel [31,89]. Studies have also indicated that capric acid is suitable for lower temperature flow and can improve the oxidative stability of biodiesel [22,177,178]. Therefore, J curcas biodiesel could be susceptible to oxidation since the oil contains a higher level of MUFA and PUFA levels (Table 7).

6.6. *Engine performance, combustion and emission characteristics*

The diesel engine performance and combustion characteristics are affected by various processes such as atomization and evaporation of the fuel, degree of fuel-air mixing, self-ignition, oxidation, turbulence induced by air-fuel jet, and heat transfer between the fuel and the surrounding gases [179]. Thus, several experiments were done for the last two/three decades to investigate the performance, combustion, and emission characteristic of diesel engines fuelled with pure or blended Jatropha biodiesels [175,180–182]. The brake power (BP), brake torque (BT), brake thermal efficiency (BTE), brake specific fuel consumption (BSFC), higher heat release rate (HHR), peak cylinder pressure (CPr), and ID were used as a response variable for evaluating the performance of diesel engines working with Jatropha biodiesel [179]. At the same time, quantitative emission CO, NOx, UBHC, and PM were used to assess the emission characteristic of the engines [176,183]. The results obtained from most experiments revealed that the performance, combustion, and emission characteristics of internal combustion engines are mainly affected by the physicochemical properties of fuelled biodiesel, biodiesel contents, CN, injection timing and combustion, oxygen contents, engine load, engine speed, fuele density and viscosity [117]; thus, various studies are critically reviewed as described below.

Kumar *et al.* [117] have investigated the emission and performance characteristics of the compression ignition (CI) engine by varying the supplied Jatropha biodiesel levels and compression ratios between 0 and 40% and 16.5:1–18.5:1, respectively. A single-cylinder engine having 3.73 kW power and variable compression ratios was used in this experiment. The result revealed that under a higher compression ratio (18.5:1) and 75% of rated loading, 34.68% higher CPr was observed when 40% biodiesel is blended with 60% of petrol diesel. The estimated peak CPr values of engine worked with all biodiesel blend levels were relatively higher than that of engine run with petrol diesel. Likewise, at a similar compression ratio (18.5:1) and rated loading (75%) conditions, the peak HHR was increased by 26.32% when 20% biodiesel was blended with 80% petrol diesel. The higher viscosity, low volatility, and higher CN of biodiesel blends have contributed to the higher CPr values. The higher combustion rate of biodiesel accelerated by its inherent oxygen molecule could have also been positively correlated with the higher CPr value of the engine [117]. On the other hand, the higher HRR of biodiesel blend could be explained by the complete combustion of biodiesel due to the presence of oxygen in the fuel. Ganapathy *et al.* [182] also justified that the higher HRR value of biodiesel is associated with greater premixed combustion of the engine. In contrast, another study showed that the HRR of biodiesel is slightly lower than petrol diesel due to its lower calorific value, lower volatility, shorter ID, and higher viscosity [179].

Jain *et al.* [180] have investigated the performance and emission characteristic of a conventional diesel engine fuelled with oxidatively stabilized and non-stabilized Jatropha biodiesel. The BSFC of diesel engines worked with stabilized biodiesel has significantly reduced over the untreated biodiesel, but higher BSFC was noted while using both biodiesels as compared to the engine fuelled with petrol diesel. Furthermore, the engine performance characterized by Paul *et al.* [184] showed that BSFC and BTE have been increased and decreased, respectively, with the use of Jatropha biodiesel as compared to petrol diesel. Similarly, Datta *et al.* [181] noted a lower BTE for CI engine fuelled with various biodiesel-diesel blends, and the BTE has significantly reduced as increasing of biodiesel percentages in the mixture. Kumar *et al.* [176] showed that blending 20% Jatropha biodiesel with 80% petrol oil resulted in less BP and higher BSFC than the engine worked using pure petrol diesel. Fattah *et al.* [175] has investigated the effect added antioxidant on the performance and emission characteristics of a four-cylinder diesel engine fuelled with 20% Jatropha biodiesel. The experimental result revealed that the respective BP and BSFC of the engine fuelled with 20% biodiesel were declined by 0.95% and 5.02% as compared to the petrol diesel. The same argument was also reported by Datta *et al.* [181], which pointed that the BSFC fuel consumption had increased when the share of biodiesel in the blend increases, while values estimated for BTE has declined with increased blended Jatropha biodiesel. The higher BSFC of biodiesel is mostly associated with its lower heating value over petrol diesel [179]. The lower BTE values might be due to the poor atomization, lower vaporization, and weak volatility behavior of Jatropha biodiesel [182]. The density and kinematic viscosity of biodiesel could also affect its BSFC [176].

Ganapathy *et al.* [182] have investigated the impact of injection timing, load torque, and engine speed on the performance and combustion characteristic of a diesel engine fuelled with Jatropha biodiesel. The BSFC, BTE, peak CPr, maximum HRR, and emission of CO, UBHC, NOx, and smoke density values were utilized as a response variable. The result revealed that advancing the injection timing from its factory setting was significantly reduced the BSFC of the engine; however, the BTE, CPr, and HRR performances were adversely affected. At constant load torque (15 N), engine speed (1800 rpm), and crack angle ejection time (340 CAD), the reduction of BSFC was estimated to be 5.1%. In contrast, at similar conditions, the estimated values for BTE, CPr, and HRR were declined by 5.3%, 1.8%, and 26%, respectively. However, at all levels of injection timing, load torque and engine speed, the BSFC of engine worked with Jatropha biodiesel was higher than petrol diesel.

The other characteristic of biodiesel that affects the knocking characteristics of the CI engine is the ID period [185], and it is directly related to the CN of the fuelled biodiesel (Section 6.3). Biodiesel, in general, has a lower ID as compared to petrol diesel. The shorter ID is mostly manifested by decreased premixed composition and longer combustion duration over petroleum [185]. The longer combustion duration behavior of engine fuelled with biodiesel is also associated with poor atomization and mixture formation. However, some studies showed that biodiesel's combustion duration is longer than petrol diesel depends on the engine load and speed [186]. The effect of blending biodiesel with petrol diesel at various ratios has been investigated in the previous study [117]. According to this study, the delay period has significantly declined with increasing of both the shares biodiesel and rated loadings. The diesel engine fuelled with 40% biodiesel/diesel showed 21.26% less ID than the performance of the same engine fuelled with petrol diesel alone. These phenomena could be explained by a complex and rapid preflame chemical reaction that occurs at higher temperature [117]. The higher cylinder temperature that exists during fuel injection causes thermal cracking of injected fuel, and then lighter compounds were produced that can shorten the ID. While Shahabuddin *et al.* [179] pointed out that the higher CN, lower compressibility, and lower density of biodiesel causes shorter ID or earlier injection timing as compared to petrol diesel.

The application of Jatropha biodiesel as a fuel source has significantly reduced the emission CO, CO_2, UBHC, and PM due to improved combustion process [176,181]. Kumar *et al.* [117] evaluated the emission reduction potential of a diesel engine fuelled with Jatropha biodiesel blended with petrol diesel at various proportions. The result revealed that at a higher compression ratio (18.5:1) and 75% rated load conditions, the emission of CO_2 was declined by 21.26% when 40% biodiesel was blended with 60% petrol diesel [117]. Ganapathy *et al.* [182] investigated the impact of injection timing on the emission of various exhaust gasses. The result revealed that at the optimum injection timing, the percentage reduction of CO, HC, and smoke levels were estimated to be 5.1%, 2.5%, 1.2%, and 1.5%, respectively. Kumar *et al.* [176] has investigated the emission characteristic of a diesel engine fuelled with 20% biodiesel, and the result showed that the respective emission of CO and UBHC were reduced by 45.45% and 25%; however, emission NOx was increased by 7.43% as compared to petrol diesel.

Khalid *et al.* [187] have tried to improve the diesel engine emission by blending Jatropha biodiesel with petrol diesel at different ratios (5–15%). The emission reduction experiment was employed using a Dynapack chassis dynamometer operated at various speeds (1500–3000 rpm) and load test conditions (0–100%). The result indicated that blending Jatropha biodiesel with petrol diesel has significantly reduced the emission of UBHC, CO, and CO_2 without compromising the other engine performances. Another study has also examined the performance, combustion, and emission characteristics of pure and blended biodiesel produced from Jatropha at a constant speed and variable load conditions [188]. According to this study, CO, UBHC, and smoke emission were significantly reduced when Jatropha was utilized in its pure form or blended with petrol diesel. The same result was reported by Kathirvelu *et al.* [183], Kumar *et al.* [176]; Fattah *et al.* [175].

The lower emission of CO, UBHC, and particulate matter in the exhaust gas emission is associated with an improved combustion process attributed to the higher oxygen content of the biodiesel [182]. The oxygen content of the biodiesel has been estimated between 10 and 11% by weight [117,181]. The lower emission of CO, UBHC, and PM is associated with complete combustion of Jatropha biodiesel accelerated by molecular oxygen. However, the emission of NOx from biodiesel combustion was significantly higher as compared to petrol diesel. The higher NOx emission is mostly associated with a lower ID, higher biodiesel oxygen content, higher composition temperature, and longer reaction time [179]. For instance, the exhaust gas temperature of a diesel engine fuelled with biodiesel and petrol-diesel was estimated to be 320 °C and 265 °C, respectively. Thus, the higher oxygen concentration in the biodiesel enhances the combustion process. The improved combustion process then raises the engine cylinder's flame temperature, which leads to higher emission of NOx [185]. It has been also speculated that faster diffusive burring of biodiesel contributed to higher emission of NOx. As a result, studies have attempted to reduce the emission of NOx emissions by implementing various measures. Exhaust gas recirculation and reduction of in-cylinder temperatures were identified as the best option to minimize NOx emissions [189]. Exhaust gas recirculation reduced the oxygen available in the combustion chamber and increases the specific heat of intake charge, resulting in lower flame temperature. The impact of injection timing on the emission of NOx has also been investigated by Ganapathy *et al.* [182], and the result indicates that retarded injection time resulted in less NOx emission.

7. Conclusions and future directions

The highest cost and severe competition with food and feed production due to biodiesel production from edible oils have perused researchers, governments, industries, and policymakers to find new low-cost and non-edible energy oil crops. Jatropha is identified as a promising feedstock for sustainable biodiesel production due to its suitable physicochemical composition, higher seed and oil yields, and lower competition with food crops. However, securing sustainable and sufficient amounts of Jatropha oil for large scale biodiesel production is not achieved yet. Thus, the implementation of new Jatropha projects has been declined, and several ongoing initiative projects have been terminated. The cultivation of Jatropha for sustainable biodiesel production is significantly affected by various factors, which could be considered as ecological, economic, social, institutional, and technological barriers. Inadequate market opportunity, small incentives from the government, absence of clear policies and legislative, ownership problem, shortage of land, limited technology in seed collection and processing, and lower agronomic performance Jatropha seed were the main constraints affecting the cultivation of Jatropha for sustainable biodiesel production. Although Jatropha comprises suitable characteristics for biodiesel production as described in EN 14214 standards, the reported physicochemical properties of Jatropha are highly dependent and significantly affected by various biotic, abiotic, and technological factors. The Jatropha*'s* seed yield is substantially lower than the anticipated yields because most lands allocated for cultivation of this plant is characterized by nutrient and moisture deficiencies. Furthermore, the yield and physicochemical properties of Jatropha oil and biodiesel are strongly correlated with the types of oil extraction and biodiesel production techniques. The performance, combustion, and emission characteristic of diesel engines fuelled with Jatropha biodiesel have been significantly affected by engine types, working process, combustion processes, and various biodiesel's fuel properties. Thus, future studies should focus on innovating, developing, and optimizing of technologies to modernize the seed collection, seedprocessing, oil extraction, and biodiesel production process. For commercial and economical viable cultivation, the agronomic performance, water and nutrients requirement, and pest and disease vulnerability of Jatropha should be investigated rather than calming as 'Jatropha is drought and pest resistant plants that can grow on degraded lands'. Intensive experimental works are also needed on the molecular and genetic improvement for securing adequate and quality feedstock for sustainable biodiesel production.

Future studies should further focus either on improving the fuel properties of Jatropha biodiesel or diesel engine modification for enhancing the BTE, BSFC, and combustion duration. The emission of NOx during biodiesel combustion should be improved since nitrous oxide (N_2O) is the most potent GHG over to CO_2. In conclusion, the potential of Jatropha for sustainable biodiesel production should be evaluated in terms of its economic, social, environmental, and technical advantages for the respective growing region before establishing a large-scale biodiesel production.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Paper II

Characterizing the potential and suitability of Ethiopian variety *Jatropha curcas* for biodiesel production: variation in yield and physicochemical properties of oil across various growing areas

Gebresilassie Asnake Ewunie [1,3]. Odd Ivar Lekang[1]. John Morken[1]. Zerihun Demrew Yigezu[2]

[1]Faculty of Science and Technology, Norwegian University of Life Sciences (NMBU), P. O. Box, N-1432, Ås, Norway

[2]College of Agriculture, Hawassa University, P. O. Box, 05, Hawassa, Ethiopia

[3]Corresponding author's, E-mail: asnake.ewunie.gebresilassie@nmbu.no

Abstract

The increased cost of biodiesel production from edible oils has motivated the researchers to find alternative non-edible oil feedstocks. *Jatropha curcas* has been identified as a promising tropical and subtropical perennial energy crop for biodiesel production. However, securing adequate *J. curcas* oil for sustainable biodiesel production is not achieved yet. Therefore, the present study sought to investigate the potential and suitability of *J. curcas* grown at different area of Ethiopia for biodiesel production using analytical, instrumental, and empirical methods. The dry weight proportion of *J. curcas* seeds, seed coats, and kernels were significantly varied across growing areas. The oil contents of kernels were reneged between 47.10-59.32%, while the free fatty acid content of all sampled oils was less than 2.7%. The estimated iodine and saponification values were ranged between 99.6-112.6 mg I_2 and 180.9-202.0 mg KOH per gram of oil, respectively. Oleic (34.2-42.2%) and Linoleic (34.8-41.8%) acids dominated the fatty acid composition of *J. curcas* oil. The biodiesel fuel properties such as kinematic viscosity, density, cold filter plugging point, and cetane numbers predicted from the fatty acid composition of oils were agreed with EN-14214 standards. The k-means cluster revealed that kernels harvested from different geographic locations were grouped in the same cluster, which indicates that the effect of altitudinal variation on the oil content of *J. curcas* was not significant. However, at too lower and higher altitudes, the kernel's oil content has significantly declined. In conclusion, Ethiopian variety *J. curcas* oil showed suitable physicochemical properties for biodiesel production. However, its yield, composition, and physicochemical properties were significantly varied across growing areas.

Keywords: Biodiesel; growing areas; Fatty acid composition; *Jatropha curcas oil*; Physicochemical properties.

1.Introduction

The sub-Saharan country, Ethiopia, with the landmass of 1.1 million km^2, has the second higher population in Africa next to Nigeria. In the rural area of the country, more than 46 million peoples are living without electricity [1]. Energy poverty of the country is further manifested by the full dependence of traditional biomasses such as direct combustion of firewood, crop residue, animal dung, and charcoals. The energy production from those traditional biomass accounts for above 95% of national energy demand, and it is utilized by more than 86.42% of rural households [2]. Unfortunately, energy production from conventional biosources is neither sustainable nor environmentally benign. Heavy dependency on the traditional biomasses have resulted in severe deforestation, land degradation and greenhouse gas emissions [3]. Meanwhile, Ethiopian transportation fuel is entirely dependent on imported oil, and the annual importing cost has been estimated to be above 2.4 billion US dollars [4]. The higher foreign currency demands, coupled with hike and variability of petroleum price, have severely affected the national economy. Besides, energy production and utilization from conventional carbon-based fuel such as petroleum, coal, and natural gas have been identified as major sources for greenhouse gas emissions [5, 6]. Thus, searching alternative energy sources such as wind and hydropower for heat and electricity production was the interest of many researchers. Likewise, petrol fuels such as diesel and gasoline consumed mainly in the transportation sector could be substituted by various biofuels. Among biofuel, biodiesel has been identified as versatile fuel for diesel engines applications [7]. However, the transition to these renewable fuels requires ensuring adequate feedstocks that do not compete with food and feed production.

In Ethiopia, several non-edible plant species have been identified for biodiesel production. *Jatropha curcas* L *(J. curcas),* Castor bean, Pongamia, Candlenut, and Croton seeds are among the predominant identified energy crops [2]. Biodiesel production from *J. curcas* has been evaluated as economically viable and environmentally sound over other edible and non-edible oil feedstocks [8]. The application of *J. curcas* biodiesel as engine fuel has been successfully tested in various diesel engines without major modification [6, 9, 10]. Thus, Ethiopia has identified 23.3 million hectares of marginal land that could be used for growing various non-edible biodiesel feedstock [11]. Other studies also showed the availability of 16.61 million ha of lands that could not be used for food production but highly suitable for *J. curcas* cultivation. According to the government report, 16.61 million hectares of land have been planted with *J. curcas* and castor bean [12]. However, securing adequate *J. curcas* oil for sustainable biodiesel production is not achieved yet.

J. curcas is a small tree or large perennial shrub of up to 5-7 m in height that belongs to the family of Euphorbiaceae [13]. It has a life expectancy of 50 years [14]. The plant is native to Central America and Mexico and later widely distributed into Africa and Asia. In Ethiopia, *J. curcas* is grown abundantly across most regions as life fence, hedge, and soil and water conservation. The plant can grow in moisture stressed and degraded land with minimal managements and inputs [15]. *J. curcas* bear seeds after 12 months of planting [6, 8]. According to Sotolongo *et al.* [16], 1 hectare of land could produce around 3500 kg of *J. curcas* fruits in which 1000 kg (29%) of the gross weight was estimated as fruit shell. The remaining 2500 kg (71%) was the dry seeds. The seed by itself has two components: the seed coat (41%) and kernel (59%). The kernel's oil content ranges between 38.7 to 58% [8, 17], while 60-65% of the seed is disposed of as press cake after oil extraction [18]. Different parts of *J. curcas* have also been used to synthesize various drugs, cosmetics, and dyes [17]. The oil has been used in the painting industry and for production of soap, biodiesel, and biolubricant*.* *J. curcas* oil contains higher unsaturated oleic and linoleic fatty acids, which are relatively suitable for the biodiesel production as compared to polyunsaturated and saturated fatty acids [19]. The oil is also characterized by quite viscous and possessed a higher cetane number [20]. Thus, the oil from *J. curcas* could be used as a fuel in standard diesel engines after reducing its viscosity through preheating, blending, pyrolysis, emulsification, or transesterification [6, 21].

Despite many advantages of using *J. curcas* as a biofuel source, in Ethiopia, there is no adequate and successful cultivation of this energy crop for large scale biodiesel production. Many *J. curcas* cultivators and biodiesel producing companies have ceased or suspended their investments. A study conducted in Ethiopia reveals that moisture stress, poor soil quality, and poor agronomic performance of *J. curcas* seed were the major identified factors for the termination of many large-scale *J. curcas* projects [22]. The economic viability of biodiesel production is highly dependent on the quantity, quality, and chemical composition of the targeted feedstock [23]. In a semi-arid environment and poor soil quality, the achievable seed yields were estimated to be 2-3 tones ha^{-1} year^{-1}, which is significantly lower than the expected yield [24]. The yield, composition, and physicochemical properties of *J. curcas* oil is strongly affected by various edaphic and agroecological factors [24]. In Ethiopia, ecological conditions are directly and indirectly governed by the altitudinal variation since the annual rainfall, mean temperature, and soil type are affected by the slope and topographic features [25, 26]. Therefore, the present study hypothesized that altitudinal variation could significantly affect the yield and physicochemical properties of *J. curcas* oil.

In different parts of tropical regions, numerous studies have been conducted to investigate the impact of agroecological variation on the seed yield and oil content of *J. curcas* seed. A study conducted by Kumar and Sharma revealed that the oil content of J. curcas seeds ranged between 40-60%, while Agyemang *et al.* [27] showed a strong correlation between oil content, dryness, and wetness of the growing environments. Kassahun *et al*. [28] showed the oil yield variation across different regions of Ethiopia. The oil content of *J. curcas* seed grown in Brazil, Nigeria, China, and Congo-Brazzaville showed a significant variation [20, 29-31]. The oil yield and its physicochemical composition are mainly affected by various biological and geographical factors such as annual rainfall, temperature, soil quality, altitude, and genetic factors [8]. Areas with 1000 to 1500 mm annual rainfall, 20 °C to 28 °C with no frost, and free-draining sand and loam soils with no waterlogging risk has been identified as an ideal environment for *J. curcas* cultivation [32]. However, most previous studies have investigated the impact of several biotic and abiotic factors either on the seed or oil yield of *J. curcas*; and the effect of those factors on the composition and physicochemical properties of *J. curcas* seed and oil are ignored. Various studies have also characterized the suitability of *J. curcas* oil for biodiesel production through transesterification reaction [33, 34]. However, biodiesel production methods and input process variables are highly dependent on the physicochemical properties of *J. curcas* oil. The biodiesel production process and type of catalyst utilized in the transesterification reaction are also affected by the free fatty acid (FFA) content of *J. curcas* oil [6].

Studs also showed that the reaction design, feedstock handling, and preprocessing activities are affected by the physicochemical properties of biodiesel feedstocks [35]. Furthermore, investigating the composition *J. curcas* oil before biodiesel production could also support the prediction of various biodiesel's fuel properties [6, 23]. Yaşar [23] showed that fuel properties such as kinematic viscosity (KV), specific gravity (SG), cetane number (CN), iodine value (IV), and various cold flow properties are significantly affected by the fatty acid composition of feedstocks. Fallen *et al.* [36] reported that the oxidative stability of biodiesel produced from feedstock with higher polyunsaturated fatty acid content was out of the EN 14214 standard. Fatty acid composition and physicochemical analysis also could play an essential role in genetic improvement for large scale biodiesel production. Furthermore, identifying the best variety for higher oil yield and better quality could assist in clonal propagation, field trials, and large-scale plantations for biodiesel production. However, to the best of the authors' knowledge, no previous studies that comprehensively characterize the potential and suitability of Ethiopian variety *J. curcas* for biodiesel production. This study, therefore, sought to examine the yield, composition, and various physicochemical properties of oil from *J. curcas* grown in different areas of Ethiopia, and the potential and suitability of the oil for biodiesel production were thoroughly examined. The present study results will be utilized as valuable information for seed zone delineations, strategies for conservation of genetic variation, prospects of improvement, and evaluation of the potential of locally adapted seed sources.

2. Materials and methods

2.1. Description of the study area

Ethiopia has ten regions, out of which Amhara, Tigray, Southern Nations-Nationalities and Peoples' (SNNP'), Oromia, and Benishangul Regions are well-known areas that potentially grow of *J. curcas*. The major *J. curcas* growing areas across these five regions were identified and mapped using previous studies [28], preliminary field visits, and stockholder interviews. Then, all the identified sites were grouped as lowland (800-1320 m.a.s.l) and midland (1320-2089 m.a.s.l) growing areas [37]. For representing the whole study area, 16 sample sites from each altitudinal range, i.e., 16 from lowland and 16 from midland growing areas, were randomly selected and mapped (Fig. 1). However, it should be noted that the stratification of growing sites based on their altitude did not follow the existing traditional agroecological classification system of Ethiopia [25] since *J. curcas* is growing in the lower altitudes. The specific name of each study site and their corresponding given code and location are presented in Table 2.

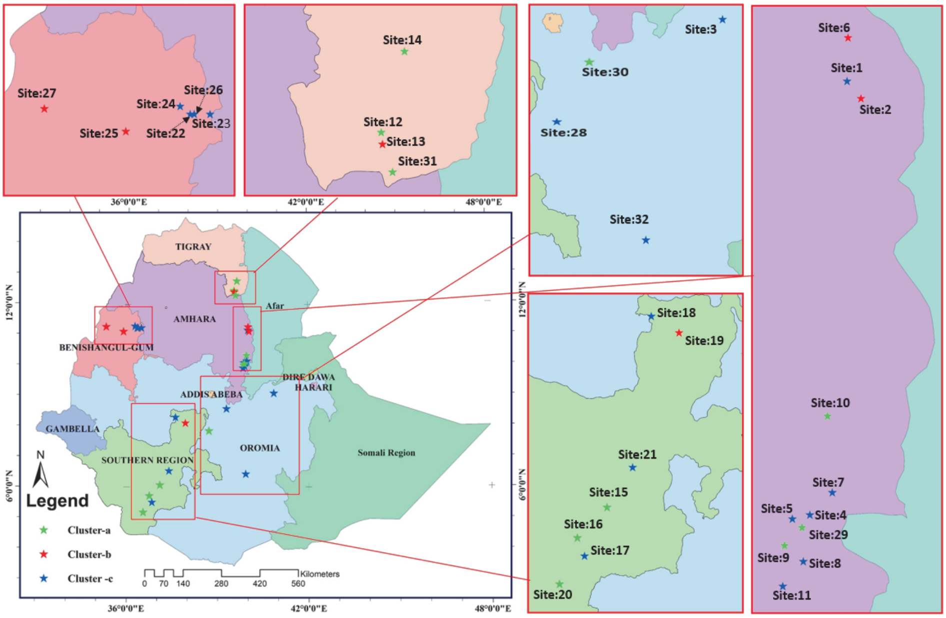

Fig. 1. Map of the study sites (star points on the map with green, blue, and red colors indicates sites showing relatively higher, moderate, and lower oil yields, respectively).

2.2. Sources of raw material and chemicals

J. curcas fruit samples with yellowish color were manually collected from randomly selected mother trees growing in all sampled study sites [8] and immediately transported to Wondo Genet College of Forestry, Wondo Genet, Ethiopia. The fruits were then dried under open-air until their moisture content was reduced to below 50% on dry weight bases. Forty fruits from each sample sites were randomly taken, and the seeds from these fruits were then separated through manual threshing. The shells and seeds were further dried separately in room temperature (25 °C) until their dry weight becomes constant for three consecutive measurements. For ensuring removal of all moisture content, the shell and seeds were further dried in an oven drier for 16 hrs at 105 °C [8]. Finally, the seed coat from 100 randomly selected seeds was carefully removed using a sharp metal stick to estimate the weight proportion of seed coat, kernel, and oil retained in the kernels [38]. The weight of seeds, seed coats, kernels, and kernel oil content were measured carefully using Shimadzu AW320 analytical balance with a precision of ± 0.0001g. The analytical grade chemicals used for necessary analysis in the study such as N-Hexane (99%), Diethyl Ether (98%), Ethanol (98%), Phenolphthalein Indicator, Potassium Hydroxide (85%), Chloroform (99.9%), Potassium Iodide (99.5%), Sodium Thiosulphate (99%), Carbon Tetrachloride (99.5%), Hydrochloric Acid (37%), and Glacial Acetic Acid (99.5%) were purchased from the local markets, Addis Ababa, Ethiopia. While, the Iodine Monochloride (Wijs solution) and starch potato were obtained from Merk (Germany) and VWR international ltd (Belgium), respectively.

2.3. Determination of kernel oil content

The kernels separated from the seeds were further dried using an oven drier for overnight at 80 °C [39, 40], and the moisture content of the kernel was determined by calculating the weight differences before and after drying. The dried kernels were then crushed using mortar and pestle in a moisture-free area, and the powder was forced to pass through a sieve with a mesh size of 1mm [41]. Then, 50 g kernel powder was accurately measured and transferred into a cellulose extraction thimble (Whatman, I.D. × H 18 × 55 mm, GE). The thimble contained the powder was dipped into the Soxhlet apparatus. Meanwhile, 300 ml of n-hexane was measured and transferred into a pre-weighed boiling flask. Since the oil yield was significantly affected by extraction temperature, time, solvent to solid ratio, and particle size of the kernel [42], a series of batch extraction was performed to define the optimum process for high oil yield. The oil extractions for composite kernel samples (sample taken from all study areas) were performed by varying the extraction temperature (60-80 °C) and time (6-9 hrs) at constant kernel particle size ($\leq$ 1mm) and solvent to solid ratio (6:1, v/m). The oil yield after each extraction process revealed that extraction employed at 70 °C for 8 hrs was identified as optimum conditions for maximum oil yield; thus, these mentioned extraction parameters were applied for all kernel samples collected across 32 study areas. However, this study did not quantify how much oil residue was left in the press cake, but there was no oil yield increment for extractions employed above 70 °C and 8 hrs. For maintaining the stability of heating during oil extraction, a water bath was used as a source of heat. After extraction, the n-hexane was removed from the oil using a rotary vacuum evaporator at lower pressure. Triplicated extractions were performed for all treatments, and average values were reported. In this study, the oil yield was calculated from the dry weight of kernel powder using Eq. (1) [8]. The oil samples obtained after extraction were kept in the refrigerator until further processing [15]. Finally, study sites were ranked chronologically based on their oil yield, then top 10 best sites that showed higher oil yield were chosen for further compositional and physicochemical property analysis.

$$Oil\ yield = \left[\frac{(W_2 - W_1)}{W_3}\right] X100 \quad (1)$$

Where; W_1, W_2, and W_3 are weights of the boiling flask, boiling flask contained the oil, and kernel powder dipped into the thimble, respectively.

2.4. Oil quality analysis

The oils extracted from *J. curcas* kernel harvested from 10 best sites (sites showing higher oil yield) were characterized for their kinematic viscosity (KV), acid value (AV), iodine value (IV), saponification value (SV), and peroxide value (PV). The AV of crude *J. curcas* oil was determined by titration with potassium hydroxide following the method described in Asmare [43], then the FFA % was estimated by multiplying the AV with 0.501 [44]. Briefly, the AV of oils was determined after dissolving five grams of oil with 25 ml of diethyl ether and ethanol mixture that have an equal volume. Then 5 droplets of phenolphthalein indicator were added into the solvent-oil mixture, and the titration was carefully performed using 0.1 N of ethanolic KOH solution until the light pink color appears. The total acidity of oil in mg KOH g^{-1} of oil was calculated using Eq. (2).

$$\text{AV} = \frac{56.1 \text{ x N x V}}{\text{Sample oil } (g)} \quad (2)$$

Where, N is the normality of alcoholic KOH, and V is the volume (ml) of alcoholic KOH used in the titration.

The IV was determined using the Wijs method [45], while the SV was estimated using the AOCS Cd 3-25 standards as described by Mohammed *et al.* [46]. For IV determination, 0.3 gram of *J. curcas* oils were dissolved in 15 ml of chloroform. Afterward, 25 ml of Wijs reagent were added to the samples, and the solutions were kept in the dark place for 60 minutes. The flask containing the mixture was tightly covered between sample preparation and dark environment storage. Subsequently, 20 ml of potassium iodide (10% water solution) and 100 ml of water were added. The solution was then titrated with standard sodium thiosulfate (0.1 N) using some drops of starch solution as an indicator. The endpoint of titration was verified by the disappearance of the blue color developed due to iodine in the Wijs solution. The blank test was conducted following the same procedures by excluding the oil sample; then, the IV was determined using Eq. (3).

$$\text{IV} = \frac{\left(\text{ml}_{\text{Na}_2\text{S}_2\text{O}_{3\text{black}}} - \text{ml}_{\text{Na}_2\text{S}_2\text{O}_{3\text{sample}}}\right) \text{x N x } 12.69}{\text{sample oil (g)}} \quad (3)$$

Where; N is normality of sodium thiosulfate ($Na_2S_2O_3$) expressed as equivalent l^{-1} and 12.69 is a constant related to the equivalent weight of iodine.

The SV of *J. curcas* oil was determined by saponifying the oil with a potassium hydroxide solution. Briefly, 2 grams of oils were accurately measured and transferred into the boiling flasks. Subsequently, 50 ml of excess ethanolic potassium hydroxide solution (0.1 N) was added into the boiling flask, and then the mixture was refluxed for 45 minutes by gentle stirring using a magnetic stirrer. The unreacted KOH was then back titrated with 0.5 N hydrochloric acid solution using 2 to 3 drops of phenolphthalein indicator until the pink color changes to colorless. The SV was then determined using Eq. (4).

$$SV = \frac{(V_b - V_S) x\ N\ of\ KOH\ x\ 56.1}{Sample\ oil\ (g)} \quad (4)$$

Where, V_b and Vs are volumes (ml) of hydrochloric acid solution utilized for blank and oil titration, respectively.

The PV of *J. curcas* oil was estimated using AOCS standards [47], whereas the KV of the oil was measured using Cannon 9721-R56 Viscometer at room temperature [48]. For determining the PV, two gram of each sample oils were placed in 250 ml conical flasks and dissolved with 10 ml of chloroform. Then 15 ml of glacial acetic acid and 1 ml of a saturated potassium iodide solution were added into the flask that contains oil and chloroform mixture. After gently shaking with hand for 1 minute, the flask was tightly closed and then placed in a dark environment for 5 minutes. Finally, 15 ml of distilled water was added, and the mixture was titrated with sodium thiosulphate solution (0.002 M) using starch solution as indicator. The blank titrations were also performed under the same conditions without oil samples, then PV was determined using Eq. (5).

$$\mathrm{PV} = \frac{\left(\mathrm{ml}_{\mathrm{Na_2S_2O_3black}} - \mathrm{ml}_{\mathrm{Na_2S_2O_3sample}}\right) \mathrm{x\ M\ x\ 1000}}{\mathrm{sample\ oil\ (g)}} \quad (5)$$

Where, M was the concentration (morality) of sodium thiosulphate

2.5. Determination of the fatty acid composition

For analyzing the fatty acid composition of *J. curcas* oils, the fatty acid methyl ester (FAME) was produced from oils sampled from ten different study sites. Briefly, 25 g of preheated *J. curcas* oil was taken and transferred into a boiling flask that contained 0.25 grams of KOH dissolved in 5.5 ml of methanol. The oil was then transesterified at 50 °C for 50 minutes by gently stirring with a magnetic stirrer. After the transesterification reaction, the mixture was dissolved with required amount of n-hexane and then gently shacked and centrifuged at 6000 rpm for 15 minutes. Finally, the fatty acid profiles of *J. curcas* FAMEs were analyzed using GC–MS Agilent Technology 7820A GC and 5977E MSD systems equipped with an autosampler following the methods described in Tsegay *et al.* [37]. More specifically, the samples for GC-MS analysis was prepared by mixing 9 µg ml^{-1} of FAME with 5µg ml^{-1} of standard decanoic acid methyl ester. Chromatographic separations were carried out using DB-1701 column with 30 m length, 0.25 mm internal diameter, and 0.25µm column phase thickness. Injection mode was splitless while helium was used as a carrier gas, and 1µl volume of the sample was injected to the inlet heated to 275 °C. The oven temperature condition was programmed to be 60 °C for initial and hold for 2 min and reached up to 280 °C. The program was separated into the rate of 20 °C min^{-1} until it reaches 200 °C, and the rate of 3 °C min^{-1} until it reaches 240 °C with zero hold time. Conditions used for the MS were a source temperature of 230 °C, 40–650 m/z scanning range, and operated in positive electron impact mode with ionization energy of 70 eV. The chromatogram and mass spectral data were processed using the instrument installed software (MS-Chem Station; Agilent Technologies, USA). Wiley's and Nist's libraries were used for identification purposes, and its quantification was calculated by the internal standard with the relationship of relative response factors.

2.6. Biodiesel fuel properties prediction

Based on the fatty acid profile, fuel properties such as degree of unsaturation (DU), long-chain-saturated factor (LCSF), CN, KV, density, higher heating value (HHV), cold filter plugging point (CFPP), and surface tension of biodiesel were predicted from *J. curcas* oil and each parameter was evaluated against the EN 14214 standards. The empirical models utilized in the present study were assumed to be reliable and robust since the prediction is highly affected by the strength of the model. The present study also assumed that, rather than the fatty acid composition, the purity and various physical properties of *J. curcas* biodiesel are comparable to those used to develop the actual models. Accordingly, various empirical models were applied to predict the fuel properties of Ethiopian variety *J. curcas* biodiesel as described below.

The DU and LCSF of the methyl ester were determined using Eq. (6), and Eq. (7), respectively [23].

$$DU = [monounsaturated\ cn(wt.\%)] + 2 * [polyunsaturated\ cn{:}\,2{,}3(wt.\%)] \tag{6}$$

$$CSF = 0.1 * [C16{:}\,0\ (wt.\%)] + 0.5 * [C18{:}\,0(wt.\%)] + 1.0 * [C20{:}\,0(wt.\%)] + 1.5 * [C22{:}\,0\ (wt.\%)] + 2 * [C24{:}\,0(wt.\%)] \tag{7}$$

The CN for the individual (pure) ($\boldsymbol{\phi}_i$) and combination (mixture) of all FAMEs ($\boldsymbol{\phi}$) detected by GC-MS were determined using Eq. (8) and Eq. (9), respectively, as described in Ramírez-Verduzco *et al.* [49].

$$\phi_i = -7.8 + 0.302 * M_i - 20 * db \tag{8}$$

$$\phi = \sum_{i=1}^{n} (\phi_i W_i) \tag{9}$$

Where $\boldsymbol{\phi}$ and $\boldsymbol{\phi}$i are the CN value of all and each FAME, respectively; db is the number of double bonds; M_i is the molecular weight of $FAME_i$, and W_i is weight percentage of each $FAME_i$ in the biodiesel.

The empirical models developed by Ramírez-Verduzco *et al.* [49] were utilized to determine the KV ($\boldsymbol{\eta}_i$), density ($\boldsymbol{\rho}_i$) and HHV ($\boldsymbol{\delta}_i$) values of each FAME using Eq. (10), Eq. (11), and Eq. (12), respectively. Besides, these values are also determined for the biodiesel, i.e., a mixture of all FAMEs (f_b) using Eq. (13).

$$\ln(\eta_i) = -12.503 + 2.496 * \ln(M_i) \,.\, 0.178 * db \tag{10}$$

$$\rho_i = 0.8465 + (4.9/M_i) * 0.0118 * db \tag{11}$$

$$\delta_i = 46.19 - (1794/M_i) - 0.21 * db \quad \mathit{(12)}$$

$$f_b = \sum_{i=1}^{n} (f_i \, w_i) \quad (13)$$

Where $\boldsymbol{\eta}_{i,}$ $\boldsymbol{\rho}_i$ and $\boldsymbol{\delta}_i$ are the KV ($mm^2\ s^{-1}$), density ($g\ cm^{-3}$) and HHV ($MJ\ kg^{-1}$) of pure FAME, respectively, while f is a function that represents any physical property; the subscripts b and i refer to the biodiesel and the pure i^{th} FAME, respectively, and w_i refers the mass or mole fraction of i^{th} $FAME_i$.

The surface tension of methyl ester synthesized from Ethiopian variety *J. curcas* oil was determined from its fatty acid composition using Eq. (14). Detail procedures about the computation of the surface tension and weight factor of each fatty acid component is described in Allen *et al.* [50]. In this study, the surface tension and weight factors for palmitoleic (C16:1) and Eicosanoic (C20:0) fatty acids were not reported. As a result, the average measured surface tension and weight factors that have been reported for other saturated fatty acids (C16:0, and C18:0) and unsaturated fatty acids (C18:1, C18:2, and 18C:3) were used for C20:0 and C16:1, respectively. However, it should be noted that some deviation could occur during mean surface tension prediction (T_m) due to utilizing the average surface tension and weight factor of these fatty acids for C20:0 and C16:1 fatty acids.

$$T_m = \sum_{i=1}^{n} W_i \delta_i Y_i \quad (14)$$

Where; T_m is the mean surface tension of the mixture ($N\ m^{-1}$); $\boldsymbol{\delta}_i$ is the surface tension of component i ($N\ m^{-1}$); Y_i is the mass fraction of component i; W_i is the weight percentage of component i.

The cold flow properties of FAME synthesized from *J. curcas* oil were predicted using the multiple regression model developed by Yuan *et al.* [51]. Among various methods, CFPP was well known and accepted methods for assessing the cold flow properties of biodiesel as compared to cloud and pour points. Accordingly, Eq. (15) was applied to predict the CFPP of FAME from the saturated fatty acid content of *J. curcas* oil.

$$CFPP = -13.688 + 0.518 * C16{:}0 + 0.778 * C18{:}0 + 3.066 * C20{:}0 \quad (15)$$

Where, C16:0, C18:0, and C20:0 are percent weight compositions of palmitic, stearic, and eicosanoic acids, respectively.

However, these empirical models described above were not validated for this study; thus, the predicted fuel properties would deviate slightly from their actual values. Some of the major factors that could potentially affect the certainty of the models are variation in structure, chain length, and double bond configuration between fatty acids reported in the present study and previous reports [52]. These structural configurations are varying from feedstock to feedstocks, and it could affect the predicted fuel properties. For instance, the model developed from longer carbon chain fatty acid showed higher viscosity and vice versa. While Knothe and Steidley [52] have found that the double-bond configuration, such as cis and trans, could affect various fuel properties of biodiesel despite having the same degree of unsaturation. It should be also noted that the empirical models utilized in the present study were developed using different types of oil whose chemical composition are slightly varied as compared to *J. curcas*. Therefore, the probable uncertainty of the model due to the mentioned factors should be considered for future utilization.

2.7. Statistical analysis

Analysis of variance (ANOVA) was carried out for all treatments using R software (version 3.6.2) to compare the mean difference in a weight proportion of various components of *J. curcas* and variation in different physicochemical properties among treatments. The ANOVA analysis was performed using a Tukey test, and the means differences were considered statistically significant if the p-value is ≤ 0.05. Pearson correlation matrix was constructed to depict the weight relationship between seed and seed coat, seed and kernel, seed coat and kernel, and kernel and its oil contents. Furthermore, hierarchical euclidean cluster analysis was performed to group similar study sites that showed the same average oil yields. The euclidean distances were calculated using the Wards method, and a dendrogram was constructed to describe the relationships within and between kernels sampled across various study sites [53]. After clustering, the number of groups (K-means) was decided based on Tukey's posthoc analysis, which displayed the same lowercase letters.

3. Result and Discussion

3.1. *Jatropha curcas* fruit and seed characterization

The proximate analysis like weight proportion of fruit, fruit shell, seed, seed coat, and kernel could be used as an essential impute for selecting a good variety of *J. curcas* for genetic improvement and ensuring adequate oil for sustainable biodiesel production. Furthermore, estimating the dry seed weight from the shell and its oil content allows the rapid prediction of the amount of oil produced per unit area of *J. curcas* plantation. The effect of growing area variation on the different components of *J. curcas* fruit is presented in Table 1. More specifically, the lower p-values (< 0.05) reveals that the impacts of growing area variation on the seed, seed coat, kernel, and oil yield of *J. curcas* were statistically significant. The higher calculated F-value and very small p-value from Table 1 showed a higher statistically significant variation of seed weight harvested across 32 different sites than variation in the kernel, seed coat, and oil weights. While, the number in bracket indicates that variation between treatments (sites) was higher as compared to variation among replications. Most of the fruits contain three seeds, which agreed with the previous study [54]. The fruit shell and seed accounts for 35.84% and 64.15% of the dry weight of the fruit, respectively, while the dry weight of 100 *J. curcas* seeds were ranged between 50.53 to 68.97 g

(Table 2). Furthermore, the seeds by itself were characterized as kernels (embryos) and seed coat. The estimated dry weight proportion of kernel relative to 100 seeds weight ranged between 49.07% (site:23) to 66.74% (site:17), while the seed coats were varied between 33.26% (site:17) and 50.93% (site:23). The shell weight estimated from Ethiopian variety *J. curcas* was higher than that of Cuba's variety [16]. The higher and lower seed weights were recorded from site:26 and 23, respectively**.** The weight of Ethiopian variety *J. curcas* seed is slightly lighter than Chinese and Indian varieties [38].

The percentage composition of seed coat was negatively correlated with the weight of seed, kernel, and oil contents, and the correlation coefficients were estimated to be 0.56, 1.00, and 0.47, respectively. Meanwhile, the correlation matrixes showed a strong positive correlation (r=0.47) between kernel weight and oil contents. Likewise, a higher correlation (r = 0.56) was observed between dry seed and kernel weights. The probable reason for variation in a weight proportion of different biomass components of *J. curcas* could be irregularity of soil type, moisture content, rainfall, planting density, management, and age groups [15]. The higher dry weight of seed indicates the potential of getting higher oil yield as the seed weight was positively correlated with the oil contents (r = 0.31).

Table 1. One-way ANOVA showing the influence of growing area variation on *Jatropha curcas* seed oil content.

Parameter	Mean square	F-test	Significance (P-value)
Seed weight per 100 seeds (g)	Between groups (31.90) Within groups (5.55)	14.58	$2.2\text{x}10^{-16}$
Kernel (% in dry seeds)	Between groups (37.03) Within groups (4.13)	8.98	$1.2\text{x}10^{-14}$
Oil content (% in dry kernels)	Between groups (31.90) Within groups (5.51)	5.79	$1.1\text{x}10^{-9}$
Seed coat (% in dry seeds)	Between groups (37.03) Within groups (4.13)	8.98	$1.2\text{x}10^{-13}$

3.2. *Jatropha curcas* kernel oil yields

In the present study, oil retained in the kernel samples harvested from 32 study sites were examined after optimizing the solvent extraction process, i.e., 70 °C extraction temperature, 8 hrs extraction time, ≤ 1mm kernel particle size, and 6:1 solvent to biomass ratio (v/m). The same approaches have been reported in previous studies [55-57]. For instance, Zhang *et al.* [56] identified 55 min extraction time, 51 °C extraction temperature, and 19:1 solvent/sample ratio at a fixed ultrasonic frequency of 40 kHz and power of 150 W. At these optimum conditions, the oil yield obtained from almond powder was determined to be 81.89% of the available oil. Balasubramanian *et al.* [55] have also examined and modeled the effect of microwave heating temperature (80 and 95 °C) and extraction time (20-30 mins) at constant Algae-water suspension (1:1 w/w). Microwave heating employed at 95 °C for 20-30 mins resulted in recovery of 76-77%

of oil retained in the Algae biomass. As a result, it could be concluded that all oil contained in the kernel could not be removed as there are no conditions that resulted in 100% oil recovery efficiency [55, 56]. This study also assumed that the oil left in the press cake is minimal and similar for all treatments since the same extraction process parameters are utilized, although the oil left in the press cake was not quantified.

The oil yield obtained from kernels harvested across the 32 different study sites were ranged between 47.10-59.32%. The maximum oil yield was obtained from site:9, while the minimum oil yield was recorded from site:19. The data presented in Table 2 illustrates that kernels collected from more than 81% of sampled sites showed higher oil content (>50%), which agreed with the previous studies [27, 29, 31, 58, 59]; however, significantly higher oil yield was obtained as compared to Mexican [60], Brazilian [30], and Chinese [20] variety. Previous studies also noted that Ethiopian *J. curcas* kernel contains higher oil content. In addition to genetic variability and environmental effect, the higher oil yield in the present study could also be associated with extraction parameters as higher temperatures, longer preheating time, and small particle sizes give a better oil yield [61].

Table 2. Comparative analyses of the average weight of 100 dry seed and weight proportion of seed coat, kernel, and kernel oil contents of *Jatropha curcas* determined across 32 different study sites (n = 3; mean ± SD).

Name of the study site (kebele)	Site code	Altitude (m.a.s.l)	100 seed's dry weight (g)	Seed coat (% in dry seeds)	Kernel (% in dry seeds)	Oil content (% in dry kernels)
Salmane[a]	Site:1	1667	59.61±1.98	37.89±1.65	62.11±0.10	54.63±1.44
Atto[a]	Site:2	1430	57.45±1.82	41.06±1.92	58.94±0.15	49.34±2.40
Metecho Kutir[g]	Site:3	1467	51.14±2.15	35.73±2.32	64.27±2.32	52.83±2.44
Wacho[b]	Site:4	1198	58.99±2.50	39.30±1.88	60.70±0.14	50.96±0.39
Tere[b]	Site:5	1108	60.60±2.20	37.55±1.98	62.45±1.98	52.60±0.43
Kurkura[a]	Site:6	1320	54.88±2.15	39.84±2.32	60.16±2.32	49.36±3.28
Bete[a]	Site:7	1263	65.12±2.68	37.65±1.44	62.35±1.44	52.99±3.24
Shewa-Robit/01 kebele[b]	Site:8	1328	66.53±2.15	34.93±1.75	65.07±1.75	53.73±1.73
Kobo kebele[b]	Site:9	1437	64.00±0.75	35.92±0.98	64.08±0.13	59.32±1.09
Efratana Gidim[b]	Site:10	1739	65.29±2.15	36.26±1.82	63.74±1.82	58.00±0.26
Asfachew[b]	Site:11	1606	59.03±2.19	37.18±2.02	62.82±2.02	51.25±1.15
Adibore Kebele[c]	Site:12	1497	57.89±0.67	37.77±0.46	62.23±0.15	56.79±1.04
Abo Tsebel[c]	Site:13	1767	56.54±1.90	41.43±1.62	58.57±0.14	48.76±3.07
Bala kebele[c]	Site:14	1573	64.09±2.15	35.11±1.82	64.89±1.82	56.05±1.65
Zenga Zelgo[e]	Site:15	1163	56.29±2.20	34.28±2.05	65.72±2.05	56.29±1.56
Chali-1[d]	Site:16	1343	68.76±2.30	34.24±1.57	65.76±1.57	55.81±1.16
Sheleuka[d]	Site:17	1134	67.20±2.15	33.26±1.67	66.74±1.67	52.56±1.30
Ableta[h]	Site:18	1875	62.84±3.01	39.03±1.99	60.97±1.99	50.87±1.36
Yibrie[h]	Site:19	2082	56.54±2.72	41.35±0.98	58.65±0.12	47.10±1.37
Dimeka[d]	Site:20	1119	56.29±2.15	34.28±2.05	65.72±2.05	55.56±0.47
Dana[e]	Site:21	1283	64.25±2.19	33.87±1.74	66.13±1.74	53.34±1.70
Ketena-2/Mender-4[f]	Site:22	1038	51.93±2.11	42.54±2.61	57.46±2.61	50.63±3.83
Du/baguna[f]	Site:23	1131	50.53±2.00	50.93±3.04	49.07±3.04	50.90±1.24
Manbuk[f]	Site:24	1171	51.06±2.20	37.24±2.43	62.76±0.43	52.87±1.31
Dilsanbi[f]	Site:25	1000	58.25±4.65	39.25±3.94	60.75±0.14	48.56±0.28
Ketena-1/Mender-49[f]	Site:26	1038	68.97±2.30	38.94±1.84	61.06±1.84	51.62±4.90
Mankush[f]	Site:27	875	62.73±4.22	40.23±3.17	59.77±0.13	47.16±2.19
Soloque Kurfa[g]	Site:28	1437	61.19±2.19	34.02±1.84	65.98±1.84	54.22±3.01
Yelen[b]	Site:29	1232	60.64±3.00	37.35±1.33	62.65±1.33	55.79±6.09
Adame Tulu[g]	Site:30	1648	67.80±2.80	37.58±1.28	62.42±0.09	57.99±1.58
Tumiga[c]	Site:31	1461	59.67±2.20	33.99±1.92	66.01±1.92	55.52±0.83
Burkitu[g]	Site:32	1181	53.54±2.15	44.68±2.36	55.32±0.15	51.30±1.36
Mean			59.99±5.56	37.96±3.90	62.04±3.90	52.96±3.76
Minimum			50.53	33.26	49.07	47.10
Maximum			68.97	50.93	66.74	59.32

[a]located in Amhara Region, Oromia Zone; [b]situated in Amhara Region, North Shewa Zone; [c]located in Tigray region, Southern Zone; [d]situated in SNNP's Region, South Omo Zone; [e]located in SNNP's Region, Gamo-Goffa Zone; [f]located in Benishangul Region, Metekel Zone; [g]located in Oromia Region, [h]situated in SNNP'S Region, Gurage Zone.

The ANOVA model with P-value of 1.1 x 10^{-9} demonstrates significant oil yield variations were noted among 32 study sites (Table 1), while the post hoc Tukey test revealed that statistically significant oil yield variations among many pairwise treatment tests. Thus, considering the oil content of kernel as a primary criterion, all study sites were grouped into three clusters. The clusters were displayed using a dendrogram (Fig. 2), and each cluster was named as cluster-a, cluster-b and cluster-c. Cluster-a and cluster-c were the largest class that contain 31% and 50% of the study sites, respectively, while cluster-b represents 19% of study sites. The oil contents obtained from cluster-a, cluster-b and cluster-c were in between 55.52-59.32%, 47.10-49.36% and 50.63-54.63%, respectively.

The K-mean clustering revealed that *J. curcas* trees grown in different geographic areas were grouped into the same class rather than trees planted in the same geographical location. This phenomenon indicates that in addition to altitudinal variation, the oil content of *J. curcas* kernel oil content was significantly affected by other site-specific factors. Furthermore, the k-mean clustering system would allow for identifying the promising *J. curcas* accession with higher oil yield for the future establishment of elite seedling seed orchard or clonal seed orchard for hybridization programmers [62].Thus, the oils extracted from *J. curcas* kernel collected from study sites listed under cluster-a were selected for fatty acid composition and various physicochemical property analysis to examine their suitability for biodiesel production.

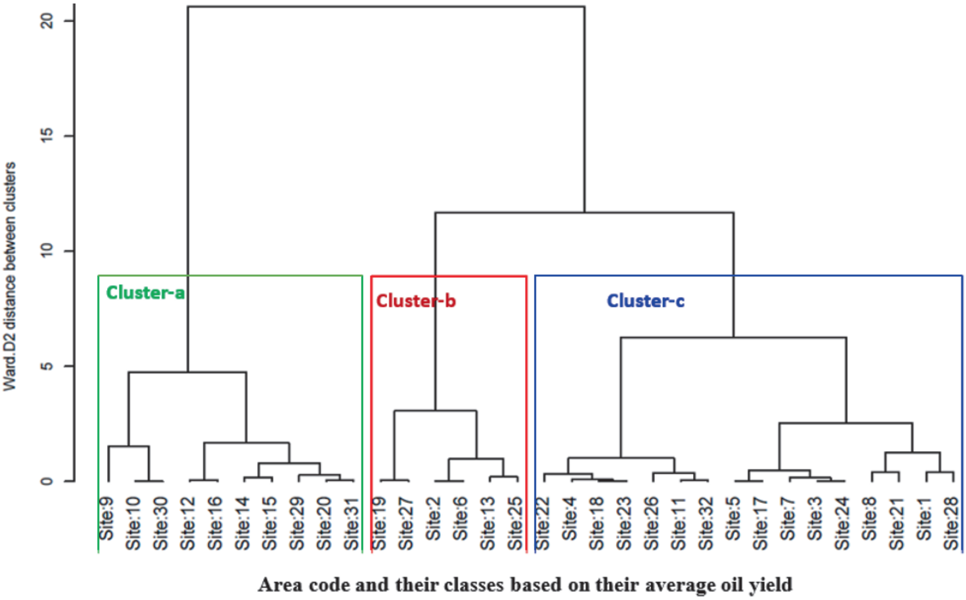

Fig. 2. Dendrogram of 32 different *Jatropha curcas* growing sites mapped based on their average oil yield (Site:1, Site:2, Site:3…denote the code given for individual study areas).

The impact of altitudinal variation on the oil content of *J. curcas* kernel was investigated for individual sites and areas categorized as lowland and high land (Fig. 3). Although the ANOVA model developed from 32 different study sites showed an insignificant effect of altitudinal variation, the kernel's oil content declined when the altitudes were getting too low and too high (Fig 3a). Generally, the accumulation of oil in *J. curcas* seeds from the 32 study sites in the present investigation followed the quadratic equation of $y = -Ax^2+Bx+C$, where A, B, and C are constants. For instance, the estimated oil yield varied between 47.16-51.61% for kernels harvested from study sites having lower altitudes (875-1038 m.a.s.l). Similarly, *J. curcas* kernels growing at higher altitudes (1767-2082 m.a s. l) contained lower oil content (47.10-50.85%). In contrast, higher oil contents were measured from *J. curcas* kernels harvested from study sites having moderate altitudes (1232-1320). However, the oil yield of Jatropha kernel collected from middle altitudes showed an irregular pattern with altitudinal variations. This could be due to the impact of other factors, such as edapho-climatic, genetic, and age factors [63], since all study areas are distributed in a different part of Ethiopia, and most sampled *J. curcas*es kernels are wild.

On the other hand, the average overall oil yield reveals that kernels collected from areas groped under lower altitude (875-1320 m.a.s.l) showed relatively less oil yield than that of kernels harvested from middle altitudes (1328-2082 m.a.s.l) (Fig. 3b). The lower oil content of kernels harvested at higher and lower altitudes could be directly associated with its fatty acid contents. Previous studies reported a decline in saturation and the unsaturation fatty acid contents with increasing and decreasing of growing altitudes, respectively [64]. Likewise, at higher altitudes, photo assimilation for biomass growth is higher as compared to oil production [54], which is clearly shown in Fig 3a. Variation in oil yield could also be associated with edapho-climatic factors as Ethiopian soil type and climate are governed by altitudinal variation [25]. Thus, the effect of altitude on *J. curcas*'s oil content could be modified by other factors, as shown in the middle altitude of growing sites. In general, drier condition increases the oil content of *J. curcas*; however, study sites with alluvial and dryland soil type caused less oil content [65]. Soil having a sufficient quantity of phosphors has also proved to increase the seed oil contents [63].

Variation in temperature could significantly affect the oil content, and its effect was reported by several authors [32, 66, 67]. *J. curcas* investigated in the present study are also wild, and its age is unknown. However, age has a significant effect on the yield and physicochemical properties of *J. curcas* oil. Studies showed that *J. curcas* gives higher oil yield after 4-5 years of plantation then starts to decline as the age further increases [24]. Soil type and moisture content were also identified as the primary factors affecting the seed yield and its oil content [15, 22]. A study conducted in China indicates that the seed weight and oil content of *J. curcas* grown in acid soils were significantly higher than those harvested in basic soils [38]. Likewise, Kheira and Atta [15] noted that drier climatic conditions increased the oil content of *J. curcas* seed as compared to a wet climate. Turinayo *et al.* [17] showed significant oil yield variation between seeds sourced from two different districts of Uganda. Agyemang *et al.* [27] reported a significant change in oil content among seeds collected across six different agroecological zones of Ghana. In general, the oil content of *J. curcas* could be affected by various abiotic and biotic factors. The reported biotic factors were genotype, pests, and diseases, while the abiotic factor includes soil type and its nutrient content, rainfall pattern and amount, soil moisture, average temperature, altitude, and agronomic practices such as planting density, pruning, thinning, and weeding [68, 69]. Besides, the maturity level during seed collection was also reported as a potential factor affecting *J. curcas* kernel oil content [8], and the result revealed that the oil content of the seed was increased with increasing of the maturity level, but again decline when the seeds were over-matured [8, 70]. Therefore, for best *J. curcas* variety selection, the wild species should be domesticated at the same biophysical conditions, and a detailed physicochemical property investigation should be carried out for suggesting the best variety for large scale biodiesel production.

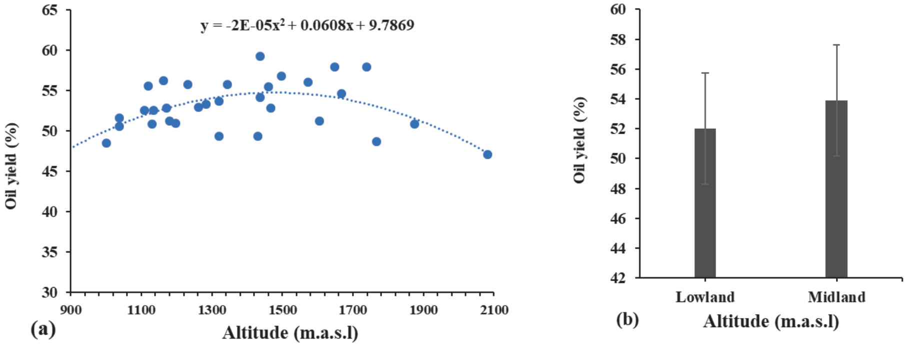

Fig. 3. Effect of altitudinal variation on the oil yield of *J. curcas* kernel.

3.3. Physicochemical property *Jatropha curcas* oil

3.3.1. Acid value and free fatty acid contents

The FFA content of vegetable oil can affect biodiesel quality and determine the type and amount of catalysts used in the transesterification process [71]. The FFA content of the oil is defined as the number of grams of fatty acid in 100 ml of oil [13], while the AV is the amount of KOH in milligram require to neutralize the FFA present in one gram of oil. As shown in Table 3, the AV and FFA content of *J. curcas* oil were in between 0.7-5.3 mg KOH g^{-1} oil and 0.4-2.7%, respectively. Results of ANOVA on the AV and FFA content of *J. curcas* oil showed a statistically significant variation among treatments at a p-value of 4.8×10^{-8}. The lower AV was obtained from *J. curcas* oil sampled from site:29, while the higher AV was recorded from kernel oil harvested from site:15. The AVs estimated in the present study were significantly lower as compared to 12.78 KOH g^{-1} reported by Silitonga *et al.* [14]. Similarly, Rodríguez *et al.* [21] showed different AVs for *J. curcas* kernel oil growing at different ecologies of Cuba.

Table 3. Physicochemical characteristics of *J. curcas* oil (n = 3; mean ± SD). Values sharing similar lowercase letters in the same column showed non-statistically mean difference among study sites at p-value of 0.05.

Site code	AV (mg KOH g^{-1})	FFA (%)	SV (mg KOH g^{-1} oil)	IV (g I_2 per 100 g oil)	PV (meq kg^{-1} oil)	KV at 25 °C (mm^2s^{-1})
Site:9	2.8 ± 0.01^{b}	1.5 ± 0.01^{b}	189.3 ± 3.97^{b}	112.2 ± 3.75^{a}	$6.1\pm1.34^{a,b}$	38.4 ± 2.34^{a}
Site:10	$1.6\pm0.02^{d,f}$	$0.8\pm0.02^{d,f}$	$191.6\pm1.20^{a,b}$	$110.5\pm3.99^{a,b}$	$4.7\pm0.30^{b,c}$	$38.2\pm1,12^{a}$
Site:12	$2.7\pm0.10^{b,c}$	$1.4\pm0.10^{b,c}$	$188.6\pm4\text{-}96^{b}$	$108.8\pm1.02^{a,b}$	$5.4\pm0.85^{b,c}$	39.0 ± 2.34^{a}
Site:14	$2.8\pm0.24^{b,c}$	$1.5\pm0.12^{b,c}$	180.9 ± 1.98^{b}	110.5 ± 1.56^{ab}	$6.5\pm0.21^{a,b}$	34.6 ± 2.52^{a}
Site:15	5.3 ± 0.39^{a}	2.7 ± 0.20^{a}	202.0 ± 1.98^{a}	112.6 ± 1.36^{a}	9.0 ± 0.21^{a}	39.3 ± 1.12^{a}
Site:16	$1.9\pm0.34^{c,d,e}$	$1.0\pm0.18^{c,d,e}$	190.8 ± 2.12^{b}	$108.2\pm0.72^{a,b}$	$3.7\pm0.92^{b,c}$	37.9 ± 2.49^{a}
Site:20	$1.3\pm0.14^{e,f}$	$0.7\pm0.07^{e,f}$	$192.2\pm2.12^{a,b}$	$99.6\pm2.87^{a,b}$	4.4 ± 1.13^{bc}	35.9 ± 2.52^{a}
Site:29	0.7 ± 0.08^{f}	0.4 ± 0.08^{f}	186.5 ± 1.98^{b}	$103.5\pm3.07^{a,b}$	2.6 ± 0.71^{c}	36.8 ± 0.45^{a}
Site:30	$2.5\pm0.14^{b,c}$	$1.3\pm0.07^{b,c}$	183.7 ± 5.95^{b}	$101.8\pm2.05^{a,b}$	$4.3\pm0.07^{b,c}$	37.0 ± 2.49^{a}
Site:31	$2.2\pm0.09^{b,c,d}$	$1.1\pm0.05^{b,c,d}$	$191.2\pm0.71^{a,b}$	$106.9\pm5.80^{a,b}$	$6.1\pm1.41^{a,b}$	37.7 ± 0.45^{a}
P-value	$4.8\ x10^{-8}$	$4.8\ x10^{-8}$	$2.7x10^{-3}$	$2.5x10^{-2}$	$1.1x10^{-3}$	$8.1x10^{-1}$

Conventional alkaline catalyzed transesterification reactions could be employed for *J. curcas* oil that contains 1-3% of FFA content [72-74]. However, oils with higher FFA (≥1%) needs more catalysts during the transesterification process [34, 74]. Moreover, higher FFA content in the oil would lead to a saponification reaction and incomplete reaction during the alkaline transesterification process [34, 75]. Therefore, if the AV of oil is beyond 4 mg KOH g^{-1}, a two-step catalytic processes, i. e., esterification followed by transesterification reactions, are needed [20]. The possible reasons for AV variation among treatments could be associated with genotype variations and environmental factors [76]. The higher FFA content of the oil could also be due to improper handling and storage of the seeds and oils [27]. Studies indicate that exposing the oil to open air and sunlight for a longer time would lead to oil degradation [6] and then to higher FFA content [77].

3.3.2. Iodine values

The IV (also called iodine number) of raw vegetable oil is almost identical to the corresponding FAME. The iodine number is a measure of the unsaturated fatty acid content of oil and biodiesel that influences the oxidation stability of oil/biodiesel when exposed to air [13]. The IV of Ethiopian variety J. curcas oils were ranged between 99.6-112.6 mg I_2 g^{-1} oil (Table 3), which agreed with EN 14214 standard. The ANOVA model with small p-vale ($2.5x10^{-2}$) indicates a significant IV variation among oil samples across various study sites. More specifically, higher IV was obtained from kernel oil sampled from sites: 9 and 15, while the remaining sites showed lower IV. The higher IV values were further supported by higher unsaturated oleic and linolenic acid contents (Table 4). Biodiesel produced from vegetable oil with a higher level of unsaturated fatty acids (higher IV) is ideal engine fuel utilized in its liquid form without solidification at a lower temperature. This justification works for Ethiopian variety *J. curcas* since the produced biodiesel's cold flow property is under EN 14214 specifications (Table 5). However, it should also be noted that oxidation reaction could be the other challenging issue during oil/biodiesel storage as oxidation stability is inversely related to the IV [6]. Moreover, high unsaturated fatty acid methyl ester would result in the polymerization of glycerides during the engine heating process, which leads to the formation of deposits and deterioration of lubricant.

3.3.3. Saponification values

Saponification is the hydrolysis process of oil with an alkaline catalyst to form glycerol and corresponding salt of fatty acids. Thus, SV represents the base required to neutralize the fatty acid generated from the complete hydrolysis of one-gram oil. The SV also indicates the nature of fatty acid (both free and esterified) present in the triglyceride [78]. Moreover, the SV indicates the average chain length of all the fatty acids present in the oil [79]. The data presented in Table 3 showed that the SV of *J. curcas* kernel oil harvested across 10 different study sites varied between 180.9 to 202.0 mg KOH g^{-1} oil. Furthermore, the ANOVA model showed that a statistically significant variation among treatments regarding their SV. Site:15 showed significantly higher SV as compared to sites: 9, 12, 14, 16, 29, and 30, while the remaining sites showed comparable SV values. The estimated SVs were in line with previous studies [27, 76]. The higher SV indicates the availability of triglycerides in the *J. curcas* oil, which is a suitable characteristic of biodiesel feedstocks [80]. However, excess SV could also result in a loss of ester yield during the transesterification process. Vicente *et al.* [81] reported that excessive saponification of triglyceride and dissolution of the ester by glycerol resulted in a loss of ester yields.

3.3.4. Peroxide value

The PV estimated from Ethiopian variety *J. curcas* oil were in between 3.7 to 9.0 meq kg^{-1} oil, and it was significantly varied among treatments with a p-value of $1.1x10^{-3}$. The maximum PV recorded from kernel oil sampled from site:15 was considerably higher as compared to sites:10, 12, 16, 20, 29, and 30 (Table 3). The higher PV indicates the deterioration of oil due to an oxidation reaction at the double bond of unsaturated fatty acids. Deterioration of the oil was also further supported by a strong correlation (r = 0.79) between estimated AVs and PVs. Most of the PVs estimated in the present study were relatively higher as compared to the values estimated for Nigeria and Indian variety [80]. Rodríguez *et al.* [21] studied the PV of *J. curcas* oil collected from two regions of Cuba, and the result indicates that the PV was significantly varied depending on growing area conditions. Another parameter that affects the direct application of *J. curcas* oil in a diesel engine is its inherent higher viscosity. The KV of Ethiopian variety *J. curcas* oils were estimated across various growing sites, and the estimated values were within the range of 34.6-39.3 $mm^2 s^{-1}$ (Table 3). However, the p-value from the ANOVA model ($8.1x10^{-1}$*)* indicates that not statistically significant KV variation was observed among the studied oil samples.

3.3.5. Fatty acid compositions

The data displayed in Table 4 illustrated the fatty acid compositions of Ethiopian variety *J. curcas* kernel oil harvested from 10 different study sites. The fatty acid composition analysis would help to predict the various fuel properties of biodiesel, such as viscosity, density, CN, IV, calorific value, lubricity, oxidation stability, and cold flow properties [6, 23]. Before the fatty acid compositions analysis, the oils were transesterified to produce easily detectable FAMEs using 1% KOH, 5:1 methanol to oil molar ratio, 50 °C reaction temperature, and 50 minutes of reaction time. Table 4 reveals the level of saturation (Cn:0), mono-unsaturation (Cn:1), di-unsaturation (Cn:2), DU, and LCSF estimated from *J. curcas* oil sampled from various study sites. Likewise, the chromatogram depicted in Fig. 4 showed the fatty acid profile of *J. curcas* oil sampled from site:10. Ethiopian variety *J. curcas* oil contains 75.80-80.30% of unsaturated and 19.70-24.2% of saturated fatty acid levels. The chromatogram from GC-MS analysis showed that the fatty acid composition of kernel oil was dominated by oleic (34.20-42.20%) and linoleic (34.80-41.80) acids. While palmitic, stearic, palmitoleic, and eicosanoic acids shared the 3rd, 4th, 5th and 6th levels of fatty acid compositions of the *J. curcas* oil, respectively. The data presented in Table 4 further showed that site:10, 14, and 31 contains relatively higher oleic acids, while sites:16 and 20 showed higher linoleic acids than the remaining study sites. The palmitic acid content of all oils sampled from all study sites was comparable, but higher stearic acid contents were observed from *J. curcas* oil sampled from site:16, 20, 29, 30, and 31. Higher saturated fatty acid contents could potentially deteriorate the cold flow properties of biodiesel, but they can also improve the CN and oxidative stability [23]. The unsaturated and saturated fatty acids proportions estimated from Ethiopian organ *J. curcas* oil/biodiesel were in line with cottonseed and algae oils [23] and very close to Congo-Brazzaville's *J. curcas* variety [31].

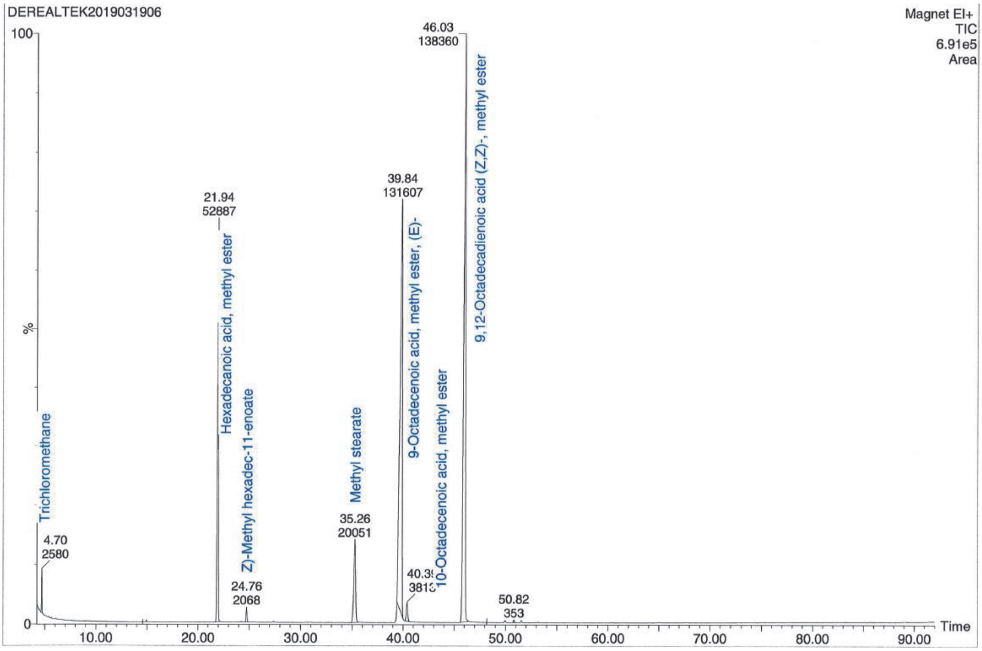

Fig. 4. Chromatogram of *J. curcas* oil (site:10) after transesterification reaction (1% NaOH, 5:1 methanol to oil molar ratio, 50 °C, and 50 minutes).

The small variation in fatty acid composition among treatments could be attributed to change in altitude [37, 64] and maturity levels [8]. The mono and di-unsaturated fatty acids were sensitive to the maturity level of seeds as compared to other types of fatty acids. Turinayo *et al.* [17] have also noted that the fatty acid composition of *J. curcas* oil could be affected by the seed size and weight. Jonas *et al.* [8] reported nearly the same amount of oleic (38.19%) and linoleic (39.24%) acids in Botswana, while the study reported by Inekwe *et al.* [80] reveals higher linoleic acids (80.07%) for Nigerian variety *J. curcas* oil. In contrast, Rodríguez *et al.* [21] reported that insignificant fatty acid composition variation among *J. curcas* oils grown at two different ecologies of Cuba.

Table 4. Fatty acid composition of *Jatropha curcas* seed oil sampled from different study sites.

Type of fatty acid	Fatty acid compassion (%) by study sites									
	Site:9	Site:10	Site:12	Site:14	Site:15	Site:16	Site:20	Site:29	Site:30	Site:31
Palmitic acid (C16:0)	15.2	14.0	14.9	14.2	14.8	14.8	14.4	14.5	14.1	13.1
Palmitoleic acid (C16:1)	0.6	0.5	0.1	0.5	0.6	0.6	0.6	0.9	0.7	0.7
Stearic acid (C18:0)	5.7	5.8	5.9	5.6	4.8	8.2	9.8	8.8	8.7	10.3
Oleic acid (C18:1)	38.8	42.2	39.2	41.8	39.8	34.5	34.2	37.8	38.1	41.1
Linoleic acid (C18:2)	39.6	37.5	39.9	37.8	39.8	41.8	41.0	38.1	38.5	34.8
Eicosanoic acid (C20:0)	0.1	0.1	0.1	0.1	0.2	ND	ND	ND	ND	ND
Proportion of saturation	20.9	19.9	20.9	19.9	19.9	19.7	24.2	23.3	22.7	23.4
long chain saturated factor [a]	4.5	4.5	4.6	4.4	4.2	5.6	6.3	5.9	5.7	6.5
Proportion of monounsaturation[a]	39.4	42.7	39.3	42.3	40.4	35.2	34.8	38.6	38.8	41.8
Proportion of diunsaturation[a]	39.6	37.5	39.9	37.8	39.8	41.8	41.0	38.1	38.5	34.8
Degree of unsaturation	118.6	117.6	119.0	117.9	119.9	118.8	116.9	114.8	115.8	111.4

[e] *etimated from the fatty acid profile of J. curcas oil/biodiesel; ND: non detected*

3.3.6. Biodiesel fuel properties

The fatty acid composition of *J. curcas* oil demonstrated in section 3.3.5 was utilized as an input variable for predicting the various fuel properties such as viscosity, density, CN, CFPP, and HHV of biodiesel synthesized from 10 sample oils (Table 5). The length of carbon chains and degree of saturation/unsaturation of the fatty acids affects the suitability of *J. curcas* oil for biodiesel production [82]. Vegetable oils with a higher level of mono and di-unsaturated (Cn:1, Cn:2), lower polyunsaturated ($Cn \geq 3$), and controlled saturated (Cn: 0) fatty acids are recognized as ideal feedstock for biodiesel production [8]. The KV of *J. curcas* oil has significantly decreased after converting the oil into biodiesel, and the predicted viscosities were ranged between 4.3 to 5.16 $mm^2 s^{-1}$. The data presented in Table 5 showed that the KVs, densities, CFPPs, and CNs of *J. curcas* methyl esters satisfied the EN 14214 standards.

Table 5. The fuel properties of *Jatropha curcas* methyl ester across different study sites.

Site code	KV ($mm2\ s^{-1}$)^a	Density ($g\ cm^{-3}$)^a	CFPP (^oC)	Surface tension ($mN\ m^{-1}$)^b	CN	HHV ($MJkg^{-1}$)
Site:9	4.296	0.877	-13.41	28.891	56.576	39.891
Site:10	4.322	0.878	-13.426	28.903	56.954	39.941
Site:12	4.661	0.875	-13.408	28.893	61.897	39.935
Site:14	4.313	0.877	-13.423	28.876	56.813	39.899
Site:15	4.289	0.877	-13.403	28.894	56.33	39.892
Site:16	4.297	0.876	-13.405	28.891	56.576	39.853
Site:20	5.164	0.877	-13.420	28.913	56.576	39.898
Site:29	4.339	0.878	-13.437	28.911	56.576	39.939
Site:30	4.338	0.878	-13.434	28.916	57.312	39.941
Site:31	4.382	0.876	-13.471	28.852	58.221	39.915
EN 14214 standards	3.50-5.00	0.86-0.90	< 5.00 (summer)> -15.00 (winter)	c	>51.00	c

^{a} KV and density measured at 20 ^oC; ^{b} surface tension measured at 40 ^oC; ^{c} not specified.

Oils with a higher level of monosaturated fatty acid content have higher CN, better oxidative stability, and lower NOx emission than oils enriched with higher polyunsaturated fatty acids [73]. In contrast, unsaturated fatty acids are more chemically unstable and susceptible to oxidation reaction than saturated fatty acids [6]. The oxidative stability of *J. curcas* oil decreased as the number of double bonds increases [8]; thus, linoleic acid is highly sensitive to degradation than oleic acid. The DU is directly correlated with the CN of methyl ester, while the cold flow property of biodiesel is significantly affected by LCSF [23]. Increasing chain length with lower branching and DU increases the CN, but it compromises *the* viscosity and cold flow properties of biodiesel. As shown in Table 5, the CNs of *J. curcas* biodiesel ranged between 56.33-58.22, which exceeded by 10.45-14.16% compared to the minimum CN (51) described in EN 14214 standards. Yaşar [23] has noted that the CN of various edible and non-edible oils are highly dependent on their fatty acid composition, and the CN linearly decreased with increasing of DU. Ramírez-Verduzco *et al.*

[49] have noted that the CN, KV, and HHV increase with increasing molecular weights, while these fuel properties were decreased as the number of double bond increases. In contrast, density is positively related to the molecular weight but inversely correlated with DU.

In contrast, the cold-flow property of biodiesel was found to be worse while utilizing feedstock with long-chain and more saturated fatty acids. The data presented in Table 5 illustrated that *J. curcas* oil grown across 10 different study sites contained higher levels of mono and di-unsaturated fatty acids and the estimated CFPPs ranged between -13.408 to -13.471 °C. Thus, the cold flow property of *J. curcas* biodiesel seemed under the EN 14214 standard. Surface tension is also one of the fundamental fuel properties that affect the atomization process in a conventional diesel engine [50]. The atomization process could be defined as the initial stage of the combustion of engine fuel, and thus the surface tension of fuel has a vital role in the fuel's combustion process. Moreover, the surface tension of the fuel is an essential parameter in the formation of oil droplets during spray atomization. The surface tension of *J. curcas* biodiesel predicted from its fatty acid composition varied between 28.85-28.92 mN m^{-1} and increases with increasing of the carbon chain, molecular weight, and DU. Moreover, the biodiesel synthesized from *J. curcas* oil also showed higher HHV (39.85-39.94 MJ kg^{-1}). HHV is the amount of heat released during the combustion of one gram of fuel to produce CO_2 and H_2O at its initial temperature, and this property is usually used to define the energy content of fuels and thereby their efficiency [49]. However, the energy content of *J. curcas* oil is relatively lower than that of petrol diesel. Thus, the lower calorific value of biodiesel is responsible for decreasing brake-thermal efficiency and brake-specific fuel consumption [6].

Conclusions

The present study was conducted to investigate the potential and suitability of Ethiopian variety *J. curcas* for biodiesel production. *J. curcas* grown across different regions of Ethiopia were characterized for seed, seed coat, kernel, and oil weight proportions. The seed coat and kernel accounts for 33.26-50.93% and 49.09-66.74% of dry weight seeds, respectively, while the dry weight of 100 randomly selected seeds varied between 50.53 -68.97 g. The oil content of *J. curcas* ranged between 47.1-59.3%, while the seed and kernel weights were positively correlated to the oil yield. Besides, the fatty acid composition and various physicochemical properties of *J. curcas* oil from the different study sites were also examined. The estimated AV (0.7-5.3mg KOH g^{-1} oil), IV (98.8-112.2 mg I_2 g^{-1} oil), and SV (180.9-202.0 mg KOH g^{-1} oil) values were significantly varied across ten selected study sites. The fatty acid composition of *J. curcas* oil was dominated by oleic (34.22-42.18%) and Linoleic (34.78-41.83%) fatty acids. Likewise, fuel priories like viscosity, density, CFPP, CN, surface tensions, and HHV of *J. curcas* biodiesel were studied using the fatty acid composition of oils, and the results revealed that all predicted fuel properties were agreed with the EN 14214 standards. Therefore, considering the higher kernel oil content and promising fatty acid composition, the present study concluded that Ethiopian variety *J. curcas* oil is suitable for biodiesel production. However, the seed yield and various physicochemical properties of *J. curcas* oil were highly dependent on the growing area conditions, but no significant effect was observed regarding the growing altitudes. Thus, future research should focus on the actual factors that affect seed yield, oil content, and various physicochemical properties of *J. curcas* kernel oil.

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Paper III

Biomass Conversion and Biorefinery
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ORIGINAL ARTICLE

Alkaline and co-digestion pretreatments: process optimization for enhancing the methane yield of Jatropha press cake

Gebresilassie Asnake Ewunie[1] · **John Morken**[1] · **Zerihun Demrew Yigezu**[2]

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Abstract

Oil extraction and biodiesel production process produce a massive amount of by-products like Jatropha press cake (JPC) and crude glycerol (CG), which could be used as a potential substrate for methane production. However, the higher lignocellulosic and nitrogen content in the JPC act as a recalcitrant and inhibitor, respectivly, for microbes that are involved in the anaerobic digestion (AD) process. Therefore, the present study aimed to enhance the methane yield of JPC by optimizing the alkaline pretreatment and co-digestion process conditions. The effects of NaOH concentration, incubation temperature, and retention time on methane and soluble chemical oxygen demand (sCOD) yields were evaluated and modeled by employing a response surface methodology coupled with central composite design (RSM-CCD). Moreover, a series of batch experiments with various feedstock concentrations (FCs) were tested to investigate the methane yield of JPC when co-digested with CG at different levels. The methane yields of all pretreated samples were significantly higher when compared with these of the untreated JPC. Pretreating the JPC using 7.32% NaOH at 35.86 °C for 54.05 h was the optimum conditions for maximum methane increment of 40.23% (353.90 mL g^{-1} VS), while co-digesting 2% CG with JPC at 2 g VS L^{-1} FC enhanced the methane yield by 28.9% (325.47 mL g^{-1} VS). Thus, the methane yield of JPC was effectively increased by alkaline pretreatment and co-digesting with CG. However, the alkaline pretreatment was relatively more effective compared with the co-digestion process.

Keywords Co-digestion · Crude glycerol · Jatropha press cake · Methane yield · Optimization · Pretreatment

Abbreviations

adj. R^2	Adjacent *R* square
APHA	American Public Health Association
NH_3	Ammonia
NH_4OH	Ammonium hydroxide
AD	Anaerobic digestion
ANOVA	Analysis of variance
BCR	Benefit-cost ratio
BMP	Biochemical methane potential
$Ca(OH)_2$	Calcium hydroxide
CO_2	Carbon dioxide
C/N	Carbon-to-nitrogen ratio
CCD	Central composite design
CG	Crude glycerol
°C	Degree Celsius
E_i	Energy input
E_o	Energy output
EPA	Environmental protection agency
η	Eta (Greek letter)
FC	Feedstock concentration
GC	Gas chromatography
g	Gram
H_2O_2	Hydrogen peroxide
ISR	Inoculum-to-substrate ratio
JPC	Jatropha press cake
K	Kelvin
kg	Kilogram
kJ	Kilojoule
kPa	Kilopascal
L	Liter
MRV	Measurement, reporting, and verification
MJ	Mega joule
m^3	Meter cubic
CH_4	Methane

✉ Gebresilassie Asnake Ewunie
asnake.ewunie.gebresilassie@nmbu.no

[1] Department of Science and Technology, Norwegian University of Life Sciences (NMBU), P. O. Box, N-1432 Ås, Norway

[2] College of Agriculture, Hawassa University, P.O. Box 05, Hawassa, Ethiopia

MC	Moisture content
R^2	Multiple *R* square
NIBIO	Norwegian Institute of Bioecnomy Research
pred. R^2	Predicted *R* square
RSM	Response surface methodology
rpm	Revolution per minute
NaOH	Sodium hydroxide
sCOD	Soluble chemical oxygen demand
TCD	Thermal conductivity detector
TS	Total solid
VFA	Volatile fatty acid
VS	Volatile solid
wt	Weight
ξ	Xi (Greek letter)
HMF	5-hydroxymethylfurfural

1 Introduction

The demand for fuel energy sources and their actual consumption across the globe are increasing drastically [1]. Fossil fuel resources supplied almost 87% of the global energy demand [2]. However, three-fourths of the world's poor households consumed only 10% of the worldwide energy supply [3]. Most of these poor households are living in net oil-importing Sub-Sahara Africa, where Ethiopia is ranked at the bottom of the energy poverty index [4]. In Ethiopia, 91% of the energy demand is satisfied by the local available traditional biomass resources [5]. However, the utilization of these conventional energy sources has resulted in severe environmental problems like forest degradation and greenhouse gas emissions. Furthermore, the entire transport energy requirement of Ethiopia is dependent on imported petroleum, with the cost of 65–80% of the total export earnings [6]. For instance, in 2018/2019 alone, 3.99 million metric tons of petroleum products were imported; and the expense of this import was more than 2.4 billion US dollars [7]. The costs associated with port rent, along with petroleum price and fluctuation, have severely affected the trade balances of the country. As a result, the Ethiopian government has launched a strategy in 2007 to assist the production and utilization of biofuels (biodiesel, bioethanol, and biogas) from different non-edible oil seeds and agro-industrial and municipal solid organic wastes [8].

Jatropha curcas L. (Jatropha hereafter), castor bean (*Ricinus communis*), Croton (*Croton macrostachyus*), and Moringa (*Moringa stenopetala*) plant seeds have been identified as promising non-edible energy plants for biodiesel production [9]. Jatropha seed is characterized by high oil content [10]. The oil is dominated by monounsaturated and polyunsaturated fatty acids [11]. Although the seeds are suitable for biodiesel production, 65–70% of them reported being disposed of as Jatropha press cake (JPC) during oil extraction [12], while 100 kg of biodiesel production generates 10–14 kg of crude glycerol (CG) [13]. Considering the availability of adequate Jatropha plantation in Ethiopia [4] and higher seed production per ha [14], biodiesel production would generate a substantial quantity of JPC (10.5–11.4 million tons) and CG (0.61–0.70 million tons) per year.

CG can be purified into high grade for various applications in cosmetics and pharmaceuticals. However, the high purification cost hinders its use in such industries and usually considered organic waste [2]. While JPC contains toxic chemicals like phorbol ester and curcin [15], thus, the cake could not be directly used as animal feed or organic fertilizer [16]. As a result, managing and controlling these organic wastes become the most challenging issue for most biodiesel producers. Detoxification and anaerobic digestion (AD) were identified as promising pretreatment methods for utilizing JPC for animal feed and organic fertilizer, respectively. With regard to this, studies have tried to investigate the biochemical methane potential (BMP) of JPC [17, 18]. However, JPC contains a considerable amount of seed husks, which are enriched with lignocellulosic materials. Liang et al. [19] showed that JPC contains 19% lignin and 27% carbohydrates, while Kumar et al. [20] measured 14% and 28% cellulose and hemicellulose, respectively. Furthermore, the carbon-to-nitrogen ratio (C/N) of JPC was reported to be 9:1 [16], which is significantly lower compared with the optimum ratio of 20:1–30:1 [21]. The digestibility of lignocellulosic biomasses for methane production is limited mainly due to the rate-limiting hydrolysis stage caused by the complex cellulose-hemicellulose-lignin networks [22]. Higher nitrogen content may cause inhibition of the methanogenic activities [23] and subsequently leads to process collapse due to the ammonia accumulation [18]. In contrast, CG is very rich in carbon, which means the mono-digestion of CG for biogas production is impossible due to nitrogen deficiency for maintaining the microbial biomass growth [2].

Pretreating the JPC using proper techniques can improve the anaerobic degradation process by bringing a structural change. The structural change would help the microbes to convert the carbohydrate polymers into simple fermentable sugars [24], whereas, co-digestion could improve the methane yield by optimizing the required C/N ratio [2, 25]. Therefore, various techniques such as mechanical [26], thermochemical [24], and steam explosion [27, 28] pretreatments were commonly used for pretreating lignocellulosic biomasses. Furthermore, the biodegradability of feedstocks with lower C/N ratio could be improved by co-digestion, solid-state AD, bio-augmentation, or nutrient supplementation [21, 23].

In the present study, alkaline pretreatment and co-digestion process were selected as a potential technique for enhancing the methane yields of JPC due to various positive characteristics compared with other pretreatment methods. Briefly, thermo-acidic pretreatments are corrosive and cause degradation of carbohydrates and lignin [29]. Biological pretreatments are expensive and time-consuming due to the slow processes, and finding a specific enzyme is complicated [21, 26, 30, 31]. Physical pretreatments are energy-intensive processes [21, 26]. The alkaline pretreatment may increase the pH of the feedstock due to the residual chemical after the pretreatment [32]. However, the remaining chemical is useful since the next step, the AD, requires an alkaline addition for controlling pH drop due to volatile fatty acid (VFA) accumulation. Moreover, alkaline pretreatment is highly effective in lignin removal by cleaving the lignin-carbohydrate linkages with minimal carbohydrate degradation [21].

Among different alkaline chemicals, NaOH is effective for enhancing the biogas yield of lignocellulose biomasses [21]. However, the efficiency of alkaline pretreatments is affected mainly by chemical concentration, temperature, and retention time [33–35]. The effect of these process variables in the alkaline pretreatment was studied by varying one variable at a time but keeping the other variables into a constant value. However, the synergistic effects from simultaneous changes of more than one process variable were not widely investigated. Failure in optimizing the effects of individual and interactive process variables would lead to insufficient hydrolysis of lignocellulosic materials or AD process inhibition due to the accumulation of degraded products [36]. To the best of our knowledge, no reports are found on the optimum condition of alkaline pretreatment, and the amount of CG that should be added for enhancing the methane yield of JPC. Thus, the present study was initiated to examine and model the effect of NaOH concentration, incubation temperature, and retention time in the alkaline pretreatment on the methane and soluble chemical oxygen demand (sCOD) yield of JPC.

2 Materials and methods

2.1 Source of raw materials

The JPC sample was kindly provided by YME Design and Manufacture Company located at Hawassa, Ethiopia. At the time of supply, the company exploded the oil from the Jatropha seed using a hydraulic press machine. The fresh JPC was dried at room temperature (27 °C) until its moisture content reduced to 10% on the dry weight basis. The dried JPC was then transferred into airtight zipped plastic and stored at 4 °C until further processing. The bacterial inoculum prepared from cow manure was collected from a biogas laboratory operated under mesophilic conditions. The dominant types of microbes living in the manure-based inoculum were identified by Ozbayra et al. [37]. *Firmicutes* and *Bacteroidetes* accounted for about 46% and 36% of the bacterial community, respectively, while the methanogenic community was dominated by methanomicrobia (65%), methanobacteria (25%), and methanoplasma (8%) archaea. The analytical grade of NaOH for the alkaline pretreatment was purchased from VWR, Oslo, Norway. The CG that co-digested with JPC was purchased from biodiesel producer company, Perstorp AB, located in Sweden.

2.2 Co-digestion of Jatropha press cake with crude glycerol

The BMP of JPC that co-digested with CG at various levels (0, 1, 2, 3, 4 wt%, on VS basis) was investigated by following a completely randomized design [2]. The actual experimental layout for the AD experiment is shown in Table 1, and each treatment was duplicated into a total of 30 batch reactors.

2.3 Alkaline pretreatment

The alkaline pretreatment was conducted by following the method described by Zhu et al. [38], with some modifications. Accordingly, JPC was allowed to pass through a sieve with a mesh size of ≤ 2.5 mm [39]. Then, 100 g of sun-dried sample (with 8% moisture content) was transferred into 1-L beakers that contain 2.64%, 4%, 6%, 8%, and 9.36% of NaOH, based on the dry weight of JPC. The samples were then adjusted into a moisture content of 82% by adding the required amount of tap water (Eq. (1)) [40]. After adding the water, all samples were gently stirred with glass road to homogenize the mixture. Then after, all beakers were covered with plastic films and fastened with plastic rings and incubated at 26.6 °C, 30, 35, 40, and 43.4 °C for 7.68, 24, 48, 72, and 88.82 h. Following the subsequent pretreatments, a subsample was taken from individual treatment and centrifuged at 6000 rpm for 20 min to separate the solid and liquid fraction for sCOD analysis. Finally, the solid/liquid mixture and liquid fractions of the samples were immediately transferred into zipped plastic bags and 20-mL vials, respectively. Both samples were stored in the freezer until the actual biogas production and sCOD analysis were started. For the control experiment, 100 g of JPC was diluted with the same amount of water without alkaline and incubated under room temperature for the maximum retention time of 88.82 h [29].

$$\text{MC required } (\%) = \left(1 - \frac{\text{Dry weight}_{\text{JPC}}}{\text{Weight}_{\text{JPC}} + \text{water}_{\text{added}}}\right) \times 100 \qquad (1)$$

where MC is moisture content and JPC is Jatropha press cake.

2.4 Biochemical methane potential assays

The effects of alkaline pretreatment and co-digestion of CG with JPC on the BMP were investigated using 57 and 32-

Table 1 The experimental layouts for co-digesting the JPC with CG at different FC and CG levels

Feedstock concentrations	CG levels (%)	CG (g VS L^{-1})	JPC (g VS L^{-1})	Inoculum (g VS L^{-1})
2 g VS L^{-1}	0.0	0.000	2.184	5.300
	1.0	0.022	2.184	5.300
	2.0	0.044	2.184	5.300
	3.0	0.066	2.184	5.300
	4.0	0.088	2.184	5.300
5 g VS L^{-1}	0.0	0.000	5.002	5.300
	1.0	0.050	4.950	5.300
	2.0	0.099	4.901	5.300
	3.0	0.146	4.853	5.300
	4.0	0.190	4.822	5.300
7 g VS L^{-1}	0.0	0.000	7.000	5.300
	1.0	0.070	6.930	5.300
	2.0	0.138	6.862	5.300
	3.0	0.205	6.795	5.300
	4.0	0.270	6.729	5.300

batch reactors, respectively. The total and working volume of the batch reactors for alkaline samples were 122 and 70 mL, respectively, while the bigger batch reactors with 530 and 350 mL of total and working volume, respectively, were used in the co-digestion process. The main reason for preferring the bigger batch reactor in the co-digestion process was the limited working volume from the smaller reactor for adding the required amount of CG. In contrast, the smaller reactors were used for the alkaline-pretreated samples due to the inoculum shortage. Moreover, the authors assumed that the effect of volume variation on methane yield is minimal since the inoculum-to-substrate ratio (ISR), incubation temperature, agitation rate, and reactor configuration were the same for all batch reactors.

The manure-based inoculum was further incubated at 37 ± 0.5 °C under the anaerobic condition for 1 week, which helps the microbes to assimilate the environment and reduce the endogenous biogas production. Following the pre-incubation, the prepared reactors were filled with the required amount of inoculum (5 g VS L^{-1}); then, 3.34 g VS L^{-1} of untreated or pretreated JPC was added into all batch reactors. Thus, in this experiment, the ISR was kept to 1.5 on a VS basis [27]. However, the amount of VS added into the bigger batch reactor (530 mL) depends on the predefined FCs, i.e., 2, 5, and 7 g VS L^{-1}. The respective working volumes of the reactors were then adjusted by diluting the inoculum and substrate mixture with tap water. For blank and positive control experiments, the same amount of inoculum was poured to each bottle, and tap water was added for required working volume: 1.5 g L^{-1} pure cellulose ($C_6H_{10}O_5$) $n < 20$ μm was added into the bottles reserved for positive control. For alkaline-pretreated JPC, triplicated bottles were prepared for samples pretreated under the corner and axial (star) points, whereas six bottles were prepared for samples pretreated under the center points (Table 2). The batch reactors arranged for co-digestion experiments were duplicated, and a total of 30 bottles ware utilized. All batch reactors were closed with a rubber stopper and aluminum crimps and then purged with pure nitrogen using a syringe for 5 min to make an anaerobic environment [41]. Finally, the reactors were incubated at 37 ± 0.5 °C and continuously centrifuged to 90 rpm. The methane yield increment (MYI) due to the alkaline pretreatments and the co-digestion processes were estimated using Eq. (2) [42].

$$\mathrm{MYI}\ (\%) = \left(\frac{\mathrm{MYI}_{\mathrm{pretreated}} - \mathrm{MYI}_{\mathrm{untreated}}}{\mathrm{MYI}_{\mathrm{untreated}}}\right) \times 100 \tag{2}$$

where MYI is methane yield increment.

2.5 Experimental design

The alkaline pretreatments were carried out for each combination of all variables (NaOH concentration, incubation temperature, and retention time) using RSM-CCD, and all pretreatments were carried out in random orders. A total of 20 treatments that containing eight factorial points, six axial points, and six replicates for the center value were applied. The values estimated from center points were used to determine the experimental error. The coded and real values were calculated using Design-Expert version 12 (State Ease Inc., Statistics Made Easy, Minneapolis, MN, USA). The lower and upper values of each factor (Table 2) were determined following the methods of Zheng et al. [43]. The data obtained from AD and hydrolysates were used to develop a second-order polynomial equation that relates the responses to the process variables (Eq. (3)). Both the experimental design

Table 2 Coded and real values of each variable used in the RSM-CCD

Variable	Symbol	Levels				
		$-\alpha$ (− 1.68)*	− 1	0	+ 1	+ α (1.68)
NaOH concentration (wt%)	X_1	2.64	4.00	6.00	8.00	9.36
Incubation temperature (°C)	X_2	26.6	30.00	35.00	40	43.40
Retention time (h)	X_3	7.68	24.00	48.00	72	88.32

*α is the distance from the axial point to the center point calculated by $2^{k\ (1/4)}$ (k is the number of independent factors being used in the alkaline pretreatment = 3)

and statistical analysis were also done using the same software. Furthermore, the second-order polynomial equation from RSM-CCD was used to define the optimum value of each process variable that could maximize the methane and sCOD yields. The linear, interactive, and quadratic effect of temperature, NaOH concentration, and retention time was evaluated using a multivariate analysis of variance (ANOVA). The contour and 3D response surface plots were drawn to depict the interactive effect between two process variables while keeping the third variable into its center value. Values for multiple R^2, adj. R^2, and pred. R^2 were used to evaluate the fitted model.

$$Y = \beta_O + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \qquad (3)$$

where Y is the response (sCOD or methane yield); X_1, X_2, and X_3 are the coded independent variables; and β_0, β_1, β_2 and β_3, β_{11}, β_{22}, β_{33}, β_{12}, β_{13}, and β_{23} are the model coefficients calculated from the experimental value.

2.6 Analytical method

2.6.1 Substrate analysis

The total solid (TS) and volatile solid (VS) contents of inoculum and JPC were determined based on the APHA standards [44]. The carbon, nitrogen, and hydrogen contents were estimated using an elemental analyzer (Thermo Flash 2000) from soil laboratory located at Norwegian University of Life Sciences, Ås, Norway. The crude protein content of JPC was estimated from its nitrogen content [45]. Karl Fisher titration was used for determining the moisture content of CG [45], and the TS was calculated by considering the moisture content. The VS content of CG was assumed to be the same with the TS value as nothing was left in the crucible when it was ignited at 550 °C for 2 h. The sCOD values for each treatment were estimated following DIN ISO 15705, which is analogs to EPA 4104 and APHA 5220 D [42]. Merck Spectroquant® COD kits with a measuring range of 500–10,000 mg L^{-1} were used in the analysis of the sCOD yields. The caloric value and crude fat content of the dried JPC were determined using a Bomb Calorimeter (IKA^R C 200) [42] and conventional Soxhlet extraction method, respectively [18].

2.6.2 Biogas analysis

The BMP of both the alkaline pretreated and co-digested JPC with CG were regularly monitored for 61 and 56 days, respectively, until the last methane production in all flasks was less than 2% of the total amount. Briefly, the biogas pressure was measured using a digital manometer (GMH 3161 Reisinger Electronics, Germany), and the concentration of CO_2 and CH_4 in the biogas was determined using gas chromatography (3000 Micro GC, Agilent Technologies, USA), equipped with a thermal conductivity detector (TCD) using helium as a carrier gas [42]. After the successive biogas measurements, the excessive pressure was released by inserting the needle into the rubber stopper. All measured gas volumes were reported at 273 K and 101.3 kPa using Eq. (4), and the specific and cumulative methane volume was calculated using the normalized methane concentrations. The endogenous methane value produced from the blank controls (inoculum) was subtracted from the total methane yield. Besides CO_2 and CH_4, the biological degradation of organic matter could generate hydrogen gas. However, considering its small fraction (5–10%) when compared with the total biogas yield [46], this study did not measure the hydrogen concentration during the AD process.

$$\mathrm{Vb} = \frac{n \times R \times 273}{P_o} = \left(\frac{dp \times V}{R \times T}\right) \times \left(\frac{R \times 273}{P_o}\right) = \left(\frac{dp \times V \times 273}{T \times P_0}\right) \qquad (4)$$

where Vb is the volume of biogas (L) at the standard condition of 273 K (0 °C), 1 atm total pressure; P_o is 1 atm; n is the number of moles; R is the ideal gas constant; V is the volume of headspace (L); T is the temperature in the incubator room (310 k); and dp is the overpressure measured in the bottle (atm) (1013.25 mbar = 1 atm).

2.6.3 Energy balance and economic feasibility

Energy balance and benefit-cost analysis are essential tools for examining the economic viability of various pretreatment techniques applied for enhancing the biogas production potential lignocellulosic material. Although all alkaline and co-digestion processes improved the methane yield of JPC, energy balance and cost analysis were done only for the optimum conditions identified for higher methane yields by using Eqs. (5) and (6) [47]. The benefit-cost ratio (BCR) analysis was performed based on the following assumptions: (1) the average market price of NaOH is assumed to be 412 € t^{-1} [48] and (2) the estimated cost of methane sale is 0.57 € m^{-3} [47]. Moreover, the optimum temperature (35.86 °C) identified in the alkaline pretreatment is nearly the same as the maximum ambient temperature (33 °C) of Hawassa City, Ethiopia, where the JPC sample was brought. Thus, we assumed that no heat energy input was needed for this alkaline pretreatment.

$$E_O = \Delta p \times \xi \times \eta \tag{5}$$

$$E_i = E_{\mathrm{NaOH}} \times n \tag{6}$$

where E_o is energy output (kJ kg^{-1} VS); E_i is energy input (kJ kg^{-1} NaOH); E_{NaOH} energy needed for 1-kg alkaline production (7 MJ kg^{-1}); Δp is net methane yield increment (m^3 g^{-1} VS); ξ is methane heating value (35.8 kJ L^{-1}); η is methane energy conversion efficiency (90%), and n is the amount of NaOH needed in the pretreatment process (kg).

2.6.4 Statistical analysis

The interactive effect between FC and CG levels on the biogas and methane yield of JPC was tested using a two-way ANOVA via R software (version 3.6.2), while the linear, interactive, and quadratic effects of NaOH concentration (X_1), incubation temperature (X_2), and retention time (X_3) were analyzed using Design-Experts (version 12) as discussed in Section 2.5. All ANOVA was carried out using Tukey's test. Thus, there were statistically significant variations at $p \leq 0.05$ between mean with different letter groups, or the mean differences were considered statistically significant if the p value is ≤ 0.05.

3 Results and discussion

3.1 Substrate compassion

The physical and chemical constituents of JPC, CG, and inoculum characterized using the methods described in Section 2.6.1 are shown in Table 3.

Table 3 Physicochemical characteristics of substrates being used in the AD process

Parameters	Types of substrate		
	JPC	Inoculum	CG
TS (%)	90.00 ± 0.01	4.40 ± 0.28	85.00 ± 0.03
VS (%)	93.36 ± 0.08	59.90 ± 0.34	–
sCOD (g L^{-1})	717.43 ± 3.19	–	1399.33 ± 6.99
Nitrogen (%)	3.99 ± 0.09	–	–
Carbon (%)	50.40 ± 2.07	–	–
Hydrogen (%)	7.17 ± 0.23	–	–
C/N ratio	13.10 ± 0.88	–	–
Crude protein (%)	24.90 ± 0.28	–	–
Crude fat (%)	9.00 ± 20	–	–
Calorific value (kJ kg^{-1})	19.87 ± 0.62	–	–

3.2 Impact of feedstock concentration and CG level on BMP of JPC

Various studies indicate that FC has a detrimental effect on the AD process and the subsequent methane recovery process [49–51]. Moreover, the higher protein, lipid, and long-chain free fatty acid concentration present in the JPC could also inhibit the activity of microbes in the digester unless the steady-state condition is maintained. In the present study, the feedstock concentration (FC) is defined as the amount of JPC and CG in VS bases added into the working volume of the batch digester (g VS L^{-1}). Significant variations in cumulative and specific methane yields were observed among treatments operated under different FCs (2, 5, and 7 g VS L^{-1}) and CG levels (0, 1, 2, 3, 4%). Most of the batch digesters run without CG produced lesser biogas and methane when compared with the treatments containing both CG and JPC (Table 4). The lower methane yield might be attributed to the higher content of lignocellulosic materials along with lower C/N ratios. The availability of higher nitrogen in the JPC could generate non-ionized and/or ammonium ion in the AD process [52]. Then, the ammonium reacts with water and forms ammonium hydroxide (NH_4OH), which alkalizes the bulk solution of the digester. The alkalized bulk solution could inhibit the anaerobic process by affecting the activity of *Archaea methanogenesis* [53]. Moreover, variation in trends and peaks of daily methane yield also noted among treatments (Fig. 1a, b, and c). Co-digesting 2% CG with JPC at 2 g VS L^{-1} FC showed a fast degradation rate, which needed only 3 days to attain the maximum methane peak value (48.9 mL g^{-1} VS day^{-1}) when compared with all other treatments (Fig. 1a).

A two-way ANOVA and Tukey post hoc test were conducted to investigate the statistical variation in biogas and methane

Table 4 The cumulative biogas and methane production (mL g^{-1} VS) of JPC co-digested with CG at different conditions

	Crud glycerol levels (%)	Feedstock concentrations		
		2 g VS L^{-1}	5 g VS L^{-1}	7 g VS L^{-1}
Biogas (mL g^{-1} VS)	0	339.91b	334.50b	344.24b
	1	360.24b	369.96b	358.42b
	2	451.43a	369.13b	364.20b
	3	382.74ab	355.54b	369.49b
	4	358.95b	391.96b	234.89c
Methane (mL g^{-1} VS)	0	252.41b	240.11b	247.98b
	1	259.49b	264.33b	249.68b
	2	325.47a	261.97b	251.11b
	3	278.46ab	250.43b	254.19b
	4	265.23b	275.69b	165.86c

The difference in lowercase letters showed a statistical mean difference due to the interactive effect between FC and CG levels. The statistical variation of means in the same row was due to the FC effect, while the mean difference in the same column was due to the CG level effect

yields among digesters operated at various co-digestion conditions. The smaller p value ($< 2.2 \times 10^{-16}$) of the ANOVA model indicates a statistically significant variation among anaerobic digesters in estimated cumulative biogas and methane yields (Table 4). The cumulative methane yield of JPC slightly declined as increasing of both the FC and CG levels (Fig. 1d). The average cumulative methane yield of digester run by adding various concentrations of CG to JPC under 2 g VS L^{-1} FC ranging between 252.41 to 325.47 mL g^{-1} VS, while the methane yield obtained from 5 g VS L^{-1} and 7 g VS L^{-1}

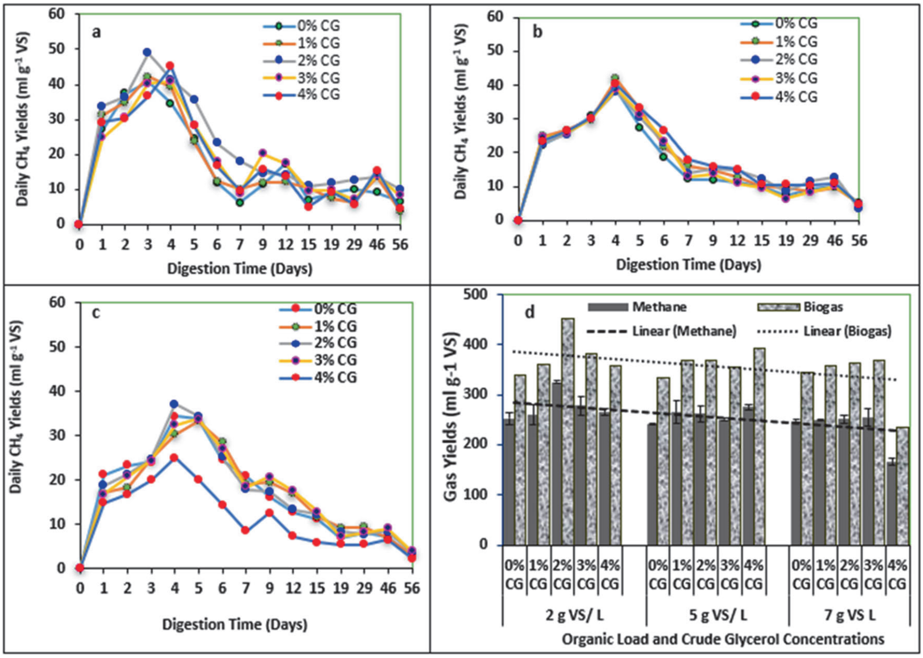

Fig. 1 The daily methane yield at FC of 2 g VS L^{-1} (**a**), 5 g VS L^{-1} (**b**), and 7 g VS L^{-1} (**c**) and the cumulative biogas and methane yields resulted from the various FC and crude glycerol (CG) levels (**d**)

FCs varied from 240.11 to 275.69 and 165.87 to 254.19 mL g^{-1} VS, respectively, depending upon the amount of added CG levels.

The highest cumulative methane yield (325.47 mL g^{-1} VS) was obtained from digester working under 2 g VS L^{-1} that contains 2% CG, while less methane yield (165.87 g^{-1} VS) was recorded from digester operated under 7 g VS L^{-1} and enriched with 4% CG. The methane increment recorded from the former treatment was 28.9% when compared with the samples containing no CG at the same FC. However, the methane yield obtained from treatment that co-digested 4% CG with JPC at 7 g VS L^{-1} FC declined by 96.23% when compared with the maximum methane yield. Similar results were reported by Schwingel et al. [2], in which the biogas and methane yield from laying hen waste were strongly dependent on the amount of added CG. Oliveira et al. [54] noted that co-digesting 2% CG with sargassum resulted in 18% more methane as compared with the control treatment, while Schwingel et al. [2] showed that co-digesting 2.6% glycerol with laying hen manure produced 21.83% more methane than the manure without glycerol. Veroneze et al. [55] indicated that inhibition of methane production from swine manure when more than 5% of glycerol was added.

The increment in methane yield might be attributed to the higher biodegradability and enhancement of the C/N ratio due to the added CG. In contrast, the lower methane yield might be associated with organic overloading, fast production of VFAs, or acidification of the digester [43]. The rapid production of VFA due to the fast hydrolysis of CG may inhibit the methanogenesis bacteria [38]; however, its effect seemed small since variation in methane concentrations among reactors was not statistically significant. Organic overloading could stimulate the microbial production of biosurfactants and mostly resulted in partial degradation of organic matter [56], which subsequently causes lower methane yield (Fig. 1d). The VS content of the digestate after 61 days of AD was higher for batch reactors operated at higher FC. The effect of overloading on methane yield could be higher since JPC contains less biodegradable protein [50]. Moreover, stable small bubbles (foams) were observed in the batch reactors run at higher FC. The foaming incidence might be associated with the surfactant nature of protein and lipid available in the JPC [57]. Thus, the foaming incidence could reduce the methane yield by producing a gas-liquid film at the top of the digester.

Variation in average cumulative methane yield was also investigated for samples with the same FC but contained different levels of CG using a single-factor ANOVA. The *p*-values of digesters working under 2, 5, and 7 g VS L^{-1} FCs were 0.02, 0.18, and < 0.01, respectively, which indicate that the effects of added CG levels were statistically significant at lower and higher FCs; however, their effects were negligible at 5 g VS L^{-1} FC. Besides the cumulative methane yield, the average methane concentrations were calculated for all samples and the values ranging from 70.7 to 73.9%. The ANOVA model showed an insignificant variation in methane concentration among treatments. Therefore, considering the economic gain of using lower FC for higher cumulative methane production, co-digesting 2% CG with JPC at 2 g VS L^{-1} FC was identified as the optimum condition for higher methane production. The optimum condition obtained in the present study was comparable with conditions suggested by Haitl et al. [58]; however, relatively higher methane yield (1058.9 mL g^{-1} VS) was reported by co-digesting 2.5% CG with hen laying manure [2].

3.3 Modeling the degree of solubilization (sCOD)

The effect of alkaline pretreatment on the compositional change of JPC was assessed using the sCOD yields as a primary indicator [41]. A regression model was fitted to determine the optimum values of the selected process variables for higher sCOD production. By referring Eq. (3), the second-order polynomial equation obtained from the experimental data was taken in terms of coded and actual value to evaluate the correlation between predictors (X_1, X_2, and X_3) and the response (sCOD) variables (Eqs. (7) and (8)). Thus, the liner, interactive, and quadratic effects of X_1, X_2, and X_3 on the degree of solubilization were investigated using RSM-CCD. As shown in Eq. (7), all coded linear and two interaction terms had a positive effect, while all quadratic terms and the interaction between X_2 and X_3 adversely affected the degree of solubilization.

$$\begin{aligned}\text{sCOD yield (coded)} = {} & 145.96 + 12.25X_1 + 6.26X_2 \\ & + 20.13X_3 - 7.67X_1^2 - 2.19X_2^2 - 8.38X_3^2 \\ & + 3.00X_1X_2 \\ & + 0.25X_1X_3 - 4.5X_2X_3\end{aligned} \tag{7}$$

$$\begin{aligned}\text{sCOD yield (actual)} = {} & -183.31 + 18.39X_1 + 7.39X_2 \\ & + 3.52\mathrm{X}_3 - 1.92X_1^2 - 0.09X_2^2 - 0.01X_3^2 \\ & + 0.30X_1X_2 \\ & + 0.01X_1X_3 - 0.04X_2X_3\end{aligned} \tag{8}$$

The adj. R^2 value of the quadratic model (0.81) was higher as compared with the values estimated for linear (0.68) and two-factor interaction (0.63) models; hence, the second-order polynomial equation was the preferred model in this study [59]. The strong correlation between experimental and model-predicted sCOD values further exhibited the suitability

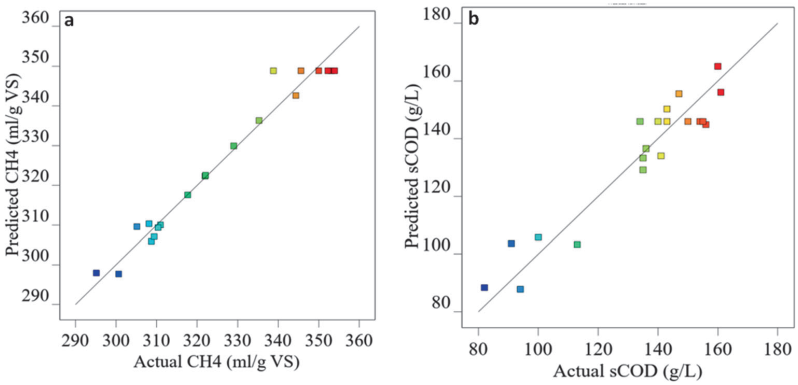

Fig. 2 Correlations between the actual and model-predicted values of methane (**a**) and sCOD (**b**)

of the model (Fig. 2b). This value lies between ± 4.3, in which all estimated values were close to the model-predicted value. The value for the coefficient of determination (R^2) was 0.90, which assured the applicability of the model for investigating the effect of these process variables in the alkaline pretreatment process. Furthermore, the p value > 0.05 indicates that there was no evidence on the model's lack of fit relative to the pure error, which further elaborated the strength and adequacy of the model to depict the correlation between predictor and response variables.

The multivariate ANOVA for the second-order polynomial equation was also done to test the strength of the model. The results suggested that the second-order polynomial equation could better describe the relationship between sCOD productions and operational variables. As shown in Table 5, the p-values ≤ 0.05 indicate the significant linear impact of NaOH concentration (X_1) and retention time (X_3), as well as the quadratic effects from the same variables (X_1^2 and X_3^2). Even though the p value of temperature was slightly greater than 0.05, it was included in the model by considering as important process variables in the solubilization of the JPC. This finding was in agreement with the report by Monlau et al. [60].

Table 5 ANOVA for the model regression representing sCOD

Source	Sum of squares	df	Mean square	F value	p value
Model	10,051.77	9	1116.86	10.14	0.001*
X_1	2049.87	1	2049.87	18.61	0.002*
X_2	534.71	1	534.71	4.86	0.052
X_3	5531.94	1	5531.94	50.23	< 0.001*
X_1X_2	72.00	1	72.00	0.65	0.438
X_1X_3	0.50	1	0.5000	0.006	0.948
X_2X_3	162.00	1	162.00	1.47	0.253
X_1^2	848.19	1	848.19	7.70	0.019*
X_2^2	69.23	1	69.23	0.63	0.446
X_3^2	1011.76	1	1011.76	9.19	0.013*
Residual	1101.23	10	110.12		
Lack of fit	751.23	5	150.25	2.15	0.211
Pure error	350.00	5	70.00		

*Statistically significant effect of process variable on the sCOD yield at 5% errors

3.3.1 Effect of independent processing parameters on the degree of solubilization

Depending on the impact of various process variables, the sCOD yield increments ranging between 200 to 488% compared with the untreated JPC (Table 6). The increased sCOD yields were comparable with the reported value for greenhouse crop waste after the alkaline H_2O_2 pretreatment [61]. The effect of three independent process variables on the sCOD yield of JPC is shown in Fig. 3b, d, and f. The impact of individual process variables on the degree of solubilization was examined by maintaining the other two variables into their center value.

The lowest sCOD yield (82 g L^{-1}) was obtained when the JPC was pretreated by adding 6% of NaOH and incubated at 35 °C for 7.6 h. In comparison, the highest value of 161 g L^{-1} was recorded at the same NaOH concentration and incubation temperature but retained for 88.4 h (Table 6). Figure 3b shows that the sCOD yield from JPC was increased by 60% when the NaOH concentration increased from 2.64 to 6% by keeping the incubation temperature and retention time to their center value. However, with a further increase in NaOH concentration from 6 to 9.36%, the rate of sCOD yield increment showed a declining trend; only 6.85% improvement was observed. The catalyst (NaOH) was highly effective in attacking the binding bond between lignin and hemicellulose [62]. Moreover, NaOH is also effective for cleaving the ester and carbon-to-carbon bonds in lignin molecules (ferulic acid) and resulted in the release of soluble compounds from carbohydrate and lignin components. In the alkaline pretreatment, the

Table 6 RSM-CCD's experimental layout and its corresponding experimental and model-predicted sCOD values (g L^{-1})

Run order	Catalyst (%)	Temperature (°C)	Time (h)	Actual value	Predicted value	sCOD in^{a}. (%)
Unt. JPC^{b}	0.00	0.00	88.60	27.40	–	0.00
1	6.00	43.40	48.00	143.00	150.30	423.00
2	6.00	35.00	48.00	134.00	145.90	390.00
3	4.00	30.00	24.00	94.00	87.80	244.00
4	6.00	35.00	48.00	140.00	145.90	412.00
5	8.00	30.00	24.00	100.00	105.80	265.00
6	4.00	30.00	72.00	136.00	136.60	397.00
7	6.00	35.00	7.68	82.00	88.40	200.00
8	8.00	40.00	72.00	160.00	165.10	485.00
9	9.36	35.00	48.00	156.00	144.90	470.00
10	6.00	35.00	48.00	143.00	145.90	423.00
11	6.00	35.00	48.00	150.00	145.90	448.00
12	2.64	35.00	48.00	91.00	103.70	233.00
13	8.00	30.00	72.00	147.00	155.70	437.00
14	6.00	35.00	48.00	155.00	145.90	467.00
15	8.00	40.00	24.00	135.00	133.40	393.00
16	6.00	26.60	48.00	135.00	129.20	393.00
17	4.00	40.00	72.00	141.00	134.10	415.00
18	4.00	40.00	24.00	113.00	103.30	313.00
19	6.00	35.00	88.32	161.00	156.10	488.00
20	6.00	35.00	48.00	154.00	145.90	463.00

[a] Increased soluble chemical oxygen demand after the alkaline pretreatments (%). [b] Untreated JPC

NaOH would dissociate into two Na$^+$ and OH$^-$ ions, and as the OH$^-$ concentration increases, the rate of hydrolysis increases consequently [63].

The incubation temperature at all levels slightly increased the degree of solubilization from JPC (Fig. 3d). The sCOD yield increased by 11.11% when the incubation temperature increased from 26.6 to 43.4 °C, which implies that the alkaline pretreatment was effective at mild temperatures. However, the effect of temperature on sCOD yield was relatively lower when compared with the other pretreatment variables. The weaker impact of temperature on the degree of solubilization might be associated with the lower temperature ranges (26.60–43.40 °C) applied in the pretreatment processes. In the same way, the effect of retention time on sCOD yield was highly significant in its lower range (Fig. 3f). For instance, maintaining the NaOH concentration and reaction temperature to their center value and then increasing the retention time from 7.6 to 48 h resulted in 78% more soluble compounds, but a further increase of the retention time caused for deterioration of the sCOD yields. The possible reason for the enhancement of sCOD for most levels of the process variables may be attributed to higher hydrolysis of the lignocellulosic materials found in JPC due to the optimum pretreatment conditions [64].

3.3.2 Effect of interactive factors on the degree of solubilization (sCOD)

Although the interaction effects among all variables were not statistically significant (Table 5), the sCOD yields were increased when the value of each process variable increased. More specifically, the amount of sCOD increased when the JPC was pretreated using higher NaOH concentration at the higher temperatures (Fig. 4b). However, a further increment of catalyst concentration (≥ 8%) and incubation temperature (≥ 40 °C) showed a declining trend in sCOD yields. Furthermore, as it is noted from the response surface curve, the effect of the catalyst was more powerful when compared with the temperature at constant retention time (48 h). On a similar vein, the degree of solubilization was increased with increasing of the NaOH concentration from 4 to 8% and retained up to 72 h (Fig. 4d). However, a further increase in NaOH concentration and retention time caused a slight reduction of sCOD yields. Figure 4f shows the amount of sCOD from the JPC increased with the corresponding increment of retention time and incubation temperature at all levels.

The increment in sCOD was associated with the solubilization of cellulose, hemicellulose, and lignin components at the optimum pretreatment conditions. In contrast, the possible reason for lower sCOD yield at severe pretreatment conditions

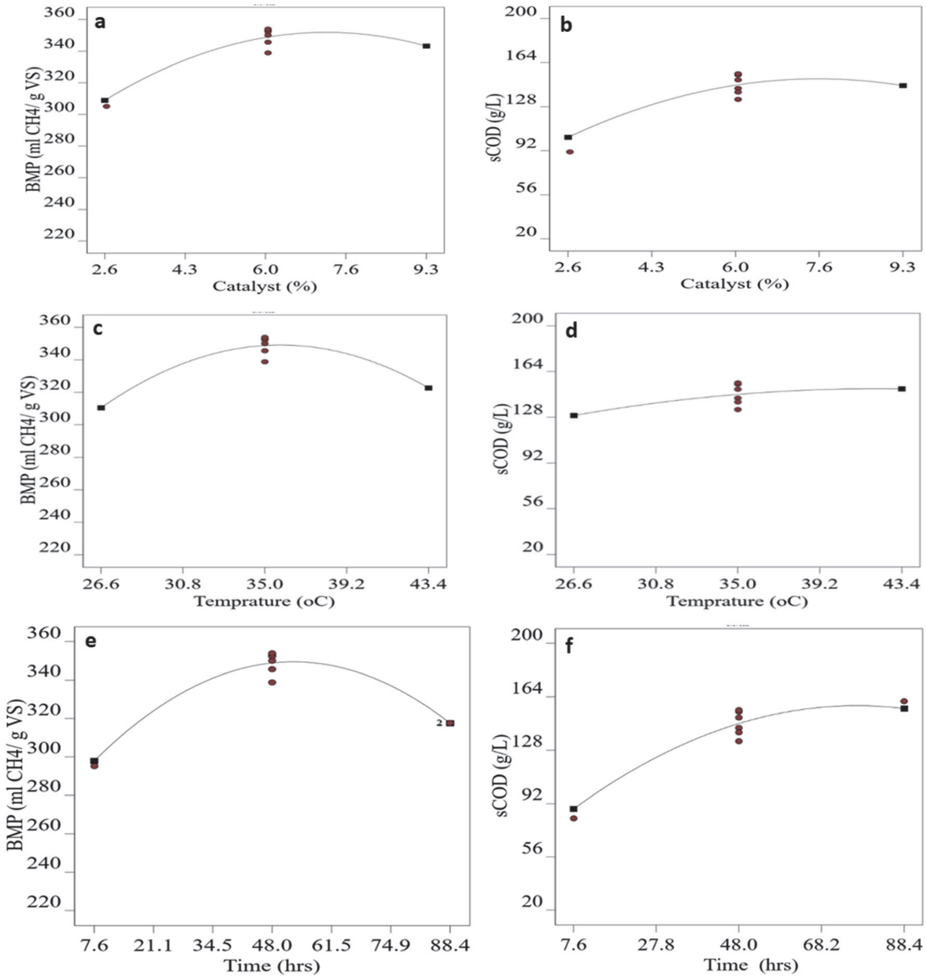

Fig. 3 Effect of individual process variable: NaOH concentration (**a**, **b**), incubation temperature (**c**, **d**), and retention time (**e**, **f**) on methane and sCOD yields. One parameter was varied while the rests were maintained to their center points

might be due to condensation and precipitation of the solubilized components [65]. Furthermore, the lower sCOD yield could also be associated with instability and formation of complex non-soluble compounds at severe pretreatments [66]. Strong alkali concentration leads to a loss of carbohydrates [67]. The loss of carbohydrates is mainly due to the peeling and hydrolytic reactions, followed by the formation of lower molecular compounds [68]. Moreover, the degradation of these lower molecular compounds at severe pretreatment conditions triggers the loss of organic carbon in the form of carbon dioxide, which could subsequently result in lower sCOD yields. Loss of soluble compounds from cellulose and hemicellulose would increase with increasing NaOH concentration and retention time [35]; this leads to lower sCOD recovery. Furthermore, at a higher temperature, the cross-link between sodium ion and lignin molecule would further decrease the solubilization of lignin during the alkaline pretreatment. As a result, the optimum operational variables for higher sCOD yield were investigated from the model developed using the actual value (Eq. (8)). According to the optimum value suggested by the RSM-CCD, pretreating the JPC with 7.84% NaOH at 37.54 °C for 64.84 h would result in the maximum predicted sCOD value of 163.31 g L^{-1}.

The optimum condition predicted for higher sCOD yields may not be suitable for higher methane production. The possible reasons could be the generation of degraded products like 5-hydroxymethylfurfural (HMF), furfural, and phenolic compounds at severe pretreatment conditions, which all are

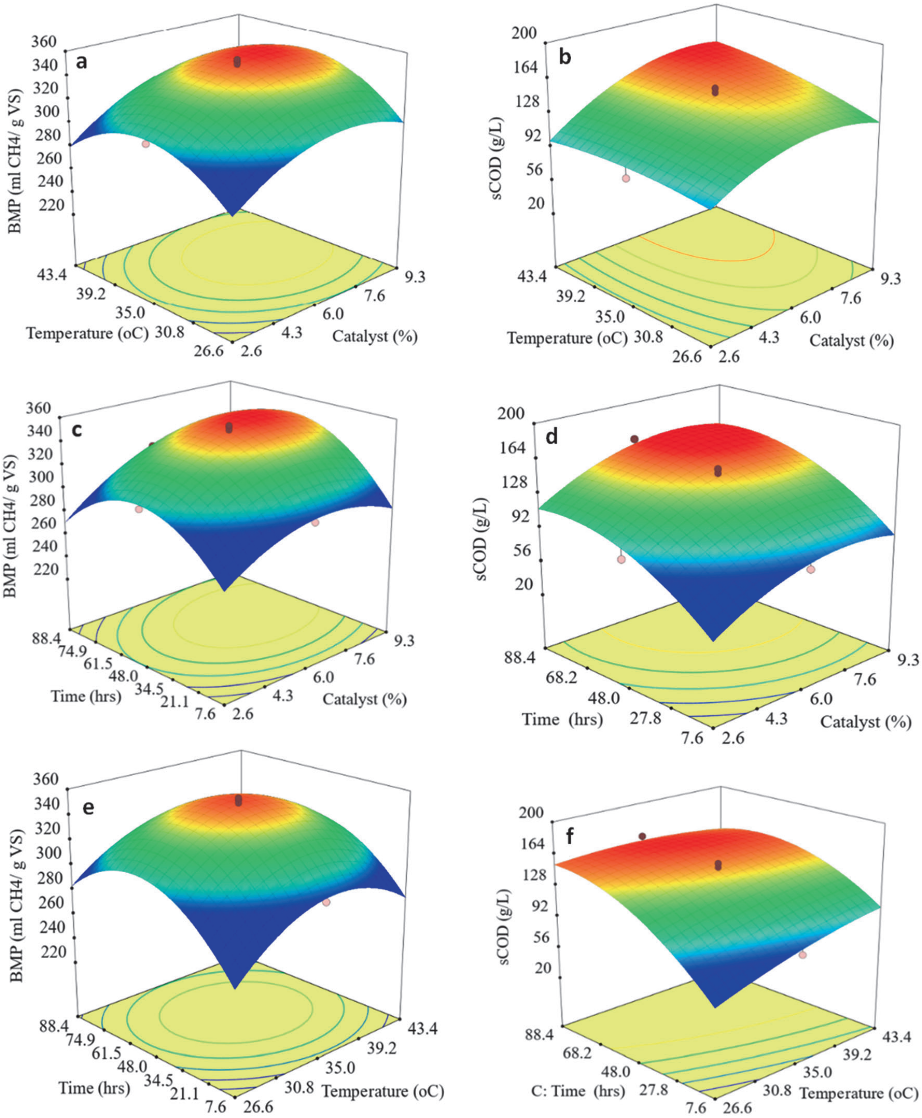

Fig. 4 The response surface plot mapped for BMP and sCOD yields; the interactive effects between NaOH and temperature (**a**, **b**), NaOH and retention time (**c**, **d**), and incubation temperature and retention time (**e**, **f**)

inhibitor and toxic for microbes involved in the AD process. Furfural and HMF at low levels (≤ 1.4 g L^{-1}) could enhance the biogas production [69]; however, at higher concentrations (≥ 2.0 g L^{-1}), they could disrupt the methanogenic activity [70, 71]. Although alkaline pretreatment is better in carbohydrate preservation as compared with acid pretreatment, the peeling reactions may lead to degradation of polysaccharides with the formation of acids like saccharinic, lactic, formic, and different dihydroxy and dicarboxylic acids [72]. In general, the effect of time was higher when it interacts with a catalyst and temperature, while the impact of the catalyst was powerful when it was combined with temperature. The lower effect of temperature might be attributed to its narrow ranges supplied in the pretreatment process. These temperature ranges were kept intentionally to ensure low energy pretreatment demand, while the lower temperature value (26.6 °C) is the real ambient temperature in Ethiopia where Jatropha is abundantly growing.

3.4 Modeling for biochemical methane potential

In addition to the sCOD, the catalyst concentration (X_1), incubation temperature (X_2), and retention time (X_3) were also selected as main process variables that could affect the methane yield of JPC. The RSM-CCD was employed to investigate the interaction between these process variables and the methane yields obtained after the alkaline pretreatments. Furthermore, RSM-CCD was used to predict the optimum pretreatment conditions for maximum methane production after 61 days of AD. Thus, using Eq. (3) as a generalized model, the actual multiple regression models were developed from the coded and actual experimental data, and the models indicated that a second-order polynomial equation was the best model that describes the relationship between these process variables and the methane yield obtained from JPC (Eqs. (9) and (10)).

$$\begin{aligned}\text{BMP (coded)} = {} & 348.85 + 9.81X_1 + 3.64X_2 \\ & + 5.83X_3 - 8.04X_1^2 - 11.45X_2^2 - 14.52X_3^2 \\ & + 1.02X_1X_2 + 2.62X_1X_3 - 1.48X_2X_3 \qquad (9)\end{aligned}$$

$$\begin{aligned}\text{BMP (actual)} = {} & -392 + 22.83X_1 + 32.77X_2 \\ & + 2.77X_3 - 20.01X_1^2 - 0.46X_2^2 - 0.03X_3^2 \\ & + 0.10X_1X_2 + 0.05X_1X_3 - 0.01X_2X_3 \qquad (10)\end{aligned}$$

where BMP is the biochemical methane potential.

Form Eq. (9), it is clearly shown that the linear effect of NaOH concentration (X_1), incubation temperature (X_2), and retention time (X_3), and the two interaction terms (X_1X_2 and X_1X_3) has a positive effect, while all quadratic terms (X_1^2, X_2^2, and X_3^2) and the interaction between X_2 and X_3 inversely correlated with the methane yield of JPC. Moreover, a multivariate ANOVA was carried out to test the strength of the model that predicted the effect of linear, interactive, and quadratic terms of the X_1, X_2, and X_3 applied during the alkaline pretreatment process. The value for multiple correlation coefficients of R^2, adj. R^2, and pred. R^2 was 0.97, 0.94, and 0.90, respectively, in which the higher R^2 value (0.97) indicates that the regression model could not explain only 3% of the total variation in methane yields. The closed correlation between all experimental and model-predicted values indicates the reliable predictive power of the model (Fig. 3a). The effect of linear terms from X_1, X_2, and X_3 and the quadratic terms of the three process variables, i.e., X_1^2, X_2^2, and X_3^2, on methane production of JPC were statistically significant (Table 7).

Table 7 ANOVA for the model regression representing the methane yields

Source	Sum of squares	df	Mean square	*F* value	*p* value
Model	7015.89	9	779.54	34.10	< 0.001*
X_1	1314.39	1	1314.39	57.50	< 0.001*
X_2	180.97	1	180.97	7.92	0.018*
X_3	464.90	1	464.90	20.34	0.001*
X_1X_2	8.38	1	8.38	0.3668	0.558
X_1X_3	55.07	1	55.07	2.41	0.152
X_2X_3	17.55	1	17.55	0.7679	0.401
X_1^2	932.23	1	932.23	40.78	< 0.001*
X_2^2	1890.31	1	1890.31	82.69	< 0.001*
X_3^2	3039.80	1	3039.80	132.98	< 0.001*
Residual	228.60	10	22.86		
Lack of fit	61.57	5	12.31	0.3686	0.851
Pure error	167.03	5	33.41		
Cor total	7244.49	19			

*Statistically significant effect of process variable on the methane yield at 5% errors

3.4.1 Effect of independent processing variables on methane yield of JPC

The estimated cumulative methane yield from untreated JPC was 252.41 mL g^{-1} VS, which is relatively higher than the reported methane yield of sunflower oil cake [60]. Even though the methane yield of pretreated JPC varied across different pretreatment conditions, all values were higher than the methane yield obtained from untreated JPC (Table 8). The increment of cumulative methane yields due to the alkaline pretreatments were ranging from 16.9% (295.10 mL g^{-1} VS) to 40.2% (353.90 mL g^{-1} VS). The lower methane yield (295.1 mL g^{-1} VS) obtained from the alkaline-pretreated JPC was comparable with the literature value of 281 mL g^{-1} VS obtained after the thermochemical pretreatment [18]. However, in addition to longer digestion time (61 days) requirement, all methane yields were lower than the report by Chandra et al. [73]. The lower methane yield after alkaline pretreatment might be associated with a lack of inherent bacteria that could digest the JPC in the AD process. This effect is strongly supported by Chandra et al. [73] in which maximum methane yield was obtained from JPC within 30 days of digestion time using active bacterial inoculum synthesized from pongamia oil seed cake.

The present study showed that the methane yield of JPC increased by 14% when the concentration of NaOH increased from 2.6 to 6% at constant incubation temperature and retention time of 35 °C and 48 h, respectively (Fig. 3a). However, at the same temperature and retention time, with further increment NaOH concentration to 9.4%, the methane yield of

Table 8 The RSM-CCD experimental layout and the corresponding experimental and model-predicted values of methane yields (mL g^{-1} VS)

Run order	NaOH (wt%)	Temperature (°C)	Time (h)	Actual value	Predicted value	MYI (%)[a]
1	2.64	35.00	48.00	305.20	309.60	20.90
2	9.36	35.00	48.00	344.30	342.60	36.40
3	6.00	35.00	48.00	349.90	348.90	38.60
4	8.00	40.00	72.00	335.30	336.30	32.80
5	4.00	40.00	72.00	310.40	309.40	22.90
6	4.00	30.00	24.00	300.56	297.70	19.10
7	6.00	35.00	88.32	317.70	317.60	25.90
8	8.00	30.00	72.00	329.00	329.90	30.30
9	6.00	35.00	48.00	353.90	348.90	40.20
10	8.00	40.00	24.00	321.90	322.30	27.50
11	6.00	35.00	48.00	352.20	348.90	39.50
12	6.00	35.00	7.60	295.10	297.90	16.90
13	4.00	30.00	72.00	309.40	307.10	22.60
14	8.00	30.00	24.00	310.90	310.00	23.20
15	6.00	35.00	48.00	338.80	348.90	34.20
16	6.00	35.00	48.00	345.60	348.90	36.90
17	4.00	40.00	24.00	308.70	305.90	22.30
18	6.00	43.40	48.00	322.10	322.60	27.60
19	6.00	26.60	48.00	308.10	310.30	22.10
20	6.00	35.00	48.00	353.00	348.90	39.90
Unt. JPC[b]	–	–	–	252.40	–	–

[a] Methane yield increment after pretreatments (%)

[b] Untreated Jatropha press cake

pretreated JPC declined by 1.33%. Similarly, Zheng et al. [43] indicated that adding 6% NaOH into the corn stover achieved 48.5% more biogas yield when compared with the untreated sample. Lower concentration of catalyst during the pretreatment condition leads to a slow rate of lignocellulosic biomass hydrolysis, which subsequently could result in less accessibility to microorganisms during the AD process [60]. Alkaline pretreatment is highly effective in the dissolution of lignin and hemicellulose [62]. However, the hydrolysate at higher NaOH concentration may contain soluble compounds from lignin that may not be metabolized by microbes in the AD process and leads to lower methane yields.

The effect of incubation temperature on methane yield from JPC is shown in Fig. 3c. Soaking the JPC with 6% NaOH and retaining it for 48 h at an incubation temperature of below or above 35 °C resulted in a slight reduction of the methane yields. The methane yield was increased by 13.24% when the applied temperature rose from 26.6 to 35 °C, but it again declined by 8% when the incubation temperature arises from 35 to 43.4 °C. The optimum temperature recorded in this study was in line with Chandra et al. [73], in which the wheat straw pretreated with 4% NaOH (g g^{-1} TS) at 37 °C for 5 days achieved 112% more methane when compared with the untreated straw.

The effect of retention time on methane yield was investigated at constant NaOH (6%) and incubation temperature (35 °C). The effect of retention time was highly significant at its lower range when compared with the upper values (Fig. 3e). The methane yield was increased by 18.22% when the retention time increased from 7.6 to 48 h. However, when the retention time further increased to 88.4 h, the methane yield increased by only 9.83%. Higher retention time may give a chance of effective hydrolysis of the lignocellulosic component of the press cake and thus leads to fast anaerobic degradation, and then followed by higher methane production [38]. However, retaining the JPC for more extended hours may result in the accumulation of inhibitor compounds such as furfural, HMF, organic acidic, and phenolic compounds, as discussed in Section 3.3.2.

3.4.2 Effect of interactive factors on methane yield of JPC

The methane yield of JPC was increased as a result of increasing the value of pretreatment conditions from their lower to the corresponding center point values (Fig. 4a, c, and e). More specifically, in the lower pretreatment conditions, the methane yield of JPC was increased when both the catalyst concentration and reaction temperature increased (Fig. 4a). However, when the amount of NaOH and incubation temperatures exceed approximately above 8% and 40 °C, respectively, the methane yield started to decline at a slower rate. Similarly, in the lower pretreatment conditions, the methane yield was increased as increasing of NaOH concentration and retention time (Fig. 4c). However, a further increment of NaOH concentration and retention time above 8% and 60 h, respectively, resulted in a decline of methane yields.

The interactive effect between incubation temperature and retention time is also shown in Fig. 4e. The curve from the response surface plot indicates that the methane yield was increased as the incubation temperature rose from 26.4 to 35 °C with the corresponding increment of retention time from 7.6 to 48 h at 6% of NaOH. When the incubation temperature and retention further increased, the methane yields started to decline at a slower rate (Fig. 4e). Comparable with the present study, Sukri et al. [74] indicated that the degradation of carbohydrates due to the higher NaOH concentration (5.25%) and retention time (90 min). In general, maximum methane yields were obtained from JPC at moderate pretreatment conditions. Therefore, the optimum conditions in an alkaline pretreatment were predicted using Eq. (10). The model indicates that pretreating the JPC using 7.32% NaOH at 35.86 °C for 54.05 h would result in maximum methane production of 353.90 mL g^{-1} VS, which the methane yield was increased by 40.23% as compared with the untreated JPC. The methane yield of JPC obtained at this optimum condition was further evaluated against the literature values, as described in Table 9.

3.5 Energy balance and economic feasibility

Regardless of the costs for biogas plant and pretreatment tank installation, the energy balance and preliminary economic feasibility of the alkaline pretreatment and co-digestion processes were studied. Moreover, the optimum temperature utilized in the alkaline pretreatment was taken as normal temperature for the area from which the sample was brought (Hawassa, Ethiopia). The net increases in methane yields (ΔP) from the alkaline and co-digestion pretreatment processes were estimated to be101.50 and 73.07 L kg^{-1} VS, respectively. Accordingly, the energy outputs (E_o) calculated from these extra methane yields were 3270.3 and 2354.3 kJ kg^{-1} VS, respectively. In contrast, the energy input (E_i) required for the alkaline pretreatment was estimated to be 548.8 kJ kg^{-1} VS, and there was no external energy needed for the co-digestion experiments. Thus, the extra energy obtained after alkaline pretreatment was almost 5.96 times higher as compared with the energy demanded by the NaOH pretreatment.

The BCR analysis was done by considering the unit price of inputs and outputs supplied in the alkaline pretreatment. The cost for one tone of NaOH was estimated to be 412 € t^{-1} [48], while selling of 1 m^3 methane can earn 0.57 € [47]. Thus, pretreating one tone VS of JPC using *7.32%* alkaline needed 0.078 t of NaOH, which costs 32.3 €. One tone VS from JPC can produce 101.5 m^3 extra methane when compared with the untreated JPC, which prices 57.86 €. Therefore, the BCR obtained by dividing the total methane selling price with NaOH market cost was estimated to be 1.79, which was positive and encourage the application of alkaline

Table 9 Effect of alkaline pretreatment on various lignocellulosic biomass as compared to the present study

Lignocellulosic biomasses	The experimental optimum pretreatment conditions	AD conditions	Gas increase (%)	References
Wheat straw	4% NaOH, 37 °C, 120 h	Batch, 37 °C, 35 days	111.60^a	[73]
Extruded grass	7.5% $Ca(OH)_2$, 10 °C, 20 h	Batch, 37 °C, 30 days	37.00^a	[75]
Wheat straw	14.2% NH_3, 51 °C, 27 h	Batch, 30 °C, 4 weeks	56.25^b	[76]
Greenhouse crop waste	1% H_2O_2, 50 °C, 6 h, 7% TS	Batch, 35 °C, 64 days	77.60^a	[61]
Rice straw	6% NaOH, 35 °C, 8 days	Batch, 38 °C, 30 days	157.48^b	[61]
Pinewood	8% NaOH, 100 °C, 10 min	Batch, 37 °C, 45 days	181.00^a	[33]
Sugarcane bagasse	8.5% $Ca(OH)_2$, RT, 4 h	Batch, 37 °C, 35 days	33.00^a	[34]
Corn stover	2% NaOH, 6% CaO, 50 °C, 0.5 h	Batch, 37 °C, 60 days	54.00^b	[35]
Jatropha press cake	7.32% NaOH, 35.86 °C, 54.05 h	Batch, 37 °C, 61 days	40.23^a	Present study

RT room temperature, *TS* total solids

[a] Methane

[b] Biogas

pretreatment at a larger scale. The same positive BCR results were obtained by You et al. [35] and Romero-Güiza et al. [47] after alkaline pretreatment of corn stover and wheat straw, respectively. The major challenge of this alkaline pretreatment was the recovery of the added NaOH. Thus, our future research may focus on the recovery of the alkaline chemicals found in hydrolysate using various methods like electrokinetic cells. The recovery of the chemical could further improve the BCR of the alkaline pretreatment.

4 Conclusions

Alkaline pretreatment and co-digestion processes were identified as effective strategies for enhancing the methane production potential of the JPC and CG. However, the efficiency of the alkaline pretreatment was significantly affected by NaOH concentration, incubation temperature, and retention time. Moreover, the methane yield in the co-digestion process was influenced by FC and CG levels. Both the methane and sCOD yields were declined at severe alkaline pretreatments. The optimum conditions predicted for higher methane and sCOD yields were significantly varied. Variation in optimum conditions defined for maximum methane and sCOD yields could be used as an indicator of process inhibition, probably due to the accumulation of degraded products at severe alkaline pretreatment. The positive energy balance and higher BCR inspire the application of the alkaline pretreatment at a larger scale for enhancing the methane yield of JPC. To reduce the downstream processing cost and effective utilization of the catalyst, the future research work in this area should focus on the recovery of NaOH from the hydrolysate using different techniques.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Paper IV

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Biochemical methane potential of Jatropha press cake: Effect of steam explosion pretreatment and co-digestion with crude glycerol

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Gebresilassie Asnake Ewunie, Zerihun Demrew Yigezu, and John Morken

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Gebresilassie Asnake Ewunie,[1,a)] Zerihun Demrew Yigezu,[2] and John Morken[1]

AFFILIATIONS

[1]Department of Science and Technology, Norwegian University of Life Sciences (NMBU), P.O. Box N-1432, Ås, Norway
[2]College of Agriculture, Hawassa University, P.O. Box 05, Hawassa, Ethiopia

[a)]Author to whom correspondence should be addressed: asnake.ewunie.gebresilassie@nmbu.no

ABSTRACT

Biodiesel production from *Jatropha curcas* generates a considerable amount of Jatropha press cake (JPC) and crude-glycerol (CG) biowastes with intense biogas production potential. However, JPC contains a larger amount of lignocellulosic materials that potentially affect the hydrolysis stage of the anaerobic digestion process, while CG significantly lacks nitrogen needed for microbial biomass growth. Therefore, the present study sought to explore the optimal steam explosion (SE) pretreatment and co-digestion conditions that can improve the methane yields of JPC with inhibitor formation reduction. The effects of different temperature-time combinations during SE on soluble chemical oxygen demand (sCOD) and methane yield of JPC were evaluated using response surface methodology coupled with central composite design (RSM-CCD). JPC was also co-digested with CG, and the methane yield of the mixture was investigated by varying the total organic loading (TOL) and CG levels. The RSM-CCD model predicated that the maximum methane yield (330.14 ml g^{-1} VS) could be achieved after exploding the JPC at 202 °C for 9.39 min, while relatively high temperature (209 °C) and retention time (13.68 min) were needed to obtain a higher predicted sCOD yield (94.48 g L^{-1}). During the co-digestion processes, the methane yields of the mixture were significantly varied, and co-digesting 2% CG with JPC at 2 g VS L^{-1} TOL was the optimum condition to obtain a maximum methane yield of 325.25 ml g^{-1} VS. Thus, considering the environmental and economic advantage of biowaste utilization, co-digesting JPC with CG was the best option for improving the methane yield of the mixture compared to SE pretreatment.

I. INTRODUCTION

Biofuels are getting attention as alternative energy sources in the transportation sector. Because of the renewable nature of the feedstocks and less greenhouse gas emissions, biofuels are considered a potential substitute for fossil fuels in the future.[1] Recent reports showed that biodiesel and bioethanol took 90% of the biofuel market share across the world.[2] Biodiesel is gaining popularity over diesel due to its various environmental advantages and easy applicability as vehicle fuel without engine modification.[3] For the last several decades, however, over 95% of biodiesel has been produced from edible oilseeds,[4,5] which adversely affected food and feed production.[6] For this reason, several studies were conducted to explore alternative nonedible oil-bearing plants that can grow on waste and non-arable lands.[7] *Jatropha curcas* L (Jatropha) is a promising tropical and subtropical species identified for biodiesel production.[8] Jatropha is ranked at third place after palm and coconut species in terms of its oil yield, and the cultivation of this plant for biodiesel production is widely expanding across many tropical and subtropical regions.[9]

The trend of biodiesel production from Jatropha has increased, and thus, surplus amounts of Jatropha press cake (JPC) and crude glycerol (CG) biowastes are generated.[10] These agroindustrial wastes are economical, abundant, and renewable and provide a unique natural resource for large-scale and cost-effective biogas production.[11] It should also be noted that the land, water, and energy requirements of agro-industrial wastes are lower and are not directly associated with food security while converting into bioenergy.[12] Energy generation from agro-industrial residues could meet more than 3%–14% of the global energy supply.[13] For instance, the global biogas consumption in 2017 was estimated to be 1.33 EJ, which accounts for more than 36.44% of the world's liquid biofuel utilization.[13] Thus, exploring and exploiting the value-added products from Jatropha residue could maintain a competitive advantage in the world market and ensure the sustainable economic return of biofuel production from Jatropha.

Studies indicated that mechanical oil extraction resulted in 60%–70% of JPC relative to the processed seed's weight,[14] while transesterification of oil for biodiesel production results in the generation of 10%–14% CG.[15] However, JPC contains toxic chemicals like curcin, saponins, phorbol ester, phytates, protease, and lectins.[16] Thus, the cake can neither be used as an animal feed nor as organic fertilizer.[17] Although CG can be purified into high-grade products and widely utilized in cosmetics, food, and pharmaceutical industries, its higher volume production combined with expensive purification cost causes the CG to be considered as hazardous organic waste.[18,19]

On the other hand, JPC and CG are identified as a potential feedstock for biogas production through anaerobic digestion.[8,17,18,20–22] However, a lower volume of methane yield (281 ml g^{-1} VS) was reported from the digestion of the press cake.[23] JPC has shown to contain 20% lignin, 14% cellulose, and 28% hemicellulose, which potentially affect the hydrolysis stage of anaerobic digestion.[23,24] Furthermore, the carbon to nitrogen ratio (C/N) of JPC was reported to be 9:1, which is significantly lower than the optimum ratios (20:1–30:1) suggested for the stable anaerobic digestion process.[25] Substrates with a higher nitrogen content may inhibit the methanogen activities[26] and subsequently lead to process collapse.[23] In contrast, too low nitrogen would mean insufficient nitrogen to maintain the microbial biomass growth.[27] Interestingly, CG contains a negligible nitrogen content (0.008%–0.037%)[10] that could be co-digested with JPC to improve the lower C/N ratio.[19] Therefore, the biochemical methane potential (BMP) of JPC could be enhanced either through pretreatment or co-digestion with CG. Pretreatment can bring a lignocellulosic structural change that could be easily available for microbes that convert the carbohydrate polymer into simple fermentable sugars.[28,29] Co-digesting the JPC with CG is also economically viable since both substrates are massively co-generated during the oil extraction and biodiesel production processes. The cost associated with the transportation of both substrates is also lower because the biogas digester could be installed near the biodiesel plant.

Various techniques like mechanical,[26,30] thermochemical,[28,31,32] steam explosion (SE),[27,33] and ultrasound[34] pretreatment methods have been employed for feedstock containing a larger amount of lignocellulosic materials, but none has been applied to JPC. The non-catalyzed SE pretreatment is recognized as a low-cost pretreatment technique with significantly lower environmental impacts since the addition of external chemicals is not necessary.[35] For instance, studies have shown that SE pretreatment needs only 30% of the energy required by mechanical pretreatment to achieve the same size reduction.[36] Lizasoain *et al.*[37] showed that explosion of corn stover at 160 °C for 2 min had improved its methane yield by 22% over the untreated sample. Other studies also indicated that SE pretreatment had increased the soluble sugar and total organic carbon content of bulrush biomass by 25% and 150%, respectively.[38] However, the effect of SE pretreatment is highly dependent on various process variables such as total solids (TSs), particle size, temperature, residence time, and pressure.[39,40]

Temperature and retention time were identified as primary factors that influence the efficiency of SE pretreatment,[40] while the methane yield of JPC in the co-digestion process depends on the level of CG and total organic loading (TOL) added into the digester.[19] However, most previous studies have investigated the effect of these process variables during SE pretreatment by varying one variable at a time while keeping the other/s to a constant value. Thus, the synergistic effects from the variation of two or more process variables at a time during the SE process were not widely studied. Non-optimized SE pretreatment may lead to incomplete hydrolysis of lignocellulosic material or cause degradation of polysaccharides and lignin, which has an inhibitory and toxic effect on the anaerobic microbes.[41] Therefore, exploring and defining the optimum temperature-time combination during SE by varying an individual and all selected process variables at a time may help to maximize the methane yield of JPC by limiting the occurrence of potential inhibitors.

As per the authors' knowledge, no studies either reported on co-digestion of JPC with CG or defined and modeled the optimum process conditions during SE pretreatment to maximize the methane yield of JPC. Thus, the present study sought to investigate and model the linear, interactive, and quadratic effect of steam temperature (X_1) and retention time (X_2) on soluble chemical oxygen demand (sCOD) and methane yield of JPC using a response surface methodology coupled with central composite design (RSM-CCD). JPC was also co-digested with CG, and its impact on BMP of the mixture was investigated by varying the TOL and CG levels. The maximum methane yields obtained due to SE and co-digestion were used as a response variable to define the optimum pretreatment conditions. Unlike the previous reports, the present study was focused on exploring the optimum SE pretreatment and co-digestion conditions that could improve the methane yield of JPC and CG without inhibitor formation or process inhibition. Moreover, variation in optimum SE pretreatment conditions predicted for maximum sCOD and methane yields was used as quick indicators of process inhibition due to polysaccharide degradation instead of conducting an expensive and time-consuming laboratory analysis. This study would be used as a benchmark for large-scale biodiesel producer companies for integrating the biogas production from JPC and CG to maximize the overall revenue obtained from Jatropha. Utilizing the biowastes for biogas production could also minimize the cost needed for waste treatments.

II. MATERIALS AND METHODS

A. Source of raw materials

Jatropha press cake was obtained from YME Design and Manufacture P.L.C, located in Hawassa City, Ethiopia. The fresh JPC was air-dried until its moisture content reduced below 10% on a dry weight basis. The dried press cake was then transferred into an airtight zipped plastic container and transported to Norwegian University of Life Sciences (NMBU), Aas, Norway. The mesophilic bacterial inoculum prepared from animal manure was obtained from a batch anaerobic digester located at NMBU. The CG that co-digested with JPC was purchased from Perstorp AB, Sweden. The SE pretreatment and batch anaerobic digestion experiments were employed at the biogas laboratory owned by NMBU, Aas, Norway.

B. Experimental design

1. Co-digestion of Jatropha press cake with crude glycerol

The effects of TOL (2, 5, and 7 g VS L^{-1}) that contains various levels of CG (0%, 1%, 2%, 3%, and 4%, on the VS weight basis) on the

BMP of the mixture were evaluated by running a series of microbial batch experiments.[33] The BMP tests were designed by employing a completely randomized design as described in the previous study.[19] The maximum methane yield obtained using the co-digestion process was then compared with the methane yield achieved at optimum SE conditions suggested by the RSM-CCD mode. Furthermore, the application of SE pretreatment and co-digestion processes at a larger scale was further evaluated against their energy balance and benefit-cost ratios (BCRs) (Sec. II B 5).

2. Modeling the impact of temperature and retention time

The most widely applied method for investigating the effect of multiple process variables for obtaining the best possible outcome is RSM.[6] It is a collection of mathematical and statistical techniques relied on fitted polynomial equations to the experimental data. Thus, the effect of SE pretreatment employed at various steam temperatures (X_1) and retention times (X_2) on the sCOD and methane yields of JPC was investigated by applying RSM-CCD methods. The independent variables (X_1 and X_2) were defined in five levels of the following ranges: temperature from 186 to 214 °C and retention time from 4.4–15.7 min, while the sCOD and methane yields obtained after SE were used as response variables. The actual and coded values of X_1 and X_2 suggested by the RSM-CCD are shown in Table I.

The RSM-CCD experimental design contains 13 experimental runs, including four-factorial, four-axial, and five replicated points. Replication of the center points was needed for estimating the standard errors of the model. The distance from the star (axial) point to the center point (0) calculated by the CCD was ±1.424. Therefore, the SE pretreatments were conducted for all temperature-time combinations suggested by the RSM-CCD, and all pretreatments were carried out sequentially as described in Sec. II B 3. Design-Expert 12 (Stat-Ease Inc., Minneapolis, USA) was used to design and analyze the experimental data and predict the empirical mathematical model to fit the experimental data points. A second ordered polynomial model was used to relate the effect of X_1 and X_2 on the sCOD and methane yields of JPC. The linear (X_1 and X_2), quadratic ($X_1{}^2$ and $X_2{}^2$), and interactive (X_1X_2) effects of these process variables were estimated and evaluated using the polynomial equation [Eq. (1)]. The degree of their impact and their statistical significance were assessed using analysis of variance (ANOVA). The contour and 3D response surface plots were drawn to display the interactive effect of X_1 and X_2. Values for multiple R^2, adj.R^2, and pred. R^2 were used to evaluate the strength of the fitted model,[42]

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2, \qquad (1)$$

where Y is the response (sCOD or methane yields); X_1 and X_2 are the coded independent variables, and β_0, β_1, β_2, β_{11}, β_{22}, and β_{12} are the model coefficients calculated from the experimental data (Table II).

TABLE I. Levels of X_1 and X_2 utilized during the SE pretreatment processes.

		Levels[a]				
Variable	Symbol	−α (−1.414)	−1	0	+1	+α (1.414)
Steam temperature (°C)	X_1	186	190	200	210	214
Retention time (min)	X_2	4.4	6	10	14.00	15.7

[a] α is the distance from the axial point to the center point calculated by $2^{k(1/4)}$ (k is the number of independent process variables applied during SE = 2).

3. Steam explosion pretreatment

In this pretreatment process, 0.35 kg of air-dried JPC was added into the SE unit (Cambi Aas, Asker, Norway) located at NMBU, Aas, Norway. The setup and working principles of this SE unit are described by Horn *et al.*[43] The reactor was preheated into the desired temperature for 10 min before adding the substrate into the SE unit.[33] JPC with a moisture content of 10% was then added into a 20 L reactor and subjected to 186, 190, 200, 210, and 214 °C for retention times of 4.4, 6, 10, 15, and 15.7 min. The temperature and retention time ranges used in the present study were adopted from various studies.[27,33,44] The moisture content and particle size of all treatments were kept constant until the actual SE experiments were employed. However, the untreated JPC was ground into ≤1 mm particle sizes prior to BMP tests. After the subsequent pretreatments, the exploded substrates were collected and cooled down to room temperature; then, small fraction samples were taken from the individual sample for sCOD analysis (Sec. II C 2). Finally, the solid/liquid mixture and filtrates were transferred into zipped plastic and small vials, respectively, and then stored at 4 °C until the actual experiments started.

4. Biochemical methane potential assay

The BMP of steam-exploded and untreated JPC was tested by running a series of anaerobic digestion in sealed small batch reactors. The total and working volumes of the batch reactors were 122 and 60 ml, respectively. Triplicate reactors were prepared for control,

TABLE II. Operational conditions assayed expressed as dimensional and dimensionless independent variables.

	X1 (°C)		X2 (min)	
Run order	Coded value	Real value	Coded value	Real value
1	−1.41	186	0	10
2	−1	190	−1	6
3	−1	190	1	14
4	0	200	−1.41	4.4
5	0	200	0	10
6	0	200	0	10
7	0	200	1.41	15.7
8	0	200	0	10
9	0	200	0	10
10	0	200	0	10
11	1	210	−1	6
12	1	210	1	14
13	1.41	214	0	10

blank, untreated, and steam-exploded JPC at corner and star points, while five reactors were prepared for JPC pretreated at the center point (200 °C, 10 min). The inoculum obtained from NMBU's biogas laboratory was further incubated at 37 ± 0.5 °C for one week under the anaerobic condition to assimilate the microbes and reduce the endogenous biogas production.[45] Following the pre-incubation, the prepared vials were filled with 16.7 g of inoculum (3 g VS L^{-1}) and then 2 g VS L^{-1} of pretreated or untreated JPC was added into the reactors that contain an inoculum; a VS ratio of 2/3 between the substrate and inoculum was maintained by varying the quantities of JPC added into each reactor that contains the same amount of inoculum.[33] For blank and control (pure cellulose $(C_6H_{10}O_5)n$, $<20\,\mu m$, Merck chemical Corp.) experiments, the same amount of inoculum was utilized without adding JPC. The working volume (60 ml) of all batch reactors was adjusted by adding tap water. All reactors were then closed with a rubber stopper and aluminum crimps and flashed with pure nitrogen using inlet and outlet syringes for 5 min to make an anaerobic environment.[46] Finally, all vials were incubated at 37 °C and then centrifuged to 90 rpm until the daily biogas production was less than 5% of the total methane production.[47] Based on the constant head-space volume of the reactors, the ideal gas law was used to calculate biogas production levels through the anaerobic digestion period,

$$\mathrm{Vb} = \frac{\mathrm{n} \times \mathrm{R} \times 273}{\mathrm{P_o}} = \left(\frac{\mathrm{dp} \times \mathrm{V}}{\mathrm{R} \times \mathrm{T}}\right) \times \left(\frac{\mathrm{R} \times 273}{\mathrm{P_o}}\right) = \left(\frac{\mathrm{dp} \times \mathrm{V} \times 273}{\mathrm{T} \times \mathrm{P_0}}\right), \tag{2}$$

where Vb is the volume of biogas (L) at the standard condition of 273 K (0 °C) and a total pressure of 1 atm; Po is 1 atm; n is the number of moles; R is the ideal gas constant; V is the volume of head-space (L); T is the temperature in the incubator room (310 K); and dp is the overpressure measured in the bottle (atm) (1013.25 mbar = 1 atm).

5. Energy balance and preliminary economic feasibility analysis of SE pretreatment

Energy balance and benefit-cost ratio (BCR) analyses are essential tools for examining the environmental soundness and economic feasibility of various pretreatment applied for enhancing the BMP of lignocellulosic materials.[48] Thus, a preliminary energy balance and BCR analysis were performed by considering various input and output components involved during the SE pretreatment and anaerobic digestion processes.[49] Multiple forms of energies such as steam heating energy (Q_{1in}), material heating energy ($Q_{2in.}$), air heating energy ($Q_{3in.}$), and reactor heating energy (Q_{4in}) were considered as input energies during SE pretreatment.[49] In contrast, the amount of energy produced from extra methane (Q_{1out}) was considered as output energy.[50] The energies required for $Q_{3in.}$ and $Q_{4in.}$ are assumed to be negligible since once the reactor starts, it will work continually; thus, the energy needed for heating the air and reactor will be zero after few seconds of processing time. Therefore, the consumed energies (Q_{1in} and Q_{2in}) during SE pretreatment were estimated following the methods in the study by Baeta *et al.*,[49] while Q_{1out} was calculated using the method in the study by Romero-Guiza *et al.*,[50]

$$Q_{1in} = Q_{im} + Q_{iw} = \left(C_p m(1-w) + C_w m w\right) * (T_e - T_O), \tag{3}$$

$$Q_{2in} = m_g\left(h_{ge} - h_{fo}\right), \tag{4}$$

where Q_{1in} and Q_{2in} are energy inputs for heating the JPC and steam (kJ kg^{-1}), respectively; h_{ge} is the enthalpy of steam at T_e (kJ kg^{-1}); h_{fo} is the enthalpy of water at T_0 (kJ kg^{-1}); C_p and C_w are the specific heat of dry JPC and water (kJ kg^{-1} K^{-1}), respectively; mg is the amount of steam in the reactor for generating the required pressure (kg); m is the amount of pretreated JPC (kg); w is the moisture content of JPC (wt.%); and T_o and T_e are the initial surrounding and holding temperature (K), respectively.

The enthalpy of water and steam at temperatures T_o and T_e, respectively, was taken from the thermodynamic table,[51] while the amount of steam (m_g) in the reactor and its partial pressure (P_g) during the SE process were estimated using the regression model developed by Baeta *et al.*[49] [Eqs. (6) and (7)],

$$p_g = \exp\left(23.40 - \frac{3994.24}{T_e - 36.27}\right), \tag{5}$$

$$m_g = \frac{18 * p_g * V}{1000 * 8.31 * T_e}, \tag{6}$$

where V is the volume of the reactor (m^3),

$$Q_{1out} = \Delta p \, x \, \xi \, x \, \eta, \tag{7}$$

where $Q_{1out.}$ is the energy output (kJ kg^{-1} TS); Δp is the net increase in methane yield (m^3 kg^{-1} TS); ξ is the methane heating value (35.8 MJ m^{-3}); and η is the methane energy conversion efficiency (90%) as described previously.[48]

The total energy-demanding cost inquired by SE pretreatment and the revenue obtained from sales of extra methane yield after SE pretreatment were considered during the determination of the BCR. More specifically, the energy balance and BCR analysis were performed based on the following assumptions: (1) the average energy market price in Ethiopia is assumed to be 0.026 €kW h^{-1},[52] (2) the estimated cost of methane sales is 0.57 €m^{-3} (Ref. 50); and (3) the specific heat capacity of dry JPC is assumed to be the same as that of corn stalk (1.62 kJ kg^{-1} k^{-1}) since no previous report on JPC.[53]

C. Analytical method

1. Substrate analysis

The various physicochemical properties of JPC, CG, and inoculum were determined following standard procedures and protocols. Briefly, the total solid (TS) and volatile solid (VS) contents of inoculum and JPC were determined based on the APHA standard methods.[54] The carbon, nitrogen, and hydrogen contents of JPC were estimated using an elemental analyzer (Thermo Flash 2000). The crude protein content of JPC was determined from its nitrogen content.[41] Karl Fisher Titration was used for estimating the moisture contents of CG to minimize the loss of easily volatile compounds during the conventional drying process,[41] and its TS content was calculated from the estimated moisture value. The sCOD value for JPC and CG was determined using the methods described in Sec. II C 2. The caloric value of dried JPC was determined using a Bomb Calorimeter (IKA^R C 200).[47] The crud fat content of dry JPC was determined using the conventional Soxhlet extraction method described in the study by Jabłoński *et al.*[23]

2. Determination of sCOD yields

The analysis of sCOD was performed using Merck Spectroquant® COD kits with a measuring range of 500–10 000 mg L^{-1}. Briefly, small fraction samples were taken from individual steam-exploded samples and then centrifuged to 6000 rpm for 10 min to separate the liquid fraction. Then, the liquid sample was further filtered through 0.45 μm pour size Whatman membrane filter paper using a vacuumed pump.[46] Finally, 1 ml of the filtered liquid was diluted with 25 ml of distilled water, and then 1 ml of the diluted sample was added to the commercial COD kit and gently shaken with a hand for a few seconds. Then, the samples were digested at 240 °C for 2 h. Triplicate analysis was performed for all treatments, and the sCOD values were estimated following the DIN ISO 15705 standard.[47]

3. Analysis of the biogas composition

For the first 11 days, daily biogas measurements were performed, and then fewer measurements were taken when needed for the rest of 47 days of digestion time. More specifically, the accumulated gas pressure inside the digester was measured using a digital manometer (GMH 3161 Reisinger Electronic, Germany). Following the subsequent pressure measurement, the biogas composition was determined using gas chromatography (3000 Micro GC, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) using helium as a carrier gas. The endogenous methane produced from inoculum was deducted from the total methane yield to calculate the net methane produced from JPC alone. The biogas volumes were calculated at standard conditions, i.e., at 273 K and 101.3 kPa using Eq. (2), while the specific methane volume was calculated using normalized methane concentrations. The effect of SE pretreatment on methane yield increment (MYI) relative to the untreated sample was calculated using Eq. (8) as described by Svensson *et al.*,[47]

$$MYI(\%) = \left(\frac{MYI_{pretreated} - MYI_{untreated}}{MYI_{untreated}}\right) \times 100, \qquad (8)$$

where MYI is the methane yield increment.

III. RESULTS AND DISCUSSION

A. Substrate composition

The JPC added into the digester contained 90.01% TS and 93.36% VS. By the time of supply, the TS and VS contents of the inoculum were estimated to be 4.4% and 59.9%, respectively. The chemical composition of JPC is dominated by carbon (50.4%), oxygen (38.44%), nitrogen (3.99%), and hydrogen (7.17%). The C/N ratio estimated from the carbon and nitrogen constituents of JPC was 12.63, which is significantly lower than the optimum values (20:1–30:1) suggested for stable anaerobic digestion process.[55] Thus, co-digesting the JPC with carbon-rich substrates such as CG could be one of the valuable options.[56] The estimated TS content of CG was 85.0%, while its VS content was assumed to be the same as the TS since nothing was left in the crucible when it was ignited at 550 °C for two hours. The sCOD yield estimated from the CG was 1399.33 ± 6.99 g L^{-1}, which agrees with the previous study.[19] The lipid and protein content of JPC was 9.00% and 24.90%, respectively, while the calorific value was estimated to be 19.87 kJ kg^{-1}. The higher VS and calorific values of JPC and CG indicate that both substrates are highly potential feedstock for biogas production.

B. Impact of TOL and CG levels on the BMP of JPC

In this study, JPC was mixed with CG since (1) both substrates are massively co-generated during oil extraction and biodiesel production processes, (2) the lower C/N ratio of JPC could be optimized when co-digested with the CG that contained a higher carbon content, and (3) the biogas produced from these biowastes can minimize the overall biodiesel production and waste treatment costs. Thus, as discussed in Sec. II B1, the effect of TOL and CG levels on the stability and cumulative methane yields of the mixture was examined by running a series of batch anaerobic experiments. The cumulative methane yield obtained by co-digesting the JPC with various levels of CG ranged between 165.87 and 325.47 ml g^{-1} VS.

A two-way ANOVA and Tukey post hoc test were also performed to investigate the specific TOL-CG combination that resulted in a significantly higher cumulative methane yield in the co-digestion process (Table III). The small P-value ($p < 0.01$) from the ANOVA model indicates that the methane yield of the mixture was significantly affected by the level of CG and TOL of the batch reactor operated at the mesophilic condition. Significant cumulative methane yield variation was observed from reactors run at lower (2 g VS L^{-1}) and higher (7 g VS L^{-1}) organic loads. The anaerobic batch reactor operating at 2 g VS L^{-1} that contained 2% CG seems the optimum condition that resulted in a higher cumulative methane yield (325.47 ml g^{-1} VS), while co-digesting 4% CG with JPC and increasing the TOL into 7 g VS L^{-1} adversely affected the methane yield of the mixture (Table III). Thus, maximum MYI (28.95%) was achieved by co-digesting 2% CG with JPC at 2 g VS L^{-1} TOL; then, it declined by 96.23% when 4% CG was co-digested with JPC at 7 g VS L^{-1} TOL.

Besides the ANOVA table, the interactive effect between TOL and CG levels on the mixture's methane yield is clearly shown in Fig. 1. All cumulative methane yields obtained from 2 g VS L^{-1} TOL and various CG levels were relatively high compared to the co-digestion experiments employed at 7 g VS L^{-1} TOL. The significant effect of the interaction of TOL and CC levels proved by the large methane yield difference observed when 2% and 4% were co-digesting at 2 and 7 g VS L^{-1}, respectively. However, no significant methane yield variation was observed from the digesters operated under 5 g VS L^{-1} TOL, while the CG level varies between 0% and 4%.

TABLE III. The cumulative methane yields (ml g^{-1} VS) obtained from the co-digestion process employed at various TOL and CG levels.

	CG levels (%)[a]				
TOL (g VS L^{-1})	0	1	2	3	4
2	252.41b	259.49b	325.47a	278.46ab	265.22b
5	240.11b	264.34b	261.97b	250.42b	275.69b
7	247.98b	249.68b	251.11b	261.11b	165.87c

[a] The difference in lowercase letters showed a statistical mean difference due to the interactive effect between TOL and CG levels at P= 0.05. The statistical variation of means in the same row was due to the CG level effects, while the mean difference in the same column was due to the TOL effects.

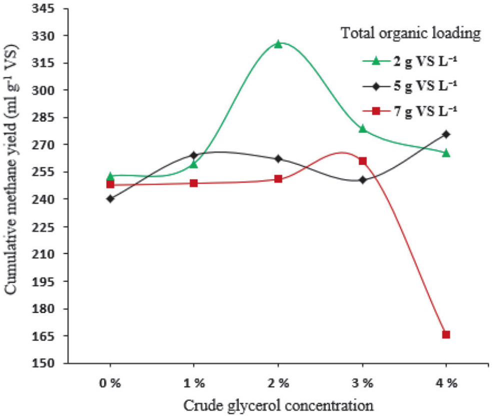

FIG. 1. The interactive effect between TOL and CG levels on the cumulative methane yield of JPC.

Defining the optimum conditions in the co-digestion process could also significantly affect the rate of degradation. The average specific daily methane yields obtained from all treatments ranged between 11.85 and 23.25 ml g^{-1} VS day^{-1}. However, the optimum condition (2 g VS L^{-1} TOL) that showed a higher methane yield (325.47 ml g^{-1} VS) was selected, and the rate of degradation was investigated by varying the CG levels between 0% and 4%. As shown in Fig. 2, the maximum methane production rate (48.9 ml g^{-1} VS day^{-1}) was observed when 2% CG was co-digested with JPC at 2 g VS L^{-1} TOL over the rest treatments. Moreover, the co-digestion process performed by mixing 2% CG with JPC seemed more stable after seven days of hydraulic retention time (HRT) and then started to fall rapidly after 32 days of HRT due to the scarcity of feeding materials for microbial biomass growth.[57]

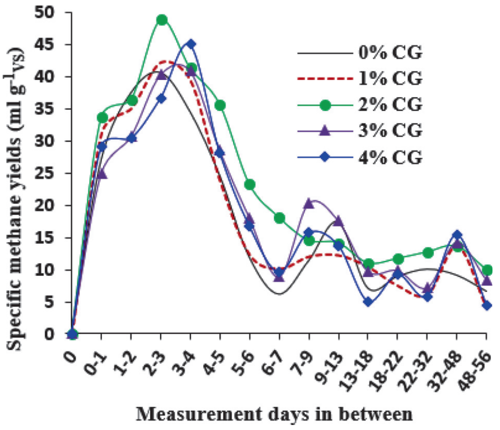

FIG. 2. The daily specific methane yields obtained at 2 g VS L^{-1} TOL, but with different CG levels.

Oliveira *et al.*[25] reported a similar result, which stated that co-digesting 2% CG with sargassum resulted in 18% more methane than the control treatments. Similarly, Schwingel *et al.*[19] showed that co-digestion of 2.6% glycerol with laying hen manure produced 21.83% more methane over the manure without glycerol. However, higher levels of CG had a detrimental effect on the performance and methane yield of the mixture. Veroneze *et al.*[58] noted that the biogas production rates from swine manure were impaired when more than 5% of glycerol was added.

Variation in the rate of degradation and cumulative methane yields in the co-digestion process could be associated with the change in the TOL, pH, C/N ratio, and toxicity levels.[57] More specifically, the higher methane yield could be explained by the co-digestion of readily biodegradable and higher energetic CG.[27] The synergic effects between JPC and CG that contained essential nutrients in the correct chemical forms and concentrations could enhance the methane yield of the mixture.[56] CG could also serve as a source of carbon and energy for microbes involved in the anaerobic digestion process.[18] However, higher organic loading mainly leads to the accumulation of volatile fatty acid (VFA), which subsequently reduces the methane yield.[25] Furthermore, higher organic loading may result in the accumulation of excess compound not being fully degraded by microbes in the digester that hamper the methane production process. The buffering capacity of nitrogen from JPC could also be depleted when adding highly concentrated CG.[57] High glycerol levels can cause tension and toxicity to bacteria, resulting in a slower rate of degradation and lower methane production.[18]

C. Modeling of methane production

The methane yield obtained from JPC was used as the primary indicator variable for evaluating the effect of SE pretreatment employed under different X_1 and X_2 values. Using the same criteria as described in Sec. II B 2, values for R^2 (0.98), adj.R^2 (0.97), and pred.R^2 (0.90) were used to evaluate the strength of the model that best explains the correlation between predictors (X_1 and X_2) and response (methane yields) variables. The significant effects of linear (X_1 and X_2), quadratic (X_1^2 and X_2^2), and interactive (X_1X_2) terms in the regression model indicate that the influence of these experimental factors on the response value was not a simple linear relationship.[59] Accordingly, a second-order polynomial model was selected as the best-fitted model. Therefore, least squares regression models were developed using both coded and actual experimental data [Eqs. (9) and (10)]. As shown in Eq. (9), the linear effect of X_1 and the interactive effect between two process variables (X_1X_2) were positively associated with the expected methane yield. In contrast, the linear effect X_2 and quadratic effect of both variables (X_1^2 and X_2^2) were negatively associated. The higher absolute values of estimates (β_i) revealed the strength of their impact on the examined response;[60] thus, the effect of X_2^2 was higher followed by X_1^2 and X_1X_2,

$$\begin{aligned} \mathit{Methane\ Yield\ (Coded)} = {} & 329.07 + 5.84X_1 - 8.27X_2 \\ & - 12.95X_1^2 - 20.71X_2^2 + 12.87X_1X_2, \end{aligned} \tag{9}$$

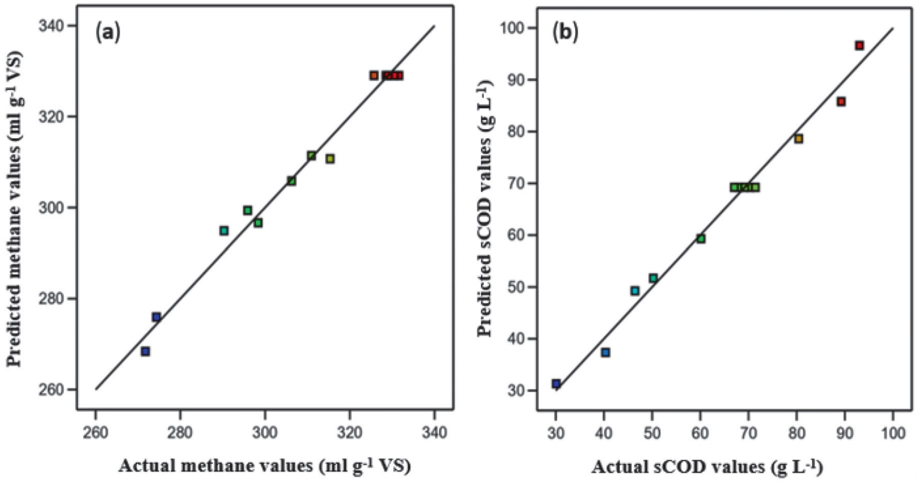

FIG. 3. The correlation between the experimental and model-predicted values of methane (a) and sCOD (b).

$$\text{Methane Yield (Actual)} = -4434.00 + 49.18X_1 - 40.54X_2 - 0.13X_1^2 - 1.29X_2^2 + 0.32X_1X_2. \quad (10)$$

The value for the coefficient of determination (R^2) obtained from the experimental data of methane yield indicates that 98% of methane yield's variability was due to SE applied at various process conditions. The closed value between R^2 and adj.R^2 demonstrates how the added variables were critical to the model.[2] The higher pred. R^2 value shows the accuracy of the model in predicting the methane yields at given X_1 and X_2 values. The predicted values obtained from Eq. (10) were close enough to the experimental values of the methane yields [Fig. 3(a)]. Thus, the maximum difference between the actual and predicted values was within ±2.76, which further demonstrates the adequacy of the model. The coefficient of variance (CV) was also estimated to be 0.0174 (less than 0.5), which indicates that the experimental data were reliable.[59] Furthermore, the F-value and P-value of the model's lack of fit were 4.82 and 0.0814, respectively, which implies that the lack of fit relative to the pure error is not statistically significant; thus, the model was adequate.

ANOVA was also employed for the same process variables as described in Sec. II B 2. The data displayed in Table IV showed that the linear, interactive, and quadratic terms of all process variables had significantly affected the methane yield of JPC. The higher F-value from the linear and quadratic effect of X_2^2 indicates that the impact of X_2 during SE was more intense than X_1. However, as shown in Table IV, the effect of X_1X_2, X_1^2, and X_2^2 on the methane yield of JPC was higher compared to X_2.

1. Effect of independent processing parameters on methane production

The effect of process variables (X_1 and X_2) during SE pretreatment was investigated by performing a series BMP test. As shown in Table V, all SE pretreatments showed a positive effect on BMP of JPC as compared to the untreated samples (247.18 ml g^{-1} VS). Thus, the average methane yields of all pretreated samples were superior, in which the increased methane yields were significantly varied between 94%–34.14% upon SE severity indexes (Table V).

The effect of each process variable was investigated by varying the value of one variable at a time while keeping the other into its center value. As shown in Fig. 4(a), the methane yield of JPC was increased upon increasing supplied X_1 up to 200 °C at a constant X_2 (10 min). However, further increment of X_1 was manifested by a reduction of methane yields. For example, the methane yield of JPC increased by 13.35% when the value of X_1 raised from 186 to 200 °C; then, it declined by 5.83% when the supplied X_1 increased to 214 °C. The effect X_2 on JPC's methane yield was also investigated by maintaining the value of X_1 into its center value (200 °C). In the same way

TABLE IV. ANOVA for the regression model presented from the methane yields.

Source	Sum of squares	df	Mean square	F-value	p-value
Model	5210.23	5	1042.05	79.87	<0.0001[a]
X_1	272.77	1	272.77	20.91	0.0026[a]
X_2	547.80	1	547.80	41.99	0.0003[a]
X_1X_2	662.74	1	662.74	50.80	0.0002[a]
X_1^2	1167.14	1	1167.14	89.46	<0.0001[a]
X_2^2	2983.14	1	2983.14	228.66	<0.0001[a]
Residual	91.32	7	13.05		
Lack of fit	71.53	3	23.84	4.82	0.0814
Pure error	19.79	4	4.95		
Cor total	5301.56	12			

[a] Statistical significance of the factors at P= 0.05.

TABLE V. The actual and model-predicted values of sCOD and methane yields.

	SEP conditions		Actual values		Predicted values		
Run order	X_1 (°C)	X_2 (min)	sCOD (g L^{-1})	CH_4 (ml g^{-1} VS)	sCOD (g L^{-1})	CH_4 (ml g^{-1} VS)	MYI (%)[a]
1	200	10	68.02	325.71	69.21	329.07	31.77
2	190	14	60.14	271.75	59.30	268.42	9.94
3	214	10	89.23	310.95	85.79	311.42	25.80
4	200	15.7	80.43	274.35	78.61	275.95	10.99
5	210	6	50.25	298.37	51.70	296.65	20.71
6	200	10	70.32	328.55	69.21	329.07	32.92
7	200	10	71.42	329.02	69.21	329.07	33.11
8	200	4.3	30.11	295.90	31.32	299.35	19.71
9	186	10	46.44	290.31	49.27	294.90	17.45
10	210	14	93.03	306.26	96.62	305.84	23.90
11	200	10	69.24	330.48	69.21	329.07	33.70
12	190	6	40.34	315.35	37.36	310.71	27.58
13	200	10	67.05	331.57	69.21	329.07	34.14
Raw JPC	0	0	ND	247.18	ND	ND	0

[a] MYI is the methane yield increment, and ND is not determined.

as the effects from X_1, the methane yield of JPC was increased by 11.21% when X_2 increases from 4.3 to 10 min. However, the methane yields significantly declined by 19.95% when X_2 further increases from 10 to 15.7 min. Furthermore, as shown in Table IV, the effect of X_2 is highly significant compared to X_1, which indicates that the accumulation of inhibitor compounds could be higher at longer pretreatment times.[27] The probable reasons for lower and higher methane yields obtained at various SE pretreatment conditions are well discussed in Sec. III C 2.

2. Effect of interactive factors on the methane yields

The data presented in Table V showed that lower methane yields (<300 ml g^{-1} VS) were obtained at both higher and lower retention times (runs 2, 4, 5, 8, and 9), whereas relatively higher methane yields (≥315 ml g^{-1} VS) were obtained after exploding JPC at 200 °C for 10 min (run1, 6, 7, 11, and 13). However, maintaining X_2 at its center value (10 min), but reducing the value of X_1 to 186 °C resulted in lower methane yields (run 9). Interestingly, supplying steam with a

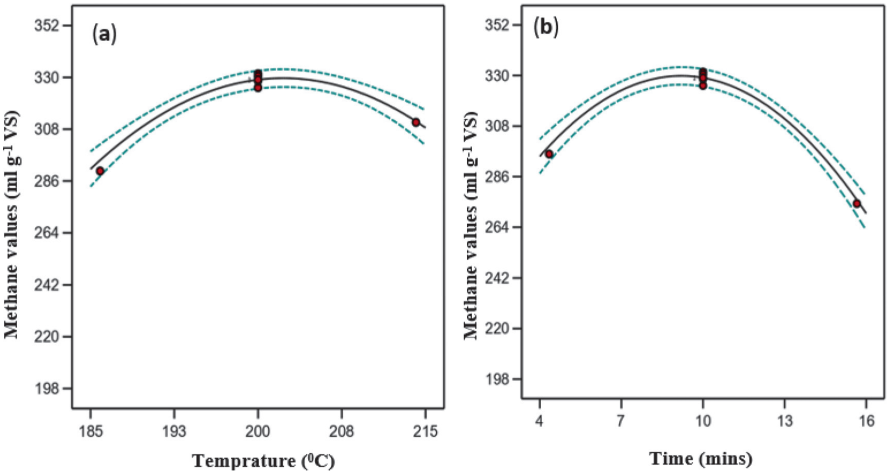

FIG. 4. Effect of steam temperature (a) and retention time (b) on the methane yields; one parameter was varied, while the other was maintained to its center value.

temperature of 190 °C and retaining it for 6 min seemed a promising condition for a higher methane yield (run 12). However, the variation in X_1 and X_2 during the SE pretreatment process did not significantly affect the methane concentration in which the values ranged between 65% and 68%.

The interactive effects between two process variables (X_1 and X_2) on the cumulative methane yield of JPC are presented using contour and 3D plots, as shown in Figs. 5(a) and 5(b). Figure 5(b) shows that the methane yield of JPC was increased when the supplied X_1 and X_2 increased from their lower values to the middle range (200 °C, 10 min) and then again declined when the value of X_1 and X_2 exceeded the center values. The reduction of methane yields due to higher X_2 seemed higher than the effect of X_1 [Fig. 5(b)]. Thus, a relatively high methane yield was achieved when JPC remained in the middle value X_2 compared to severe pretreatment conditions. The possible reason for the higher methane yield in the middle range of pretreatment conditions (200 °C, 10 min) might be higher solubilization of hemicellulose over pretreatments carried out at lower and severe pretreatment conditions. Less severe pretreatment conditions imply incomplete hydrolysis of lignocellulosic material, which leads to lower biodegradation and methane yields.[60]

On the other hand, harsher pretreatment conditions cause loss and degradation of cellulose and hemicellulose.[6] Degradation of carbohydrate and lignin during SE pretreatment may result in the production of aliphatic acids (primarily acetic acid and formic acid), furan aldehydes (5-hydroxymethylfurfural and furfural), and phenolic compounds, which are inhibitors and toxic for microbes involved in the anaerobic digestion process.[27,33,41] As expected from SE pretreatment, the lignin structure is always altered and partially degraded. However, at harsher pretreatment conditions, condensation reaction may occur within degraded lignin compound and between lignin and furfurals. Furthermore, at higher X_1 and X_2, polymerization of degraded products might occur, which might lead to a pseudo-lignin formation that could block the accessibility of carbohydrates for microbial degradation.[61]

Therefore, defining the optimum conditions in the SE pretreatment process would be indispensable for enhancing the methane yield of JPC without inhibitor formation. As a result, the optimum operational variables in the SE pretreatment process were predicted using the RSM-CCD model developed from the actual experimental values [Eq. (10)]. Thus, exploding the JPC at 202 °C for 9.39 min was identified as optimum conditions for maximum methane production (330.14 ml g^{-1} VS), which exceeded by 33.56% compared to the methane yield of untreated JPC.

D. Modeling of sCOD yields

The least squares regression models for linear, quadratic, cubic, and two-factor interaction (2FI) were fitted using RSM-CCD without any bias using the sCOD values obtained from the experimental data.[62] The cubic model was aliased. The adj.R^2 value for the quadratic model was 0.974, which is significantly higher than 0.867 and 0.842 estimated for 2FI and linear models, respectively. As a result, the second-order polynomial model was selected as the best-fitted model to depict the correlation between predictors (X_1 and X_2) and response variable (sCOD) using the criteria described in the study by Subroto *et al.*[42] Therefore, the following least squares regression models were developed from the coded and actual experimental values,

$$sCOD\ Yield(Coded) = 69.21 + 12.91X_1 + 16.72X_2 + 0.84X_1^2 - 7.12X_2^2 + 5.75X_1X_2, \tag{11}$$

$$sCOD\ Yild(Actual) = -324.39 + 3.22X_1 - 15.64X_2 - 0.01X_1^2 - 0.45X_2^2 + 0.14X_1X_2. \tag{12}$$

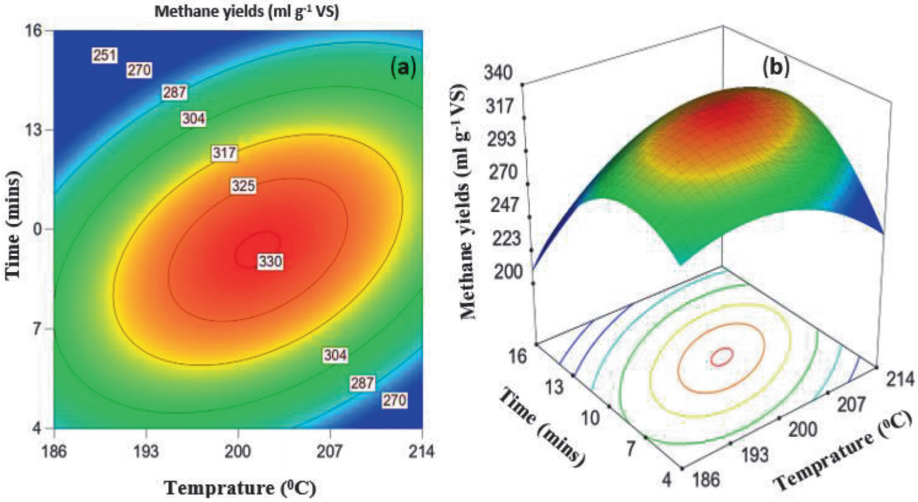

FIG. 5. The contour (a) and response surface (b) plots for methane yields of JPC.

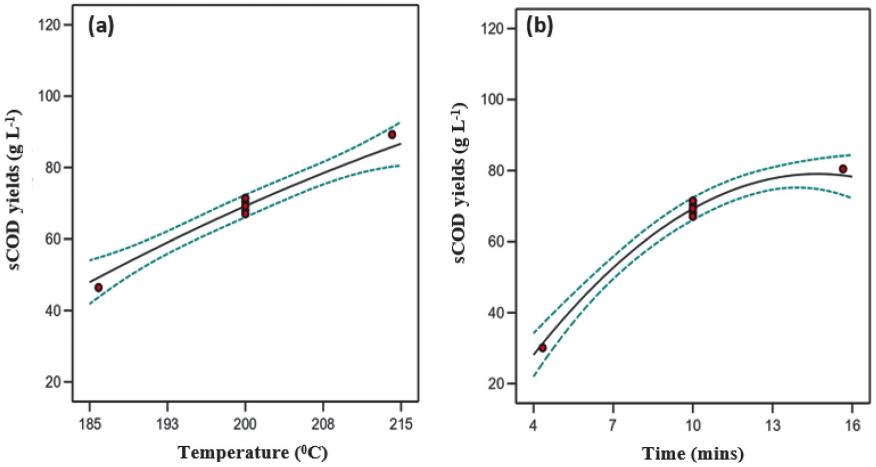

FIG. 6. Effect of steam temperature (a) and retention time (b) on the sCOD yield of JPC; one parameter was varied, while the other was maintained at its center value.

The value for the coefficient of determination ($R^2 = 0.985$) obtained from the experimental value of sCOD indicates that the model could not explain only 1.5% of the variability in sCOD yields. The close relationship between R^2 (0.985) and adj. R^2 (0974) further explains the importance of all process variables in the model as described previously.[2] A closed value between adj. R^2 and pred.R^2 (0.910) shows us how the model is strong enough to predict the sCOD yields at a given value of X_1 and X_2 during the SE pretreatment process [Fig. 3(b)].

As shown in Eq. (11), the linear and interactive effects of X_1 and X_2 were positive and statistically significant at 95% of the confidence interval. However, a quadratic effect of X_2^2 was found to be negative and statistically significant, while the sCOD yield of JPC was associated positively with the quadratic effect of X_1^2. Furthermore, as it is noted from the coefficients of the regression [Eq. (11)], the effect of X_2 was more important than X_1.

1. Effect of independent processing parameters on the sCOD yields

The effect of the individual process variable on sCOD was investigated following the same mechanisms as described in Sec. III C 1. An increase in X_1 implies a substantially higher degree of solubilization, which resulted in higher sCOD yields. As shown in Table V, the estimated sCOD yield ranged between 31.11 and 93.03 g L^{-1}. The higher value of sCOD was recorded when JPC was exploded at the temperature of 210 °C for 14 min (run 10), while the lower yield was obtained at 200 °C and 4.34 min (run 8). As shown in Fig. 6, the sCOD yield increased with increasing X_1 at all levels, i.e., from 186 to 214 °C. The effect of X_1 on the degree of solubilization was higher in its lower ranges. For instance, the sCOD yield from JPC was increased by 49.68% when the value of X_1 increased from 186 to 200 °C. However, with a further increase in X_1, the increment in the sCOD yield was relatively low; only a 28.32% increment was observed when X_1 increases from 200 to 214 °C.

The higher F-values displayed in Table VI show the higher effect of X_2 on the sCOD yield compared to X_1. At a constant X_1 value (200 °C), the sCOD yields were increased with the increment of X_2 from 4.34 to 10 min; however, with a further increase in X_2 from 10 to 15.7 min, the sCOD yields showed a declining trend. For instance, the sCOD output was increased by 122.46% when the value of X_2 increased from 4.34 to10 min at a constant X_1 value (200 °C), but the increment reduced to 15.71% with a further increment of X_2 from 10 to 15.7 min. The higher sCOD yield of JPC after SE pretreatment may be associated with the solubilization of carbohydrates and lignin.[40] In contrast, the lower sCOD at severe pretreatments could be attributed to instability and formation of complex non-soluble compounds.[62]

TABLE VI. ANOVA for the regression model with the sCOD yields.

Source	Sum of squares	Df	Mean square	F-value	p-value
Model	4055.18	5	811.04	92.49	<0.0001[a]
X_1	1334.23	1	1334.23	152.15	<0.0001[a]
X_2	2235.91	1	2235.91	254.98	<0.0001[a]
X_1X_2	132.02	1	132.02	15.06	0.0061[a]
X_1^2	4.92	1	4.92	0.5606	0.4784
X_2^2	352.97	1	352.97	40.25	0.0004[a]
Residual	61.38	7	8.77		
Lack of fit	49.18	3	16.39	5.38	0.0689
Pure error	12.20	4	3.05		
Cor total	4116.56	12			

[a] The statistical significance of factors at 5% error.

Studies have shown that at severe SE pretreatment processes, a considerable amount of volatile components from hemicellulose could be lost.[63] Furthermore, at harsher pretreatment conditions, the hydrolysate may contain soluble components from degraded carbohydrates, lignin, and other extractives, which can potentially reduce the methane yield during the anaerobic digestion process.

2. Effect of interactive factors on the degree of solubilization (sCOD)

The interactive effect between X_1 and X_2 was demonstrated by plotting the two process variables with the sCOD yield using the counter and three-dimensional surface plots (Fig. 7). As shown in Table VI, the interactive effect between X_1 and X_2 on the sCOD yield was statistically significant, while Fig. 7(b) shows that the sCOD yield increased with the increase in both X_1 and X_2. With the initial increase in X_2, the sCOD yield increased rapidly and reached a maximum value at the midpoint of X_2 (approximately 10 min). Then, it started to fall again at a slower rate with a further increment of X_2. However, the effect of X_1 was constant, in which the sCOD yield was continuously increased with the increasing X_1. Furthermore, the 3D plot shows that the effect of X_2 on the sCOD yield was higher compared to X_1. An increase in the degree of solubilization at the harsher pretreatment might be associated with the progressive hydrolysis of the various organic matter present in the press cake.[62] At the same time, the probable reasons for lower sCOD yield at extremely higher SE pretreatment conditions are described in Sec. III D 1.

RSM was employed to define the optimum X_1 and X_2 that resulted in a higher sCOD yield. The RSM-CCD model predicted that exploding the JPC at 209 °C for 13.68 min would result in a maximum sCOD yield of 94.48 g L^{-1}. These optimum conditions were comparable with previous reports,[33] in which a maximum enzymatic release of glucose and xylose values was obtained when the Salix biomass was exploded at 210 °C for 10 min. However, optimum conditions suggested for the maximum sCOD yield are relatively high compared to the optimum conditions defined for the higher methane yield. Thus, the hydrolysate used for sCOD analysis might be mostly dominated by hemicellulose since the methane production efficiency of sugar from hemicellulose is lower than that of cellulose.[40]

Moreover, the hydrolysate obtained at harsher pretreatment may also contain inhibitor compounds like furans, hydroxymethylfurfural (HMF), pyrroles, phenols, enols, and carboxylic acids.[41] For instance, Horn *et al.*[33] showed that a higher concentration of HMF and furfural when the supplied temperature during SE pretreatment exceeded about 210 °C, while Lopez-Linares *et al.*[40] showed that the hydrolysate obtained from rapeseed straw was affected by acetic acid, formic acid, furfural, HMF, and phenol compounds. Furfural and HMF are formed mostly from degraded fructose and glucose, respectively, while phenolic compounds are generated due to lignin degradation at harsher pretreatment conditions.

E. Energy balance and economic feasibility

Although all SE pretreatments improved the methane yield of JPC over the untreated sample, the energy balance and BCR analysis were performed only for the optimum conditions that have been identified for a higher methane yield. As described in Sec. II B 5, the preliminary energy balance and economic feasibility of biogas production from steam-exploded JPC were investigated by identifying the main input and output components during SE pretreatment and anaerobic digestion process. Thus, the input energies included energy required to heat the raw material (JPC) to the targeted temperature (202 °C) and energy consumed to generate the required amount of steam. The amount of steam required for treating one ton of JPC was estimated to be 0.15 tons. The data displayed in Table VII showed that the energies needed for heating

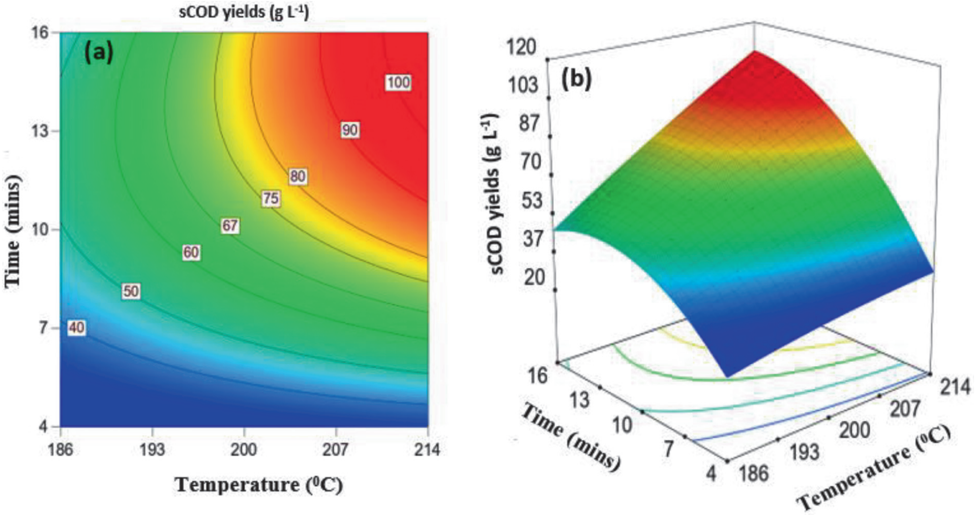

FIG. 7. The contour (a) and response surface (b) plots of the sCOD yield.

TABLE VII. The preliminary energy balance and economic feasibility of SE pretreatment applied on JPC.

Energy balance and methane yields[a]	Alkaline pretreatment (202 °C, 9.39 min)	Co-digestion process (2 g VS L^{-1} TOL containing 2% CG)
Methane produced from untreated JPC (m^3 ton^{-1} JPC)	230.77	230.77
Methane production from pretreated JPC (m^3 ton^{-1} JPC)	308.22	303.86
Net increase in the methane yield (ΔP) (m^3 ton^{-1} JPC)	77.5	73.1
Energy required for heating JPC (MJ ton^{-1} JPC)	331.45	0
Energy consumed for steam production (MJ per 0.15 ton steam)	402. 28	0
Energy produced from ΔP [MJ $(m^3)^{-1}$ ton^{-1} JPC]	2497.05	2355.28
Preliminary economic feasibility[a]		
Energy costs needed for heating of one ton of JPC (€/)	2.39	0
Energy costs for steam generating (€ per 0.15 ton steam)	2.9	0
Revenue obtained from sales of ΔP (€ ton^{-1} JPC)	44.18	41.67
Benefit-cost ratio (BCR)	8.33	undefined

[a] ΔP is the extra methane yield obtained after pretreatments (methane yield obtained from pretreated JPC-methane yield produced from untreated JPC); the BCR for the co-digestion process is undefined since zero cost was estimated for the pretreatment process.

the bulk mass of JPC and steam were significantly lower than the energy produced from the extra methane yield obtained after the pretreatment. Thus, the energy produced from the additional methane yield exceeded by 3.4-fold compared to the sum of energy consumed during the SE pretreatment process. However, it should be noted that the energy balance analysis did not include the energy needed for heating the air inside the reactor and the reactor itself since once the reactor starts, it will work continually; thus, the energy required for heating the air and reactor assumed to be zero after few seconds of retention time.[49]

The preliminary BCR analysis was also done by considering the unit price of input and output energies during the SE pretreatment and anaerobic digestion process. The revenue obtained from the sale of additional methane yield was considered as income. In contrast, energy expenditure required for heating the JPC and steam was admitted as a cost. The total energy required for pretreating one ton of JPC was estimated to be 733.73 MJ that costs 5.30 € (Table VII), while one ton TS from JPC can produce 77.50 m^3 extra methane over the untreated JPC, which prices 44.18 €. Thus, the BCR obtained by dividing the revenue gained from the sales of extra methane yield to the sum of energy cost during the pretreatment process was estimated to be 8.33. Thus, the positive BCR motivated the application of SE pretreatment at a larger scale. However, the actual energy cost could be higher than the present estimation since all multi-stage energy requirements of SE pretreatment are not included.[49] Despite the fact that positive energy balance and BCR were obtained after SE pretreatment, the co-digestion process was found to be more effective over SE pretreatment due to the following reasons: (1) the co-digestion process consumed much less energy since no energy demand for pretreatment; (2) the co-digestion process creates an opportunity for utilizing both JPC and CG biowastes generated the during biodiesel production process, and (3) a statistically insignificant MYI difference was observed between SE pretreatment and co-digestion processes.

F. Scale-up of laboratory analysis to a medium-scale biogas plant

The biogas yield, energy balance, and economic return analyzed at the laboratory scale may not be precisely the same as large scale production since industrial biogas plants often do not operate at their optimum conditions.[64] However, this study assumed that the BMP of JPC and CG digested at the laboratory and industrial scale is the same. Thus, we took a medium scale biodiesel plant instilled in Ethiopia for estimating the biogas production potential of JPC and CG residues generated during oil extraction and biodiesel production processes. The annual oil extraction and biodiesel production potentials of this plant were estimated to be 102.93 l and 96.36 tons, respectively.[65] The type of oil extraction method used in the plant is a screw press machine that can process the whole Jatropha seed without further processing. The average oil extraction efficiency of the screw press is assumed to be 35% of the dry weight of Jatropha seeds.[14] Moreover, we considered that the average CG production after the transesterification reaction is 12% of the oil being used.[15] Thus, 294.09 tons of dry Jatropha seed is needed to produce the above-indicated oil amount, which could release 191.16 and 12.35 tons of JPC and CG, respectively. Thus, considering the various physicochemical properties of JPC and CG, as shown in Sec. III A, it would be possible to produce 0.528 and 0.531 million meter cubic of methane from steam-exploded and co-digested JPC, respectively.

IV. CONCLUSIONS

The recalcitrant nature of lignocellulosic materials and ammonia inhibition that occurs during anaerobic digestion of JPC provide motivation for the design of proper pretreatment techniques. SE pretreatment and the co-digestion process were identified as promising strategies. The optimum pretreatment and co-digestion process conditions that can improve the methane yield of JPC and CG without process inhibition were critically examined and modeled. The methane yields of all steam-exploded samples were higher over the untreated JPC, while the methane yield obtained in the co-digestion process was significantly affected by TOL and CG levels. The efficiency of SE for enhancing the methane yield of JPC was significantly affected by the applied steam temperature and retention time. The RSM-CCD models developed from the experimental data were found to be adequate and reliable, and most of the linear, interactive, and quadratic effects of steam temperature and retention time were significantly correlated with sCOD and methane yields. Higher sCOD yields were obtained relatively at severe pretreatment conditions, while the methane yields declined with increased severity factors. Thus, variation in optimum conditions predicted for the maximum sCOD and methane yield could be used as a quick and new indicator for the occurrence of inhibitors due to degradation of lignin and polysaccharides at severe pretreatment conditions. Moreover, the methane yields in the co-digestion process declined upon increasing both the TOL and CG levels. The co-digestion experiment employed by mixing 2% CG with JPC and maintaining the TOL into 2 g VS L^{-1} was identified as the optimum conditions for a higher methane yield. The positive energy balance and higher BCR inspired the application of the SE pretreatment and co-digestion process at a larger scale. However, energies consumed for heating the air and reactor itself are not considered during the preliminary energy balance calculation. Moreover, a considerable amount of energies could be lost during the rapid decomposition of steam, air, liquid water, and dry biomasses. Based on the finding of this study, the authors recommended that (1) JPC should be co-digested with GC for improving the methane production potential of both substrates; (2) biodiesel production should be integrated with biogas production for ensuring sustainable biofuel production from Jatropha; and (3) future studies should focus on characterizing the digestate generated after anaerobic digestion before using as organic fertilizer.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Paper V

Biochemical methane potential of *Jatropha curcas* fruit shell: comparative effect of mechanical, steam explosion and alkaline pretreatments

Gebresilassie Asnake Ewunie[1,3], Zerihun Demrew Yigezu[2], John Morken[1]

[1] Department of Science and Technology, Norwegian University of Life Science (NMBU), P. O. Box, N-1432, Ås, Norway

[2] College of Agriculture, Hawassa University, P. O. Box, 05, Hawassa, Ethiopia

[3]Corresponding author's E-mail: asnake.ewunie.gebresilassie@nmbu.no

Abstract

Jatropha curcas is a promising tropical and subtropical plant species for biodiesel production that can reduce the competition between food and energy production. Jatropha seed processing for oil extraction usually generates considerable amount of *Jatropha curcas* fruit shell (JCFS), which can be considered as a potential substrate for biogas production rather than being discarded as solid waste. However, the higher lignocellulosic constituents in JCFS potentially affects the biological degradation process. Thus, applying suitable pretreatment techniques in advance of anaerobic digestion could enhance the biodegradability and methane yield of JCFS. In this study, the effect of mechanical, steam explosion (SE), and alkaline pretreatments on the chemical composition and methane yield of JCFS was examined at various process conditions. As compared with the untreated sample, grinding the JCFS into a particle size of less than 1mm increased the methane yield by 74.23%, while at the optimum SE pretreatment process (160 °C, 5 min), the methane yield was increased by 54.75%. The alkaline pretreatment was relatively less effective over the other pretreatments; 44.05% methane yield increment was achieved after soaking the JCFS with 7.32% NaOH at 36 °C for 54 hrs. The effect of SE on compositional change depends on the severity factor in which severe pretreatment conditions were adequate for solubilizing the hemicellulose but resulted in higher pseudo-lignin and lower methane yields. In conclusion, all pretreatments processes have significantly increased the methane yield of JCFS as compared to the untreated JCFS; however, mechanical pretreatment was more effective than SE and alkaline pretreatments.

Keywords: *Jatropha curcas* fruit shell; Steam explosion; Alkaline pretreatment; Methane; Mechanical pretreatment.

1 Introduction

The world energy profile shows an immense gap between energy supply and demand [1], and more than 85% of the energy requirement is derived from fossil fuel [2] with numerous environmental problems such as greenhouse gases (GHGs) emission and environmental pollutions. To ensure sustainable energy production and less GHGs emission, production and utilization of biofuels such as biodiesel, bioethanol, and biogas are getting a great deal of attention from the scientific communities and industries. Various studies have been conducted to explore alternative and sustainable energy sources such as lignocellulosic materials, non-edible oils crops, agro-industrial organic wastes, wastewater sludge, and municipal solid organic wastes [3,4].

Jatropha curcas L. (*J. curcas*) has been identified as a promising tropical and subtropical plant species for biodiesel production [5], which can reduce the competition between food, feed, and energy production. Besides its higher oil content with suitable chemical composition, *J. curcas* is highly adaptable to moisture stress and degraded areas [6] . These characteristics make the plant to be a good candidate for biodiesel production. Thus, the application of both *J. curcas* biodiesel as engine fuel have been tested successfully, and many large and small scale biodiesel plants are built across the world [7]. However, seed processing for oil extraction and biodiesel production usually generates massive volumes of *J. curcas* residues such as *J. curcas* fruit shell (JCFS) and *J. curcas* press cake (JCPC) [8,9]. The sum of JCFS and JCPC accounts for above 80% of the dry fruit weights [10]. More specifically, JCFS alone shares 34-40% of the dry fruit weight [11], while JCPC weights 60-70% of the dry seed's heft [12]. These residues can neither be used as an animal feed nor as organic fertilizer because of poisonous chemicals such as crucin and phorbol ester [13]. As a result, open disposal of these residues may adversely affect the environment unless adequately managed.

On the other hand, JCFS and JCPC contain essential organic constituents, which can be used as a potential substrate for biogas production. Studies indicate that the energy production potential of JCPC and JCFS exceeded by threefold as compared with the oil or biodiesel [10]. However, for the last few years, much attention was given for *J. curcas* oil and biodiesel only, which accounts for about 30% of the energy content or 17% of the dry fruit biomasses [14]. The potential of JCPC for biogas production has been examined in our previous study [4]. In addition, other studies have tried to investigate the potential of the JCFS as an energy source through direct combustion [15], gasification [11], bio-briquettes production [16], and pyrolysis processes [17]. However, various environmental and technical challenges have been encountered during the conversion of JCFS to the mentioned energy forms.

Direct combustion of 100% JCFS was impossible, even after spreading kerosene oil over it [15]. Direct combustion of JCFS was also characterized by flame front instabilities and short combustion periods due to its higher ash content [18]. Moreover, considerable indoor and outdoor air pollutants were emitted during the direct combustion of this biomass **[**19**].** The higher ash content of JCFS causes fusion at a temperature of above 750 °C during the gasification process [16]. Thus, JCFS was found to be unfitted for gasification when the temperature in the oxidation zone reached 900–1000 °C. At higher temperatures, the ash would react and form a slag in the combustion grate, which reduces the plant throughput during the combustion process [20]. Despite the fact that JCFS contains a high content of volatile solid (VS) that can facilitate the ignition in the pyrolysis process, the excess VS generates large amounts of polluting gases during the combustion process [21]. Singh *et al*. [16] showed that the ash left after bio-briquettes combustion could be used as potential fertilizer, but most of the essential nutrients were burned and emit into the air [9]. In contrast, JCFS digestate obtained after biological degradation was identified as a useful biofertilizer [22]. Therefore, anaerobic digestion may be the best alternative solution for waste stabilization, along with mitigating the above technical and environmental problems. However, a limited number of studies are reported on the biochemical methane potential (BMP) of JCFS. Adinurani *et al.* [23] estimated the methane yield of JCFS as 10.67 and 16 ml g^{-1} of TS under batch and semi-continuous reactors, respectively. While Dhanya *et al.* [24] showed that 1 kg of dry JCFS can produce 162.52 l of biogas; however, the estimated methane yields were significantly lower than other agro-industrial organic wastes [4,25].

The lower methane yields of JCFS could be due to the recalcitrant nature of lignocelluloses materials [14]. Visser *et al.* [26] estimated the cellulose, hemicellulose, and lignin contents of JCFS to be 26.5%, 14.5%, and 22.1%, respectively. The abundance of these polysaccharides and lignin in JCFS may act as a protective barrier that hinders anaerobic micro-organisms from accessing the carbohydrates [25]. Thus, proper pretreatment could disintegrate the complex cellulose-hemicellulose-lignin networks for enhancing the digestibility and methane yield of JCFS. The choice of the pretreatment method depends on the physicochemical properties of the material [27], its efficiency [25], environmental soundness [28], and economic advantage [29]. In fact, variations in methane yield and compositional change could also be perceived by pretreating the shell with different techniques since each method acts on different parts of the material [27]. Therefore, examining the effects of various pretreatment methods on a specific substrate is essential for a better understanding of the impact of single pretreatment on the particular type of material.

In the present study, mechanical, steam explosion (SE), and alkaline pretreatment were selected as the potential techniques for enhancing the methane yields of JCFS due to various positive characteristics. Mechanical and SE pretreatments are environmentally sound since the addition of external chemicals is not necessary [30]. Mechanical pretreatment, such as milling, can alter the internal ultrastructure of the biomass, increase the accessible surface area, reduce the degree of cellulose crystallinity, and decrease the degree of cellulose polymerization [31]. The non-catalyzed SE pretreatment is recognized as a low-cost option [30] and requires 70% less energy than physical pretreatments [32]. Heating the lignocellulosic material with a high temperature combined with a sudden pressure release results in a significant explosive decompression of lignocellulosic biomass [33]. Alkaline pretreatment is highly effective in lignin removal by cleaving the lignin-carbohydrate linkages with minimal carbohydrate degradation [2]. Unlike acid pretreatment, the chemical left after the alkaline pretreatment is useful since the next step (anaerobic digestion) requires an alkaline addition for controlling the pH drop due to volatile fatty acid (VFA) accumulation [34]. Therefore, the pretreatment techniques mentioned above are hypothesized to be suitable methods to enhance the methane yield of JCFS. However, their efficiency could be affected by various process conditions.

Mechanical pretreatments are mainly dependent on particle size, shape, and moisture content of the biomass [35], while SE pretreatment is profoundly affected by temperature and processing time [32]. Moreover, the efficiency of alkaline pretreatment is influenced by chemical dosage, incubation temperature, and retention time [4]. Thus, unoptimized pretreatment may lead to insufficient hydrolysis of lignocellulosic material or cause degradation of polysaccharides and lignin that have an inhibitory and toxic effect on anaerobic micro-organisms [36]. To the best of our knowledge, there are no studies that investigate and compare the effect of various pretreatment methods on the digestibility and methane yield of JCFS. Therefore, the present study aimed to examine the effect of mechanical, SE, and alkaline pretreatments at various processing conditions on the methane yield and biochemical composition of JCFS.

2 Materials and methods

2.1 Source of raw materials

The *J. curcas* fruits were harvested in November 2019 from Adami Tulu Agricultural Research Site, located in Oromia Region, Ethiopia (7°51′38″ N and 38°42′45″ E). At the time of harvesting, the fruits were matured with a yellowish colour. After harvesting, the fruits were immediately transported to Wondo Genet College of Forestry and Natural Resource, Ethiopia. The fruits were then sun-dried until their moisture content reduced to 50%. Finally, JCFSs were separated from the seeds manually and then further sun-dried for five days until its moisture content reaches 10% by weight bases [14]. The dried JCFSs were transferred into an airtight zipped plastic bag and then transported to the Norwegian University of Life Science, Aas, Norway, for pretreatment and BMP analysis. The microbial inoculum used in the BMP analysis was obtained from the batch anaerobic digester running using cow manure at a mesophilic temperature (38 °C). Then the inoculum was screened to remove large solid particles and impurities using a 2 mm sieve size and further incubated anaerobically at 37 °C for seven days to reduce the endogenous biogas production.

2.2 Mechanical pretreatment and particle size determination

The mechanical pretreatments (grinding) could increase the pore size and surface area of lignocellulosic biomass [37]. The crystallinity and degree of cellulose polymerization could also be significantly reduced after grinding the biomass into smaller particle sizes [38]. Therefore, in this study, JCFSs were crushed into a smaller particle size (denoted as ground-JCFS) using a coffee grinder (DeLonghi-KG 40). This type of grinder is called 'blade grinder' containing two stainless steel blades (radius =3.25 cm) configured in the opposite direction that works with power and spinning speeds of 170W and 50 Hz, respectively. On the other hand, the JCFS that partitioned into eight small equal parts using a regular scissor was designated as untreated-JCFS (Fig.1). More specifically, the chipped JCFS was added into a 0.35 l DeLonghi-KG 40 grinder, and the grinding was performed for 3 minutes. The milled biomass was then transferred into ISO stainless steel sieve with a mesh size of 1 mm and forced to pass through this sieve using a shaker (Edmund Buhler GmbH), and all ground JCFS was passed through the specified mesh size. The sieved biomass retained on the receiver was then transferred into airtight plastic bags for storing at room temperature until the actual particle size distribution, chemical analysis, and BMP tests were started.

Fig. 1 JCFS with different particle sizes: Ground-JCFS is with an average particle diameter of 581.9 µm, while the average particle size of untreated-JCFS is estimated to be 5000 µm

2.3 Particle size distribution analysis

Laser diffraction is a well-established technique used to measure particle size distributions across many industries, and its application in biomass sample has been assessed effectively [39-41]. Thus, this study utilized a Beckman Coulter laser diffraction (LS 13 320) for determining the particle size distribution of JCFS that passed through a sieve with a mesh size of 1 mm. The detection capacity of LS 13 320 is ranging between 0.4-2000 µm. The biomass sample was dispersed in Milli-Q® water; thus, this particle size distribution analysis method is called a wet method [41]. The opening to the measurement flow loop is 2 mm; thus, particles larger than 2000 µm never circulate and are never measured. The particle size distribution was then characterized by the median diameter (d50), mean diameter, d10, and d90. The average particle diameter estimated by the laser diffraction was used for assessing the effect of grinding on the methane yield of JCFS [42].

2.4 Alkaline pretreatment

The alkaline pretreatment was conducted using the optimum pretreatment condition identified in our previous report [4]. Briefly, the dried JCFS was chipped into a small size and then allowed to pass through a sieve with a mesh size of ≤ 5 mm. Then, 100 g of dry biomass was measured and transferred into one litter beaker containing 7.32% NaOH on the dry weight of JCFS. The samples were then diluted with tap water until obtaining 82% moisture contents [4]. After adding the water, the mixture was homogenized using a glass rod. Finally, the beakers were covered with plastic films and fastened with a plastic ring and then incubated at 36 °C for 54 hrs. The pretreated JCFS was immediately transferred into zipped plastic bags and stored in the freezer until the actual biogas production and compositional analysis started. For the control experiment, 100 g of JCFS with the same particle size was diluted with the same amount of water without alkaline and then incubated under room temperature for 54 hrs [43].

2.5 Steam explosion pretreatment

The JCFS was pretreated using the SE unit designed by Cambi AS (Asker, Norway) and situated at the Norwegian University of Life Science, Aas, Norway as described previously [44]. The moisture content and particle size of JCFS were kept constant until the actual SE pretreatment process started. Briefly, before adding the JCFS into the SE unit, the reactor was preheated to the desired temperature for 10 min [4]. Then without any size-reduction (as received from the field), 0.4 kg of dry JCFS was added into a 20-litre pressure vessel tank and pretreated by supplying steam from the electric steam boiler (Parat, Flekkefjord, Norway). Since no previous studies on SE of JCFS are available, the pretreatments were carried out at wider temperature ranges between 160 to 220 °C, using intervals of 20 °C, and each temperature was maintained for 5, 10, 15, and 20 mins**.** The supplied temperature during SE pretreatment was indirectly controlled by regulating the reactor's pressure using a manometer connected to the automatic valve [25]. After each SE pretreatment, the exploded biomasses were collected from the removable bucket, cooled down to room temperature, and then stored in airtight plastic bags at 4 °C until the compositional change and biogas production tests carried out. The supplied temperature and retention time during SE pretreatment were combined into a single vale (R_o), commonly referred as severity factor (SF), using Eq.1 [25].

$$\log(R_O) = \log\left\{t * exp\left[\frac{T-100}{14.75}\right]\right\} \quad (1)$$

Where, R_o is the reaction ordinate, t is the reaction time (in mins), T is the pretreatment temperature (°C), and 14.75 is the activation energy value under conditions where the process kinetics are first order, following the Arrhenius law.

2.6 Batch experiment to test biogas potential

The BMP of untreated and pretreated JCFS samples was tested in batch serum bottles with a total and working volume of 530 and 300 ml, respectively. The same volume of inoculum, i.e., 48.34 ml (5g VS l^{-1}) was added to all bottles. Then, excluding the bottles reserved for the control test, 3.34 g VS l^{-1} of untreated or pretreated JCFS was added into all bottles. The inoculum-to-substrate ratio (ISR) was maintained to be 1.5 (based on the VS bases) as suggested previously [45]. The control batch bottles that contained inoculum alone were used to correct the endogenous biogas production. Duplicated bottles were prepared for each treatment, and all bottles were flushed with pure nitrogen gas for 5 mins to maintain the anaerobic environment. Finally, all batch reactors were sealed with a septum and aluminum caps and incubated inside the shaker (Multitron Standard, Infors HT, Switzerland) at 37± 0.5 °C and continuously centrifuged at 90 rpm for 64 days until the daily biogas yield was ≤ 3% of the total biogas production. The methane yield increment (MYI) due to various pretreatment was estimated using Eq. (2) [4].

$$\mathrm{MYI}\ (\%) = \left(\frac{\mathrm{MYI}_{\mathrm{pretreated}} - \mathrm{MYI}_{\mathrm{untreated}}}{\mathrm{MYI}_{\mathrm{untreated}}}\right) * 100 \qquad (2)$$

2.7 Analytical method

2.7.1 Physicochemical and compositional analysis of JCFS

The dry matter (DM) and volatile solid (VS) contents of JCFS were determined based on the APHA standard method [46]. Briefly, the DM content of the biomass was determined by drying the samples at 105 °C until a constant weight was measured. The ash and VS contents were then determined after oxidizing the dried biomass at 550 °C for 2 hrs in a muffle furnace. The carbon, nitrogen, hydrogen, and oxygen contents of JCFS were taken from a previous study [47]. The caloric value of the dried JCFS was determined by Bomb Calorimeter (IKA® C 200) [4]. The cellulose, hemicellulose, extractive, and lignin content of the biomass were estimated using the thermogravimetric analysis (Netzsch STA 449F1) connected to a Bruker Tensor FTIR following the method described in Yang *et al.* [48]. Furthermore, the types of sugars from hemicellulose and their concentration were estimated using one-step fast acid hydrolysis [49]. Accordingly, 0.3 ± 0.01 g of milled dry samples were measured and hydrolyzed with 87 ml of 4% sulfuric acid at 121 °C for one hrs. The liquid was then drawn and centrifuged at 14600 rpm for 5 mins and pipetted into 500 ml polyethylene high-performance liquid chromatogram (HPLC) vials (Grace, Deerfield, IL). The samples were then stored at 4 °C until analysis of sugar content started. For soluble sugar content analysis, the individual treatment along with appropriate calibration standards (arabinose, galactose, glucose, mannose, and xylose) was run on a Waters Alliance HPLC system (Model e-2695, Waters Corporation, Milford, MA) employing an Aminex HPX-87H column (Bio-Rad Laboratories, Life Science Research, Hercules, CA). The samples were processed at an eluent of 5 mM sulfuric acid with a flow rate of 0.60 ml min^{-1} using a refractive index (RI) detector (Model 2414, Waters Corporation). The chromatograms were recorded and processed with Empower 1 2 software (Waters Corporation).

2.7.2 Biogas composition and calculation

The biogas produced from the individual batch reactor was regularly monitored by measuring the gas pressure in the reactor's headspace using a digital manometer (GMH 3161 Reisinger Electronic, Germany). While, the concentration of CO_2 and CH_4 in the biogas was measured using gas chromatography (3000 Micro GC, Agilent Technologies, USA), equipped with a thermal conductivity detector (TCD) [50]. More specifically, the gases were separated using two parallel capillary columns (MolSieve 5 Å PLOT, 10m × 0.32 mm ×12 µm, and PLOT Q, 10m × 0.32 mm × 10 µm) connected to the TCD by using helium as a carrier gas. The injector and column temperatures for MolSieve 5 Å PLOT capillary were maintained at 90 and 70 °C, respectively, while the PLOT Q column was operated at 50 and 45 °C, respectively. Before each biogas measurement, the GC was calibrated using a certified biogas standard consisting of 65% CH_4, and 35% CO_2. After the successive biogas measurements, the excessive pressure was released by inserting a needle into the rubber stopper to prevent over pressurization and solubility of CO_2. The measured overpressure, reactor's headspace volume, and normalized methane concentration were used as input variables during methane volume calculation [4]. All measured gas volumes were reported at 273 K and 101.3 kPa pressure. The endogenous methane produced from the control (inoculum) was deducted from the total methane yield, while the digestibility of the sampled biomass was determined based on the ratio of the cumulative methane yield to the theoretical methane yield [51]. The theoretical BMP of JCFS was estimated using Buswell's equation (Eq. 3) that relayed on the elemental composition (C, H, O, N) of the biomass as described previously [52]. At the same time, the biodegradability (Bd) of the JCFS was estimated using Eq. (4) [53].

$$\mathrm{TMP} = \frac{22.4 * (a/2 + b/8 - c/4 - 3d/8)}{12.017 * a + 1.0079 * b + 15.999 * c + 14.0067 * d} \tag{3}$$

$$\mathrm{Bd} = \left({}^{\mathrm{EMY}}\!/_{\mathrm{TMP}}\right) * 100 \tag{4}$$

Where, TMP and EMY are total methane potential and experimental methane yield (ml g^{-1}VS), respectivly; the coefficients a, b, c, and d are content of each element, equal to the ultimate analysis-based mass divided by the element molar mass: a = mass/C molar mass = mass/12.0107; b = mass/H molar mass = mass/1.0079; c= mass/O molar mass = mass/15.999; d = mass/N molar mass = mass/14.0067.

2.7.3 Kinetic analysis

The modified Gompertz equation (Eq. 5) was used to investigate methane production kinetics during batch fermentation [50]. For evaluating the accuracy of predictions, the coefficient of determination (R^2) and root mean square error (RMSE) were calculated.

$$Y = A_O \exp\left\{-exp\left[\frac{R_{max}e}{A_O}(\lambda - T) + 1\right]\right\} \tag{5}$$

Where: Y: cumulative methane production at a time t (ml g^{-1} VS); A_0: maximum methane yield (ml g^{-1} VS); R_m: maximum methane production rate (ml g^{-1} VS day^{-1}); λ: length of lag phase (day); *e:* Euler's constant (2.71828).

2.7.4 Statistical analysis

The significant effect of various pretreatment conditions on the biochemical composition and cumulative methane yield of JCFS was tested using either linear regression or one-way analysis of variance (ANOVA) via R software (version 3.6.2). All ANOVA was carried out using a Tukey's test. Thus, the mean differences were considered statistically significant if the p-value is ≤ 0.05.

3 Results and discussions

3.1 Composition of untreated JCFS

The JCFS used in the present study contains a considerable amount of dry matter (DM) and volatile solid (VS) contents (Table 1). The higher DM content could allow storage of the JCFS for longer times, while the higher VS content indicates the potential of JCFS for biogas production. The VS content obtained in the present study was comparable with the literature values estimated for similar biomass [16], wheat straw, and coconut shell [54]. The theoretical methane potential of JCFS calculated from its chemical constituents (Eq.3) was estimated to be 364.5 ml g^{-1} VS. However, this estimation may be slightly higher than the actual methane yield since 5-10% of the DM content would be converted to bacterial biomass during the anaerobic digestion process [25] The C/N ratio of the JCFS was comparable to the optimum values (20:1-30:1) suggested for stable anaerobic digestions processes [2]. The estimated calorific value was relatively lower than the energy content determined from JCPC [4]. The data displayed in Table 1 showed higher cellulose, hemicellulose, and lignin contents, which inquire a pretreatment work before anaerobic digestion is employed.

Table 1 Physicochemical constituents of untreated JCFS

Parameters [a]	Estimated values	References
DM (%)	88.58	This work
VS (%)	83.00	This work
Nitrogen (%)	1.63	[47]
Carbon (%)	43.37	[47]
Hydrogen (%)	5.19	[47]
Oxygen (%)	49.80	[47]
C:N ratio	26.60	This work
Cellulose (%)	29.83	This work
Hemicellulose (%)	13.95	This work
Lignin (%)	37.33	This work
Calorific value (kJ kg^{-1})	18.02	This work

3.2 Particle size distribution of milled JCFS

Fig. 2 shows the particle size distribution of milled JCFS after plotting the particle size (in µm) against corresponded volumetric fractions [55]. The estimated particle size was in between 0.4-1822 µm. According to the setup of the sieve analyzer used in the present study, the biomass should contain particle sizes of equal or less than 1000 µm. However, Fig. 2 showed that 20% of the total volume is measured from particles having more than 1000 µm, and the graph was a monomodal and skewed negatively into the left. The logical assumption is that the elongated particles have passed the sieve during the screening process [39]. This implies that the shape of the particles after grinding was not only spherical but could also contain an elongated cylindrical and circular structures. Likewise, the median diameter (d50) and mean diameter calculated from particle size distribution were estimated to be 433.6 and 581.9 µm, respectively. The d50 value tells us that 50% of the population was greater than 433.6 µm, and the remaining 50% was smaller than these values [41]. The other two parameters that characterize the particle size distribution were d10 and d90 [40], whose estimated values were 49.4 and 1410 µm, respectively. The d10 and d90 values represent the 10th and 90th percentile of the total particle's volume [40].

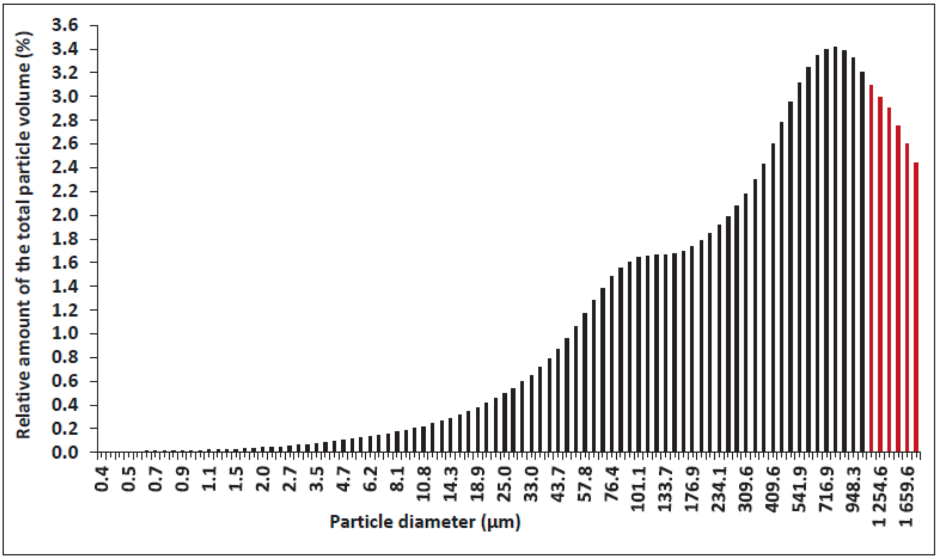

Fig. 2 Distribution of the relative volumes of various size classes of particles in milled JCFS (peaks highlighted with red color indicates the proportion of volume estimated from particles having more than 1000 µm diameter)

3.3 Effect of SE and alkaline pretreatments on JCFS's composition

3.3.1 Loss of biomass (VS)

The impact of alkaline and SE pretreatment on JCFS's chemical composition was analyzed as mechanical pretreatment does not significantly alter the chemical constituent of the biomass [56]. Moreover, all chemical compositional change due to pretreatment was examined after grinding the samples to tiny powders due to incomplete acid hydrolysis and combustion of coarse samples [49]. Each treatment was having the same particle size prior to chemical composition analysis; thus, it was impossible to investigate the effect of grinding on JCFS's chemical constituent.

The data presented in Table 2 showed that all pretreated samples exhibited lower DM content, which was in between 19.58-34.52%, less than that of the untreated JCFS (88.58%). Reduction in DM content was attributed to the direct steam injection into the material to attain the desired pressures and temperatures. Higher temperatures and longer pretreatment times needed more steam injection; therefore, more water was added into the samples. A least square regression model showed that a moderate negative correlation (R^2 = 0.20) between SF and DM content of JCFS. Similarly, the VS contents of exploded treatments ranged between 74.48 to 82.62% (on DM bases), which were relatively lower as compared with the untreated JCFS (83.00%). Excluding samples pretreated with NaOH, the least-square regression model showed a strong negative correlation (R^2 = 0.77) between SF and VS, and higher VS loss was noted at severe pretreatment conditions (Table 2). For instance, the biomass loss at lower pretreatment condition (160 °C, 5 min) was 3.25%; however, the loss progressively increased to 6.86% at the most severe pretreatment condition (220 °C, 20 min). Furthermore, JCFS was characterized by higher ash content over most agro-industrial biomass residues such as JCPC [4], miscanthus [57], and wheat straw [58], but comparable with sunflower cake [59] and rice straw [60]. The ash content of the untreated JCFS was 17%; however, pretreated JCFS showed higher ash content, which ranged between 17.32 to 25.52% (Table 2). The ash content of the alkaline pretreated JCFS was higher than all other treatments, and it was exceeded by 50.13% as compared with the untreated JCFS. The higher ash content of the pretreated sample could be associated with the loss of carbohydrate, and pseudo-lignin production during the pretreatment process.

Table 2 The physicochemical constituent of pretreated JCFS at various process conditions

Treatments (°C, mins)	SF	DM	VS	EXT	CEL	H.CEL	Lignin
		(% mass fraction of FM)	(% mass fraction of DM)				
160, 5	2.47	28.12	80.39	1.76	28.72	12.57	37.33
160, 10	2.77	27.71	80.73	1.69	29.80	11.60	37.65
160, 15	2.94	23.93	81.14	1.72	30.37	10.94	38.11
160, 20	3.07	25.18	80.31	1.68	28.18	11.26	39.19
180, 5	3.05	34.52	82.62	1.88	30.23	10.27	40.24
180, 10	3.36	31.29	80.17	1.63	30.68	9.19	38.66
180, 15	3.53	28.38	80.15	1.58	28.96	10.36	39.25
180, 20	3.66	24.37	79.54	1.64	29.07	10.43	38.40
200, 5	3.64	27.65	78.52	1.60	28.28	10.94	37.70
200, 10	3.94	31.15	78.07	1.66	27.51	10.07	38.83
200, 15	4.12	27.40	78.79	1.62	30.27	6.61	40.29
200, 20	4.25	23.03	78.63	1.74	30.09	6.15	40.64
220, 5	4.23	25.95	77.60	1.70	30.15	6.27	39.48
220, 10	4.53	27.12	77.85	0.14	31.44	5.57	40.71
220, 15	4.71	22.14	77.07	0.29	30.69	4.43	42.66
220, 20	4.83	19.59	77.67	0.69	32.03	3.79	41.16
Alkaline pretreated	-	19.58	74.48	2.68	23.57	16.18	32.06

SF: severity factor; DM: dry matter; VS: volatile solids; FM: fresh matter; EXT: extractives; CEL: cellulose and; H-CEL: hemicellulose.

The probable reason for VS loss during SE could be associated with the escaping of easy volatile compounds due to the degradation of hemicellulose [61]. Exploding the biomass above 195 °C mostly results in an exothermic degradation of sugars, which further promotes the loss of volatile compounds [25]. Besides SE, variation in purity among treatments [62] and the release of readily volatile compounds during conventional oven drying process [63] could also affect VS loss estimation. Moreover, some chemical reactions that occurred to attain the targeted temperature level were not considered during SF determination; thus, it would indirectly affect the VS calculation. Therefore, the use of Karl Fisher Titration for determining the DM content can solve the problem associated with the VS loss estimation [36], while condensing the volatile compounds released during the abrupt pressure drop of SE could significantly reduce the loss of

VS [25,3]. In contrast, the main reason presumed for VS loss during the alkaline pretreatment is lignin solubility and cellulose degradation, as clearly shown in Table 2, and the same result was reported in the previous study [64].

3.3.2 Cellulose, hemicellulose and lignin content

Table 1 showed the estimated carbohydrates and lignin content of untreated JCFS, while Table 2 presented the effect SE and alkaline pretreatments on the chemical composition of JCFS. The cellulose and hemicellulose content of the untreated JCFS was comparable with previous reports [20,16]; however, the cellulose content of steam-exploded JCFS was relatively higher over the untreated sample. The least-square regression model showed that a moderate positive correlation (R^2 = 0.22) between SF and cellulose contents. The increased cellulose content at severe pretreatment intensity mainly associated with the dissolution and degradation of hemicellulose [57]. In contrast, JCFS pretreated with NaOH showed significantly lower cellulose content as compared with the untreated and steam-exploded JCFS. The reduction of cellulose could be attributed to its dissolution by the thermochemical pretreatment processes. Previous studies also showed that cellulose dissolution was mainly due to excessive NaOH concentration rather than a higher temperature [57]. It should also be noted that at higher NaOH concentrations, cellulose could be degraded and result in significant loss of carbon. This justification further supported by this study since 11.43% of VS was lost after the alkaline pretreatment in comparison to the untreated JCFS (Table 2).

As expected, the effect of SE pretreatment on hemicellulose was higher than on cellulose and lignin. The hemicellulose content of steam-exploded JCFS has linearly declined with increasing of the SFs (R^2= 0.81). Significantly lower hemicellulose contents (≤ 6.61%) were recorded when the SFs were above 4.12 **(**Table 2). The steam generated from the boiler combined with acetic acid produced from the acetyl groups of the biomass can accelerate the hydrolysis of hemicelluloses, and then result in the release of easily biodegradable sugars. Xylose, mannose, galactose, glucose, and arabinose were the major hemicellulose's monomeric sugar (Fig. 3), which were comparable to the previous study [65]. The xylose, mannose, and galactose sugars were analyzed together since they have similar retention time during the HPLC detection [49]. However, xylose was the dominant sugar as for other most lignocellulosic biomasses. Moreover, the hydrolysis of hemicellulose mostly followed by defibrillation effects [25], which subsequently increased the availability of cellulose for microbial degradation. However, it should also be noted that severe pretreatment conditions would cause the degradation of hemicellulose [57]. As shown in Fig. 3, all monomer sugar contents from hemicellulose have declined with increasing of the SFs. The arabinose fraction was degraded entirely at the last two SFs. Contrastingly, the hemicellulose content of the alkaline pretreated JCFS increased by 28.96% over the untreated sample. The probable reason for higher hemicellulose content from NaOH pretreated sample could be associated with cellulose and lignin contents reduction since each component was quantified based on their mass fractions [50].

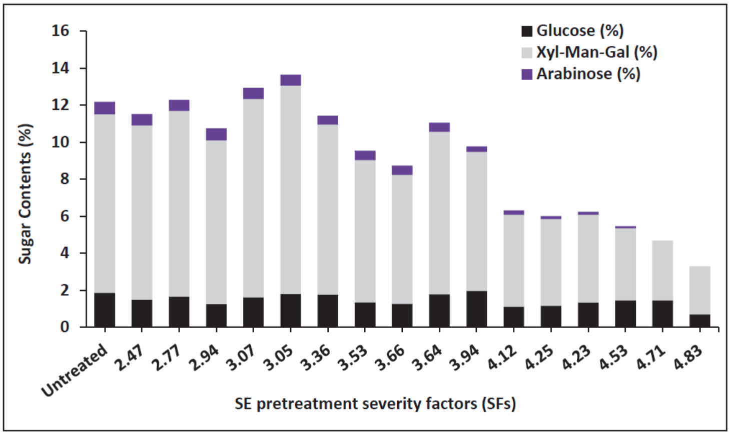

Fig. 3 Chemical composition of the untreated and steam-exploded JCFS; Xyl-Man-Gal denotes the xylose, mannose and galactose, respectively, estimated using HPLC

The lignin content of untreated JCFS was estimated to be **37.33%,** which was significantly higher as compared with other lignocellulosic materials such as wheat straw (17.4%), rice straw (13.3%), *Eucalyptus globulus* (25%), and sugarcane bagasse (22.1%) [30]. Thus, the higher lignin, cellulose, and hemicellulose contents have motivated to take a delignification measure before the anaerobic digestion process. As a result, mechanical, SE, and alkaline pretreatments were applied for disintegrating this lignocellulosic chain. The lignin content of JCFS was reduced by 16% after the alkaline pretreatment. However, samples after SE showed higher pseudo-lignin content over the untreated JCFS. Moreover, the linear regression model showed that a positive and moderate correlation (R^2 = 60) between lignin content and SFs. The lignin content increment could be attributed to the condensation and re-polymerization reaction between degraded compounds (e.g., furans), which increases the acid-insoluble lignin fraction called pseudo-lignin [61]. The higher lignin content could also be directly correlated with degradation and loss of hemicellulose and cellulose since lignin was estimated relative to the mass of carbohydrates [57].

3.3.3 Extractives

The extractives components obtained from the untreated and pretreated JCFS are presented in Table 2. These extractives components can be either non-structural and/or structural constituents [66]. The extractives derived from the untreated biomass were estimated to be 1.76%, while exploded JCFS contained relatively lower extractives than the untreated JCFS. These extractive values determined in the present study were significantly lower as compared with 42.3%, 42.5%, and 31.3% reported by Marasabessy *et al.* [67], Garcia *et al.* [65] and Martín *et al.* [68], respectively. The extractives mainly contain lipids, phenolic compounds, terpenoids, fatty acids, resin acids, steryl esters, sterol, and waxes [66]. The simpler chemical structures, along with smaller molecular weight, assist the extractive to be ready for biodegradation. However, the extractives generated from degraded products like phenolic and furan compounds may inhibit the anaerobic digestion process since they are very toxic. Furthermore, it should be noted that the condensation reactions between extractives and lignin causes formation pseudo-lignin, which could hinder the enzymatic hydrolysis of the pretreated materials.

3.4 Specific biogas and methane potential

3.4.1 Effect of particle size

The effect of particle size reduction on the BMP of JCFS is presented in Fig.4. The specific methane yield obtained from the untreated JCFS was estimated to be 200.53 ml g^{-1} VS, which was relatively lower as compared with other agro-industrial residues such as JCPC [4] and reed biomass [25], but higher over sugarcane bagasse [69] and wheat straw [58]. As shown in Fig. 4, mechanical pretreatment clearly influences the specific methane production potential of JCFS. The MYIs due to particle size reduction was estimated to be 74.23% as compared to untreated JCFS (Fig. 4b). The analysis of variation (ANOVA) with p-value of < 0.001 showed a significant methane yield variation between the two treatments. The variation was significantly higher after six days of anaerobic digestion. The higher methane yield (349.56 ml g^{-1} VS) was obtained when the average particle diameter of JCFS was reduced to 581.9 µm. The highest methane yield obtained in the present study was comparable with the literature values reported by Gunaseelan *et al.* [70]. Furthermore, the total methane yields obtained from ground-JCFS was equivalent to 95.90% of the theoretical methane yield of JCFS.

The digestion time needed to produce 80% of the total methane potential of biomass is defined as T_{80} [57], which is one of the crucial indicators to assess substrate biodegradability. It is noted that ground-JCFS needed only 25 days for producing more than 80% of the theoretical methane potential of JCFS due to the fast degradation rate (Table 3). In comparison, relatively longer digestion time (> 64 days) was needed for untreated-JCFS to attain more than 80% of the theoretical methane yield of JCFS. This indicates that the hydrolysis stage was essential for enhancing the BMP of the JCFS and the days needed for anaerobic digestion was shortened due to mechanical pretreatments. As shown in Table 3, mechanical (grinding) pretreatment has significantly improved the maximum rate of methane production. The higher R^2 values showed the strength of the model and closeness between measured and predicted methane values. Although a higher lag phase was noted from ground-JCFS, significantly maximum rate and higher methane yields were recorded than untreated-JCFS. Relatively similar R^2 and RMSE values were reported in the previous study [50].

Table 3 Parameters of the modified Gompertz model fitted using experimental data

Treatments	Measured yield (ml g^{-1} VS)	Calculated values from the model [a]				
		CH_4 yield (ml g^{-1} VS)	Max. rate (ml g^{-1} VS day^{-1})	lag time (days)	R^2	RMSE
Ground-JCFS	349.56	349.48	19.25	1.65	0.996	8.25
Untreated JCFS	200.55	200.25	8.67	0.00	0.992	5.64

[a] R^2 and RMSE were calculated from the average values of measured and model-predicted methane values over 64 days of anaerobic digestion, Max. is maximum.

As shown in Fig. 4a, both treatments produced a maximum specific methane yield within 6th to 11th digestion days. The lag time for grounded-JCFS was estimated to be 1.65 days and produced relatively similar methane yield until the 6th day of anaerobic digestion as compare to untreated-JCFS. However, the degradation rate from ground-JCFS was exponentially projected between the 6th to 11th days and sustained relatively to be higher until 25 days of digestion time. Furthermore, as shown in Fig. 4a, the degradation rate of both ground and untreated-JCFS has declined after 25 days of hydraulic retention time (HRT) due to the scarcity of feeding materials for micro-organism growth, and the becomes stable after 32 days of digestion time.

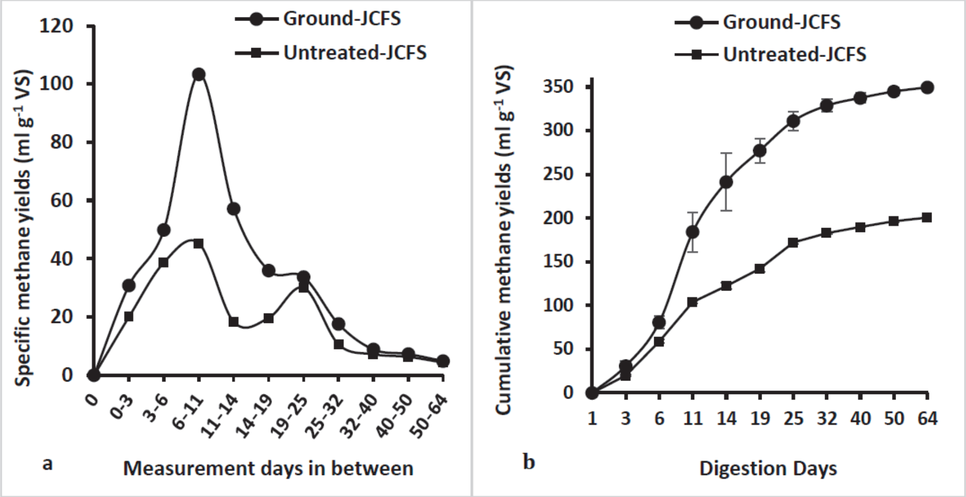

Fig. 4 The specific (a) and cumulative (b) methane yields obtained from ground and untreated JCFS

The possible reason for higher biogas and methane yields obtained from milled samples could be attributed to particle size and cellulose crystallinity reduction. As it is known, cellulose is hydrophilic, but less soluble in water due to its larger size and crystallinity. Moreover, the crystalline nature of cellulose increases its resistance to biological degradation. Thus, particle size reduction improves the cellulose exposure for microbial and enzyme degradation [2]. Besides, grinding could also alter the inherent ultrastructure of JCFS, which increases the accessible surface areas, reduce the degree of cellulose crystallinity and polymerization [31]; all processes then improved the methane yield of ground-JCFS as shown in Fig. 4b.

3.4.2 Effect of alkaline pretreatment

In this study, JCFS was pretreated with NaOH at the optimum pretreatment conditions identified for JCPC [4]. Accordingly, soaking the JCFS with 7.32% NaOH solution at 36 °C for 54 hrs resulted in 459.87 and 288.61 ml g^{-1} VS of biogas and methane yields, respectively. The alkaline pretreatment caused a significant dissolution of lignin (Table 2), which indicates the breakdown of complex lignocellulosic networks [57]; thus, higher methane yield was obtained after the alkaline pretreatment. The methane yield obtained after the alkaline pretreatment was increased by 43.90% over the untreated JCFS. However, the achieved methane yield was only 79.18% of the theoretical methane potential of JCFS. The time needed to attain T_{80} was more than 64 days, which proved that either the hydrolysis or methanogenesis stage was still a rate-limited process. Thus, the alkaline pretreatment was found to be less effective than the optimum conditions defined for mechanical and SE pretreatments (Table 4). Moreover, the data displayed in Table 4 showed that the untreated and alkaline-treated JCFS produced relatively lower methane yield over the untreated and pretreated JCPC, respectively.

The lower methane yield obtained after the alkaline pretreatment might be because of the unoptimized pretreatment process since we utilized variables defined for JCPC pretreatment [4]. However, the physicochemical constituents determined for JCPC and JCFS were slightly varied. Unlike the SE process, the hemicellulose content of alkaline-treated JCFS was increased by 15.95% over the untreated JCFS, while the cellulose content was significantly reduced after NaOH pretreatment (Section 3.2.2). It has also been reported that the cellulose content of biomass deteriorates when alkaline concentration exceeds a specific dosage [57], which leads to lower methane yields.

Table 4 Effect of various pretreatments on the methane yield of *Jatropha curcas* residues

Pretreatment type	*J. curcas* Residue[a]	Optimum pretreatment conditions	Methane yields (ml g^{-1} VS)	MYI (%)[b]	References
Mechanical	JCFS	581.9 μm	349.56	74.32	This work
Alkaline	JCFS	7.3 % NaOH, 35.9 °C, 54.1 hrs	288.6	43.9	This work
SE	JCFS	160 °C, 5 mins	310. 3	54.7	This work
SE	JCPC	201.5 °C, 9.4 mins	330.1	33.56	[4]
Alkaline	JCPC	7.3% NaOH, 36 °C, 54.05 hrs	353.9	40.23	[4]
Co-digestion	JCPC	2% CG with JCPC at 2g VS L^{-1} TOL	325.5	28.9	[4]
Untreated	JCFS	-	200. 5	-	This work
Untreated	JCPC	-	247.2	-	[4]

[a] JCFS: *Jatropha curcas* fruit shell; JCPC: *J. curcas* press cake; TOL: total organic loading; [b] MYI: methane yield increments due to the corresponding pretreatment conditions.

3.4.3 Effect of steam explosion

The biogas and methane yields obtained from steam-exploded JCFS found in between 294.51-555.39 and 179.49-310.32 ml g^{-1} VS, respectively. As shown in Fig. 5, the biogas and methane yields of JCFS have linearly decreased with increasing of the SFs. The pretreatments employed at the lower SFs (2.47-3.64) resulted in relatively higher cumulative biogas and methane yields compared with the untreated samples. The MYI determined at various pretreatment intensities varied between 1-54.75%. The higher MYI (54.75%) was obtained when JCFS exploded at 2.47 SF (160 °C, 5 min). However, the methane yields have significantly declined when the pretreatment intensities were getting more severe. In the last two severe pretreatment conditions (200 °C, 10 min, and 200 °C, 20 min), the methane yields were decreased by 2.63 and 11.72%, respectively, compared with the untreated JCFS. Moreover, the effect of SE pretreatment on methane yield enhancement was significantly lower than that of mechanical pretreatments. Briefly, all cumulative biogas and methane yield obtained from all steam-exploded JCFS were significantly lower as compared with the gas yields obtained after mechanical pretreatments.

The lower biogas/methane yield at severe pretreatment conditions might be due to cellulose, hemicellulose, and lignin degradation. The primary reaction during the SE pretreatment process is solubilization and disintegration of hemicellulose and lignin, respectively. Thus, the hemicellulose content was progressively reduced (Table 2 and Fig. 3), which could increase the accessibility of cellulose for microbial degradation. However, at severe pretreatment conditions, the hydrolytic reaction may also cause exothermic degradation of monomeric sugars. Degradation of carbohydrates mainly manifested by the formation of furfural and 5-hydroxymethylfurfural [33]. This justification further supported by Fig. 3, in which the acid-soluble sugar fractions from the hemicellulose were reduced with an increase of the SFs. Besides, the degradation of hemicellulose could be explained by the production of various organic acids, which could significantly reduce the pH of the digester [50]. At the severe pretreatment conditions, re-polymerization and condensation of degraded products could increase the pseudo-lignin contents (Table 2), which negatively affects the overall biogas production process [25]. Therefore, all degraded compounds and secondary lignin could inhibit methane production by adversely affecting the activity of anaerobic micro-organisms. Similarly, previous studies showed that lower biogas and methane yields with increasing pretreatment temperature and retention time during the SE pretreatment processes [44,61].

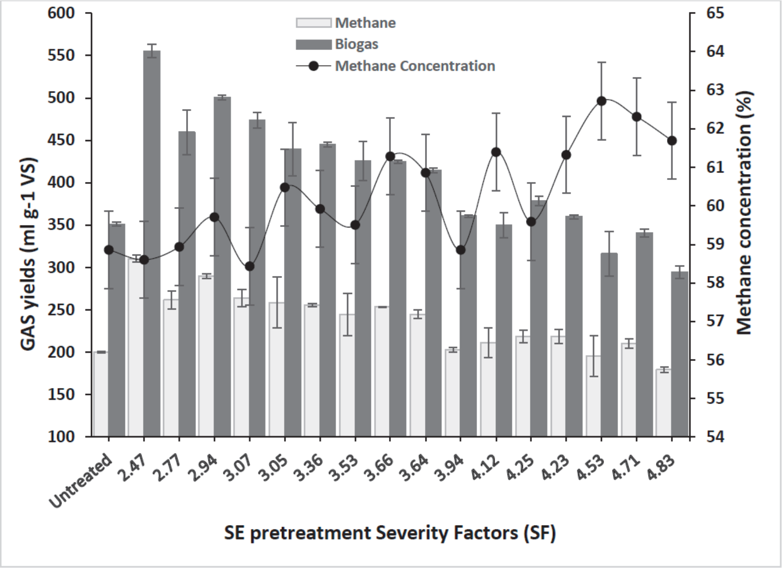

Fig. 5 Cumulative biogas and methane yields with standard deviations obtained from steam-exploded JCFS after 64 days of anaerobic digestion

Besides the cumulative methane yield, the average methane concentration was calculated for all treatments, and the values ranged between 58.43 to 62.72%. The ANOVA model with p-value < 0.002 indicates a significant variation in methane concentration among treatments upon changing pretreatment intensities. As shown in Fig. 5, the methane concentration increased with increasing of SFs. The probable reason for higher methane concentration at the severe pretreatment conditions could be associated with the lower pH due to the production of acids like acetic, formic, and levulinic acids from the acetyl group of the biomass at severe pretreatment conditions [71]. At a lower pH, CO_2 is highly soluble and could be changed into carbonic acid and bicarbonate ions (HCO^3) in the liquid phase [72], but the rate of methane solubility is significantly lower as compared with CO_2 [73]. This justification was further supported by a significant variation in the rate of CO_2 ($R^2 = 0.91$) and CH_4 ($R^2 = 0.86$) reduction as the same SF increments.

Conclusions

Considering the optimum conditions obtained in the present study, mechanical, SE, and alkaline pretreatment increased the methane yield of JCFS by 74.23%, 54.75%, and 43.92%, respectively compared with the untreated sample (200.55 ml g^{-1} VS). The mechanical pretreatment (grinding) was more effective for enhancing the methane yield of JCFS. Steam explosion pretreatment was efficient for hemicellulose solubilization, but also resulted in higher pseudo-lignin contents; thus, the methane yields were significantly declined at higher SFs. In contrast, the lignin content of JCFS was significantly reduced after the alkaline pretreatment. However, the methane yield obtained after the alkaline pretreatment was relatively lower than that of mechanical and SE pretreatments. Thus, mechanical pretreatment was found to be more effective for enhancing the methane yield of JCFS over SE and alkaline pretreatments. Compared with most lignocellulosic biomasses, the lignin content of JCFS is significantly higher; therefore, future research should focus on the investigation of other pretreatment methods that could simultaneously reduce the higher hemicellulose and lignin content of JCFS.

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Declaration: Not applicable

Conflicts of interest: The authors declare that they have no conflict of interest.

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Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no

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