Norwegian University of Life Sciences
Faculty of Science and Technology

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Source-separation and On-site Wastewater Treatment: A Combined Treatment and Resource Recovery Facility towards a Circular Economy

Kildeseparasjon og lokal behandling av avløpsvann: Et kombinert behandlings- og ressursgjenvinningsanlegg for en sirkulær økonomi

Melesse Eshetu Moges

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Foreword

This thesis is based on an Industrial PhD project sponsored by Ecomotive AS, and the Norwegian Research Council to develop a combined wastewater treatment and resource recovery facility. This PhD thesis work is one of the examples to demonstrate the importance of the University-Industry link for skill development and exploit the synergies of scientific and technological competences. A University-Industry link is a collaborative approach where both the university (Norwegian University of Life Sciences, NMBU), the company (Ecomotive AS) and the trainee (PhD candidate) are benefited. The university gains financial support from the company and networks where students carry out practical training and applied research. The company is benefited through developing its human resources equipped with applied scientific research and training. The company also enhances competitiveness by advancing its innovative technological developments. The trainee (industrial PhD candidate) gains basic and applied scientific know-how through education, innovative research and technology development from the university and professional expertise from the company and learn how to succeed and contribute for the advancement of the company in the business environment.

Moreover, the knowledge, relationships and experiences that the PhD candidates earn can help them boost their employability. As such this Industrial PhD research aimed to contribute to the scientific and industrial responses to avert the current and future challenges of sanitation, water, energy, and food insecurity by developing a more resource-oriented and sustainable way of managing municipal wastewater. Through collaborative scientific research work, a prototype combined treatment and resource recovery facility is developed. The results of this research and produced scientific documentation revealed a way towards the next generation of source-separating wastewater treatment systems. It will, therefore, strengthen the company's fulfilment of its objectives and help it to meet its future target for the development of a green economy based on decentralized, sustainable sanitary solutions. This prototype has also been used as a means of demonstration and teaching aid for students. Thus, the collaboration between academia and industry enhances knowledge sharing, financial support, and communication and contributes to the development of a win-win-win situation, so that results are maximized.

Melesse Eshetu

Summary

The world's water specialists agree to work on increased resource recovery from wastewater and reduced emissions to the environment. Source-separated wastewater treatment systems, which provide opportunities for local recycling and utilization of resources, are appropriate alternatives to conventional treatment of mixed wastewater in centralized treatment systems. This work includes the development of technical solutions for the treatment of source-separated blackwater, as well as studies of processes related to the treatment of black and grey wastewater from student housing inhabiting 48 students at the Norwegian University of Life Sciences (NMBU), Ås, Norway. Furthermore, the possibility of local resource utilization through biogas production and in the production of microalgae biomass based on nutrients recovered from wastewater is assessed. The main objectives of this work have been to i) develop a technical solution for a combined treatment and resource recovery in a closed cycle that creates opportunities for the circular economy, and ii) assess the efficiency of the various unit processes in terms of resource recovery and residual emissions.

In the first section, a compacted greywater treatment system with different post-treatment solutions was investigated (***Papers I and II***). Separation of blackwater alone resulted a reduction in organic matter (OM), total suspended solids (TSS), nitrogen (N) and phosphorus (P) of 64%, 75%, 85 and 88%, respectively. Treatment of the greywater by sludge separation and biological treatment in a porous medium with attached biofilm, and post-treatment by fine sand filtration (***Paper I***) resulted in low emission concentrations: <2 mg O_2 L^{-1} (BOD); <2 mg TSS L^{-1}; <0.1 mg P L^{-1} and <5 *Escherichia coli* (*E.coli*) 100 mL^{-1}. In a separate experiment, the post-treatment of the biofilter effluent with biochar and fine Filtralite ***(Paper II)*** also gave very low emission concentrations. The results showed that biological treatment plant in combination with post-treatment are appropriate solutions where the emission requirements are strict, especially in areas close to drinking water sources and where treated water can be used for watering salads and general irrigation of outdoor areas without restrictions. Separation of the blackwater greatly contributes to this.

The second section of this study focused on the development and evaluation of a combined treatment and resource recovery facility for source-separated blackwater. A hybrid reactor, which integrates an up-flow sludge blanket and the anaerobic baffled reactor was developed. The performance of this reactor was tested, as an integrated treatment system for source-separated blackwater. The effects of load and feed pulses on system performance were evaluated in terms of stability, effluent quality, the removal efficiency of organic and suspended particulate matter, biogas production and methane yield (***Paper III***). The results showed that the total COD removal efficiency stabilized above 78 % within less than 120 days. The configuration of the reactor provided sufficient sludge expansion volume, solid-liquid separation, and biogas production rate ranged

from 0.52 to 1.16 L d^{-1} (L reactor volume)$^{-1}$. At steady state, the methane concentration ranged from 67 - 82 % with an average conversion rate of 0.69±0.2 and 0.73±0.2 g CH_4-COD g^{-1}COD*in* for reactor I and reactor II, respectively.

Paper IV demonstrates a combined blackwater treatment and nutrient-recovery strategy for the recovery of a more dependable source of plant nutrients. The anaerobically treated blackwater effluent, rich in NH_4-N and PO_4-P, was treated in a sequence of upflow and downflow filtration columns using granular activated carbon, Cocos char and Polonite. The flow rate was set at 600 L m^{-2} day^{-1}. Filtration of the anaerobically treated effluent through activated carbon removed over 80% of the residual organic matter, more than 90% of suspended solids and turbidity while releasing more than 76% NH_4–N and 85% of PO_4–P in the liquid phase. The treatment train also removed total coliform bacteria (TCB) and *E. coli*, achieving concentrations below the detection limit after the integration of 11 W ultraviolet (UV) light. This integrated technological approach ensured simultaneous nutrient recovery as a nutrient solution, inactivation of indicator organisms, and reduction of organics. The treated and hygienized nutrient-rich water can be applied for various end-use options.

One of the challenges for the treated nutrient-rich water, if not used close to the area of treatment, is storage and transportation. In order to address this challenge and diversify the value-added byproducts and end-use options, a nutrient recovery study was carried out using microalgae (***Paper V***). A preliminary study revealed that the growth of *Chlorella sorokiniana* with 10% of the treated blackwater as a substrate in a continuous culture resulted in complete uptake of NH_4-N and PO_4-P. The N and P removal rate at steady state reached up to 99.2 mg NH_4-N L^{-1} d^{-1} and 8 mg PO_4-P L^{-1} d^{-1}, respectively, with a corresponding average biomass yield on the energy of 0.29 g (mole photons)$^{-1}$. The 10% treated blackwater substrate, however, had a low concentration of Mg and trace elements essential for microalgae growth. Low concentration of Mg was observed as the limiting factor for using diluted treated blackwater solely as a substrate. Therefore, the supplementation with Mg and trace elements was required. Moreover, the nitrogen in the treated effluent is mainly available as NH_4-N. A higher concentration of NH_4-N and its oxidation during substrate storage could increase the NO_2-N concentration, which inhibited the growth of *C. sorokiniana* at a concentration of above 50 mg L^{-1}.

In conclusion, results from this thesis demonstrated the value of domestic wastewater as a source of alternative nutrient-energy-water resources. The development of an integrated treatment and resource recovery facility for a source-separated sanitation system could provide a healthy local environment, social and economic payback for households and communities, and contribute to green development and food security.

Sammendrag

Verdens vannspesialister er enige om å arbeide med økt ressursutvinning fra avløpsvann og reduserte utslippene til miljøet. Kildeseparerte avløpsbehandlingssystemer, som gir muligheter for lokal resirkulering og ressursbruk, er et aktuell alternativ til konvensjonell behandling av blandet avløpsvann i sentraliserte behandlingssystemer. Målet med denne undersøkelsen var å undersøke og utvikle en ny og bærekraftig tilnærming der ressursene i avløpsvann behandles, gjenvinnes og gjenbrukes i områder nær opprinnelseskilden. Dette arbeidet omfatter utvikling av tekniske løsninger for behandling av kildeseparert svartvann, samt studier av prosesser knyttet til behandling av svart og grått avløpsvann fra studentboliger med 48 studenter ved Norges Miljø- og Biovitenskaplige Universitet, (NMBU) Ås, Norge. Videre vurderes muligheten for lokal ressursutnyttelse gjennom biogassproduksjon og i produksjon av mikroalgenbiomasse basert på næringsstoffer som gjenvinnes fra avløpsvann. Hovedmålene med dette arbeidet har vært å: i) utvikle en teknisk løsning for kombinert behandling og ressursutvinning i en lukket syklus som skaper muligheter for den sirkulære økonomien; og ii) vurdere effektiviteten av de ulike enhetsprosessene med hensyn til ressursutvinning og gjenværende utslipp.

I den første del ble et kompakt renseanelegg for rensing av gråvann med ulike etterpoleringsløsninger, ble undersøkt (**Artikkel 1 og 2**). Utsortering av svartvann representerte alene en reduksjon i organisk materiale (OM), totalt suspendert stoff (TSS), nitrogen (N) og fosfor (P) på henholdsvis 64%, 75%, 85 og 88%. Behandling av gråvannet ved slamavskilling og biologisk behandling ved umettet filtrering i porøst medium med fastsittende biofilm, samt etterpolering ved filtrering i finsand (**Artikkel 1**), resulterte i lave utslippskonsentrasjoner: <2mg O_2L^{-1} (BOD); <2mgSS L^{-1}; <0,1mgP L^{-1} og <5 *Escherichia coli* (*E.coli*)100mL^{-1}. Den andre alternative etterpoleringsløsninger ble studert ved filtrering i kolonner fylt med biokull og Filtralite (**Artikkel 2**). Disse metodene ga også svært lave utslippskonsentrasjoner. Dette viser at biologiske gråvannsrenseanlegg i kombinasjon med etterpolering er en aktuell løsning der utslippskravene er strenge, blant annet i nedbørfeltet til drikkevannskilder og at renset vann kan brukes til vanning av salat og til generell vanning av utearealer, uten restriksjoner. Separering av svartvannet bidrar i stor grad til å oppnå dette.

Den andre delen av denne avhandlingen er fokusert på evaluering av et kombinert behandlings- og ressursutvinningsanlegg for kildeseparert svartvann. En hybridreaktor, bestående av et oppstrøms slamteppe og en anaerob baffel reaktor for behandling av kildeseparert svartvann ble evaluert. Effektene i reaktoren ble undersøkt med forskjellig tilførsel (feed pulses) i termer av effektstabilitet, utløpskvaliteter, fjerning av organisk- og partikulært materiale, biogass produksjon og metan utbytte, variasjoner i svartvannet tatt i betrakting (**artikkel III**). Resultatene viste at total COD-fjerning stabiliserte seg over 78%

innen mindre enn 120 dager. Reaktorens konfigurasjon viste seg å gi tilstrekkelig slamlager, væske/faststoff- separasjon og biogass produksjonen som varierte fra 0,52 til 1,16 L d^{-1} (L reaktorvolum)$^{-1}$. Ved steady-state varierte metankonsentrasjonen fra 67 - 82% med en gjennomsnittlig konverteringsrate på 0,69 ± 0,2 og 0,73 ± 0,2 g CH_4-COD g^{-1} CODin for reaktor I og reaktor II.

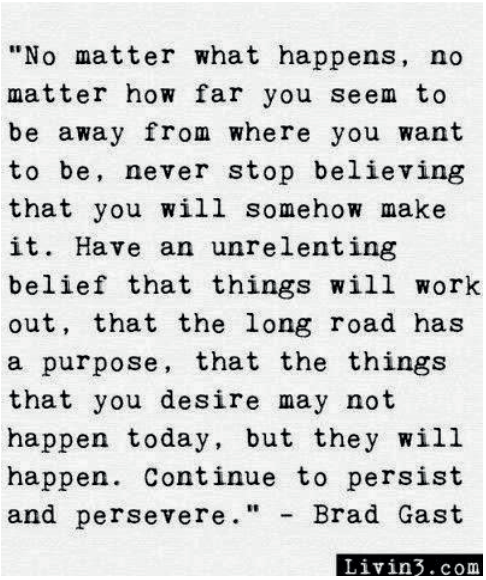
Artikkel IV Viser en kombinert strategi for behandling og gjenvinning av næringsstoffer i svartvann som en kilde til plantetilgjengelige næringsstoffer. Anaerobt behandlet svartvann, rikt på NH_4-N og PO_4-P, ble behandlet i kolonner i en sekvensiell oppstrømnings- og nedstrøm filtrering gjennom granulert aktiv kull, Cocos kull og Polonite. Hydraulisk overflatebelasting ble satt til 600 L m^{-2} d^{-1}. Filtrering gjennom aktivt kull fjernet over 80% av det gjenværende organisk materialet, mer enn 90% suspendert faststoff og turbiditet, og frigjorde over 76% NH_4-N og 85% PO_4-P i væskefasen. Behandlingen fjernet også TCB og *E. coli*, og oppnådde konsentrasjoner under deteksjonsgrensen etter at en 11 W ultrafiolett lampe (UVC) ble tilført som en integrert del av filtersystemet. Samlet fører denne tilnærmingen til næringsstoffgjenvinning som en næringsløsning, inaktivering av patogener og reduksjon av organiske stoffer. Det behandlede, hygieniserte - og næringsrike vannet kan brukes til ulike formål.

En utfordring for det behandlede næringsrike vannet, er lagring og transport. For å løse denne utfordringen og utvide mulighetene for gjenbruk av næringsstoffene ble det utført en studie ved hjelp av mikroalger (**artikkel V**). En foreløpig studie viste at veksten av *Chlorella sorokiniana* med 10% av behandlet svartvann som substrat i en kontinuerlig kultur resulterte i fullstendig opptak av NH_4-N og PO_4-P. Oppnådd N og P-fjerningen ved steady state var 99.2 mg NH_4-N L^{-1} d^{-1} og 8 mg PO_4-P L^{-1} d^{-1} med et tilsvarende gjennomsnittlig biomasseutbytte på 0.29 g (mol fotoner)$^{-1}$. Behandlet svartvann hadde imidlertid en lav konsentrasjon av Mg og sporstoffer som er viktig for mikroalgeveksten. Dette ble observert som en begrensningsfaktor ved å bruke det behandlede svartvannet som eneste vekstsubstratet. Derfor var det nødvendig å supplere substratet med Mg og sporstoffer. Dessuten er nitrogenet i det behandlede svartvannet hovedsakelig i form av NH_4-N. En høy konsentrasjon av NH_4-N og dets oksidasjon ved lagring vil kunne øke andelen av NO_2-N, som ble observert å hemme veksten av *Chlorella sorokiniana* når konsentrasjonen kom over 50 mg L^{-1}.

Som konklusjon viste resultatene fra denne avhandlingen verdiene i avløpsvann som en kilde til alternative NEV ressurser (næringsstoff, energi og vann). Utviklingen av et integrert behandlings- og ressursutvinningsanlegg basert på kildeseparert avløpsvann vil kunne bidra til et sunt lokalt miljø, sosial og økonomisk tilbakebetaling for husholdninger og lokalsamfunn, og bidra til å oppnå en grønn utvikling og matsikkerhet.

Dedication

This thesis is dedicated to those who are suffering from chronic pain 24/7. Living with pain is a big challenge. But, if you **Use** your talents, resources, dreams, and visions as your daily positive energies, you will **Learn** how to live with chronic pain. You may not be free from the pain, but you will succeed and achieve your dream one day.



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First of all, I would like to thank the Almighty God who gave me a second life. With a little Faith, He gave me a lot of Love and Hope. Faith, Love and Hope are the three pillars of my Life.

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pain we have every day for the last 17 years, Every Step of The Way was astonishing. This has to do with the blessing and love we got from God who gave us the strength and the wisdom of learning how to live with chronic pain. Yes, if LOVE, HOPE and FAITH surround you, chronic pain cannot stop you from using your resources. Thanks also to our kids Natnael and Mehiretab who gave me the energy when I am weak, make everything and every moment in my life shine, and for their continuous support and prayers and love; my life has no meaning without you since you are the largest part of it. Thank you, and I love you all very much.

Melesse Eshetu Moges

Dec. 2018, ÅS, Norway.

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Appended Papers

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List of Acronyms

AD	Anaerobic digestion
ANOVA	Analysis of variance
ARM	Antimicrobial-resistant microorganisms
BOD	Biochemical oxygen demand
BPR	Biomass production rate
BW	Blackwater
COD	Chemical oxygen demand
CODf	Filtered chemical oxygen demand
CODt	Total chemical oxygen demand
DBM	Dry biomass
E. coli	*Escherichia coli*
GWT	Greywater treatment
GWTP	Greywater treatment plant
HLR	Hydraulic loading rate
HRT	Hydraulic retention time
IE	Inhabitant equivalent
MPN	Most probable number
MWWTP	Municipal wastewater treatment plant
N	Nitrogen
NH_4-N	Ammonium nitrogen
NIBIO	Norwegian Institute of Bio-Economy Research
NIVA	Norwegian Institute for Water Research
NMBU	Norwegian University of Life Sciences
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
OD	Optical density
OLR	Organic loading rate
OSGWTP	On-site greywater treatment plant
P	Phosphorus
PBRs	Photobioreactors

PLC	Programmable logic controller
PO4-P	Phosphate (Orthophosphate)
P tot	Total phosphorus
PVC	Polyvinyl chloride
R I	Reactor I
R II	Reactor II
TCB	Total coliform bacteria
TSS	Total suspended solids
SRT	Solid retention time
STP	Standard temperature and pressure
UASB	Upflow anaerobic sludge blanket
UV	Ultraviolet
VFA	Volatile fatty acid
WWTP	Wastewater treatment plant (conventional)

List of publications

This thesis is based on the following five appended papers. The published papers are reproduced with permission from the publishers.

Paper I

Moges, M.E., Todt, D., Eregno, F.E. and Heistad, A., 2017. *Performance study of a bio-filter system for on-site greywater treatment at cottages and small households***.** *Ecological Engineering*, 105: 118-124. https://doi.org/10.1016/j.ecoleng.2017.04.060

Paper II

Moges, M.E., Eregno, F.E. and Heistad, A., 2015. *Performance of biochar and filtralite as a polishing step for the on-site greywater treatment plant*. *Management of Environmental Quality: An International Journal*, 26(4): 607-625. https://doi.org/10.1108/MEQ-07-2014-0101

Paper III

Moges, M. E., Todt, D., Janka, E., Heistad, A., & Bakke, R., 2018. *Sludge blanket anaerobic baffled reactor for source-separated blackwater treatment***.** *Water Science and Technology*. https://doi.org/10.2166/wst.2018.411

Paper IV

Eshetu Moges, M., Todt, D.; Heistad, A. 2018. *Treatment of Source-Separated Blackwater: A Decentralized Strategy for Nutrient Recovery towards a Circular Economy. Water*, 10(4), 463. https://doi.org/10.3390/w10040463

Paper V

Melesse Eshetu Moges, Arve Heistad and Thorsten Heidorn. *Optimization of Nutrient Recovery from anaerobically treated blackwater and improving effluent quality through Microalgae biomass production.* (*Manuscript in preparation*)

Related Publication but not in the PhD thesis

Eregno, F.E., **Moges, M.E**. and Heistad, A., 2017. *Treated greywater reuse for hydroponic lettuce production in a green wall system: Quantitative health risk assessment*. Water, 9(7), 454. https://doi.org/10.3390/w9070454

1. Background and Research Motivation

Major challenges facing the modern society in the twenty-first century are related to water quantity and/or water quality issues, mainly caused by population growth, industrialization, intensive food production practices, increased living standards, climate change and poor water use strategies. In 1950 the total world population was 2.6 billion, with 750 million living in urban centres and 1.8 billion in rural areas (Schnitzler 2013). The global urban population has skyrocketed from 29 % (750 million) of the total population in 1950 to 55 % (4.2 billion) today (United Nations 2018). By 2050 the world population is projected to be 9.2 billion inhabitants with 6.3 billion, i.e. 68 % of the total population, living in cities and only 2.9 billion remaining in the countryside (United Nations 2018) (Fig. 1). This trend in urban population means the global demand for food and feed, water and energy would continue to grow.

Feeding a world population of 9.2 billion people in 2050 would require raising overall food production. The agricultural and energy productions are, therefore, expected to increase by roughly 60% and 80%, respectively in 2025 (Alexandratos and Bruinsma 2012, OECD 2012). Although agriculture remains as the largest overall water user, it is becoming obvious that water demand for industrial use and energy generation is increasing considerably and much faster (UN-Water 2018). The expansion of municipal water supply and sanitation systems also contribute to the rising demand. Spatial and temporal variations of water cycle dynamics could also arise from climate change, which aggravates the inconsistencies between water supply and demand (WWAP 2017).

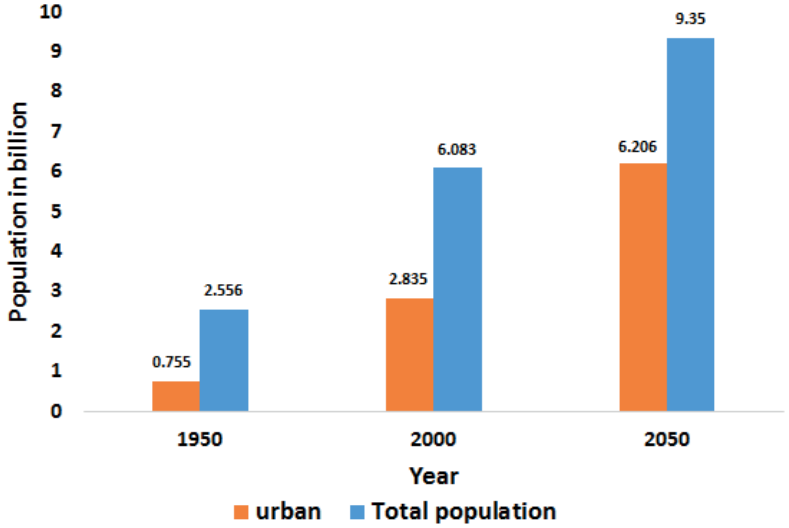

Figure 1. Urban and total population (Source: World Urbanization Prospects: The 2014 Revision (UNPD 2014).

The Environmental Outlook baseline scenario projected future global water demand to increase by 55% from about 3 500 km^3 in 2000 to nearly 5 500 km^3 in 2050 (OECD 2012). Figure 2 presents the water demand in 2000 and the projected water demand in 2050 for domestic, manufacturing, thermal energy generation, and irrigation. The major increase in water demand accounts mainly for manufacturing (+400%) followed by for energy generation (+140%), and for domestic water use (+120%). Water demand for irrigation purpose is projected to be less (OECD 2012).

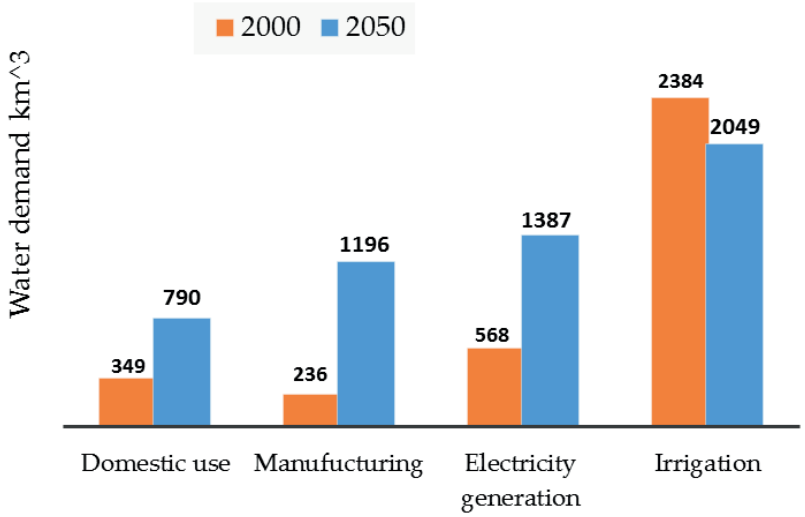

Figure 2. Global water demand for domestic use, manufacturing and electricity generation and irrigation: Baseline, 2000 and 2050 (Adopted from OECD (OECD 2012)).

At the same time, wastewater generation from the different sectors is going to grow proportionally. Out of the estimated current global freshwater withdrawals of 3,928 km³ per year, 56% (2,212 km³ per year) is released into the environment as wastewater in the form of municipal and industrial effluent and agricultural drainage water (WWAP 2017). With the current trend and projection of urban population growth, wastewater management and disposal in cities will continue to be a major social, environmental and economic challenge. Wastewater management of the growing urban and periurban population requires the development of new and long wastewater discharge networks and wastewater treatment facilities. Development of such new sewer systems is, however, difficult to realize in most parts of the world especially in those regions where the major urban population growth occurs.

1.1. Challenges with the present wastewater management approach

Most modern cities established centralized sewer systems with a network of collection pipes for transporting domestic wastewater to a municipal wastewater treatment plant, which greatly improved public health and environmental

quality (Wilderer and Schreff 2000, Larsen et al. 2016). However, these systems require enormous volumes of freshwater to transport the small volume of human excreta from the toilet to the wastewater treatment plant (Langergraber and Muellegger 2005, Larsen et al. 2016, McConville et al. 2017), it also strongly depends on high investment costs, and stable institutions, as well as long planning horizons and extensive use of resources (Larsen et al. 2016).

Today, wastewater management faces emerging concerns like energy efficiency, resource recovery, greenhouse gases emission, and stringent effluent standard requirements. At the wastewater treatment plant, a lot of energy, mainly for aeration, is needed to remove organic compounds and nutrients. However, these organic compounds in wastewater represent a potential energy source and the nutrients as valuable fertilizers (Guest et al. 2009, Verstraete et al. 2009). Moreover, the nutrients from the toilet are highly diluted by wastewater from other sources such as stormwater and by groundwater intrusion. Hence, high levels of energy and large amounts of chemicals are needed for processing to recover these resources. Furthermore, with long-distance transportation, significant amounts of nutrients are also lost before reaching the treatment plant through leakages and overflow resulting in environmental pollution.

Concerns are also growing in water-scarce areas that continued dependence solely on centralized sewer systems may not be optimal for sustainable water resource management (Gikas and Tchobanoglous 2009). Unless different scenarios for wastewater management is devised this current linear model of resource flow that follows a 'take-use-dispose' pattern (Korhonen et al. 2018) and end-of-pipe treatment may not be ideal for serving the 6.3 billion urban inhabitants in 2050, living in an increasingly resource and energy constrained world. The increased discharges of inadequately treated or untreated wastewater (which is the case in most parts of the world) contribute to contamination of surface waters, groundwater, and water in coastal areas (WWAP 2017). The contamination of freshwater and coastal ecosystems, threaten food security,

access to safe drinking and bathing water, and providing major health and environmental management challenges (Corcoran 2010). In conventional wastewater collection and transportation systems, micro-pollutants are highly diluted and present in the ng/L range (Schwarzenbach et al. 2006). Thus, the current wastewater treatment systems cannot remove these small fractions of micropollutants and have become the main concern in the aquatic environment (Joss et al. 2006, Schwarzenbach et al. 2006). Although the risks that these micropollutants may pose are not well assessed and understood (Schwarzenbach et al. 2006, Schirmer and Schirmer 2008), few studies have reported feminization of fishes at ng/L levels (Gibson et al. 2005), impacts on developmental processes and reproduction (Arnnok et al. 2017).

Recent studies have also shown an increasing trend of consumption of pharmaceuticals. From 2000 to 2015 the global antibiotic consumption, increased by 65%, from 21.1 to 34.8 billion defined daily doses (DDDs) with the major increase coming from the low and middle-income countries (Klein et al. 2018). This has a direct relation to the growing concern of antimicrobial-resistant microorganisms. Although antimicrobial resistance develops naturally over time, usually through genetic changes, the misuse and overuse of antimicrobials are accelerating this process. In many places, antibiotics are overused and misused in people and animals mainly as growth promoters in animals or used to prevent diseases in healthy animals. Wastewater effluents are, therefore, the main point sources of emission of these pollutants in recipient water bodies.

These aspects of global change and challenges justify the need for swift planning and execution of strategic, reasonable and effective management and countermeasures against deteriorating water security (Burek et al. 2016). To offset the rising challenges to water, food and energy security from the increasing population growth, wastewater pollution and climate change, in one hand and protecting the ecosystem, on the other hand, it is vital to work on closing the resource loops in cities with innovative solutions or advanced technologies. Thus,

as the world continues to urbanize, successful management of urban growth in all the social, economic, and environment is crucial for sustainable development. This management should take into account the wastewater issue in the water-food-energy nexus and utilize new opportunities for transforming the resources in wastewater (mainly water, nutrients, organic matter and energy) into valuable assets.

1.2. New perspectives for domestic wastewater

In today's practice, cities and households are considered as net consumers of mainly water, nutrients, and energy. Increased demand for water, energy and food by the growing population coupled with the necessity for a simultaneous reduction of the environmental impact of wastewater has increased the need for innovative solutions such that communities and households can be considered as production units of nutrients, energy and water. From a global perspective, nearly 20% of the manufactured nitrogen and phosphorous end up in the domestic wastewater (Batstone et al. 2015, Matassa et al. 2015). Moreover, micropollutants such as pharmaceutical residues, hormones, personal care products and household cleaning chemicals, which are considered as emerging water quality concerns also, end up in the same wastewater stream.

About 80 % of the nutrients, up to 70 % of the organic matter, and most of the pathogens in domestic wastewater, however, comes from a small fraction (~1%) of the wastewater stream, which is human urine and faeces, hereafter called blackwater (Langergraber and Muellegger 2005, Kujawa-Roeleveld and Zeeman 2006, Vinneras et al. 2006, Todt et al. 2015). Besides, about 70% of the pharmaceuticals including antibiotics and their metabolites and almost all hormones and endocrine disrupting compounds are excreted through urine and the rest in faeces (Kapusta 2007). Therefore, the concentrations of these micropollutants in blackwater may range from μg/L to mg/L (De Mes et al. 2007, Butkovskyi et al. 2015). Most personal care products and household cleaning

agents, on the other hand, constitute the greywater fraction (Hernández Leal et al. 2010, Butkovskyi et al. 2016). Recent studies halve also shown the presence of some pharmaceutical residues in greywater (Butkovskyi et al. 2015). Thus, the blackwater fraction of domestic wastewater is the major sources of organic matter, nutrients, pathogens, and micropollutants (Kujawa-Roeleveld and Zeeman 2006, de Wilt et al. 2016). Separating and concentrating this domestic wastewater stream in a small volume is one of the management approach in the new perspective of domestic wastewater treatment. This allows separate recovery of useful resources and effective removal of harmful pollutants.

The concept of seeing wastewater as a resource (Otterpohl et al. 2002, Larsen et al. 2009, Otterpohl and Buzie 2011, Zeeman and Kujawa-Roeleveld 2011, Leal Lucía et al. 2017) and introducing a closed-loop system in wastewater management (Winkler 2011, Vasconcelos Fernandes et al. 2015, Davis et al. 2016) will help in protecting water bodies from eutrophication and pollution, ensuring long-term food security and shifting to a circular economy. This represents compelling objectives for water-, energy- and nutrient-management strategies (Zoboli et al. 2016). In this regard, domestic wastewater could be a key platform towards closing the urban resource loop and contributing to green development by improving both economic and environmental goals simultaneously (Winkler 2011).

With the notion of circular resource flow and proper management, domestic wastewater could be a potentially affordable and sustainable source of water, energy, nutrients, organic matter and other useful by-products. There exist, therefore, opportunities from domestic wastewater that could be exploited for green development, social well-being and ecological health (Corcoran 2010). Effective management of wastewater as an alternative source of water for different end-use options is essential for future water security. Moreover, recycling nutrients or extracting energy from wastewater can also bring in new opportunities for income generation and expand the resource base available to

poor households (Winblad and Simpson-Hebert 2004) at the same time reducing the negative impact on the urban ecosystem.

1.3. Composition of domestic wastewater

Wastewater generated from households consists of various fractions each with specific characteristics with respect to volume and patterns of flow, composition and concentration of flow. Wastewater contains approximately 99.9% water, and the rest 0.1% is organics and inorganics in suspended and dissolved solids form (Von Sperling 2007). This 0.1% is responsible for water pollution and the main reason for the need for treatments. Understanding the composition and volume of the different streams is important for the design of the treatment system.

The wastewater streams can be grouped into blackwater (originating from the toilet and contain flushing water, faeces, toilet paper and urine), and grey water (originating from kitchen, showers and/or bath, laundry, etc.). These wastewater fractions seem to vary considerably between different locations both in terms of volume and compositions. Table 1 presents the differences in household water use and total wastewater production per capita per day in different countries.

Greywater represents up to 70% of the total water consumed in a household (Otterpohl et al. 2003, Kujawa-Roeleveld and Zeeman 2006) while most of the rest is used up for toilet flushing and released as blackwater. Treatment and reuse of greywater close to the source will have a significant role in reducing the overall water consumption (Friedler 2004). The quantitative and qualitative characteristics of household greywater seem to vary significantly depending on residents' habits, the quality and quantity of water supply, the activities in the household, the number and the age distribution of household members, lifestyles, and water use pattern and the climate of the area (Eriksson 2002, Hernandez et al. 2007, Eriksson et al. 2009, Donner et al. 2010).

Table 1. Wastewater generated from different household uses (L/p/d)

Use	Norway [a]	Denmark [b]	Germany [c]	The Netherlands [d]	Greece [e]
Hand basin				5.3	8.6
Bath/shower		43	48.3	51.1	33.9
Kitchen/food preparation		25	5.4	9.3	12.2
Laundry		17	16.1	15.4	21.3
Dish washer, cleaning, other		15.7	16.1	7	6.6
Total greywater	123.9	85	84.9	86	82.6
Toilet flushing	4.8*	27	36.8	34.6	59.4
Total	128.7	119	121.7	119.2	142

*Kaja student dormitories (vacuum toilet) [a](Todt et al. 2015); [b] (Revitt et al., 2011); [c] (BMU/UBA 2018); [d] (van Thiel 2017); [e] (Antonopoulou et al. 2013).

Although conceived to be less contaminated, greywater constitute up to 55% of the daily organic load of the municipal sewage, contain significant concentrations of detergents and salts (i.e. boron, sodium and chlorides) (Rose et al. 1991), personal care products (PCP) (Eriksson et al. 2003, Hernández Leal et al. 2010, Butkovskyi et al. 2014), some pharmaceutical residues (Butkovskyi et al. 2015) and faecal coliforms of about 10^4-10^8 CFU/100 ml (Eriksson 2002, Ottoson and Stenström 2003). Greywater may pose health risks and exhibit negative environmental and aesthetic effects. Thus, the design and development of a greywater treatment facility should take all the above factors into considerations to reduce the negative impacts on human health and the environment.

Similarly, blackwater which constitute from about 4% (with vacuum toilets) to 40% (conventional toilets) of the total volume of household wastewater, contain

up to 70% of the organic material in domestic wastewater and 80 to 92% the nutrients mainly nitrogen, phosphorus and potassium and pathogens (Zeeman 2012, Todt et al. 2015) and micropollutants (de Graaff et al. 2011b, Butkovskyi et al. 2015).

1.3.1. Source-separation – as a basis for resource recovery and pollution control

Recent concerns over environmental sustainability and the need for resource recovery have encouraged the promising development of the concepts of Ecological sanitation (Jenssen et al. 2003, Otterpohl 2003, Langergraber and Muellegger 2005) and Resource-oriented or New sanitation (Zeeman and Kujawa-Roeleveld 2011, Tervahauta et al. 2013). This approach sees domestic wastewater no more as a waste to be treated and disposed but recognized its value as a resource, providing opportunities for recovery of water, energy, nutrients, and valuable materials (Verstraete et al. 2009, McCarty et al. 2011, Bae et al. 2014, Stazi and Tomei 2018) with potentials contributing to the circular economy. This recognition brought a nutrient-energy-water paradigm by shifting from wastewater treatment with the end-of-pipe concept to a combined treatment and resource recovery system (Guest et al. 2009). The basis for this concept is source-separation of the different domestic wastewater streams (Tervahauta et al. 2013).

The larger volume called greywater is less contaminated and can be treated more efficiently for further non-potable use or safe discharge. If the greywater fraction is collected and treated on-site and used for non-potable local use, a significant reduction in water consumption and wastewater generation can be achieved. Based on the Danish water use statistics, up to 43 per cent of potable water could be saved by recycling greywater (Revitt 2011). It is also indicated that reusing treated greywater for conventional toilet flushing and laundry can reduce the drinking water consumption by 42 % (Hernandez 2010). Reusing the treated water also has implications in terms of energy savings. The operational cost of a

water supply system in terms of energy include the cost of energy used for pumping from the sources and pumping stations and the cost of water treatment for use. The energy used to supply one cubic meter of water can vary from 0.25 to 4.5 kWh/m^3 depending on the source of water supply, i.e. surface and groundwater, respectively (Puleo et al. 2015).

Similarly, the energy requirement for sewage water transport and treatment lies between 0.42 and 0.93 kWh/m^3 (Frijns et al. 2008). If we assume that about 0.5 kWh/m^3 energy is used to supply fresh water to a household and the household water consumption is reduced by up to 25%, by introducing a vacuum or very low flush toilet, a substantial amount of energy can be saved. For operating a vacuum sewer system approximately 10 kWh, electrical energy is needed per capita per year (Todt and Jenssen 2015). Similarly, an additional amount of energy can be saved on the wastewater side. If the greywater is treated locally and replace up to 40% of the freshwater demand, up to 26 kWh of energy per capita per year can be saved.

Moreover, source-separation avoids unnecessary and uneconomical waste dilution. The key principle of source-separation is that energy and nutrients (mostly contained in blackwater) can be recovered more efficiently from concentrated streams (Capodaglio 2017). Although the blackwater fraction of domestic wastewater is the major sources of pathogens, and micropollutants (Kujawa-Roeleveld and Zeeman 2006, de Wilt et al. 2016), the minimal amount of water use through vacuum or low flush toilet yields a more concentrated stream from which it is more cost-effective to remove the harmful micropollutants (de Wilt et al. 2016). Thus, using the source-separation approach, the blackwater stream of domestic wastewater can be collected separately using low flush toilets (e.g. vacuum toilet) and processed with specific treatment systems for energy and nutrient recovery. In the current practices of wastewater treatment, the energy potential of the wastewater is only partially recovered. The activated sludge treatment process consumes substantial amounts of energy for aeration. During

the oxidation process, the chemical energy present in COD is also lost as metabolic heat (Frijns et al. 2013). With the source-separation concept and the use of vacuum or low flush toilets, a substantial amount of energy and water can be saved. The system allows concentrating the wastewater organics in a small volume (i.e. blackwater) which increase both the energy use and recovery efficiency.

Anaerobic treatment of the concentrated blackwater stream can yield net energy and be a source of nutrients (Zeeman et al. 2008). For instance, taking the organic matter (CODt) content of 29520, 4710 and 2580 mg/L for 1, 6 and 9 L flush volume, respectively (Gao et al. 2019), the theoretical potential energy from COD corresponds to energy densities of 103, 16, and 9 kWh/m^3, respectively. If we consider an overall conversion to electricity efficiency of 25% (i.e. assuming 70% methane conversion rate and 35% CHP electricity conversion efficiency, and a conversion factor of 0.35 m^3 CH_4/kg COD, 35.9 MJ/m^3 CH_4 and 0.278 KWh/MJ), the energy value of source-separated blackwater will be 25.6, 4.0 and 2.2 kWh/m^3 for the 1, 6, and 9 L flushed blackwater, respectively. This value is several times higher than the energy requirement at municipal wastewater treatment plant (MWWTP), which is in the range of 0.3-0.5 kWh/m^3 (Mizuta and Shimada 2010). Anaerobic wastewater treatment, therefore, allows energy production, resource recovery and upstream energy savings.

The potential energy embraced in domestic wastewater is even more significant if a holistic approach to water, nutrient and energy recovery and reuse is considered. This is due to the fact that, domestic wastewater contains three energy-related characteristics: the energy resource contained in wastewater organics, the external fossil-fuel energy requirements for the production of equivalent amounts of the fertilizing elements N and P in wastewater and the energy that might be gained from wastewater's heat content (Rittmann and McCarty 2001). Moreover, the quantity of digested sludge resulting from anaerobic digestion is much less in volume and is more stabilized. Hence, the

stabilized sludge requires less management for further use than with traditional activated sludge treatment systems. This, in turn, has a highly significant cost as well as energy benefits (McCarty et al. 2011). Recovery and use of nutrients from anaerobically treated blackwater could, therefore, contribute to the reduction of fossil fuel consumption in manufacturing fertilizers (Rittmann and McCarty 2001). Furthermore, the energy consumption for removal of N in the wastewater treatment plant can be reduced when the N load to the treatment plant is reduced. Therefore, overall indirect energy gain is high.

Source-separation, as a result, opens up an excellent opportunity to adopt a circular metabolism (Wielemaker et al. 2018) both in cities and rural areas, to recover, reuse and recycle resources contained in domestic wastewater. Taking those aspects into consideration source-separation of the wastewater streams and their respective targeted wastewater treatment will boost the circular resource flow. Hence, implementing appropriate techniques for efficient use and recovery of water, energy and nutrient locally, and their use in urban food production will enhance the water-food-energy security. Moreover, this approach can also help for source control of pathogens and emerging contaminants before they are diffused into the ecosystem.

The focus of this research is to develop a combined treatment and resource recovery system based on the concept of source-separation and resource recovery. The New Sanitation concept developed in The Netherlands has shown the importance of technological developments in treating domestic wastewater and recovery of resources (Zeeman and Kujawa-Roeleveld 2011, Zeeman 2012, Tervahauta et al. 2013). The development of separate treatment technologies aiming at separate flows fit for reuse or recycling will maximize the benefits of domestic wastewater while minimizing negative impacts on the environment and health risks. This further increases public acceptance and strengthen the new perspectives of domestic wastewater management in the realm of water - nutrient - energy nexus.

1.4. Treatment and reuse approaches for source-separated wastewater

1.4.1. Source-separated greywater treatment

Various greywater treatment technologies have been applied and examined in the last decades to obtain affordable treatment systems that meet the local discharge and/or recycling requirements. Treatment systems including constructed wetlands (Jenssen et al. 2003, Jenssen and Vråle 2003, Jefferson 2004, Gross et al. 2007, Jenssen 2010), and compacted treatment systems such as membrane bioreactors (MBR) (Friedler et al. 2006, Lesjean and Gnirss 2006) have been practised with promising achievements. Anaerobic treatment of greywater was also studied (Elmitwalli and Otterpohl 2007, Ghunmi et al. 2008, Abu-Ghunmi 2009). Although the performance in the removal of COD is relatively poor (Abu-Ghunmi 2009), the anaerobic step was suggested as a pretreatment (Elmitwalli and Otterpohl 2007) to be followed by aerobic treatment. By combining the advantages of aerobic and anaerobic processes, a study was made on a combined anaerobic and aerobic system (Abu-Ghunmi 2009, Hernandez 2010). The system consisting of a sequence of an upflow anaerobic sludge bed reactor (UASB) and a sequencing batch reactor (SBR) operating at short hydraulic retention time showed a COD removal efficiency of 89%, which was comparable to the aerobic treatment with a 90% removal efficiency but with lower energy requirement (Hernández Leal et al. 2010).

Though several of these technologies are promising, most of them suffer from limitations in relation to either operation, maintenance, area and cost (Schwemer and Wolfgang 2016). Most of the poor performances of household on-site systems are also related to the skill of the users in the operation and management of the systems. In most cases of household on-site treatment systems, the owners of the houses are in charge of these facilities and most do not have any in-depth knowledge of the processes for the successful operation of the treatment system (Wilderer and Schreff 2000). The owners should get the appropriate training to

accomplish the operation, and people with adequate training must also be assigned for supervision and control of the treatment facility to sustain the performance of the systems. The management structure is, thus, a key factor for the performance of small-scale treatment systems.

Moreover, small wastewater treatment systems for the decentralized application must provide advanced wastewater treatment such that water recovery and reuse are focused. The systems must, however, be highly effective, robust, easy to operate, and affordable. A treatment system is considered efficient if it produces the required effluent quality, simple in operation with minimum maintenance, and affordable due to its low energy consumption and low operational and maintenance costs (Wendland et al. 2006, Abu-Ghunmi 2009). Thus, in addition to reducing health risk and aesthetic problem, on-site treatment can help to optimize resource recycling and re-utilization and minimize energy and operation costs (Friedler 2004).

1.4.2. Anaerobic treatment of source-separated blackwater

Anaerobic digestion (AD) is a mature technology which involves different groups of microorganisms to decompose and convert organic matter into biogas (Rittmann and McCarty 2001). Anaerobic digestion process, governed by different groups of microorganisms, is a multi-step process consisting of four main stages in series: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Batstone et al. 2002, De Mes et al. 2003). The schematic diagram in figure 3 shows the different processes involved in anaerobic degradation and the rate-limiting steps.

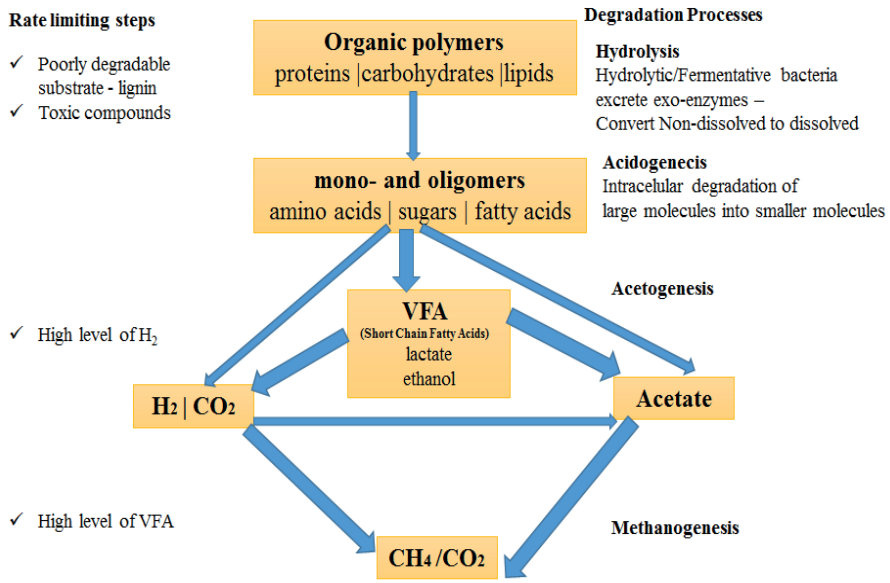

Figure 3. Processes of anaerobic degradation (adopted and modified from Khanal S. K. (Khanal 2011)).

A thorough understanding of both the chemistry and microbiology of the anaerobic process is a prerequisite for the anaerobic systems to be used successfully implemented (Rittmann and McCarty 2001). Through hydrolysis complex proteins, carbohydrates, and fats converted into simpler molecules such as amino acids, sugars, and fatty acids. These simpler molecules are then fermented to form fatty acids and hydrogen (acidogenesis). The fatty acids are oxidized further to acetate and hydrogen (acetogenesis). Finally, two different methanogenic groups convert acetate and hydrogen to methane (methanogenesis) (Vögeli 2014). Acetate-forming (acetogenic) bacteria which grow in a symbiotic relationship with methane-forming bacteria (Gerardi 2003) convert volatile fatty acids (VFAs) into acetic acid, carbon dioxide and hydrogen to supply the methane production process. Otherwise, the accumulation of unionized VFA inhibits methanogenesis and cause process failure (Weiland 2010). At the same time, the high level of hydrogen is a limiting step for acetogens (Gerardi 2003). Acetate-forming bacteria which are obligate hydrogen producers survive only at very low concentrations of hydrogen in the environment. They

can only survive if their metabolic waste, hydrogen, is continuously removed. Methanogens, thus, consume the hydrogen and keep the partial pressure of hydrogen low for the acetogens to survive and function (Gerardi 2003).

On the other hand, methanogens are known to be highly sensitive to their environment in terms of temperature, pH, and the concentrations of certain chemical compounds (ammonia, unionized VFAs) (Manser 2015). Moreover, basic considerations such as the composition and strength of wastewater, the volume, activity and adaption of the inoculum, operation parameters like loading rate, retention time and liquid mixing are all important in the design and operation of the anaerobic treatment (Weiland and Rozzi 1991).

In such a multi-step and complex process, the kinetics of the slowest step will account for a limiting step of the overall kinetic process. In most cases, either hydrolysis or methanogenesis become a rate-limiting step. Hydrolysis is often limited if the substrate is rich with insoluble or poorly biodegradable organic solids, while the rate-limiting step in the digestion of solubilized organic matter is methanogenesis (Tomei et al. 2009). If hydrolysis is a limiting step, the accumulation of suspended solids in the reactors is prevalent, resulting in decreased methanogenesis and removal efficiencies (Miron et al. 2000). Under these conditions, the application of short hydraulic retention time (HRT) is virtually impossible and hence a long sludge retention time (SRT) is needed for stabilization of the solids and sludge inside the upflow anaerobic sludge bed (UASB) reactor (Zeeman and Lettinga 1999). Based on this knowledge it was decided to search for a technological solution that could handle short HRT with high feed particulate content.

With decades of experiences in AD and novel technological developments, the application potential of the high-rate anaerobic reactor systems expanded to a more extreme type of wastewaters (Van Lier et al. 2015). In recent studies, the feasibility of anaerobic treatment of source-separated blackwater characterized

by high suspended solids was successfully demonstrated at the lab- and full-scale in The Netherlands using UASB with relatively short HRT (de Graaff et al. 2010a, Tervahauta et al. 2013, Cunha et al. 2018b, Zeeman et al. 2008). The application of UASB septic-tank (Kujawa-Roeleveld et al. 2005) and continuous flow stirred tank reactor (CSTR) (Wendland et al. 2007) to concentrated source-separated blackwater have also been investigated. UASB septic-tank is an upflow mode system designed for the accumulation and stabilization of sludge (Zeeman and Lettinga 1999, Kujawa-Roeleveld et al. 2005, Kujawa-Roeleveld and Zeeman 2006). However, it requires long HRT and thus a larger reactor volume. On the other hand, CSTR is a continuously fed reactor where the liquid and the solid including biomass, are completely mixed (Zeeman and Kujawa-Roeleveld 2013) and is characterized by a long HRT and short SRT (HRT=SRT) (Wendland et al. 2007). Moreover, the performance of CSRT in terms of CODt and CODss removal is low compared to UASB and UASB septic-tank. In UASB and UASB septic-tank, good removal efficiency of organic matter was achieved due to the establishment of a dense sludge bed at the bottom of the reactor, in which all biological processes take place (Kujawa-Roeleveld and Zeeman 2006).

The novel development in UASB reactors, however, received great interest due to their high loading capacity and low sludge production (van Lier 2008, Zeeman et al. 2008). The upflow mode provides sufficient contact between anaerobic sludge and incoming substrate of the wastewater, thereby increasing the physical removal of suspended solids and biological conversion of dissolved organic compounds (Luostarinen and Rintala 2005). The high-rate UASB technology relies on the inoculation of anaerobic granular sludge, growth of granular sludge and the application of an internal gas-solid-liquid (GSL) separation system to retain biomass and formation of good settling sludge aggregates (Zeeman and Lettinga 1999, Van Haandel et al. 2006, van Lier et al. 2008). Another important feature and more relevant factor in the design of UASB to achieve high biomass retention is the height of the reactor (Lettinga and Pol 1986). The height should

be sufficient to provide enough sludge bed height, to avoid channelling of suspended particles and to keep liquid upflow velocity within maximum permissible limits. Based on this it was assumed that an adaptation of the granular sludge bed process to handle high particulate feeds in a compact design was required for conditions relevant for Norwegian applications.

1.4.3. Nutrient recovery from anaerobically treated blackwater effluent

The estimated total emissions of phosphorus and nitrogen from all the Norwegian municipal wastewater plants, including estimated leakage losses, in 2016 was 1530 tons for total P and 19880 tons for total N (Berge et al. 2017). The total nutrient discharge to the environment is even likely to be higher at the source, considering the numerous smaller point emissions that are not captured by these figures. As stated earlier, the main source of these nutrients is the blackwater stream of the domestic wastewater and in particular from urine. Hence, the nutrients (particularly N, P and K) from source-separated blackwater of the domestic wastewater stream are the main sources of pollution, but if managed and recovered they are potential resources.

Recovery of these nutrients with appropriate technology can provide a vast range of benefits. These include (i) reducing eutrophication problems in downstream water bodies, (ii) providing a potential source of fertilizer which generates agricultural and economic value by reducing the reliance on chemical fertilizer (Verstraete et al. 2009), (iii) reducing the energy used to produce an equal amount of chemical fertilizer (McCarty et al. 2011), and (iv) avoid advanced nutrient removal processes, including nitrification, denitrification, reducing greenhouse gas emission, and phosphorus elimination. However, the efficiency and cost-effectiveness of the nutrient recovery methods as well as the management strategy should be considered particularly at the small-scale level.

Since biomass production under anaerobic condition is low, after anaerobic digestion of the source-separated blackwater, most of the nutrients are largely

conserved in the liquid phase. Phosphorus and nitrogen subsequently can be recovered from the anaerobic effluent by physical-chemical processes such as ion-exchange, electrodialysis, nanofiltration or reversed osmosis (van Voorthuizen et al. 2008) and by precipitation as struvite (Maurer et al. 2006, de Graaff et al. 2011a) or Ca-P (Tervahauta et al. 2014b, Cunha et al. 2018b). Struvite precipitation is usually used for simultaneous recovery of phosphorus and nitrogen. However, due to the high N/P atomic ratio of the anaerobically treated blackwater, the fraction of nitrogen that can be recovered through struvite is limited (Vasconcelos Fernandes et al. 2015) unless additional P and Mg is added to the stoichiometric level of the N in the digestate. Phosphorus recovery from concentrated wastewater through struvite precipitation requires a high pH (>8) and the extra addition of magnesium to form $MgNH_4PO_4$ (de Graaff et al. 2011a).

The most common Mg sources used in struvite precipitation studies are salts of Mg, such as MgCl2, MgSO4 and MgO. It has been, however, reported that the cost of high-grade Mg compounds contributes up to 75% of overall production costs, limiting large-scale use economically not viable (Dockhorn 2009). Using magnesium alloy (electrochemical magnesium dissolution) as an alternative source of struvite crystallization has also been reported feasible to recover phosphate (Hug and Udert 2013, Huang et al. 2016). Electrochemical magnesium dosage involves the direct dissolution of the magnesium in the solution and requires no mechanical feed mechanism. Such a system is particularly interesting for decentralized reactors (Hug and Udert 2013).

A coupled electrochemical process, electrochemical precipitation of struvite followed by electrochemical decomposition of the struvite formed, has been evaluated as a feasible mechanism for simultaneous P recovery and N removal (Huang et al. 2016). The solid obtained from the calcination of $MgNH_4PO_4$ (MAP) could be reused for the removal/recovery of aqueous ammonium. It was suggested that $MgHPO_4$ was a key compound for the recycling of MAP for the removal/recovery of aqueous ammonium (Sugiyama et al. 2005). The calcination

process, however, may require high energy input. Calcium phosphate granule formation in the anaerobic treatment of blackwater has also been investigated as an alternative P recovery method (Tervahauta et al. 2014b, Cunha et al. 2018a, Cunha et al. 2018b). Addition of Ca in a UASB reactor during the treatment of source-separated BW increased the accumulation of P and stimulated formation and growth of Ca-P granules, without affecting the treatment performance (Cunha et al. 2018a). Ca-P granulation could, however, be influenced by the bicarbonate and Ca concentration in the incoming blackwater (Cunha et al. 2018b). Moreover, the thickness and composition of the outer biofilm on the Ca-P granule and formation of CaCO3 may hinder the recovery of P.

On the other hand, ammonia stripping has been applied at full scale for N recovery (Maurer et al. 2003). Ammonia stripping, however, requires a large energy and chemical inputs. Recently, use of microbial electrochemical technologies (METs) for N recovery from urine has shown to be technically and economically viable, opening the path for novel decentralized systems focused on nutrient recovery and reuse (Ledezma et al. 2015). Microbial fuel cell (MFC) is a bioelectrochemical technique which utilizes electrochemically active bacteria (EAB) or electrogenic bacteria to convert biodegradable COD to electrons and form an electric field on the anode (Kim et al. 2015). The electrons produced by EAB are then transferred to the cathode using the potential gradient between the anode and the cathode. Localized pH increases near the cathode would produce a shift of ammonium ions to ammonia, resulting in nitrogen losses due to ammonia volatilization through the cathode (Kim et al. 2008). Ammonia released from the liquid-gas boundary via volatilization can be recovered by subsequent absorption into an acid solution (Kuntke et al. 2012). This system recovers only N and has to be integrated and preceded by a struvite reactor to recover both N and P. Moreover, various parameters such as electrodes, materials, configuration, biocatalyst, reaction kinetics, fabrication and operational costs, resistance for electron transfer etc. will critically govern the performance of microbial catalyzed

electrochemical systems and poses challenges towards up-scaling and practical applications (Butti et al. 2016).

The other alternative is to optimize complete nutrient recovery (N, P, K and micronutrients) through microalgae biomass production (Tuantet et al. 2014a) or through concentrating the nutrient solution by nitrification and distillation (Udert and Wächter 2012). The later, however, require more energy input for nitrification and distillation (Udert and Wächter 2012, Fumasoli et al. 2016). However, for reliable operation of these processes, the feed should be free of particles, colloidal material, and as low as possible in soluble organic matter (Tanninen et al. 2005). Using selective adsorbent (such as Cocos char and activated granular carbon) for the removal of residual organic matter, total suspended solids, turbidity and possibly micropollutants and pathogens, both N and P can be recovered in the liquid phase as a nutrient solution. This has not been studied as nutrient recovery option from anaerobically treated effluent but could be the ideal solution if the nutrients are used for local food/feed production close to the source as well as for hygienized and purified sources of N and P if concentration of the nutrients (through struvite or other methods) in small volume is needed.

1.4.4. Application of microalgae for wastewater treatment and nutrient recovery

In the past few decades, tremendous efforts have also been put into the research of microalgae cultivation in wastewater. The cultivation of microalgae in wastewater offers the combined advantages of treating the wastewater and simultaneously producing algal biomass. The biomass can further be exploited for protein complements and food additives (for aquaculture, animal and human feed), an energy source such as biogas and biofuels, agriculture (fertilizers and soil conditioners), pharmaceuticals, cosmetics and other valuable chemicals. The use of a wide range of microalgae species such as *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas* and *Spirulina* for treating domestic

wastewater has been reported (Olguín 2003, Chinnasamy et al. 2010, Kong et al. 2010, Wang et al. 2010b) and effectiveness of this method was found to be promising. Studies showed positive results regarding the potential of utilizing microalgae to remove nitrogen, phosphorus, and other elements (especially heavy metals) from wastewater (Cai et al. 2013).

As compared to the typical agricultural, municipal, and industrial wastewater, AD effluents have relatively lower levels of carbon as most of the organic carbon is converted to methane and microbial biomass during the anaerobic digestion (Wang et al. 2010a). The AD effluent, however, retains high concentrations of dissolved nutrients mainly ammonium nitrogen and orthophosphate. Hence, the removal of nitrogen from such effluent, with very low carbon/nitrogen (C/N) ratio, can often be limited in conventional wastewater plants (WWTPs) because organic carbon is a limiting factor for denitrification. Recent developments in innovative nitrogen removal pathways such as shortcut nitrification/ denitrification (Ruiz et al. 2006, Gao et al. 2009, Gao et al. 2010) simultaneous nitrification/denitrification (Helmer and Kunst 1998, Yilmaz et al. 2008, Virdis et al. 2010), and the nitritation-anammox process (Fux et al. 2002, Vazquez-Padin et al. 2009, de Graaff et al. 2010b, Vlaeminck et al. 2012, Lackner et al. 2014) can remove nitrogen with low or zero dosage of organic carbon sources (Sun et al. 2010). These processes, however, do not encourage N recovery as a resource.

Converting nutrients contained in AD effluent into microbial biomass like microalgae may, therefore, be an efficient way to recover nutrients for use as biofertilizer, fish food or as a resource coupled to biofuel production. Moreover, local recovery of N and P reduces the need for mineral fertilizers and the associated or indirect energy and transportation cost. The use of microalgae as an integrated technology offers both N, P, K and other macro and micronutrient recovery. The nitrogen in the AD effluent is mainly available as ammonium (Singh et al. 2011), and a dilution of the AD effluent may be needed before feeding

to the microalgae to avoid the potential inhibition of algal growth due to a high ammonium concentration and turbidity (Wang et al. 2010c).

A recent study, however, demonstrated the possibilities of growing microalgae on non-diluted urine and anaerobically treated blackwater (Tuantet et al. 2014a, Vasconcelos Fernandes et al. 2015). *Chlorella sorokiniana* was able to grow on concentrated human urine with up to 1.4 g NH_4^+-N L^{-1} (Tuantet et al. 2014a) at a pH lower than 8.0, although, maximum growth was obtained, on 20 times diluted urine with additional trace elements (Tuantet et al. 2014b). A post-treatment of urine and anaerobically treated blackwater may, however, be required to reduce the residual organic matter, suspended particles, and turbidity which again improve the light transmission and reduce the light energy demand. Moreover, the post-treatment may reduce micropollutant and heavy metal load to the microalgae and pathogen contamination. This is, therefore, one of the aims of the present study to evaluate if this can be achieved.

1.5. Source-separating sanitation approach in rural Norway

In Norway, about 16 % of the population is connected to over 335,000 on-site wastewater treatment systems (Berge and Chaudhary 2015). In addition to the 335,000 on-site systems for rural residents, more than 420,000 recreational houses are currently found in rural Norway (Berge et al. 2017). In 2016, the total discharges of phosphorous and nitrogen in Norway, for the whole wastewater sectors including estimated leakage losses, was around 1530 tons of P and 19880 tons of N (Berge and Sæther 2018). The on-site treatment plants, <50 pe, contribute to about 24 % of the total P discharge and about 15 % of the total N discharge (Berge and Sæther 2018).

Septic tanks without further treatment or discharges without treatment account for 54% of all systems and are found along the western coast where the recipients are less sensitive to phosphorus discharges. In more sensitive recipients with

strict regulations on phosphorus discharges we find soil infiltration systems (29%), sand filters (6%), biological/chemical package treatment plants (4%), various types of source separation systems with collection of black and/or greywater (5%) and constructed wetlands and other undefined solutions (2%) (Berge et al. 2017). However, in areas where the soil cover is limited or not suitable for infiltration, particularly in the mountain areas and along the coast, where a large fraction of the cottages are located, other solutions are needed. As pointed out by Johannessen (Johannessen 2012) approximately 2/3 of the package treatment plants under operation do not meet the required outlet quality, partly because of insufficient operation/maintenance.

As a supplement to soil infiltration systems and package treatment plants, subsurface flow constructed wetlands (CWs) with bio-filters as a pretreatment was demonstrated in cold climate for the treatment of domestic wastewater (Jenssen et al. 1993, Maehlum et al. 1995). These CWs, utilizing Filtralite® P, a light-weight expanded clay aggregate with high phosphorus sorption capacity (Ádám et al. 2007), showed excellent performance (Jenssen et al. 2005). However, appropriate selection of filter bed media and their grain size is essential. Moreover, the performances of such systems depend on the composition and lifetime of the filter bed materials and area requirements (Drizo et al. 2002).

The CW´s in Norway require 7–10 m^2 wetland area per person according to Norwegian design guidelines. In order to reduce the footprint, a compact version of the CW was constructed with reduced filter volume contained in a fiberglass tank. The compact filter system performed well for the expected 5 years of operation, limited by the phosphorus sorption capacity (Heistad et al. 2009). The study by Heistad also showed a high removal of faecal indicators as well as bacteriophages. This is of particular interest in rural areas where the households have individual drinking water wells. A study of compact filter bed systems with Filtralite® P as a filter material in the Nordic countries, as an alternative to the larger CWs, showed stable and consistent performance (Jenssen 2010). The

integrated biofilter is essential for stable performance in cold climates and the principles of biofiltration as a pretreatment has been utilized in soil infiltration systems (Heistad et al. 2001) and in CW´s treating greywater (Jenssen and Vråle 2003).

With the aim to serve single households/cottages in sensitive recipient areas the knowledge obtained from using pretreatment bio-filters and constructed wetlands in Norway was utilized in the design and development of a compact on-site greywater treatment system using septic tank and aerobic biofilter succeeded by a secondary clarifier, with final discharge to local deposits or shallow sand filter trench (Heistad et al. 2006). The results obtained from more than 8 years of full-scale laboratory tests have shown stable performance with respect to the removal of organic matter, nutrients and faecal indicators (Moges et al. 2014). The small size of the treatment plant facilitates installing of this type of bio-filter systems on small lots or under difficult ground conditions with sensitive recipients. These systems are, therefore, ideal for cottages, single houses or cluster of houses in remote areas. However, most of the treatment systems are designed to remove P, and a comprehensive study to assess treatment efficiencies and effluent quality with a resource recovery concept in a rural configuration of a source separating sanitary system has not been implemented. Moreover, post-treatment system using column filtration, to mimic soil infiltration trench, need to be tested to study the application of these systems in vulnerable areas and where discharge requirements are very stringent, and to assess the potential of greywater for non-potable local reuse.

On the other hand, most of the treatments related to blackwater are a collection in sealed tanks which are then emptied periodically and transported to the central wastewater treatment systems. These sealed tanks could be sources of uncontrolled emission to the environment, particularly during emptying and transportation. Discharging the concentrated blackwater into the central wastewater treatment plant may also cause temporal nutrient and organic shock

loads to the wastewater treatment system. Source-separation sanitation with energy and nutrient recovery through the integration of AD has not been tested in this region. This could be a viable opportunity for reducing the environmental impacts from these on-site wastewater treatment systems and at the same time to recover energy and nutrients at the source of waste generation. Although the UASB reactor is considered proven and might be the best candidate for treating concentrated blackwater, its use is currently rare in Norway. The high tower feature of the UASB might be a critical issue especially when the reactors should be installed in basements or in low sheds discreetly integrated into the terrain. The height of such rooms is generally designed low to minimize heat loss during the long cold season. Moreover, an adaptation of the granular sludge bed process to handle high particulate feeds in a compact design was needed to be tested in the Norwegian conditions. Hence, designing and developing alternative compact and efficient high-rate AD reactor with enhanced solid retention capacity for treating concentrated blackwater in small volume reactor is required for conditions relevant for Norwegian applications.

2. Approaches and objectives of the thesis

Except for pathogens and micro-pollutants, the bulk volume and components of domestic wastewater are renewable resources. The question is how do we strategically manage domestic wastewater towards sustainable means of resource recovery? In what ways can we integrate new concepts and technologies to achieve both resource recovery and environmental and public health safety? Moreover, how can we enhance the sustainability of these activities in domestic wastewater management?

The development of separate treatment technologies aiming at the separate flows of domestic wastewater streams fit for reuse or recycling enables us to identify, quantify and recover value-added resources from domestic wastewater. Circular flows of these resources within the sources of origin enhance the sustainable management of water, nutrients and energy. The recovery of energy and nutrients from wastewater results in a reduced organic matter and nutrient release and thus alleviate the major problems related to environmental degradation such as eutrophication and pollution of drinking water sources (Carey et al. 2016).

The approach in this study is, therefore, to consider households and communities as a source of sustainable production units of water, energy, nutrients and food from the wastewater they generate. Targeted treatment of source-separated wastewater streams at household or community level will shift the wastewater-related problems to a source of value creation. Despite the basic knowledge on source-separated sanitation, efficient and affordable technology solutions for a combined treatment and resource recovery facility and to create opportunities for the circular economy are still under development. Only few successful demonstrations and full-scale systems are developed, mainly in The Netherlands (Kujawa-Roeleveld 2005, de Graaff 2010, Zeeman and Kujawa-Roeleveld 2011,

Tervahauta et al. 2013, Leal Lucía et al. 2017, Zeeman et al. 2008) and Germany (Otterpohl 2002, Wendland and Oldenburg 2003, Otterpohl and Buzie 2011).

Implementation of these new perspectives of domestic wastewater management systems in the realm of water - nutrient - energy nexus are, therefore, utmost important. In the Norwegian rural settings, the current small on-site wastewater treatment systems contribute to 24% of the total P discharge and about 15% of the total N discharges into the environment. The existing source-separated systems (5%) are used in sensitive areas or areas unsuitable for infiltration, in order to reduce the local discharges, not for the purpose of reuse. To our knowledge no full-scale source-separated, on-site system is currently in operation with the purpose of resource recovery. Post-treatment steps for greywater recovery for non-potable local reuse and on-site blackwater treatments have not yet been applied. Hence, designing and developing an alternative compact and efficient high-rate AD reactor with enhanced solid retention capacity for treating concentrated blackwater in small volume reactor is suggested as an integrated part of source-separation sanitation system. By developing and integrating efficient post-treatment schemes into the source-separation sanitation systems a circular resource flow can be achieved on a local scale. This study, therefore, contributes to the current knowledge of transforming the domestic wastewater streams into reusable resources.

Recovery of energy and nutrients from wastewater aiming at no or minimum chemical and energy inputs will contribute to the local needs of energy and nutrients. The recovered nutrients could be recycled to agriculture for food or feed production. Moreover, waterbodies could be more effectively protected from organic matter, nitrogen and phosphorus inputs. These dual goals make source-separation with targeted treatments of the domestic wastewater streams a very attractive option for sustainable management of water and wastewater. The study also aims to contribute to fill the knowledge gaps in technology

development with respect to the major challenges in reaching a widespread use of source-separation system.

The overall objective of the research project was twofold, i) to develop a combined treatment and resource recovery facility for a systematic closure of local resource flows with potential contribution towards a circular economy and ii) to assess the performance of the combined processing units for an efficient on-site treatment and in-situ resource recovery from source-separated domestic wastewater streams.

The specific objectives of this study and each of the appended papers are

- To assess treatment efficiencies and effluent quality of a biofilter system for on-site greywater treatment, and to study the application of post-treatment systems (Fig. 4) for water reuse applications and for vulnerable areas where discharge requirements are very stringent. **(Paper I and II)**

- To evaluate the application of an upflow sludge blanket anaerobic baffled reactor for source-separated blackwater and assess the effects of load and feed pulses on its performance in terms of initial adaptation, stability, effluent quality, the removal efficiency of organic and suspended particulate matter, biogas production and methane yield. **(Paper III)**

- To develop a combined treatment and resource recovery unit for processing blackwater and assess residual OM, TSS, and pathogen removal efficiency and nutrient recovery potential of different filter media **(Paper IV)**

- To assess the nutrient recovery efficiency of *Chlorella sorokiniana* and its potential to improve effluent quality (**Paper V**)

2.1. Scope and structure of the thesis

The thesis is based on five papers studied on source-separated domestic wastewater streams from student dormitories inhabiting 48 students at NMBU, Ås, Norway. The study focused on combining and developing integrated processing units for a source-separating sanitation system to achieve efficient treatment and resource recovery. These integrated processes include applications of the following four units. i) Compacted greywater treatment systems for water recovery and safe discharge (**Paper I & II).** ii) Anaerobic treatment of source-separated blackwater for energy recovery (**Paper III**). iii) Liquid digestate treatment with anaerobic carbon filtration coupled with UV for sanitized liquid nutrient recovery (**Paper IV).** And iv) Use of microalgae for optimization of nutrient recovery and improving effluent quality (**Paper** V). Figure 4 presents an overview of the scope of the research approach and system description in this thesis.

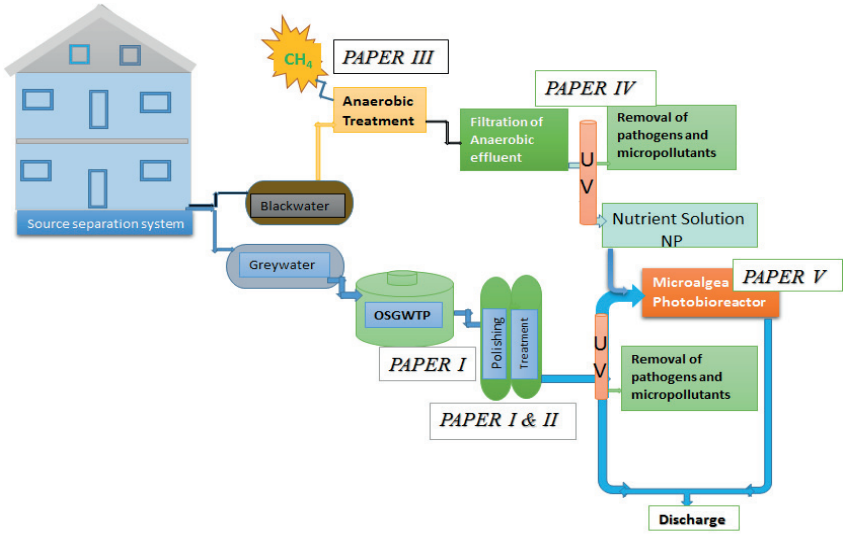

Figure 4. An overview of the research approach and system description in this thesis.

The first section of the thesis examines the effect of source-separation on the efficiency of on-site greywater treatment plants (**Paper I**). The organic and

nutrient loads are the most important factors that determine the design and efficiency of on-site wastewater treatment systems. The mass load of organic matter, total suspended solids, and nutrients were measured, and results discussed. Since the majority of nutrients, organic matter, pathogens, pharmaceuticals and hormones originate from a very small fraction of wastewater called blackwater, separation of this stream from the rest of the wastewater stream would allow for simplified greywater treatment for water recovery or safe discharge. The impact of varying loading conditions, which may be caused by pump failure and power break, have also been tested and the performance of the system evaluated. In the second part of this section, post-treatment steps using soil infiltration system and saturated column filtration units (**Paper I and II**) were integrated and studied to establish affordable mechanisms to reduce the risks during overloading or overflow conditions and unintended discharges. Moreover, the performances of post-treatment systems were also evaluated for unrestricted water reuse and for applications in vulnerable areas where discharge requirements are very stringent.

In the second section of the thesis, the separately collected blackwater was used as the feedstock for a laboratory scale anaerobic digester. The performance of a hybrid upflow sludge blanket anaerobic baffled reactor was studied with respect to load and feed pulse lengths (**Paper III**). Intensive data were collected and analyzed to evaluate the effects in terms of stability, effluent quality, the removal efficiency of organic and suspended particulate matter, biogas production and methane yield.

The third part of the thesis deals with developing a combined treatment and nutrient recovery facility to establish mechanisms for a more dependable source of plant nutrients from the liquid phase of anaerobically treated blackwater (**Paper IV)**. Different filter media were tested and evaluated for selective removal of organic matter and suspended particles while retaining the nutrients in the liquid phase. An integrated post-treatment unit was developed from which a

hygienically safe and aesthetically good nutrient solution (liquid fertilizer) was produced.

The last part of this thesis focused on the optimization of nutrient recovery and improving effluent quality of anaerobically treated blackwater. One of the main challenges for the produced nutrient solution in **Paper IV** is storage and transportation for use in agricultural fields if not used close to the source. The aim of this last part of the thesis is, therefore, to develop a sustainable processing unit to capture and store nutrients discharged from anaerobically treated effluents and produce value-added resources (biofertilizer, bioenergy, etc.). Thus, a flat panel photobioreactor was used to cultivate the unicellular green microalgae species *Chlorella sorokiniana* strain CHL176 obtained from NIVA on treated source-separated blackwater as a substrate in continuous culture. Biomass production and nutrient removal rates were assessed (**Paper V**). Nutrient recovery from anaerobically treated blackwater assessed as the amount of N and P retained in the algae culture and challenges discussed.

Finally, the key conclusions of the thesis and outlooks for future work are presented.

3. Materials and Methods

The study was conducted based on different bench-scale laboratory experiments at the Norwegian University of Life Sciences (NMBU), Ås. Both source-separated greywater and blackwater collected from the Kaja student dormitories with 48 inhabitants at NMBU transported in a separate pipe to the laboratory and used as a feed source for the greywater treatment plant and the anaerobic reactor, respectively. The supply system is described in detail in Todt et al. (Todt et al. 2015). As the main objective of this thesis is to develop a combined processing unit for efficient on-site treatment and in-situ resource recovery, sequences of experiments were undertaken based on the source-separated system installed at Kaja. The recovery of both water, energy and nutrients from domestic wastewater were investigated using biological and physical processes.

3.1. Experimental set-up for the different experiments

3.1.1. Source-separation and on-site treatment of greywater (Paper I and II)

In this section, the performance of the biofilter system and two alternative post-treatment options were studied. The study used a greywater treatment GWT system (Ecomotive-A02, Ecomotive AS, Runde, Norway) designed for cottages and small households (Heistad, 2008). The GWT system encompasses a sequence of a primary settler, an unsaturated fixed-film biofilter and a secondary clarifier. For the fixed film biofilter lightweight clay aggregates (LWA) having a diameter of 10–20 mm (Filtralite, Saint-Gobain Byggevarer AS, Alnabru, Norway) is used. The filter bed has a thickness of 500 mm. The raw greywater was fed into the Ecomotive-A02 GWT system using a peristaltic dosing pump. After primary settling, the greywater was distributed over the biofilter in intermittent pulses via full cone nozzles as described by Heistad et al. (Heistad et al. 2006). The dosing pump was controlled by a level switch in the primary settler and a timer giving the pulse length and intervals. The filter is designed for a nominal load of

650 L d^{-1}. The GWT system was loaded based on the European test protocol for package treatment plants (NS-EN 12566-3:2005 + A2:2013) (Norge 2013) with a diurnal distribution of hydraulic load (Table 2).

The effluent of the biofilter system was also further studied with two alternative post-treatment options with the aim of applications in the sensitive areas where discharge permits are very stringent and for unrestricted use in water-limited areas. The first alternative post-treatment options illustrate infiltration trench as a polishing step for the GWTP effluent. The study used columns representing discharge points in an infiltration trench with a single-hole in the perforated disposal pipe that is placed on the top of the infiltration trench in the actual disposal system (**Paper I**).

Table 2. Diurnal distribution of greywater into the GWTP. (From Paper I)

Timeframe	Volume fraction (%) of daily load
0:00–07:00	no load
07:00–09:00	40
09:00–12:00	15
12:00–19:00	no load
19:00–21:00	30
21:00–0:00	15

The second alternative works for areas where infiltration trench is not an option for several reasons. In this experiment, the effluent from the biofilter (before entering the second clarifier) was pumped at a constant flow rate of 280 $Lm^{-2}d^{-1}$

with a multichannel peristaltic pump to the experimental columns. The columns were fed continuously in upward saturated flow mode as opposed to the biofilter unit and infiltration trench column, which were fed intermittently under unsaturated conditions (**Paper II**). Figure 5 shows the schematic diagram of the experimental set-up. The COD, TSS, N, P and indicator organisms (TCB and *E. coli*) in the influent and effluent samples were analyzed every week. Details of the method description and analytical procedures are outlined in the corresponding papers (**Paper I and II**).

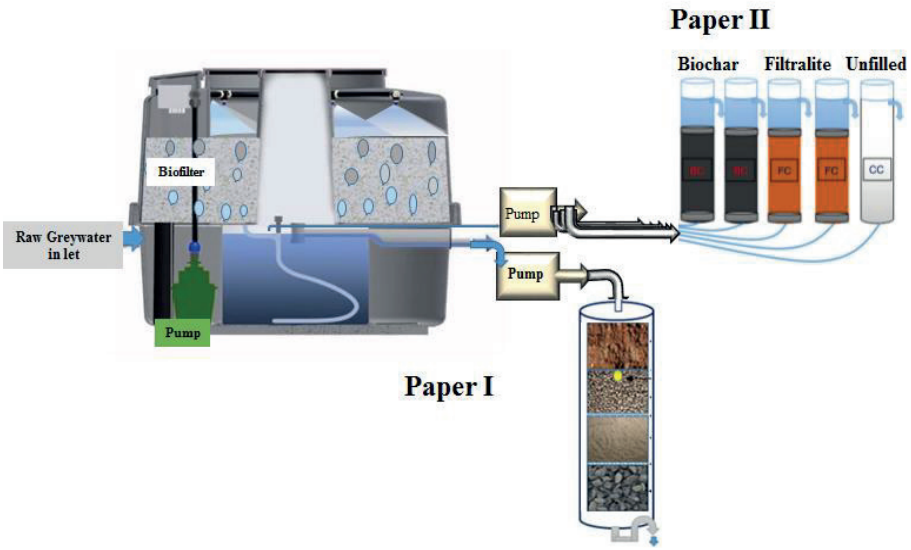

Figure 5. Schematic diagram of the experimental set-up for **Paper I and II**

3.1.2. Anaerobic treatment of source-separated blackwater (Paper III)

A new prototype of upflow sludge blanket anaerobic baffled reactor was tested for the treatment of source-separated blackwater. Source-separated blackwater from Kaja student dormitory was used for this study. The blackwater was transported to the laboratory facility with an impeller pump (40U, Tsurumi Europe GmbH, Düsseldorf, Germany). The experimental set up consists of a continuously stirred raw BW storage tank, a buffer tank and two cylinder-shaped

laboratory scale two stage sludge blanket anaerobic baffled reactors with a working volume of 16.4 L each (Fig. 6).

The reactors were fed intermittently with 16 pulses per day at a hydraulic loading rate (HLR) of 6 L d^{-1} and a hydraulic retention time (HRT) of about 3 days. Two different pulse lengths, 12 and 24 seconds per pulse, were applied for Reactor I and Reactor II, respectively. Chemical oxygen demand (COD), TSS, VFA, pH, NH_4-N, PO_4-P, TCB, and *E.coli* were analyzed weekly at different sampling points. Biogas production was measured continuously. Methane and CO_2 gas were measured on weekly bases. Details of the reactor configuration, method description and analytical procedures are outlined in **Paper III**.

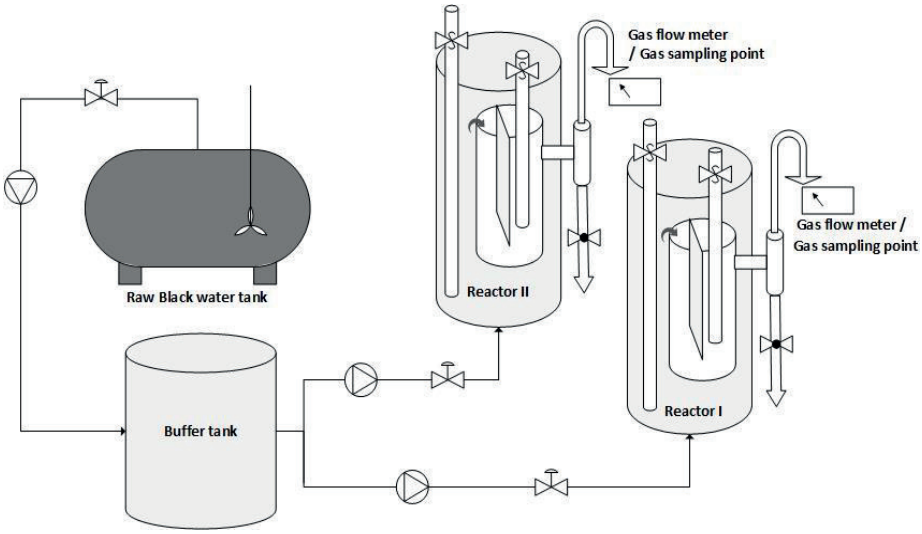

Figure 6. Flow scheme of the experimental set-up for anaerobic treatment of source-separated blackwater. P indicates pumps and the valve signs indicate sampling points (**Paper III**)

3.1.3. Post-treatment of anaerobically treated blackwater effluent for nutrient recovery (Paper IV)

Anaerobic digestion alone does not provide the necessary requirement in terms of nutrient recycling. Developing mechanisms for the removal of residual organic contaminants including pathogens and micropollutants from anaerobically treated blackwater, while keeping essential plant nutrients in the liquid-phase, is vital as a source of value creation and for reducing both health-related and environmental risks. Anaerobic filtration followed by unsaturated downflow filtration was carried out with three filter materials to assess their performance in removing residual organic matter, total suspended solids, and pathogens while retaining N and P in the liquid phase as a nutrient solution.

A UV light chamber of 290 mm long and 55 mm diameter with a working volume of 0.9 L and an 11 Watt UV lamp was installed at the outlet of the best performing columns to evaluate the removal of indicator organisms in the nutrient solution. The retention time of treated effluent in the UV chamber was about 3 h. Figure 7 displays the schematic flow of the experimental set-up for the filtration of anaerobically treated blackwater effluent in a sequential upflow and downflow filtration system in two replicates for the three treatments: coarse Polonite (ø 2.8–4.0 mm), granulated activated carbon (ø 0.5–1.4 mm) and Cocos char (ø 0.35–1.18 mm). Details of the reactor configuration, method description and analytical procedures are outlined in **Paper IV**.

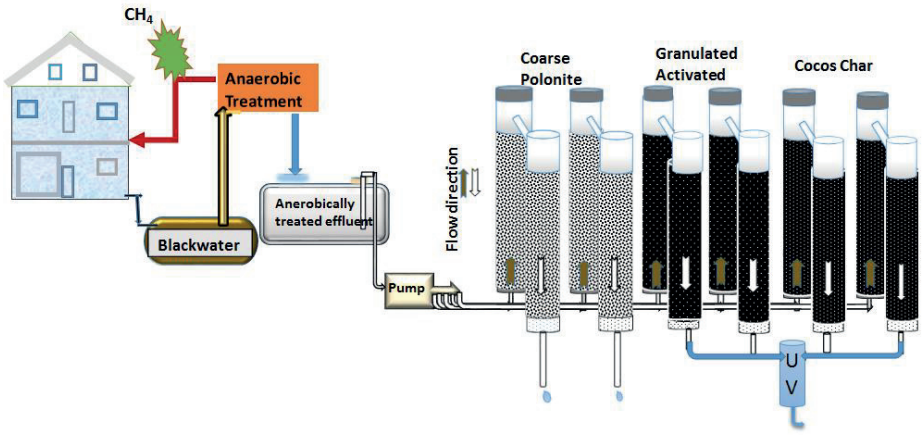

Figure 7. Schematic flow of the experimental set-up for the filtration of anaerobically treated blackwater effluent. (From **Paper IV**)

3.1.4. Optimization of nutrient recovery and improving effluent quality (Paper V)

The treated and hygienized nutrient solution from anaerobically digested blackwater effluent was collected for application in microalgae biomass production. The purpose of this experiment was to optimize nutrient recovery, concentrate nutrients in a small volume and produce effluent that meets the environmental discharge limit. The experiment was conducted at the Vollebekk microalgae laboratory, Ås in collaboration with NIBIO. Two flat panel photobioreactors (PBRs) with an outer dimension of 240 ×360× 40 mm (W×H×D) and a respective inner dimension of 180× 300 × 30 mm were used in this experiment (Skjånes et al. 2016). *Chlorella sorokiniana* was cultivated in continuous culture (chemostat) under a controlled temperature of 37 ^{o}C and a pH of 7. The PBRs were continuously illuminated with LED panels on illuminated surface areas of 0.054 m^{2} with an average light intensity of 1450 µmol photons m^{-2} s^{-1}. The culture volumes were kept at 1.3 L. After running the culture with a defined medium as a control, a 10% and 20% solution of treated blackwater was used as a source of substrate for the rest of the experiment, respectively. Daily samples were analyzed for optical density (OD_{750}), biomass and dissolved inorganic nutrients NH_4-N, NO_3-N, NO_2-N and PO_4-P. The growth of *Chlorella sorokiniana*

was monitored by optical density (OD) and dry biomass weight (DW). Biomass productivity, biomass yield on light energy and N and P removal rates were determined. Details of the reactor configuration, method description and analytical procedures are outlined in (**Paper V**).

3.1.5. Statistical analysis

Wherever necessary, the basic features of the data set were described using descriptive statistics. Due to the nature of the samples, mean values are markedly different from the median values indicating a positive or negative skewness of the data distribution. The geometric mean was used for microbial result interpretations. Unstacked One way ANOVA was used to measure the overall variation between and within treatments. An alpha level of 0.05 used to determine statistical significance for all analyses. All statistical analyses were performed using Minitab 17 statistical software (State College, PA: Minitab, Inc.). The grouping information among the different treatments was determined using Tukey and Fisher methods.

4. Results and Discussion

4.1. Characterization and treatment of source-separated greywater at Kaja student dormitory

At Kaja student dormitories the greywater production was estimated to be about 150 litres per student per day, with long and frequent showers accounting for a major part (Jenssen and Vråle 2003). Figure 8 shows the annual domestic wastewater stream flow by volume when considering an average excretion of 1.5 L urine and 0.2 L faeces per person per day and 4 L flushing water per day using a vacuum toilet. About 550 L of urine and 75 L of faeces can be produced per person per year.

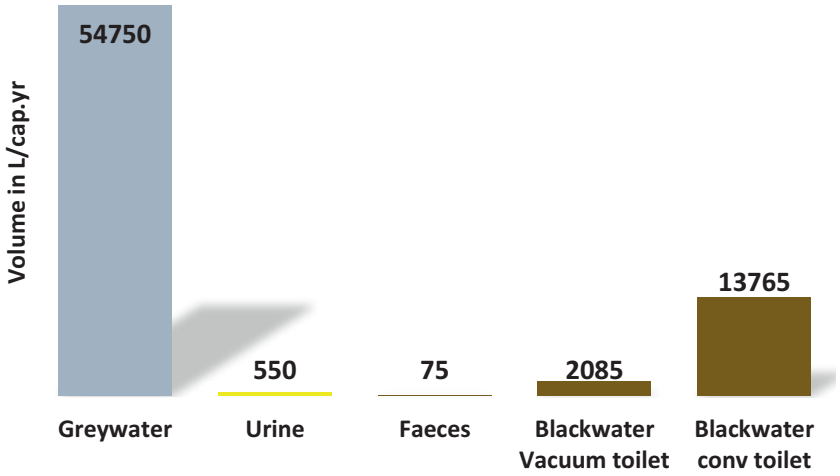

Figure 8. Annual domestic wastewater stream flow by volume

A large fraction of organic matter, N and P and almost all of the pathogens, pharmaceutical residues and hormones are contained in the urine and faeces representing only 1% by the volume fraction of the total domestic wastewater or in the blackwater stream, which represent 4% of the total domestic wastewater volume when vacuum toilet is used. Hence, with a vacuum toilet, about 96% by

volume of used domestic water (greywater) is relatively less polluted. Using a conventional toilet with 36 L flushing water per person per day 80% by volume of used water would be relatively less polluted. Moreover, when using a vacuum toilet 90% of toilet water consumption, which corresponds to 24% of the total water consumption per person, can be saved.

The average total COD, BOD, TSS, total N and total P in the studied greywater were 267 ± 71, 137 ± 38, 82, 14 ± 3 and 1.2 ± 0.3 mg L^{-1}, respectively. The concentration of COD and P in this greywater were lower when compared to the concentrations of these parameters in other places such as The Netherlands (Hernandez Leal et al. 2007, Hernández Leal et al. 2011), Germany (Elmitwalli and Otterpohl 2007), Sweden (Palmquist and Hanæus 2005) (Table 3). A high level of consistency in organic and nutrient loads (pollutant loads) at different places is not to be expected given that greywater flows, and composition of greywater is inherently variable as a result of differences in water consumption, household activities, etc. (Eriksson 2002). The greywater production at Kaja is estimated to be more than 120 L $p^{-1}d^{-1}$ (Todt et al. 2015) and hence resulted in low COD and nutrient concentration (Table 3). On the contrary, the high COD and nutrient concentration of greywater in The Netherlands may be due to the lower greywater production, i.e. 60–70 L p^{-1} d^{-1} (Hernandez Leal et al. 2007). The P load per capita (0.15-0.2 g p^{-1} d^{-1}) for our greywater is much lower than the P load reported in the literature which ranged from 0.4 to 0.6 g p^{-1} d^{-1} (Vinneras et al. 2006, Meinzinger and Oldenburg 2009). The low P concentration from Kaja student dormitory may have been resulted from higher water consumption, but the low P load is most likely a result of the absence of P-containing dishwashing detergents (Todt et al. 2015).

Table 3. Concentrations of COD, BOD, total N and total P in greywater for greywater in this study compared to other places.

Parameters	This study	A previous study on this greywater [a]	The Netherlands (Sneek) [b]	Sweden [c]	Germany [d]
CODt mg/L	249 - 309	250 - 300	724	588	640
BOD_5 mg/L	138	140 - 160			
Tot N mg/L	12 - 17.8	16 - 19	26.3	9.7	27.8
Tot P mg/L	1.15 - 1.5	1.3 - 1.6	7.2	7.5	9.8

[a] (Todt et al. 2015), [b](Hernández Leal et al. 2011), [c](Palmquist and Hanæus 2005), and [d] (Elmitwalli and Otterpohl 2007)

The concentrations of total coliform bacteria (TCB) and *E. coli* in the raw greywater were 6.7 ± 0.4 and 6. 2 ± 0.3 log 100 ml^{-1}, respectively. Other greywater studies reported comparable high concentrations on TCB ranging 7.2–8.8 log 100 ml^{-1}, but lower numbers for *E.coli* ranging from 3.2–6.0 log 100 ml^{-1} (Ottoson and Stenström 2003). The presence of high concentrations of TCB and *E. coli* do not necessarily reflect contamination from toilets. A recent study using a concentration of coprostanol, a biomarker formed by the intestinal microflora, showed a 3.1 log lower *E.* coli concentration in greywater than in combined household wastewater (Ottoson and Stenström 2003). Hence, the indicator parameters of TCB and *E. coli* used by this study most likely overestimated the concentration of faecal pathogens in greywater by 3 log_{10} (Ottosson 2003). Moreover, a faecal sterol coprostanol and gene markers of human-related *Bacteroides* analyzed in our black- and greywater showed a significantly lower concentration in greywater, 0.02% of the concentration for coprostanol and 0.001% for *Bacteroides*, respectively, than in blackwater (Oliinyk et al. 2015, Eregno et al. 2018). Furthermore, the intestinal *Enterococci* was about 3 log_{10} lower in the greywater compared to the blackwater (Eregno et al. 2018). Source-separation of blackwater would result in a 3 log_{10} reduction of indicator

organisms. A majority of the detected TCB and *E.coli* in our greywater are not likely faecal origin but could rather originate from the kitchen, where high concentration up to 7.4 $\log_{10}$ was also reported elsewhere (Naturvårdsverket 1995) or due to the re-growth of particular coliform species in sewer pipes (Manville et al. 2001).

4.1.1. On-site treatment of the source-separated greywater

Separation of the blackwater from the rest of the domestic wastewater stream produces greywater with reduced levels of nutrients, suspended solids, and organic matter, pathogens, and other micropollutants (pharmaceuticals and hormones). This fraction of the domestic wastewater, which encompasses the lion share of the total volume, can be treated with a proper treatment system to a reusable quality. The results from **Paper I** revealed that separate collection of blackwater substantially reduced the mass loads of BOD, COD, TSS, N and P in the greywater accounting for 64%, 61%, 75%, 85 and 88%, respectively (Fig. 9).

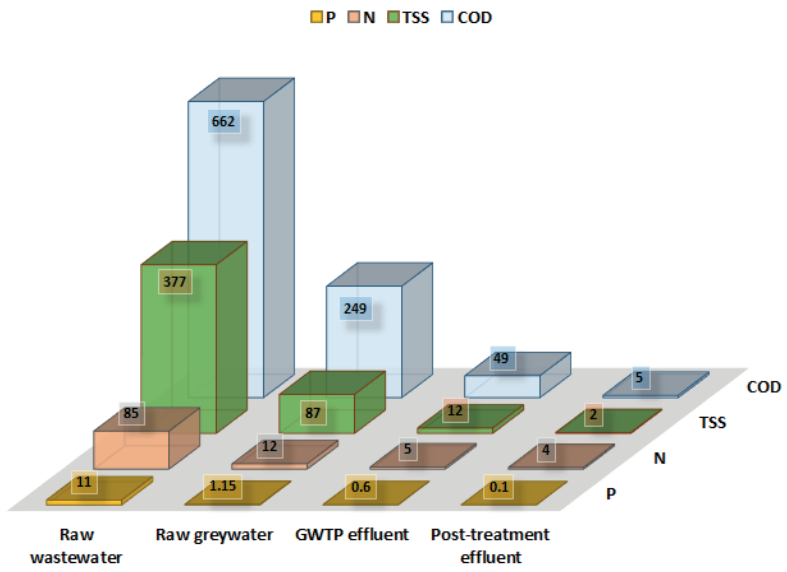

Figure 9. Calculated mean concentration for the raw combined wastewater and measured average concentration in the raw greywater, the effluent of greywater

treatment plant (GWTP) and bottom of infiltration trench Post-treatment for COD, TSS, N, and P in mg/L (modified from **Paper I**).

On the other hand, the A02-GWTP reached a removal efficiency of more than 88%, 86%, 49% and 55% for COD, TSS total P and total N, respectively. The system provided high removal efficiency compared to the rotating biological contactor (RBC) followed by a sedimentation chamber and sand filtration which resulted in 75 % to 78% removal of total COD (Friedler and Galil 2003, Friedler et al. 2006) and a 64% and 70% COD removal efficiency obtained using a UASB reactor (Elmitwalli and Otterpohl 2007, Hernández Leal et al. 2011). The RBC and subsequent sedimentation and sand filtration gave, however, a better BOD removal (96%) (Friedler et al. 2006). The results from this system were also comparable with the aerobic treatment using a sequential batch reactor (SBR) and the combined anaerobic-aerobic treatment (using UASB SBR in a sequence) which showed a COD removal of 90 and 89%, respectively (Hernández Leal et al. 2010). Together with a separate collection of BW a total removal efficiency of 93%, 95%, 96%, and 94% was achieved for BOD, total COD, TSS, and P, respectively.

Moreover, the impact of overloading on the removal efficiency of the system was evaluated by comparing periods with 100% nominal loadings with periods of 150% loading. Overloading with 150% of the nominal loading did not show a significant difference on the removal of TSS and total P tot ($p > 0.05$) while, a significantly lower ($p < 0.001$) removal efficiency was observed for organic matter. Regardless of the reduced organic matter removal, the filter achieved an average removal efficiency of 70% for both BOD and COD during the 150% loading periods, which proves the high stability of the fixed-film biofilter systems (Fig. 10).

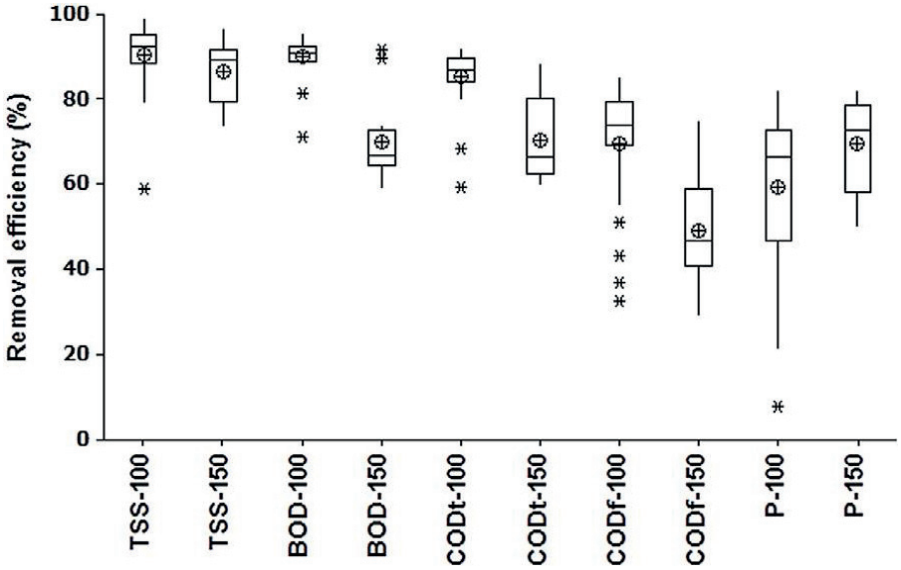

Figure 10. Removal efficiency of greywater treatment plant for TSS, BOD, total COD (CODt), filtrated COD (CODf) and total phosphorus (P) at 100% and 150% nominal load (25;50:75 per cent quartiles in the box plots with 95% quartiles in the error bars; average is indicated in the point plot) (From **Paper I**).

The effluent from the studied GWTP had a BOD of about 14-23 mg/L, COD 55 mg/L, TSS of 13 mg/L, Tot-P of 0.57 mg/L and Tot-N of about 5-10 mg/L. The effluent quality complies with the Norwegian discharge limit, which sets maximum permissible limits of 1 mg L^{-1} of P and 25 mg L^{-1} of BOD (Heistad et al. 2006). The effluent also fulfils the discharge requirements of the European Union Directives (EC 2018) and in most parameters for restricted use according to the WHO Guidelines for the safe use of greywater (Mara and Kramer 2008). However, the removal efficiency of indicator microorganisms was not satisfactory for unrestricted reuse according to the WHO guideline (WHO 2006) or to discharge to sensitive recipients. Only a 1 to 2 log reduction of *E. coli* was observed, which is in line with the findings with rotating biological contactor followed by sand filtration (Friedler and Galil 2003). Potentially high pathogens concentrations in the greywater may pose increased health risks. Although the present Norwegian regulations do not define discharge limits for indicator

organisms, the risks of faecal contamination are prominent and should not be overlooked (Stenström 2013). Therefore, the treatment system should be followed by a post-treatment step including UV disinfection.

4.1.2. On-site post-treatment of treated greywater

The choice of a post-treatment depends strongly on the characteristics of treated effluent mainly pathogens, on local standards set by authorities for reuse of treated effluent or discharge into the environment. For sensitive recipients that are close to drinking water sources as well as unrestricted reuse applications, multiple barrier systems including a post-treatment in an infiltration trench or other filtration units may be needed to minimize the health-related risks.

The post-treatment infiltration step in this study raised the total coliform and *E. coli* removal efficiency of the system up to 4.8 and 4.7 $\log_{10}$ reductions, respectively (**Paper I)**. Similarly, the results from **Paper II** indicate that the process of polishing using biochar, filtralite and unfilled considerably improved the effluent quality of the system. Biochar performed best in removing residual organic matter, total N, turbidity and odour. Filtralite was superior in removing P. The contribution of the polishing step in removing TCB and *E. coli* was remarkable. Biochar and Filtralite polishing filters contributed to raising the removal efficiency of the A02-GWT system from 1.49 $\log_{10}$ TCB up to 3.75 $\log_{10}$ and from 1.51 $\log_{10}$ to 4.21 $\log_{10}$ for *E coli* (**Paper II)**. The final effluent of the greywater treatment train in Paper I showed a TSS of <2 mg $^{-1}$L, and total P < 0.1 mg P L^{-1}, BOD <2 mg O_2 L^{-1} and *E. coli* < 5 MPN/100 ml. These values are comparable with the results obtained from greywater treatment using a submerged membrane bioreactor (Fountoulakis et al. 2016) and fulfil the US standard (NSF/ANSI 350-2012) (NSF/ANSI 2011) and Australian greywater reuse guideline (Environmental-Health-Directorate 2011). By integrating the polished effluent from biochar with UV, safe water for local unrestricted reuse, which can satisfy the WHO guideline (WHO 2006), can be produced.

4.2. Treatment of source-separated blackwater in a hybrid upflow sludge blanket anaerobic baffled reactor (USBABR) (Paper III)

This part of the study gives an insight into the performance of a new prototype upflow sludge blanket anaerobic baffled reactor (USBABR) as an integrated treatment system for source-separated blackwater (**Paper III**). The treatment concept is using the same principles as an upflow anaerobic sludge blanket (UASB) (Lettinga et al. 1980, Lettinga and Hulshoff Pol 1991, Zeeman et al. 2001, de Graaff et al. 2010a). Like the UASB reactor, this sludge blanket anaerobic baffled reactor uses upflow velocity for mass transfer, for influent–sludge contact and for releasing trapped gas. However, it differs from standard UASB by integrating two upflow zones in series, next to each other to obtain a low total height, as an anaerobic baffled reactor (ABR) arrangement. A solid-liquid separator included in each of the two ABR chambers with a similar purpose as the gas-solid-liquid (GSL) separator in the standard UASB configurations. In the USBABR tested, gas and liquid left the reactor in the same pipe, so a final separation took place outside the reactor.

The USBABR design intended to reduce the need for a larger height to diameter ratio while also retaining and degrading particles from the particle-rich feed at OLR and SRT similar to that of the UASB. It is an aim of the present study to evaluate if this can be achieved. The evaluation is carried out in a setting where the USBABR is preceded by a buffer tank that can serve as a pre-hydrolysis step and minimize the start-up period. The USBABR design is such that it does not require mechanical stirring except for that obtained by the feed pump.

The study evaluated effects of load and feed pulses on the performance of the reactor in terms of initial adaptation, stability, effluent quality, the removal efficiency of organic and suspended particulate matter, biogas production and methane yield. The operational conditions of the sludge blanket ABR and the performances in terms of COD removal and biogas production are indicated in

table 3 in comparison with the operational characteristics and performances of UASB, UASB-septic tank and CSTR. The removal efficiencies in terms of COD (78% for CODt and 89-91% for CODss) are quite comparable to the results obtained with that of the UASB reactor (de Graaff et al. 2010a, Cunha et al. 2018b, Zeeman et al. 2008) and UASB-septic tank (Kujawa-Roeleveld et al. 2005) and higher than CSTR (Wendland et al. 2007).

Table 4. Comparison of the operational conditions and performance of USBABR with the existing, proven technologies.

Parameter	This study USBABR		(de Graaff et al. 2010a) UASB	(Zeeman et al. 2008), Sneek UASB	(Kujawa-Roeleveld et al. 2006) $UASB_{ST}$	(Wendland et al. 2007) CSTR
	RI	RII				
Reactor volume (L)	16.4	15.7	50	50	140	200
Flow rate Q (L/d)	6	6	5.57	6	7	7
Upflow velocity (m/h)	1.5†	0.7††	0.76			
HRT (d)	2.8	2.8	8.7	8.3	29	20
OLR (g COD/L.d)	2.3	1.6	1	1.4	0.42	0.45
Start-up period (d)	120	90	200			90
SRT (d) $VSS_{(COD)}$	132	131	254	180	>365	20
Temerature (°C)	28	28	20	25	20	20
CODt R.Eff (%)	78	80	78	78	78	61
CODss R.Eff (%)	89	91	93	87	94	59
CODf R.Eff (%)	66	66				
Methanization %	69	73	54		60	60
CH4 (L/cap/d)	6-13	7	10	14	8,1	10

†lasts only for 12 and ††24 seconds per pulse with 90 min long pulse intervals. USBABR (upflow sludge blanket anaerobic baffled reactor, UASB (upflow anaerobic sludge blanket), $UASB_{ST}$ (upflow anaerobic sludge blanket septic tank), and CSTR (continuously stirred tank reactor)

The HRT used in this USBABR is about three times shorter than the standard UASB reactor. Moreover, the applied volumetric loading rate in the sludge blanket ABR (1.6-2.3 kg COD/m3/d) is higher than the UASB reactor (1.0 kg COD/m3/d) (de Graaff et al. 2010a), and much higher than applied in the UASB-septic tank (0.42 kg COD/m3/d) (Kujawa-Roeleveld et al. 2005) and CSTR (0.45 kg COD/m3/d) (Wendland et al. 2007). The USBABR has a smaller footprint requiring only 16 L volume reactor per person compared to the 50 L in a UASB reactor, 140 L CSTR and 200 L in UASB-septic tank reactor. The reactor configuration also allowed efficient solid-liquid separation and sufficient sludge retention.

4.2.1. Estimation of solid retention time in the USBABR

Solid retention time (SRT) is the key parameter affecting biochemical and physical properties of sludge and ultimately determining the amount of hydrolysis and methanogenesis in a UASB system at certain temperature conditions (Halalsheh et al. 2005). Previous studies have shown that the minimum SRT to achieve methanization and stabilization of the sludge is estimated to be 75 days at 25 °C (Zeeman and Lettinga 1999, Halalsheh et al. 2005). The SRT in this study was estimated based on the assessment of the sludge profile taken 204 days after the reactors started or 126 days after the end of startup period using the following equation (de Graaff et al. 2010a).

$$SRT = \frac{Sludge_{reactor}}{(Sludge_{wasted} + Sludge_{washedout})} \qquad 1$$

Where $Sludge_{reactor}$ is the amount of solids in the reactor (g VSS), $Sludge_{washedout}$, is the amount of solids that washed out with the effluent (gVSS/d) and $Sludge_{wasted}$ is the amount of solids that was wasted manually (gVSS/d).

The solid retention time of 132 d was estimated from effluent VSS_{COD} measurements (1g VSS = 1.42 g CODt (Zeeman and Lettinga 1999)). The real SRT in this reactor, however, is probably more than 132 days. The depth of the sludge

bed after 200 days was about 16 cm, which was still at 2/3 of the active reactor volume. Moreover, no excess sludge was removed, except for small samples for VFA analysis. During the first 18 weeks of the stable conditions, only 1.1 L and 1 L of sludge were removed manually. Based on these facts, and the data indicated from the results of TSS and CODss (Fig. 11 and 12), the stable performances of the reactors for more than 400 days without any regular sludge removal, suggests the actual SRT to be much longer than 132 days. As the effluent COD is characterized by more dissolved COD fractions (0.55 compared to 0.21 in the inlet) than particulate fractions (only 0.45 compared to the inlet 0.79), using VSS from effluent total COD, in this case, may overestimate the effluent sludge concentration and thereby underestimate SRT. The low and stable TSS (Fig. 11) and particulate COD (CODss) (Fig.12) in the effluent for more than 400 days after the end of the startup period revealed that the sludge was not washed out rather successfully retained within the reactors. This demonstrated that by changing the geometry inside the reactor, it was possible to reduce the height and volume of the reactor such that efficient sludge retention can be achieved while at the same time providing stable performances comparable to that reported for more standard UASB.

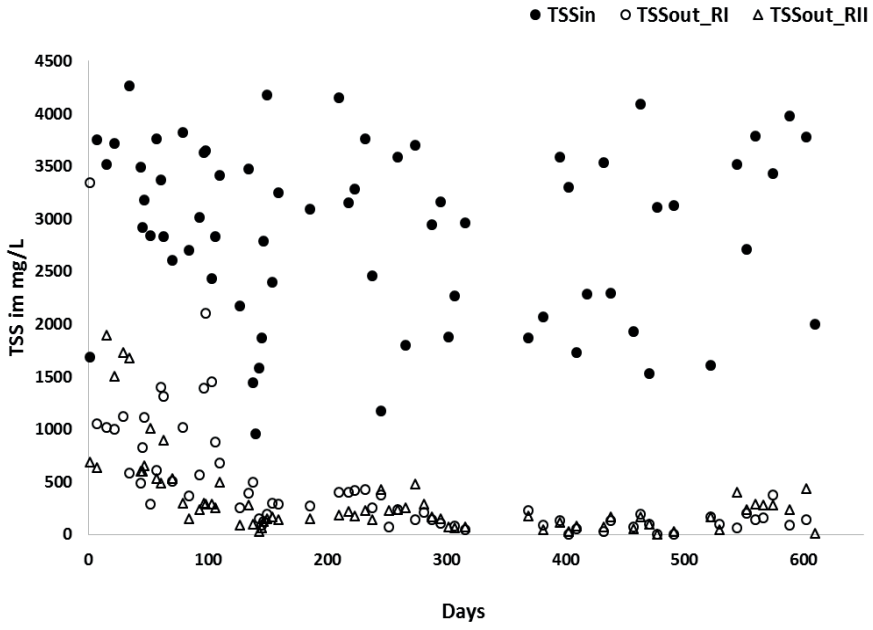

Figure 11. Inlet and effluent TSS during the startup and stable period

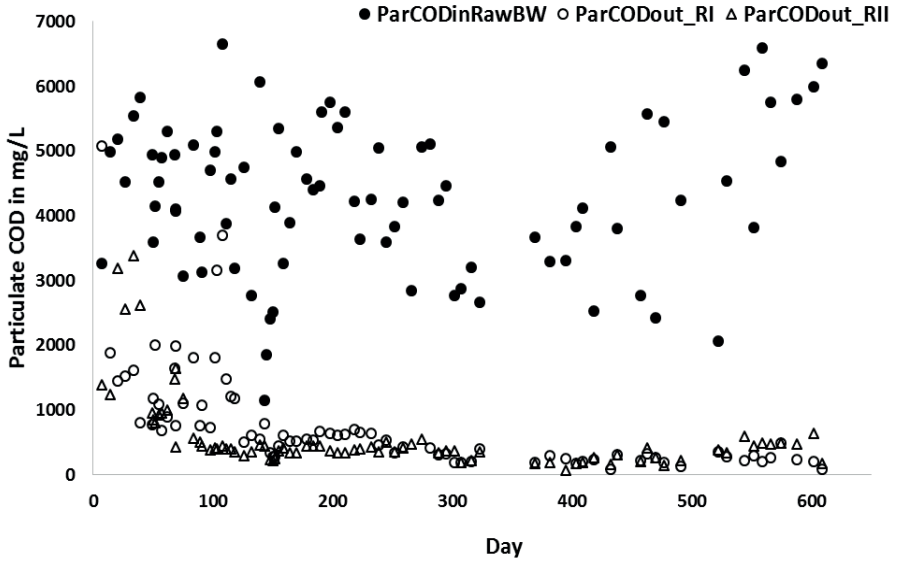

Figure 12. Particulate COD (CODss) concentration in the influent and effluent during the startup and stable period

4.2.2. Sludge bed development

For assessment of sludge bed development, making a sludge profile and determining the total quantity of sludge in the reactor it is recommended to install several sampling ports over the height of the reactor (Lettinga and Hulshoff Pol 1991). The reactor in the current study was made from a non-transparent Polyvinyl chloride (PVC) and was only 31 cm in height with an active reactor height of 24.5 cm. Due to the short height of the reactor; two sludge-sampling ports were placed on the top of the reactors, one for in the first compartment and the other in the second compartment of the USBABR. Hence, regular assessment of the sludge bed development was not possible, as it required the opening of these sampling ports.

However, after 200 days the sampling ports were opened, and a tube with a locker at the top was used to sample the sludge profile (Fig. 13). The sludge height after 200 days was about 16 cm (2/3 of the active reactor volume) in the sludge blanket compartment and 11 cm in the ABR compartment (half of the active ABR volume) with an average concentration of 43.5 g VS/L and 30.8 g VS/L for RI, respectively; and 39.4 and 36 g VS/L for RII chamber 1 and chamber 2, respectively. The fact that regular sludge withdrawal did not take place and the observed sludge height after 200 days is still at 2/3 of the active reactor volume indicated the slow buildup of the sludge bed. Looking into these particular cases, a longer SRT with about 3 days of HRT demonstrated the potential achievement of a further reduction in reactor height and volume. Moreover, lower concentration of VFA in the effluent in combination with higher total COD and suspended COD removal indicated the higher methanogenic potential of the reactor (Kujawa and Zeeman 2005).

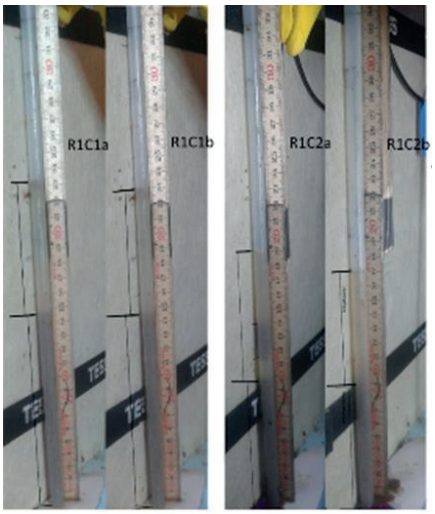

Figure 13. Sludge profile taken 200 days after the experiment started.

The geometry of the SBABR provides optimal biomass solids retention comparable to UASB reactor conditions despite a high overall reactor-loading rate. The relatively low sludge concentration in this second compartment of the reactor suggests that space is available for extra expansion of the sludge bed.

Even though the USBABR was not inoculated with anaerobic granular sludge at the start, the sludge bed at steady state, produced up to 5 mm diameter granules (Fig. 14). Although the characteristics of these granules have not been assessed in the present study, the formation of such granules is a prerequisite for proper anaerobic process operation (van Lier et al. 2016) and enhances the stable performance of the reactor. It has been reported that an introduction of calcium at concentrations from 150 to 300 mg/l enhanced the biomass accumulation and granulation process in UASB reactors fed with 4000 mg/L COD wastewater (Yu et al. 2001). In recent studies, formation of calcium phosphate (Ca-P) granules in the treatment of source-separated blackwater in UASB reactor showed the possibility of recovering P while facilitating improved methane production (de Graaff et al. 2011a, Cunha et al. 2018b). The formation of granules in the present study (Fig. 16) may not be due to Ca-P precipitation because of the low Ca content

in the influent BW (< 40 mg/L) and the fact that more than 86 % of total P is conserved in the effluent. This implies that only 14% of the P ended up in the anaerobic sludge which is low compared to about 40% obtained in other studies (Kujawa-Roeleveld et al. 2005, de Graaff et al. 2011a).

Figure 14. Active granules formed at the bottom of the first and second upflow sludge-blanket compartment of the ABR

The formation of granular and flocculent sludge enables the reactor to be operated for a long period without frequent excess sludge removal. However, infrequent sludge removal may be advantageous to avoid excessive sludge accumulation in the reactor. The longer SRT offers stabilized sludge and, thus, dewatering of sludge could be easy when sludge is removed. This also implies that sludge handling and disposal problems are minimized. The configurations of the reactor allow effective solid-liquid separation, mass transfer capacity for both feed pulses tested the possibility of achieving a high solid retention time (SRT) and high efficiency of organic matter removal.

4.2.3. Effects of organic loading rate (OLR) and feed pulse length

The effects of OLR and feed pulse length was pronounced at the early stage of the process. The removal efficiency of total COD during start-up phase varied from 24 to 67 % with an average of 48 % in Reactor I (RI) and from -4 to 74 % with an average of 36 % in Reactor II (RII) (Fig. 15 top). The reactor with longer feed length had a lower removal rate than with short feed pulse.

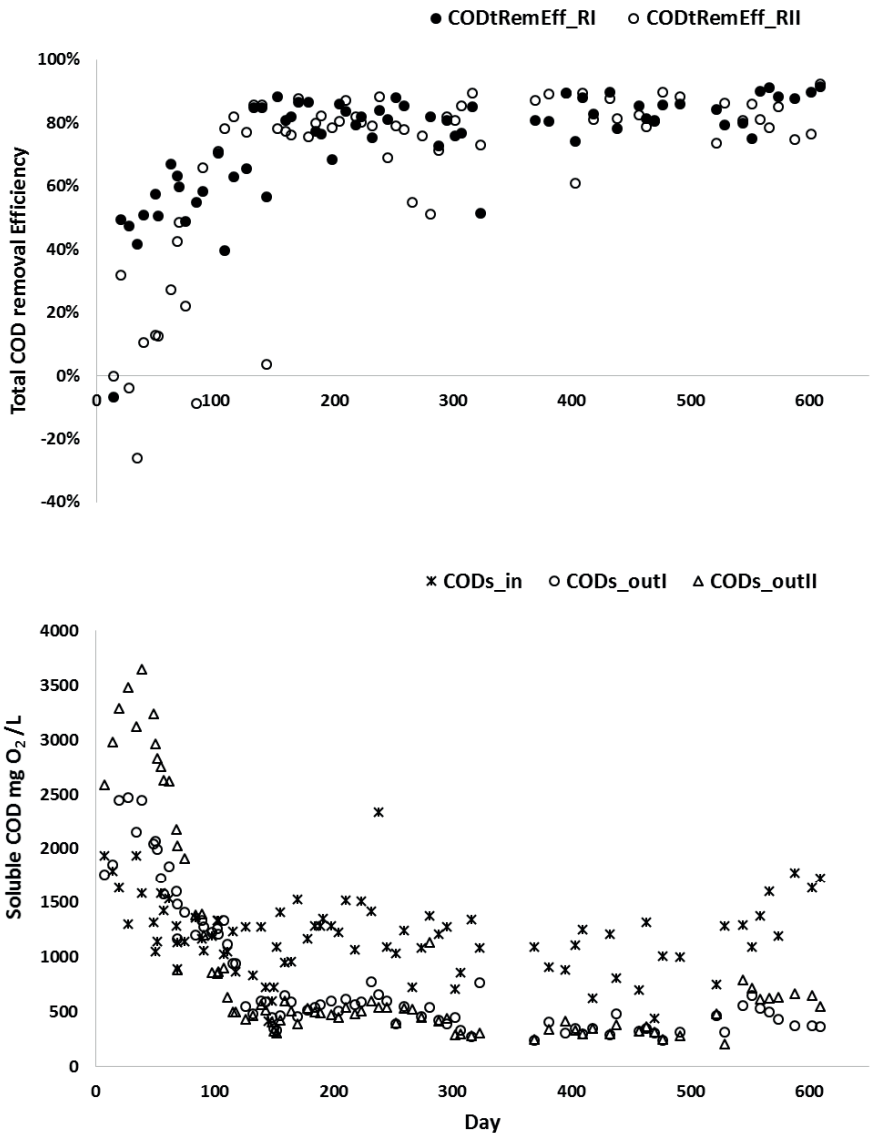

Figure 15. Total COD removal efficiency (CODt RemEff %) in RI and RII (top), and measured soluble COD (CODs) in and out of the reactors (bottom) during the start-up and after the stable performance (From Paper III)

This might be attributed to the effect of operational conditions on the level of hydrolysis. Less turbulent feeding gives more time for the particulate organic fraction inside the reactor which may lead to a greater hydrolysis of particulate COD and subsequent release of the solubilized fraction before converted into

methane. The soluble COD (CODs) removal efficiency was, therefore, negative for the first three months (Fig. 15 bottom). This increase in the dissolved fraction of the COD in the effluent was also accompanied by increased VFA values suggesting greater hydrolysis of the particulate organic matter. This high VFA in the effluent also indicates that the methanogenesis was the rate limiting step.

In this particular setup, hydrolysis started in the buffer tank. At the bottom of the buffer tank, a substantial drop in pH was observed (from an average of 9.12 ±0.38 in the equalization tank to 7.17 ± 0.56 at the bottom of the buffer tank). Similarly, the average filtered COD at the bottom of the buffer tank during this startup period was 4320 ± 2246 mg/L compared to the average filtered COD of 1382 ± 385 mg/L in the equalization tank. Thus, based on the equation described in Halalshes et al. (2005) and assuming no CH_4 is produced in the buffer tank, the average level of hydrolysis at the bottom of the buffer tank was estimated to be 68%. Besides the information from the filtrated COD, the VFA results before and after the reactor illustrate the strong hydrolysis process in the reactor as well. Taking the inlet COD from the buffer tank, the calculated hydrolysis level for the first 18 weeks of the stable condition showed about 72 and 74 % for RI and RII, respectively.

Moreover, the particulate COD fraction (CODss) in the raw blackwater was on average 0.79 which is in line with the values reported in the literature (de Graaff et al. 2010a). The effluent particulate COD fraction was, however, on average 0.45 and 0.44 in RI and RII respectively, which is by far lower compared to the influent CODss (Fig. 16). During this period, hydrolysis was not rate-limiting rather methanogenesis is lagging behind due to the slow growth rate of methanogenic bacteria resulting in incomplete degradation of the hydrolyzed organic matter and surplus concentration of dissolved organics in the effluent. This effect, however, declined with time and reached stable condition after 120 and 90 days for RI and RII respectively (Fig. 15 bottom). The sludge bed in the long-pulse fed

reactor stabilized in about 90 days, whereas, the short-pulse fed reactor took 120 days.

After the stable sludge bed developed, both the particulate and soluble organic fraction removal efficiencies were increased and stabilized with an average removal efficiency of 89 and 91 % for particulate COD and 66 % for the soluble fraction in RI and RII, respectively. The high hydrolysis of particulate organic matter and improved methanogenic activity of sludge resulted in a higher removal efficiency of the particulate substrate CODss. Moreover, higher biological conversion of the soluble substrate into biogas (~70%) increased the efficiency of soluble COD removal. At steady state, the low and stable effluent total suspended solids (Fig. 11), and VFA concentration indicated the stability of the performance of the system. The variation in the concentration of these parameters over time at the start-up phase was an indication of process instability.

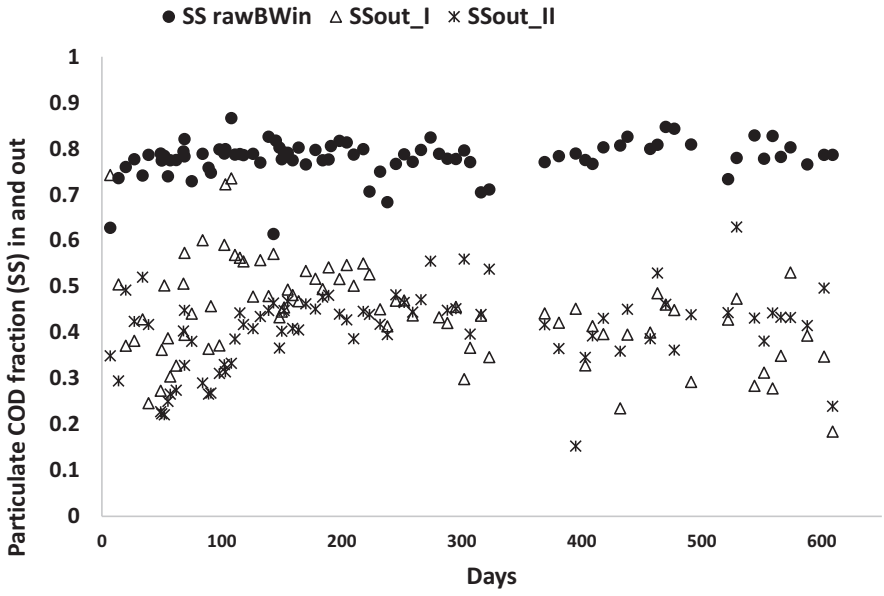

Figure 16. Particulate COD fraction (CODss) in the inlet and effluent

4.2.4. Effluent quality

As expected, at the startup period, the effluent had higher values for most of the measured quality parameters. The effluent sludge settling rate measured, using the Imhoff sedimentation cone, at 5 minutes and 30 minutes of sedimentation time was similar for both RI and RII. The effluent sludge from both reactors settled within five minutes. Thus, the change in the volume of effluent sludge between 5 and 30 min sedimentation time was insignificant ($p=0.81$ for RI and $p=0.66$ for RII). The settled effluent sludge volume was higher for RI than in RII except for the first few days. The higher effluent sludge volume in RI can be related to washout of active biomass due to higher upflow velocity (1.5 m h^{-1}) introduced during the short feed pulse. In immature reactors, higher upflow velocity may cause instability and removal of more biomass to the effluent, which is especially the case at the startup stage in RI, requiring a longer time to reach a steady state. However, both were close to zero after the stable condition reached.

Similarly, at the start-up phase, the total VFA concentrations in the effluents were higher (with an average of 893±473 mg/L for RI, and 1700±561 mg/L for RII) than the raw blackwater (440±234 mg/L) and reached peak values after two months in both RI than RII. This reveals that due to the slow growth rate of methanogenic archaea, the establishment of methanogenesis was lagging behind the hydrolysis/acidogenesis process and most of the VFA produced due to hydrolysis and fermentation leaves the reactor. Higher effluent VFA was observed in RII than in RI as less of the solubilized organic matter in this reactor is converted to methane.

Effluent VFA decreased sharply towards the end of the start-up period and reached below 90 mg/L. VFA concentrations below 150 mg/l as COD used as criteria for the stability of the anaerobic process (Wendland et al. 2007). Lower concentration of VFA in the effluent in combination with higher total COD removal indicated the higher methanogenic potential in the reactor (Kujawa and

Zeeman 2005). This low VFA concentrations in the effluent indicated that the process was stable and uninhibited in spite of high nitrogen concentration (Wendland et al. 2007). The acetate produced was converted into methane indicating the good establishment of methanogenesis after the stable condition is attained. The low effluent VFA concentrations and the stable pH value, therefore, prove that the digestion process is stable at a HRT of 3 days.

The effect of differences in feed pulse length on effluent quality was observed during the start-up period. At steady state, the average TSS, CODt and CODs in the influent were 2778±918, 5326±1537 and 1140±349 mg/L respectively. The average concentration of these parameters in the effluent during this period of operation was 189±130, 833±291, and 459±132 mg/L for RI and 169±115, 819±300, and 459±161 mg/L for RII, respectively. The removal efficiencies of the two reactors demonstrated no significant effects of organic load variation and feed pulse length on effluent quality after a stable condition was attained. The results of TSS, CODt, CODs, and VFA removal efficiencies were similar in both reactors at a confidence interval of 95 % with p-values of 0.241 and 0.197 for TSS and COD, respectively.

The removal efficiencies of USBABR in terms of COD (78% for CODt and 89-93% for CODss) are in line with the results reported using the standard UASB reactor (de Graaff et al. 2010a, Zeeman et al. 2008) and in UASB-septic tank (Kujawa-Roeleveld et al. 2005). Likewise, the effluent concentrations of NH_4-N (926 ± 113 mg/L for RI and 959 ± 188 mg/L for RII), and PO_4-P (84 ± 12 and 87 ± 17 mg/L for RI and RII, respectively) in both reactors were comparable but much higher than the concentrations in the raw blackwater (851 ± 174 mg/L NH_4-N and 60 ± 17 mg/L PO_4-P). The results were also comparable with effluent NH_4-N and PO_4-P from UASB-septic tank (Kujawa-Roeleveld et al. 2006) and CSTR where an increase in ammonium by 5 to 15% in the effluent was obtained (Wendland et al. 2007). Similarly, 91 % of the nitrogen, mainly as ammonium, and 61 % of phosphorus were conserved in the effluent of anaerobic treated source-separated blackwater

(de Graaff et al. 2010a). The higher effluent PO_4-P in the present study could be due to the diluted nature of the raw blackwater. A previous study using UASB on more diluted blackwater has also shown much higher phosphorus conservation (of 95%) in the liquid effluent (van Voorthuizen et al. 2008). The increase in effluent nutrient concentrations is caused by the conversion of organically bound nitrogen and phosphorus by hydrolysis (Kujawa-Roeleveld et al. 2005). This is one of the advantages of high rate AD as a pretreatment for renewable nutrient recovery.

4.2.5. Biogas production, potential methane recovery and COD mass balance

The average total COD during the experimental period was 5532 mg L^{-1}. This is very low compared to the average concentrations obtained from previous experiments on this blackwater (8900-11400 mg L^{-1}) (Todt 2015) and much lower than reported in the literature (9500-19000 mg L^{-1}) (de Graaff et al. 2010a, Zeeman et al. 2008). Biogas production in this source-separated blackwater ranged from 8.6 to 19 Ld^{-1} in RI and 6 to 10 Ld^{-1} for RII, with an average methane content of 70 ± 6 % and 74 ± 8 %, respectively. Accordingly, an average of 1.60 ± 0.06 g O_2 COD d^{-1} L^{-1} reactor volume and 1.20 ± 0.02 g O_2 COD d^{-1} L^{-1} reactor volume was converted to CH_4 in RI and RII, respectively. This translates into a methane conversion rate of 69 % and 73% relative to the inlet COD load and was relatively higher than a 54% methanization obtained in a UASB reactor (de Graaff et al. 2010a) and 60% with $UASB_{ST}$ (Kujawa-Roeleveld and Zeeman 2006) and CSTR (Wendland et al. 2007).

The high variation in biogas production may be related to the variation in the incoming raw blackwater (mainly due to dilution) which also showed significant variation in COD and TSS during the operational period. Residual COD fractions in the effluents represent 17 % and 20 % in RI and RII, respectively. On the other hand, the amount of COD retained or accumulated as biomass in the reactors were 14 % for RI and 5 % for RII implying slow build-up of the sludge bed. Lower

retained COD in RII is attributed to the higher conversion of COD to methane and more effluent COD. This suggests that, at steady state, slow feeding might have resulted in more contact with the active biomass in the sludge bed than the fast feeding allowing more biogas to generate. The entrapped gas can cause a gas lift, which may also force the light floccules to be transported to the top of the reactor and eventually leave the reactor. In the 18 weeks of the stable performance period, only 1.1 and 1 L of sludge was removed from RI and RII, respectively, This is beneficial from the operational point of view, as it demonstrates low sludge production and thus little withdrawal of excess sludge.

Considering the average daily biogas production of 12.5 L with 75% CH_4 content, the potential energy in the form of methane recovery from this source-separated blackwater will, therefore, be about 34 kWh/p/y (with a conversion factor of 35.6 MJ/m^3 CH_4 and 0.278 kWh/MJ). The energy gain in the form of electricity and heat is 11.5 kWh $p^{-1}y^{-1}$ and 17.3 kWh $p^{-1}y^{-1}$, respectively (with 85% efficiency of combined heat and power CHP (40% electricity and 60% heat) (de Graaff et al. 2010a)). The energy consumption by Jets vacuum toilet has been measured at 0.002 kWh for each toilet visit (WRS 2001). The annual energy consumption for flushing vacuum toilet is estimated to be about 3 kWh by considering four toilet visits per person per day to produce a 6 L BW. Assuming the heat recovered from methane is used to heat the reactor, a net 8.5 kWh $p^{-1}y^{-1}$ electricity can be gained. A higher electricity gain was obtained in BW treatment using a UASB reactor (de Graaff et al. 2010a). This may be due to the higher initial COD concentration in their blackwater.

It is also important to note that not all the CH_4 that is produced in the anaerobic treatment can be captured as potential energy. A significant amount could be lost in the effluent as dissolved methane. In the present study, dissolved methane was estimated once using the salting-out method with an average value of 10.42 mg/L and 12.49 mg/L for RI and RII, respectively. Due to technical difficulties, a further determination of dissolved methane was not continued. However, towards the

end of the experiment, a master student was involved to study the removal of dissolved methane using a moving-bed biofilm reactor (MBBR) and activated sludge reactor (ASR). In his study, it was stated that the average dissolved methane in the effluent of the USBABR was 1.05 mmol/L (16.8 mg/L) (Peterer 2018). This corresponds to a loss of 0.17 L methane per day which is low compared to the total daily methane production. However, a loss of 16.8 mg/L of methane is still considerable if emission to climate is considered. A higher value of dissolved methane up to 40 % of the total methane production has been reported in the literature (Souza et al. 2011). For climate change concerns, CH_4 must not be allowed to escape to the atmosphere but should be collected and used (McCarty et al. 2011). Methane is known to be 25 times more powerful than carbon dioxide in terms of the greenhouse effect and the high dissolved methane in the anaerobic effluent would increase risks of its release into the environment (IPCC 2007, Liu et al. 2013). Dissolved methane would also lead to the reduced energy efficiency of the anaerobic process (McCarty et al. 2011, Liu et al. 2013). Considering dissolved methane in the process of anaerobic treatment of source-separated sanitation treatment is therefore very important both in terms of efficient energy recovery and emission control.

4.3. Combined treatment and nutrient recovery approach for anaerobically treated blackwater effluent. (From Paper IV)

This part of the study (**Paper IV**) presents a systematic and multi-barrier approach to develop a combined treatment and resource recovery facility for processing source-separated blackwater. The system promotes closed-loop flows of resources and nutrients within the area close to the source of origin with novel post-treatment step and establishes mechanisms to contribute to a circular economy. Among the tested materials, granular activated carbon (GAC) was effective in selectively removing the residual organic substances, suspended solids, odour and colour while releasing most of the dissolved nutrients in the liquid phase. Figure 17 displays the concentration of NH_4-N, PO_4-P, macronutrients (Na, Mg, S, K and Ca) and micronutrients in the raw blackwater (BW), effluents of the anaerobic reactor (UASBII), Cocos char filter effluent, granular activated carbon effluent, and polonite treated effluent.

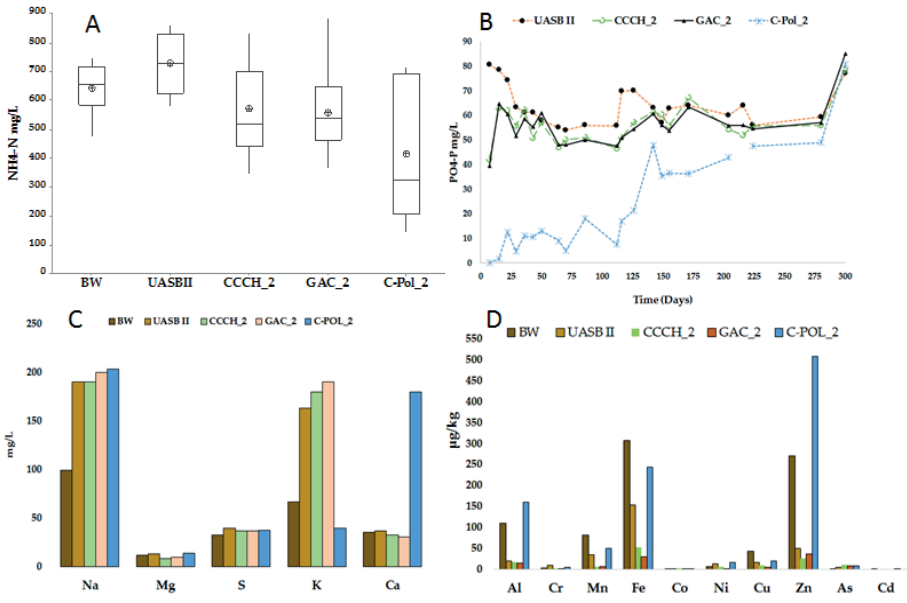

Figure 17. A) NH4-N concentration in mg/L, B) PO4-P concentration in mg/L, C) Macronutrients in mg/kg and D) Micronutrients and heavy metal concentration

μg/kg in raw blackwater (BW) and from effluents of AD reactor (UASB II) and post-treatment columns (CCCH_2 = Cocos char, GAC_2 = Granulated Activated Carbon, and C-POL_2 = Coarse Polonite). (From **Paper IV**)

More than 75% of the NH_4–N and more than 85% of the PO_4–P from the anaerobic effluent was released into the liquid phase of both carbon-based filter treatments. Similarly, a substantial amount of soluble K was also released in the liquid phase. Recovery of these valuable nutrients as liquid fertilizer, therefore, adds value to the circular economy and at the same time reduces its impact on environmental pollution. The risk of heavy metal in the raw blackwater, as well as the treated blackwater effluent, is negligible (WHO 2004) as indicated in figure 17-D. The heavy metal concentration in blackwater was by far lower than their presence in sewage sludge, livestock manure and artificial fertilizer, and comparable results were reported in the Netherlands (Tervahauta et al. 2014a). However, the concentration of Mg and other micronutrients are very low and could be limiting factors when used as a nutrient solution.

Although rich in plant nutrients, the major concern in the treatment and direct reuse of anaerobically digested blackwater is the associated health risk from pathogens. Anaerobic treatment systems are not designed to remove pathogens to a level that meet the required regulations (Chernicharo 2006). Disinfection mechanisms need to be integrated for the effluent from the anaerobic reactor to comply with local regulations for reuse or discharge and control of the health risk from pathogens. Integration of UV into the system resulted in the removal of *E.coli* to levels below the detection limit. Figure 18 shows the effects of the treatment chain - anaerobic digestion, filtration and UV light on the removal *E. coli*.

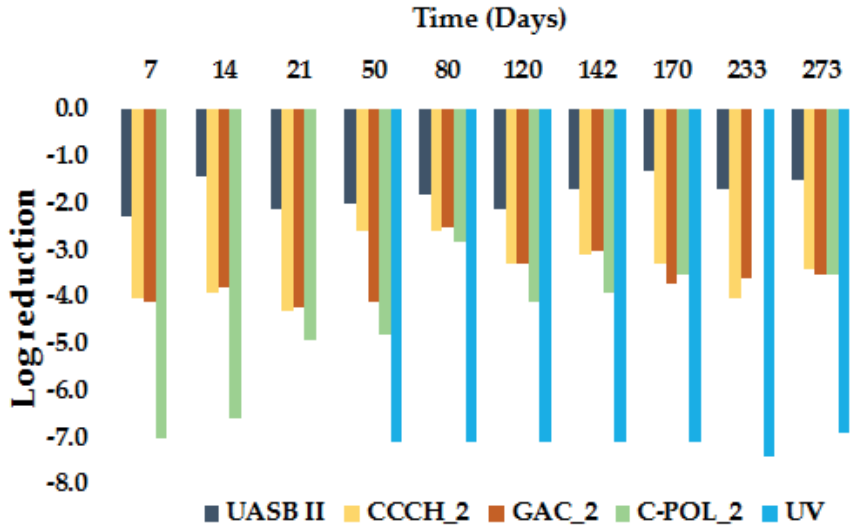

Figure 18. Effects of the treatment chain - anaerobic digestion, filtration and ultraviolet (UV) light on cumulative E. coli removal (where UASBII= AD effluent, CCCH_2 = Cocos char, GAC_2 = Granulated Activated Carbon, and C-POL_2 = Coarse Polonite). (From Paper IV).

These integrated technological approaches ensure synchronized nutrient recovery as a nutrient solution, pathogen inactivation, and reduction of active organic substances. Hence, by integrating the treated greywater system with the treated nutrient-rich solution from blackwater a source of value creation for various end-use options can be achieved with hygienic safety, aesthetics, environmental tolerance and economic feasibility. The nutrient recovery and recycling from blackwater not only replaces or supplements P (in particular) and N but also indirectly conserves energy and water used to produce an equivalent amount of these nutrients as chemical fertilizers.

4.4. Optimization of nutrient recovery and improving effluent quality (Paper V)

The hygienically safe and aesthetically good nutrient solution produced through an integrated post-treatment unit (**Paper IV)** can be used as a source of liquid fertilizer. However, the large agricultural areas required for application of such nutrient solution often result in long transportation distances. Hence, the large volume of the nutrient solution produced which constitute more than 95% of the anaerobically treated blackwater, will be challenged by the storage and transportation of the nutrient solution for use in agricultural fields. This requires, therefore, mechanisms for the up concentration of the nutrient solution in a small volume, such as, by a nitrification/distillation reactor for complete nutrient recovery (Udert and Wächter 2012). This, however, requires more energy input for the nitrification and distillation process (Udert and Wächter 2012, Fumasoli et al. 2016). Other technological options such as struvite (de Graaff et al. 2011a) and calcium phosphate (Tervahauta et al. 2014b, Cunha et al. 2018a) precipitation can be used to recover P. However, such precipitation does not allow N recovery. After P recovery as struvite or Ca-P, a high concentration of N still will remain in the effluent. A recent study revealed the effective removal of ammonium using microbial fuel cell (Kuntke et al. 2012).

The complete recovery of nutrients was suggested through the cultivation of microalgae with treated source-separated blackwater (Vasconcelos Fernandes et al. 2015) and concentrated urine (Tuantet et al. 2014a). The post-treatment of anaerobically treated source-separated blackwater with activated granular carbon resulted in a nutrient solution with colourless, odourless and very low turbidity, TSS, and residual organic matter (**Paper IV**). This might increase the light use efficiency of the microalgae in the photobioreactor. Such pretreatment also aids in removing pathogenic bacteria which could have contaminated the microalgae. Moreover, filtration through activated granular carbon and UV light

may reduce the micropollutant load into the photobioreactor and thereby in the microalgae biomass.

Although, growth of C. *sorokiniana* was possible with undiluted urine (Tuantet et al. 2014a) and anaerobically treated blackwater (Vasconcelos Fernandes et al. 2015), in this study *Chlorella sorokiniana* strain (CHL176) was found to be susceptible to the accumulation of NO_2-N and dilution was necessary. The productivity of *C. sorokiniana* with a 20% treated blackwater, with increased ammonium concentration was challenged by the nitrification process in the substrate storage tank. The biomass concentration of *C. sorokiniana* increased from 350 mg L^{-1} of the first day to 1200 mg L^{-1} in the 4th day with 20% treated blackwater. However, the biomass concentration declined and reached 170 mg L^{-1} on day 6 where the culture becomes pale yellow. This was assumed to be associated with an increased nitrite concentration in the stored substrate. The long retention time of the substrate, while stirring to supply a homogenized medium to the culture, resulted in partial oxidation of the NH_4-N and thus the nitrite concentration in the substrate gradually increased. A nitrite concentration of 72 mg L^{-1} resulted in bleaching out of the culture and higher effluent concentrations of NH_4-N, NO_2-N, and NO_3-N. The nitrite problem was not observed when the culture grew at lower substrate ammonium concentration (10% treated blackwater). To prevent *C. sorokiniana* from the possible toxic effects of nitrite, a short substrate retention time without further oxygen supply (stirring), and anaerobic conditions in the feeding tank to prevent oxidation of NH_4-N were tested (**Paper** V). In both conditions, the growth of *C. sorokiniana* was increased and maintained for long.

Preliminary results showed that *Chlorella sorokiniana* strain (CHL176) grown in a continuous culture using 10% - 20% treated blackwater as substrate, assimilated N and P at a rate of up to 212 mg NH_4-N L^{-1} d^{-1} and 35 mg PO_4-P L^{-1} d^{-1}, respectively (**Paper** V). The N and P removal rate obtained in this study was relatively higher than reported in anaerobically treated source-separated

blackwater in the Netherlands (Vasconcelos Fernandes et al. 2015) but much lower than from undiluted urine (Tuantet et al. 2014a). An average of up to 2.1 g biomass $L^{-1}d^{-1}$ or 50.4 g dry matter $m^{-2}d^{-1}$ was produced in the short light path of 30 mm of a flat panel photobioreactor with a continuous irradiance of an average of 1450 μmole photons m^{-2} s^{-1}. This corresponds to a biomass yield on energy of 0.4 g per $(\text{mole photon})^{-1}$. The biomass yield in this study was low compared to the results reported in the literature (Cuaresma et al. 2009, Tuantet et al. 2014a). The high biomass yield obtained by Tuantet et al. (2014a) and others may be due to the high surface to volume ratio of the photobioreactor used. Moreover, the photobioreactor used in those studies had a smaller light path (5 and 10 mm (Tuantet et al. 2014a)) which is 3 to 6 times smaller than the light path used in the present study. The length of the light path and the surface area to volume ratio could be the main factors for differences in biomass yield.

The nitrogen and phosphorus removal efficiency with 5 times dilution were 78% and 99.5%, respectively, and are similar with the results obtained from 5 times diluted urine at a light intensity of 1500 μmole photons m^{-2} s^{-1} (Tuantet et al. 2014a). Similarly, high recovery of nitrogen (75%) and phosphorus (100%) was also reported when growing *Chlorella sorokiniana* from anaerobically treated BW (Fernandes et al. 2017). The biomass yield on substrate was also calculated for N and P. Figure 19 presents the biomass yield on N ($\mathbf{Y}_{X/N}$), P ($\mathbf{Y}_{X/P}$), and energy ($\mathbf{Y}_{X/E}$) at steady state with an average value of 7.81 ±1.25, 46.83±7.83and 0.37 ±0.03, respectively.

The biomass yield on light in this study was higher than those observed in anaerobically treated blackwater (Vasconcelos Fernandes et al. 2015, Fernandes et al. 2017) but below reported on urine (Tuantet et al. 2014a) and the optimal value reported for *Chlorella sorokiniana* (Zijffers et al. 2010). Nutrient removal yields on light are directly related to the surface area of photobioreactor required to treat a certain amount of wastewater. Low overall biomass yield on light is an indication of high surface area requirement which will increase photobioreactor

investment costs (Vasconcelos Fernandes et al. 2015) and require an increased footprint.

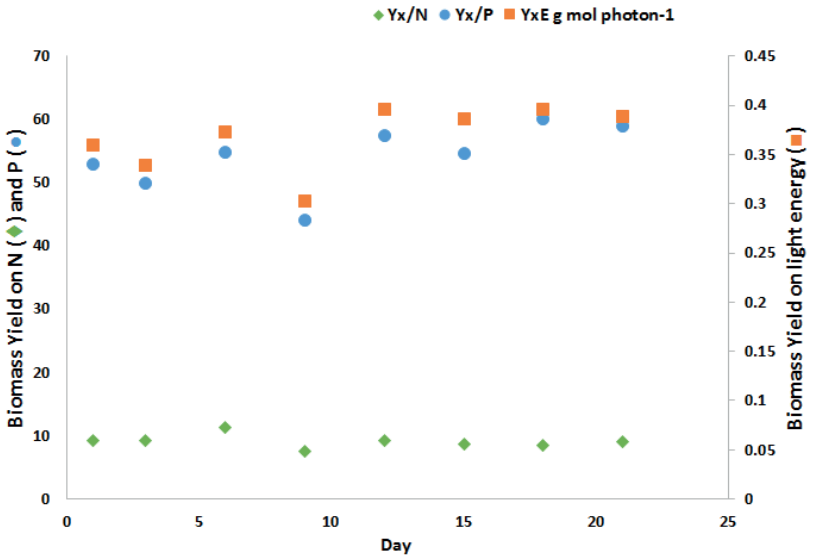

Figure 19. Biomass yield on N, P and light at the steady state

Low concentration of Mg and micronutrients in the treated blackwater (**Paper IV**) are limiting factors for the production of microalgae biomass using treated blackwater as substrate. Supplementation of these nutrients to the treated blackwater improved biomass production within a few hours and resulted in complete uptake of NH_4-N. This phenomenon has also been observed when concentrated and hydrolyzed urine was used as a substrate (Tuantet et al. 2014a). The complete retention of N and P in the microalgae biomass also resulted in an effluent quality that fulfils the discharge permit limit and can be either safely discharged or reused for non-potable use.

Microalgae are, therefore, one option to effectively assimilate and recover nitrogen (N) and phosphorus (P), as well as other macro- and micronutrients, and convert these nutrients into value-added uses. This efficient recovery of nutrients from treated blackwater via microbial biomass has the potential to enable a biobased circular economy (Matassa et al. 2015) and avoid the challenges of

storage and transportation of the liquid fertilizer for use in agricultural fields. However, the light and harvesting energy demands have to be evaluated with other options. In a circular economy concept, the microalgae grown on treated wastewater can be used as a slow-release fertilizer (Fuchs and Drosg 2013) or may be further processed for high value-added products such as bioplastics, cosmetics, colour dyes, etc. For direct consumption as a source of protein or animal feed, the careful analysis of pharmaceutical and heavy metal residues is needed (de Wilt et al. 2016, Butkovskyi et al. 2017).

4.5. The contribution of the new perspectives of domestic wastewater to food security and green development

Domestic wastewater is both a challenge and an opportunity. While the main sanitation objectives are the protection of public health and the environment, researchers in the last few decades also focused on the resources contained in domestic wastewater such as nutrients, energy and water (Larsen and Gujer 1996, Zeeman and Lettinga 1999, Otterpohl 2003, Larsen et al. 2009). The reduction of water use in sanitation systems, greywater treatment for reuse, recovery and recycling of nutrients (particularly P) and recovery of energy from human excreta are other important goals addressing the need to close the resource loop (Cordell et al. 2011).

About 80 to 96% of domestic used water at the household level is less polluted and can be reclaimed with appropriate and efficient treatment systems. At the same time, the major challenges in the domestic wastewater come mainly from a small fraction (urine and faeces representing only 1 % by volume) of the total domestic wastewater (Fig. 8). Except for pathogens, pharmaceutical residues and hormones, the bulk volume and components of this fraction is water, organic matter (source of energy) and nutrients that are recoverable renewable resources. The challenge is, thus, related to developing innovative technologies that maximize the resource potential and minimize the risks. The small size (by

volume) of the blackwater enabled us to develop an effective method of resource recovery and containment and removal of the risk with a relatively low cost.

Figure 20 presents an illustrative overview of the works and results of this thesis. It demonstrates the treatment chain and values of domestic wastewater as a source of alternative nutrient, energy and water resources. Source-separated blackwater treatment train produces energy (electricity and heat) and sanitized nutrient solution (N, P, K). The greywater treatment chain produces sanitized water for non-potable purposes. The bottles at each step indicate the quality of the treated effluents, and at the final stage a good aesthetic quality with very low turbidity, colourless, odourless, and hygienized nutrient solution and water are produced. Thus, properly managed and treated domestic wastewater can be regarded as a NEW-resource (Nutrient-Energy-Water) platform for sustainable sanitation, food security and green development. Implementation of these systems leads households or communities to be production units of nutrients, energy and water. This opens up new opportunities for social engagement and empowerment with new ways of achieving mainly the economic, environmental and social benefits of domestic wastewater management and urban agriculture.

With the concept of source-separation, the large fraction of the less polluted greywater will be separately collected and treated as described in **Paper I & II**. The treated water can be used as an alternative water source for non-potable uses. The post-treatment filtration step in this study resulted in efficient removal of residual organic matter, turbidity, nutrients and indicator micro-organisms (**Paper I**). Moreover, biochar and Filtralite polishing filters improved *E. coli* removal efficiency of the A02-GWT system from 1.51 log10 to 4.21 log10 (**Paper II**). By integrating the polished effluent from the two systems with UV, safe treated water for local unrestricted reuse, which can satisfy the WHO guideline (WHO 2006), can be produced. Reusing such treated greywater, for example, for conventional toilet flushing and laundry, can reduce the drinking water

consumption by 42 % (Hernandez 2010). This will have a significant contribution in water-scarce areas.

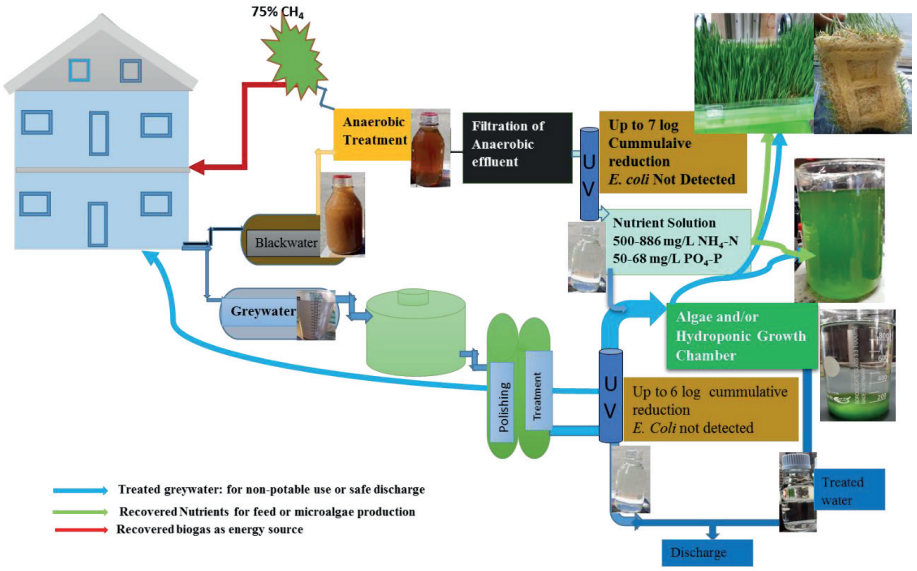

Figure 20. On-site domestic wastewater management with a focus on resource recovery towards a circular economy

Additionally, the integrated approaches of separate collection of blackwater with vacuum or low flush toilets and the development of a novel combined treatment and resource recovery facility (**Paper III, IV and V**) demonstrate how technological innovation in cities could contribute the recovery of energy and nutrients for local use. A net electricity gain of 8.5 kWh $p^{-1}y^{-1}$ can be obtained as the potential energy recovery from methane (**Paper III**). The power consumption of the greywater treatment system was determined to be 0.3 kWh/m^3 and taking into account an average greywater production of 108 L per day per person (Todt et al. 2015), which corresponds to 13 kWh $p^{-1}y^{-1}$ (**Paper I**). The electricity gain from anaerobic treatment of source-separated blackwater (**Paper III**) can, therefore, cover more than 50% of the energy demand for greywater treatment. Another 4.7 kWh $p^{-1}y^{-1}$ can also be gained from water saving by using a vacuum toilet. Thus,

the energy gain from water saving and methane recovery can cover the energy demand for greywater treatment.

The results of **paper IV** shows that using selective adsorbent (such as Cocos char and activated granular carbon) for the removal of residual organic matter and possibly micropollutants, simultaneous recovery of both N and P was possible in the liquid phase as a nutrient solution. This could be an ideal nutrient recovery option which can be used for local food/feed production. Moreover, up-concentration of nutrients from the recovered nutrient solution (**Paper IV)** might be necessary to overcome the challenges of storage and transportation of the liquid fertilizer. Although several options are available for the recovery of P e.g. in the form of struvite (de Graaff 2010, Ronteltap et al. 2010, Hug and Udert 2013), or calcium phosphate (Tervahauta et al. 2014b, Cunha et al. 2018a), the simultaneous recovery of both N and P could be achieved by using microalgae (**Paper V**). The assimilation of both N and P in microalgae biomass resulted in a high effluent quality which is fulfilling typical discharge limits for treated wastewater.

Moreover, using the hygienized nutrient solution recovered from the source–separated blackwater (**Paper IV**) as a source of nutrient and the treated greywater (**Paper I & II)** as source of water, a hydroponic salad and animal feed production can be designed (Fig. 20). Concerns from pharmaceutical and personal care product residues in the by-products, however, remain high (Hernández Leal et al. 2010, Butkovskyi et al. 2015) and the risks associated with the exposure to these micropollutants should be assessed (Hernández Leal et al. 2010) whenever reuse is planned. In relation to microbial and heavy metal risks, a recent study on hydroponic production of lettuce using treated greywater and urine (as source of nutrient) in a green wall structure has shown that both the microbial (based on the QMRA) and heavy metal health risks were not significant (Eregno et al. 2017).

Nutrient, energy and water recovery from source-separated domestic wastewater streams in cities could, therefore, contribute to urban agriculture with a positive impact on society, the economy as well as on the environment. With this system of circular resource flow aiming at zero-waste in cities, periurban and rural areas, not only resources, which have contributions to the achievement of green development and food security, are recovered, recycled and reused, but also public health and the environment can be sustainably protected.

5. Conclusion

The combined processing units and technological pathways used in this study demonstrated their potential to treat source-separated domestic wastewater streams. This approach facilitates recovery and local reuse of resources originating from domestic wastewater while reducing the undesirable impact on the ecosystem. Based on the work presented in this thesis, the following conclusions can be drawn with the respective processes and applications:

- Separate collection of BW from the rest of household wastewater streams resulted in a significant reduction of the organic and nutrient loads to greywater. Treatment of the greywater using a compacted biological treatment plant could be an appropriate solution where soil infiltration is restricted and especially in areas close to drinking water sources. Overloading the system up to 150% of the nominal loading did not affect the removal efficiency of the system for TSS and total P_{tot} ($p > 0.05$). However, for organic matter, lower removal efficiency was observed. For unrestricted reuse applications or discharge in sensitive areas such as near drinking water sources, a separate collection of blackwater in combination with a multiple barriers approach for the treatment of greywater including filtration as post-treatment is recommended to minimize the related health risks. **(Paper I and II)**

- Sludge blanket anaerobic baffled reactor as pre-treatment system for source-separated blackwater resulted in about 80% removal of organic matter and more than 90% TSS at steady state. Feed pulse length influenced the early phase of the AD process significantly, but similar performances were observed at steady-state. Biogas production ranged from 6 to 19 L d^{-1} and achieved an average methane conversion of 0.69 and 0.73 g CH_4-COD $g^{-1}COD_{in}$ at steady-state for short and long feed pulses respectively. The net energy gain in terms of electricity from this system

was 8.51 kWh $p^{-1}y^{-1}$ (30 MJ $p^{-1}y^{-1}$). Moreover, the configuration of the reactor resulted in a better solid-liquid separation, sufficient sludge expansion volume, and longer sludge retention (>132 days). The USBABR require only 16 L volume reactor per person and, hence, has a smaller footprint. **(Paper III)**

- The post-treatment system designed for effluents of anaerobically treated blackwater achieved a high effluent quality in terms of organic matter, TSS, turbidity and indicator bacteria. Both carbon-based filters removed 80% of the residual organic matter, more than 90% of residual TSS, and 93% of the turbidity and UV_{254} absorbance from the effluent of the anaerobically treated blackwater while retaining the majority of N, P and K in the liquid phase as a highly valuable liquid fertilizer. The system also overcomes the challenges of source-separated blackwater including the unpleasant aspects of smell and aesthetics, the need for long-term storage for disinfection, and the risks of unwanted precipitation of phosphorus compounds. Phosphorus and ammonium recovery from blackwater in this way, in turn, reduces the unwanted enrichment of surface water, thereby reducing the associated environmental impact. **(Paper IV)**

- *Chlorella sorokiniana* strain CHL176 can assimilate and recover N and P at a rate of up to 235 mg NH_4-N L^{-1} d^{-1} and 45 mg PO_4-P L^{-1} d^{-1} at a dilution rate of 0.06 h^{-1}, and a 1:10 dilution to 1:5 dilution of treated blackwater under ideal growth conditions. Mg and trace elements are limiting factors when using diluted treated blackwater as a substrate and supplementation is necessary. After microalgae harvesting, the effluent from 10% treated blackwater substrate fulfils the discharge permit limit. **(Paper V)**

Overall, this project outlines the development of integrated/combined processing units to treat domestic wastewater and at the same time recover valuable

resources. The integration of source-separated sanitation, anaerobic digestion of blackwater, and microalgae biomass production may deliver a win-win-win solution for domestic wastewater treatment challenges. It addresses issues of water and wastewater management, energy and nutrient recovery, avoids contamination of water bodies and emissions of odours and greenhouse gases, and microalgae biomass can be used as a raw material for numerous purposes depending on the biomass quality and quantity. The separate treatment technologies aiming at the separate flows fit for reuse or recycling have shown good performances. The treatment chain successfully removed COD, TSS and indicator pathogenic microorganisms. Integration of the different treatment units, thus, maximizes the benefits of domestic wastewater by recovering resources while minimizing negative impacts on the environment and associated health risks. The final effluent from both greywater and blackwater streams can be used for non-potable purposes in water shortage areas and also meet the discharge permit requirements for sensitive areas.

Outlooks

The results from this thesis demonstrated the potential of source-separated greywater for local water reuse and the separate blackwater treatment for energy and NPK recovery as a nutrient solution. However, the value chain and innovative logistics to return the recovered nutrients to farmlands or use as a raw material for the industrial processes like Haber-Bosch have to be considered in the future. Moreover, awareness creation and marketing strategies towards the recovered nutrients has to be made. Setting up service enterprises and implementing income-generating measures, which will facilitate the adoption of source-separation systems in the urban, and periurban areas should be sought. Future research should also focus on the legislative requirements for safe use of by-products from the recovered resources. Unfortunately, the knowledge gaps regarding the presence and fate of pharmaceuticals and their metabolites in this

treatment chain make it difficult to adequately determine the level of the risks associated with reuse options and the potential added-values of treated domestic wastewater. Although, recent studies have demonstrated that AD was not found to be effective in removing persistent pharmaceutical and personal care product residues (de Graaff et al. 2011b, Butkovskyi et al. 2015), the combined effect of the AD in the USBABR, activated granular carbon filtration, and UV treatment on micropollutants needs further investigation. Furthermore, life cycle impacts for ecosystem quality and human health and techno-economic (life-cycle cost) analysis integrated with quantitative microbial-pharmaceutical risk assessment have to be carried out to validate the recycling process and safe reuse of by-products.

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Appended Papers

Paper I

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Performance study of biofilter system for on-site greywater treatment at cottages and small households

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ABSTRACT

The current contribution of microbial pathogens and nutrient discharge into the environment from inefficient on-site wastewater treatment systems has raised concern in many areas due to the pollution of the nearby water recipient. To overcome this challenge, a novel and more robust proven treatment systems are required. This paper aims to assess the performance of a source separating wastewater management system for the removal of organic matter, total P, total suspended particles and *E. coli*. The system is a multi-stage approach including – a separate collection of blackwater (BW) and greywater, followed by on-site greywater treatment system in a fixed-film biofilter and finally a soil infiltration system used as a polishing step before discharging into the environment. The separation and collection of BW resulted a notable reduction for chemical oxidation demand (COD), biochemical oxidation demand (BOD), total suspended solids (TSS), nitrogen (N) and phosphorus (P) accounting for 64%, 61%, 75%, 85 and 88%, respectively. The overall removal efficiency of the system for the above-mentioned parameters reached over 90% at the biofilter effluent and more than 95% at the bottom of the constructed infiltration column. For coliform bacteria and *E. coli*, the overall system reached a reduction of 4–5 $\log_{10}$ units of which the major reduction was observed in the infiltration columns. The effluent quality from this source-separating and multi-barrier biofilter treatment system complies with the Norwegian discharge limits. The assessment results reveal that this system can be used in drinking water source catchments with minimum environmental and health related risks.

1. Introduction

The European water directive regulates treatment and discharge of wastewater based on the eutrophication sensitivity of the watersheds within a particular area. While greater efforts have been taken on the improvement of centralized wastewater systems in urban centers, little attention was so far given to rural areas, which are accounting for a notable fraction of the total wastewater production, especially in the northern countries. Based on statistics from 2015 (Berge and Chaudhary, 2015), 16% of the Norwegian population are not connected to central sewerage systems. Likewise, about one million residents in Finland, 20% of the total population, and over one million vacationists lives in houses that are not connected to the municipal sewer network (Lehtoranta et al., 2014). In addition to the 330,000 on-site systems for rural residents, more than 420,000 recreational houses are currently found in rural Norway (SSB, 2016) with the result that none-severed rural areas contribute with 24% of the wastewater production in the country. In 2014, the estimated nutrient discharges from rural households were approximately 350 tons for phosphorus and 3010 tons for nitrogen (Berge and Chaudhary, 2015) which is a significant fraction to affect the recipient water sources. Similar trend was also observed in Finland (Lehtoranta et al., 2014). In addition to eutrophication effects, on-site wastewater systems may pose a health risk to consumers of drinking water by spreading of pathogens to raw water catchments or by direct contamination of local wells. Hence rural wastewater management needs to be improved in order to sustain or improve the environmental quality and to protect human health.

Especially recreational houses that increased substantially both in number and size as well as in standard of sanitary facilities contributes with an increasing challenges in terms to rural wastewater management (Kaltenborn et al., 2009; Rye and Berg, 2011). Geological conditions on high mountain areas, where a majority of the cottages are located, often limit the applicability of soil infiltra-

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tion, which is the dominant type of wastewater treatment (29%) in rural Norway (SSB, 2016). On particular places package treatment plants were installed as an alternative, but these systems were also shown to struggle with the highly varying loading conditions and limited maintenance, resulting into frequent malfunction periods with high discharge of pollutants (Schwemer and Wolfgang, 2016). Novel and more robust wastewater treatment systems need therefore to be developed to handle the increasing environmental pollution from Norwegian recreation homes.

Source separating sanitation was pointed out as a potential solution to meet the challenges in rural and recreational wastewater management (Jenssen et al., 2016). In this approach, only the so called greywater originated from kitchen and washing facilities is treated locally while the notably higher polluted blackwater originated from toilets is collected and transported to a centralized treatment or recovery facility. Many different kinds of on-site wastewater and particularly greywater treatment systems have been developed and tested worldwide. Moreover, in countries like the USA and Australia, where regulations for the use of GW have been well established based on issues associated with public health and potential environmental impact, GW treatment for non-potable use is highly encouraged (Oron et al., 2014). However, the bulk of small-scale GW treatment systems currently proposed are either simple filtration systems providing minimal treatment, or are treatment systems, which are not designed to handle the differences in both flow and composition and are therefore not suitable (Gross et al., 2007). Others are complex treatment processes incorporating sedimentation tanks, bioreactors, ultra- and nano-membrane filtration, coagulants, and direct disinfection, which are more costly in terms of energy, operation and maintenance.

A multi-stage process consisting of several partially redundant treatment steps in series offer a relatively high treatment stability despite the variable loading rate. At present only little data are available on such source separating sanitary systems and these are mainly gathered from larger-scale pilot installations in urban regions (Todt et al., 2015). This study performed a comprehensive experiment to assess treatment efficiencies and effluent quality for each particular treatment step in a rural configuration of a source separating sanitary system. Post treatment system using column filtration to mimic soil infiltration trench was carried out to study the application in vulnerable areas and where discharge requirements are very stringent.

2. Methods

2.1. Source separating sanitary system

This study was done with greywater (GW) and blackwater (BW) supplied by a student dormitory with 48 inhabitants. The BW collected with vacuum toilets having a flushing volume of 1.2 l and the greywater is collected and pumped separately into two separate stirred storage tanks in the laboratory. More details are given in Todt et al. (2015). For both wastewater fractions (GW, BW), grab samples were taken from the particular stirred storage tank. The concentration in a putative mixed raw sewage (C_{raw}) was calculated considering an average BW fraction of 5.5% on the total wastewater volume as determined by Todt et al. (2015). This calculation was done with help of random variable algebra considering the measured concentrations ranges for greywater (C_{GW}) and blackwater (C_{BW}) as normal (COD, BOD, TSS, P) or log-normal (Coliform bacteria) distributed random variables, while a constant value was taken for the volume fraction of blackwater (f_{BW}) to avoid ratio distribution (Eq. (1))

$$C_{raw}(\mu,\sigma) = C_{GW}(\mu,\sigma) * (1 - f_{BW}) + C_{BW}(\mu,\sigma) * f_{BW} \quad (1)$$

Table 1
Diurnal distribution of greywater into the GWTP.

Time frame	Volume fraction (%)
0:00–07:00	no load
07:00–09:00	40
09:00–12:00	15
12:00–19:00	no load
19:00–21:00	30
21:00–0:00	15

2.2. Greywater treatment system

The study used a greywater treatment GWT system (Ecomotive A02, Ecomotive AS, Runde, Norway) designed for cottages and small households (Heistad, 2008). The GWT system encompasses a sequence of a primary settler, an unsaturated fixed-film biofilter and a secondary clarifier. For the fixed film biofilter lightweight clay aggregates having a diameter of 10–20 mm (LWA) (Filtralite, Saint-Gobain Byggevarer AS, Alnabru, Norway) is used. The filter bed has a thickness of 500 mm. After primary settling, the greywater is distributed over the biofilter in intermittent pulses via full cone nozzles as described in Heistad (2008). The dosing pump was controlled by a level switch in the primary settler and a timer giving the pulse intervals. The filter is designed for a nominal load of 650 l d^{-1}, which results into a surface load of 282 mm d^{-1}. The biofilter is supposed to serve for a longer period, but to sustain its efficiency, a resting period of two or three weeks in a year is required.

The GWT system was loaded based the European test protocol for package treatment plants (NS-EN 12566-3:2005 + A2:2013) with a diurnal distribution of hydraulic load (Table 1). Feeding of the GWTP was performed with a peristaltic pump (Bredel SPX, Watson Marlos, Falmouth, UK) and hydraulic load was monitored with a flow meter (Optiflux2000, Krohne, Duisburg, Germany). Grab samples were taken from the effluent of the secondary clarifier. The power consumption was monitored with a power meter connected to the 230 V AC supply of the GWTP.

The data from the GWTP were collected from April 2013 to Mai 2016. In total, the system was in operation for 458 days in four continuous periods lasting from 28 to 223 days related to different experiments and performance tests that were conducted with the system. The latter included different sequences with overload, underload and simulated power breaks as outlined more in detail in Table 2.

2.3. Infiltration trench as a polishing step for the GWTP effluent

To gather more data on the recommended post polishing in an infiltration trench, a column experiment was establish (Reiakvam, 2016). During this period, the GWTP was operated with nominal load. The experiment encompassed two parallel columns having a diameter of 600 mm. Each column represents a discharge point in an infiltration trench with a single-hole in the perforated disposal pipe that is placed on the top of the infiltration trench in the actual disposal system. The infiltration material used in this experiment consists of 150 mm drainage layer of 11–22 mm crushed granite stone at the bottom and sequentially overlaid by 150 mm of 0.2-1.0 mm fine sand dominated by silicon dioxide in the form of quartz and 150 mm of 2–4 mm LWA (Filtralite, Saint-Gobain Byggevarer AS, Alnabru, Norway). Single geotextile cover separated the layers and the trench is covered with 200 mm of till soil (sandy loam) at the top to mimic backfill (Fig. 1). Each of the infiltration columns was loaded with GWTP effluent with peristaltic pumps at an actual flow rate of 2.5 l h^{-1}. The infiltration took place via a pipe having 6 mm inner diameter to the center of the column on the top of the LWA layer, giving a total filtration depth of 450 mm (Fig. 2). Loading of the

Table 2
Loading sequences.

Loading sequence	Hydraulic load (L d^{-1})	number of periods	total length	number of samples
Nominal load (100%)	650	8	435 days	50
Overload (150%)	975	2	10 days	7
Underload (50%)	325	1	12 days	3
Power break	650	4	8 days	8
Loading breaks	no load	4	647 days	4[a]

[a] Samples were taken within the first 3 days after restarting load.

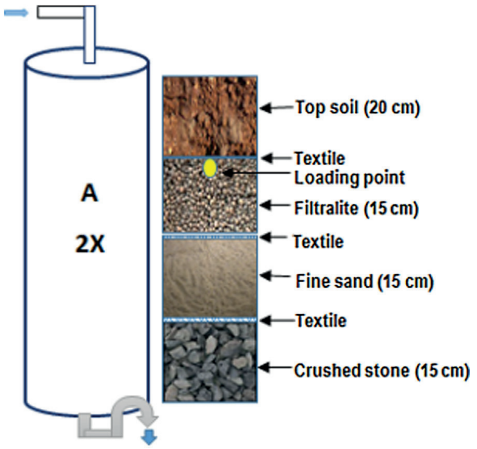

Fig. 1. Cross section of an infiltration trench column consisting the following layers: 200 mm of top soil (excavated at Ås, Norway); 150 mm of lightweight aggregate LWA 2–4 mm; 150 mm of fine sand 0.2-1.0 mm; 150 mm of crushed granite stone 11–22 mm, giving a total filtration distance of 375 mm.

columns coincided with the operation periods of the biofilter in the GWTP. The latter were determined to have a total length 15 h d^{-1} at the nominal load of 650 l d^{-1}. This implies that the filter has a long resting time, which allows sufficient time for drainage. This will prolong the lifetime of the infiltration system. Considering these figures, each of the columns reached a hydraulic load of 37.5 l d^{-1} corresponding to 150 mm d^{-1} over the whole column cross section area (Reiakvam, 2016).

Sampling from the infiltration columns was carried out in three 3-day sampling periods. The first period (P1) started 20 days, the second sampling period (P2) after 41 days and the third sampling period (P3) 118 days after the infiltration started. Analysis was done, based on grab samples taken from the center of the bottom plate via a drainage pipe having 15 mm diameter. For P1 and P2 the drainage pipe was open to the atmosphere representing deep unsaturated zone. For P3, the out let pipe is bent upwards with a water lock of 50 mm in order to simulate a ground water level at the bottom of the column, which keep the same pressure on the top of the ground water table in the actual field.

2.4. Lab analysis and mass load calculations

Grab samples were taken from inlet, GWTP outlet and final effluent under the normal loading and different stress events. BOD_5 was analysed with a manometry respirometric method (Oxitop, WTW, Weilheim, Germany). For COD, total phosphorus (P), total nitrogen (N) spectrophotometric test kits (Hach-Lange, Berlin, Germany) were used. Total suspended solids (TSS) were determined with 1.2 μm glass fiber filters (Whatman GF-C, GE Healthcare, Little Chalfont, UK). Filtrated COD was taken from the filtrate. *E. coli* was determined following the standard analytical methods (American Public Health Association (APHA), 2005) using Colilert 18 test kits (IDEXX Laboratories Inc, Maine, US).

The obtained reduction efficiency (R_{eff}) for mass or cell numbers load within the different treatment steps are calculated based on the average values that have been determined for a putative combined raw sewage (C_{raw}) and the corresponding sampling place X C_X for each of the parameters (Eq. (2)).

$$R_{eff} = C_x * (1 - f_{BW}) / C_{raw} \quad (2)$$

3. Results and discussion

The performance of the source separating sanitary system was assessed by evaluating its removal efficiency for organic matter, TSS, total P and indicator microorganisms. The subsequent effect is the result of a combination of biological and mechanical processes. Fig. 2 shows the average concentration of COD, BOD, TSS, P_{tot} and TC and *E. coli* for the combined sewage, raw greywater, GWTP effluent and infiltration trench effluent and the mass load reduction at each level. The mass load reduction line indicates the removal efficiency for each treatment step. For those parameters, an overall treatment efficiency of more than 90% was reached at the effluent of the fixed-film biofilter and more than 95% at the bottom of the constructed infiltration columns (Fig. 2). For coliform organism, the overall system reached a reduction of 4–5 log of which the major reduction was observed in the infiltration columns (Fig. 2).

3.1. Separation and collection of blackwater

The separation and collection of BW resulted into notable reductions for COD, BOD, TSS, N and P accounting for 64%, 61%, 75%, 85 and 88%, respectively (Fig. 2), which again is within a comparable range to the figures reported by other studies (Meinzinger and Oldenburg, 2009; Vinneras et al., 2006). The reduction of TCB and *E.coli* on the other hand was surprisingly low, only accounting for 0.5 log and 0.1 log for TCB and *E.coli*, respectively (Fig. 2). This is due to the high concentration of TCB and *E. coli* in the raw greywater of 6.2 ± 0.4 and 6.7 ± 0.3 log 100 ml^{-1}, respectively. Other GW studies reported comparable high concentrations on TCB ranging 7.2–8.8 log 100 ml^{-1}, but lower numbers for *E.coli* ranging from 3.2–6.0 log 100 ml^{-1} (Ottoson and Stenström, 2003). However, TCB and *E.coli* encompass both fecal and none-fecal organism (Ottosson, 2003; Ottoson and Stenström, 2003). A recent study showed that the mean concentration of coprostanol, a biomarker formed by the intestinal microflora, was 3.1 log lower in GW than in combined household wastewater. The fecal load estimated with the biomarker coprostanol in GW is 0.04 g person^{-1} d^{-1} which is 2.1–3.2 log lower compared to 5.4 g and 65 g person^{-1} d^{-1} when using the indicator bacteria *E.coli* and *Fecal enterococci* (Ottoson and Stenström, 2003). Hence, the indicator parameters TCB and E.coli used by this study likely overestimates the concentration of fecal pathogens in GW by 3 log (Ottosson, 2003). A majority of the detected TCB and *E.coli* in our GW are therefore likely not fecal origin but rather originated from the kitchen where high concentration up to 7.4 log were also reported elsewhere (Naturvårdsverket,

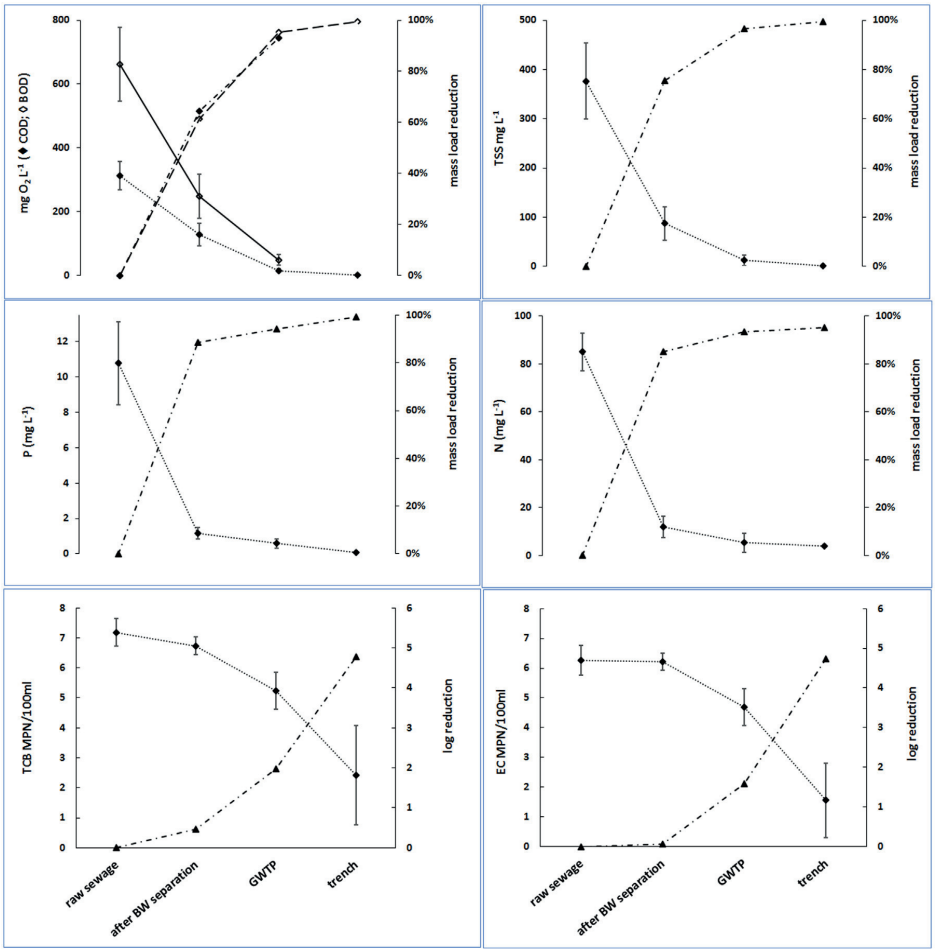

Fig. 2. Calculated mean and average concentration for the combined sewage and measured mean and average concentration in the raw combined sewage, effluent of greywater treatment plant (GWTP) and bottom of infiltration trench (♦) and Calculated mass load and cell number reduction for separating blackwater, greywater treatment plant (GWTP) and infiltration trench (▲) for COD, BOD, TSS, N, P, total coliform bacteria (TCB) and *E.coli* (EC).

1995) or re-growth of particular *Coliform* species in sewer pipes (Manville et al., 2001). The latter likely occurred also in our GW sewer system which has a long hydraulic retention time of 36 h or more (Todt et al., 2015), with an average temperature of 15 °C.

These findings are supported by another study (Oliinyk et al., 2015) on our GW using quantitative PCR (qPCR) analysis for human specific *Bacteriods* and *Enterococci* in the BW and GW. The results showed that the number of gene copies was 3.7 and 1.5 log lower in GW than in BW for *Bacteriods* and *Enterococci*, respectively. Hence, in terms of fecal pathogens having human origin, a separation of BW likely results into 1–4 log reduction. More research is needed to assess the distribution of different pathogenic organism in the wastewater fractions and related health risks more in detail. In addition, a potential regrowth and decay of different, pathogenic and none-pathogenic microorganism across a sewer or treatment system has to be addressed more in detail.

3.2. Onsite treatment of greywater in a fixed-film biofilter

The concentration of raw greywater was 137 ± 38 mg O_2/L for BOD, 267 ± 71 for COD mg O_2 L^{-1}, 14 ± 3 mg L^{-1} for N_{tot} and 1.2 ± 0.3 mg L^{-1} for P_{tot}. No notable difference to our earlier sampling period (Todt et al., 2015) could be identified for these parameters, indicating that the GW composition remains constant over time. As evaluated in our previous study (Todt et al., 2015), load and composition of our GW is comparable to other studies in Europe, except for P, which is slightly lower, likely due to the absence of dishwashing machines at the dormitories. Detergents for dishwashing machines became the major source of P in GW after the introduction of phosphate free laundry agents.

Referring to raw GW, the GWTP reached a removal efficiency of 80%, 88%, 86%, 49% and 55% for BOD, COD, TSS total P and total N, respectively (data not shown). Together with a separation and collection of BW a removal efficiency of 93%; 95%; 96%; and 94% was obtained for BOD, total COD, TSS, and P, respectively. These figures are the average over the whole sample period, encompass-

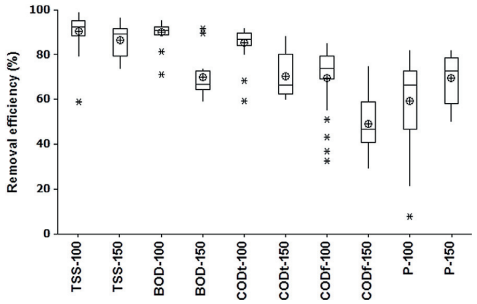

Fig. 3. Removal efficiency for TSS, BOD, homogenized COD (COD_t), filtrated COD (COD_f) and total phosphorus (P) of a greywater treatment plant at 100% and 150% nominal load (25;50:75 percent quartiles in the box plots with 95% quartiles in the error bars; average is indicated in the point plot).

ing also periods with overloading, under loading and power breaks (Table 2). In periods with nominal load, the removal efficiency of the GWTP reached more 90% for BOD, COD and TSS and close to 60% for N and P. However, a reduced P-removal efficiency has to be expected on locations using phosphate in dishwashing agents, possibly in a range of 70–80% considering the range of P concentration reported by other GW studies (Palmquist and Hanæus, 2005; Meinzinger and Oldenburg, 2009). In Norway, use of P containing detergents are prohibited and the raw greywater contain therefore low P, in our case less than 1.2 mg/L. In other areas where P containing detergents are in use the P concentration in the effluent may still be above the permissible limit. An additional polishing step for P-removal may therefore be needed on particular places. In such cases, reactive filter materials are increasingly used as post-filtration treatment to ensure removal of residual P. The filter materials for instance enriched lightweight aggregate LWA, Filtralite P® and Shell-sand (Adam et al., 2007), biochar and Filtralite (Eshetu et al., 2015) and Polonite® (Gustafssn et al., 2008) can be mentioned as efficient polishing filter. The power consumption of the system was determined to 0.34 kWh m^{-3} hydraulic load (data not shown). By taking into account an average GW production of 108 l d^{-1} per person (Todt et al., 2015) this corresponds to 13 kWh y^{-1} $capita^{-1}$, which is almost one order of magnitude lower than 93–217 kWh y^{-1} $capita^{-1}$ that has been reported for onsite treatment of combined sewage (Straub, 2008).

The impact of overloading on the removal efficiency of the system was evaluated by comparing periods with 100% load to periods with 150% load (Fig. 3). Overloading with 150% of the nominal loading did not show significant difference on the removal of TSS and total P_{tot} ($p > 0.05$) while, a significant lower ($p < 0.001$) removal efficiency was observed for organic matter. The high filter surface loading rate of 423 mm d^{-1} resulted into a lower contact time with the biofilm, which again could have reduced the degradation of organic substrates as reflected by the lower removal efficiency for BOD as well as filtered COD. Regardless the reduced organic matter degradation, the filter still achieved an average removal efficiency of 70% for both BOD and COD during the 150% loading periods, which proves the high stability of fixed-film biofilter systems.

3.3. Post treatment

Unsaturated soil infiltration systems commonly used for the disposal of treated greywater as post treatment step. In this study, the columns represent discharge points in an infiltration trench with a single-hole in the perforated disposal pipe that is placed on the top of the infiltration trench in the actual disposal system. Short-circuiting is a common phenomenon in unsaturated infiltration but the configuration of the filter media, the development of biofilm and the presence of geotextile layers allow a uniform distribution with time. This is demonstrated from the results of bacterial removal, which was increased from period 1 to period 2 and 3.

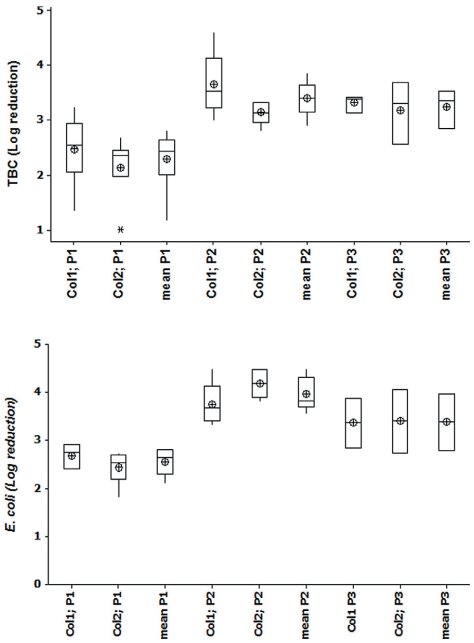

Fig. 4. Reduction of total coliform bacteria (TCB, upper panel) and *E. coli* concentration in each of the two filter column replicates (Col1, Col2) and overall mean (mean) for three sampling periods P1, P2, P3.

The results from the effluent polishing experiment further indicate a significant reduction of 85–90% for BOD, TSS and total P across the filter columns. As a result, a TSS of <2 mg/L, and total $P < 0.1$ mg P L^{-1}, BOD <2 mg O_2 L^{-1} was achieved. Determination of total coliform bacteria and *E. coli* from the columns effluent in three periods showed significant reduction. Average TCB and *E. coli* log reduction during in the first period was 2.4 and 2.5 respectively. The reduction increased by more than 1 log after three weeks of operation. The average TCB and *E.coli* log reduction in the last two periods were 3.4 and 3.8, respectively (Fig. 4). The increase in log reduction of *E. coli* and TCB in the second and third period could be due to development of biofilm and an improved water distribution in the columns. This has been shown to increase the pathogen removal efficiency in filter systems (Heistad et al., 2009). Therefore, the polishing filtration step raised the total coliform and *E. coli* removal efficiency of the system up to 4.8 and 4.7 log reduction, respectively. This is in agreement with a previous study with biochar and Filtralite polishing filters (Eshetu et al., 2015). The issue of micropollutants is one of the most important concern to be considered for any on-site wastewater treatment. A separate study on heavy metal analysis in the system have shown a significant reduction of their concentration in the effluent (data not shown here). Moreover, separate blackwater collection allows concentration of organic micropollutants, particularly pharmaceuticals and hormones, in a significantly lower volume in the blackwater (Butkovskyi et al., 2017; De Graaff et al., 2011). Since most organic micropollutants are biodegradable, the significant reduction in the BOD of greywater may suggest a lower level of micropollutants in the effluent. However, due to the presence of some persistent

Table 3
Average effluent quality in this study compared to present limits for discharge and reuse.

Standards	Applicability	BOD_5 mg/L	Tot P mg/L	TSS mg/L	E.coli MPN/100 ml
Average effluent GWTP + infiltration trench	discharge to sensitive recipients	<2*	<0.1	<2	<5
Average effluent GWTP	discharge to none-sensitive recipients	12	0.6	14	10^4–10^5
Norwegian discharge limit (Miljø Blad 100, 2010)	discharge household wastewater	<20	<1	–	–
US standard (NSF/ANSI 350-2012)	reuse of greywater	10	–	10	14
Australian Guideline (2011)	reuse of greywater	<20	–	<30	<30

* Most samples had BOD5 value below the detection limit.

organic substances in greywater (Hernandez et al., 2007), low BOD may not necessarily indicate low concentration of micropollutants. Hence, further thorough investigation of the pharmaceutical and personal care products in the raw greywater and effluents are needed.

The biofilter system is efficient to remove most of the organic and particulate matter and waxy substances. The effluent that flows into the infiltration column, therefore, does not contain suspended solids that may cause clogging. No sign of clogging was observed throughout the experiment period. However, with time and development of biofilm on the surface of the geotextile layer an impediment could occur. The experiment is running further to assess the lifetime of the filtration column and associated occurrence of clogging through time.

The quality of effluent from the GWTP complies with the Norwegian discharge limit for discharge of treated household wastewater to sensitive recipients (Table 3). However, in terms of pathogen removal, only a 1–2 log reduction was observed. Although the present Norwegian regulations do not define discharge limits for indicator organisms (Table 3), the risks of faecal contamination from blackwater should not be overlooked (Stenström, 2013). Without post polishing, the effluent of the GWTP do not fulfil the requirements for present reuse standards (Table 3) and may be rather critical for sensitive recipient that are close to drinking water sources. For sensitive recipients as well as reuse applications, a multiple barrier approach including a post polishing in an infiltration trench is needed in order to minimise the related health risks (Table 3).

4. Conclusion

- It was observed that separation of BW from the rest of household wastewater streams resulted into a significant reductions for COD, BOD, TSS, N and P accounting for 64%, 61%, 75%, 85 and 88%, respectively. Separate treatment of GW in a biofilter further reduced the concentration of organic matter and nutrients to discharge limit levels. Together with a separation and collection of BW a removal efficiency of 93%, 95%, 96%, and 94% was achieved for BOD, total COD, TSS, and P, respectively.
- Overloading the system up to 150% of the nominal loading did not affect the removal efficiency of the system for TSS and total P_{tot} ($p > 0.05$). However, lower removal efficiency was observed for organic matter.
- In terms of removal of indicator organisms, further treatment is a necessity. Infiltration trench or filtration columns as effluent polishing can significantly reduce the microbial concentration in the effluent. Overall system reached up to 5-log reduction of coliform bacteria, of which the major reduction was observed in the infiltration columns.
- For reuse applications or in drinking water areas a separate collection of blackwater in combination with a multiple barrier approach for the treatment of greywater including soil infiltration as final polishing is recommended in order to minimise the related health risks.

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Paper II

Moges, M.E., Eregno, F.E. and Heistad, A., 2015. *Performance of biochar and filtralite as a polishing step for on-site greywater treatment plant*. *Management of Environmental Quality: An International Journal*, 26(4): 607-625. https://doi.org/10.1108/MEQ-07-2014-0101

Performance of biochar and filtralite as polishing step for on-site greywater treatment plant

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Abstract

Purpose – The purpose of this paper is to investigate the performance of biochar and fine filtralite as a polishing filter material in further removing organic matter, phosphorous, nitrogen, turbidity and indicator microorganisms from effluents of a compact greywater treatment plant (GWTP).

Design/methodology/approach – A filtration experiment was carried out using columns filled with biochar and fine filtralite as filter material and unfilled column as a control. The effluent from the GWTP was pumped using a peristaltic pump at a rate of 280 l/m2-d and was fed in upward flow into the columns. The quality parameters of the raw greywater, effluents from the GWTP and the polishing columns were studied for six months of operation period.

Findings – The results indicate that the process of polishing considerably improved the effluent quality of the system. Biochar performed best in removing organic matter, total N, turbidity and odor. Filtralite was superior in removing P. The contribution of the polishing step in removing total coliform bacteria (TCB) and *Escherichia coli* (*E. coli*) was remarkable. Additional log reduction of 2.18, 2.26 and 1.81 for TCB and 2.26, 2.70 and 2.01 for *E. coli* was obtained compared to the GWTP due to biochar, filtralite and control column, respectively.

Practical implications – This study demonstrates the opportunities for improving the performance of decentralized greywater treatment systems by integrating locally available polishing materials to achieve a better quality effluent.

Originality/value – The present study identifies efficient polishing system for decentralized and compacted greywater treatment system. The recommended polishing materials potentially improve the quality of effluents and add social, economic and environmental values.

Keywords Biochar, Biofilter, Filtralite, Greywater, Wastewater treatment

Paper type Research paper

1. Introduction

Water is a key determinant for ecosystem services and function, which benefit human well-being. Social, economic and environmental developments largely depend on the quantity and quality of water. With the current trend of population growth, urbanization, industrialization and economic expansion, the amount of water required for the different services (water for drinking, sanitation, production, processing and recreation) increases considerably. At the same time, the amount of wastewater generated and discharged from the different sectors increases proportionally. The latter has a harmful effect on the ecosystem and human health and has become a growing global concern. However, there are also opportunities from wastewater that could be exploited for green development, social well-being and ecological health (Corcoran *et al.*, 2010).

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The opportunities from wastewater are many and include nutrient, energy and water recovery, reuse and recycling. The challenge for utilizing these opportunities from wastewater relies on the availability of safe, efficient and affordable wastewater treatment technologies. Finding such appropriate solutions to the recovery, reuse and recycling of resources from wastewater require innovation by holistic, integrated and participatory approaches.

Properly treated and managed greywater recycling is an integral part of water demand management, promoting the preservation of high quality freshwater as well as reducing pollutants in the environment (Al-Jayyousi, 2003). Greywater consists of 60-70 percent of the in-house domestic water demand (Friedler and Hadari, 2006). Based on the Danish water use statistics, up to 43 percent of potable water could be saved by recycling greywater (Revitt *et al.*, 2011).

Greywater should be, therefore, a central focus for sustainable social, environmental, economic and political development to fill the gap between the increasing demand and the scarce water supply. Eco-friendly and low-cost on-site greywater treatment technologies are becoming essential for reducing the negative impacts of wastewater on the environment. In addition to reducing health risk and aesthetic problem, on-site treatment can help to optimize resource recycling and reutilization, and minimize the energy and operation costs (Friedler, 2004).

Novel reactive filter media such as filtralite and Filtalite P® (Adam *et al.*, 2007a; Jenssen *et al.*, 2010; Heistad *et al.*, 2006), Polonite® (Gustafsson *et al.*, 2008; Renman, 2008), Shell sand (Roseth, 2000; Sovik and Klove, 2005) and Zeolite (Chen *et al.*, 2006) have been used for efficient removal of P in constructed wetlands (CWs) and compact filters. Lightweight expanded clay aggregates have also shown extremely high P sorption capacity (Heistad *et al.*, 2006; Adam *et al.*, 2007a, b). This material is also found to have a very high hydraulic conductivity (Heistad *et al.*, 2006). The performance of filtralite on nitrogen removal is, however, not much satisfactory (Kasak *et al.*, 2011; Jenssen *et al.*, 2010).

In the last two decades, biochar received much attention as a possible means to improve ecosystem services, sequester carbon and mitigate climate change (Lehmann, 2007, Sohi *et al.*, 2010; Laird, 2008; Lehmann *et al.*, 2006). In addition to sequestration of carbon and improvement of soil conditions, studies have also shown the potential of biochar as a low-cost adsorbent to commonly environmental pollutants (Cao *et al.*, 2009; Yao *et al.*, 2011).

Recent research on the potential of biochar to reduce the pollution effects of P, N and other organic pollutants of wastewater showed a promising outcome (Dalahmeh, 2013). According to Cao *et al.* (2009), biochar also showed a strong affinity for a number of heavy metal ions and were effective to remove metal contaminants from wastewater. Similarly, biochar derived from dairy manure was effective in removing heavy metals such as Pb, Cu, Zn or Cd, from wastewater (Xu *et al.*, 2013). A study by Cao and Harris showed appreciable competence of biochar derived from dairy manure to adsorb Pb completely and 77 percent of Atrazine from aqueous solution (Cao and Harris, 2010).

As compared to the natural soil organic matter, the sorption capacity of biochar is high and is estimated to exceed by a factor of 10-100 (Cornelissen *et al.*, 2005). However, only limited researches have been carried out to investigate the ability of biochar to remove organic matter and nutrients, particularly ammonium and phosphate from water (Hossain *et al.*, 2011; Uchimiya *et al.*, 2010, Yao *et al.*, 2011). In addition, most studies regarding pollutant adsorbent capacity of the biochar is based on batch experiments and using artificial wastewater. Batch experiments, however, can lead to

misinterpretation and overestimation of the phosphorus retention capacity of the materials (Drizo *et al.*, 2002; Ádám *et al.*, 2005).

The performance of biochar on greywater treatment using real wastewater and at a larger scale is not well documented. The purpose of this work was, therefore, to carry out a column filtration experiment as a polishing step to a commercially available on-site greywater treatment plant (GWTP) system (Ecomotive A02-GWTP): to examine the long-term performance of biochar and filtralite NC 0.8-1.6 mm in further removing organic and inorganic pollutants including P and N, odor, turbidity and hygiene indicator microorganisms, and to evaluate the feasibility of the system for possible reuse of treated wastewater effluents.

2. Materials and methods

2.1 Filter material

Two filter materials, biochar and filtralite, were selected for investigation. The biochar used in this experiment was purchased from Carbon Terra GmbH, Augsburg, Germany. The physic-chemical characteristics of the biochar are indicated by the company and were not further analyzed here. The particle size of the biochar used ranges from 2 mm to 5 mm in diameter. It was thoroughly homogenized and washed several times with tap water to remove fine particles in order to avoid clogging during the filtration process.

The filtralite, with particle size between 0.8 mm and 1.6 mm, is an inert ceramic material produced by burning of clay at 1,200°C followed by crushing and sieving (Weber, 2010). This filter material has a porous structure and a high surface area, with a dry particle density of between 1,000 and 1,200 kg/m^3. It is mainly used in drinking water treatment plants as a component in two-media filters. The filtralite was also washed with tap water several times to remove the finest substances.

Both filter materials are characterized by a very good hydraulic conductivity, especially after washing out the fine particles from the biochar. The two filter materials were packed to a height of 0.60 m in a 0.14 m diameter and 1 m long acryl column in two replicates. In order to avoid clogging at the inlet side, a 2.5 cm thick layer of gravel was placed at the bottom of the columns. Another layer of gravel was placed on top of the filter materials to prevent floating of the biochar and fine filtralite during the initial stage of the experiment. A total of five columns, two for biochar, two for filtralite and one unfilled control column placed upright close to the Ecomotive A02-GWTP unit.

2.2 Experimental set up of the treatment system

Source separated greywater, derived from bathrooms, laundries, hand washing basins, dish washing machines and kitchens in a student dormitory, that serve 48 inhabitants, was collected in a septic tank and pumped into a stirred stainless steel tank in the laboratory. The raw greywater was fed into the Ecomotive A02-GWTP by using peristaltic pump controlled by frequency converters and PLC (Eshetu *et al.*, 2014). After sedimentation and biofiltration in A02-GWTP, the effluent from the biofilter (before entering the second clarifier) was pumped with a small peristaltic pump to the inlet of the experimental columns at a constant flow rate of 280 L/m^2-d.

The columns then were fed continuously in upward saturated flow as opposed to the biofilter unit, which is fed intermittently under unsaturated condition. Figure 1 shows the schematic diagram of the experimental set-up.

2.3 Influent and effluent quality analysis

Water samples from the inlet, the outlet of the biofilter and the effluents of the polishing filters (biochar, filtralite and unfilled column) were taken for analysis once every two weeks. Electrical conductivity (EC), pH, turbidity, total phosphorus (P_{tot}), particulate orthophosphate, total chemical oxygen demand (COD*t*), total Nitrogen (N_{tot}) were analyzed according to the Standard Methods for the examination of water and wastewater (American Public Health Association, A.W.W.A. and Water Environmental Federation (WEF), 2005) using Hach Lange® DR3900 spectrophotometer. Odor and color were analysed subjectively in both the influent and effluents immediately after sampling. Color was subjectively determined based on the purity and clarity of the effluents, whereas odor was determined based on the strength of the smell. Comparison was made based on whether the smell of effluents is very strong odor (offensive), strong and no smell. At a later stage analysis of ammonium (NH_4-N), nitrate (NO_3-N) and nitrite (NO_2-N) were included. In addition, a limited number of total suspended solids (TSS) and biochemical oxygen demand (BOD_5) tests were carried out.

Total coliform bacteria (TCB) and *Escherichia coli* (*E. coli*) were routinely enumerated in greywater and treated effluents using the Colilert-18/Quanti-Tray Method™ (IDEXX, USA). A ten-fold dilution series were prepared from the samples and one pack of Colilert-18 reagent was added to the 100 ml diluted sample in a sterile and transparent vessel and carefully mixed. The sample/reagent mixture was then poured into a Quanti-Tray/2000, sealed in a Quanti-Tray sealer and incubated at 36°C for 20 hrs. The bacteria were enumerated using IDEXX Quanti-Tray/2000 MPN Table. A 6-Watt fluorescent UV lamp was used to count *E. coli*.

2.4 Statistical analysis

The basic features of the data set were described using descriptive statistics. Due to the nature of the samples, mean values are markedly different from the median values indicating a positive or negative skewedness of the data distribution. Geometric mean, as it also describes the central tendency of the data set, was used for microbial result interpretations. Unstacked One way ANOVA using Minitab 16 was used to measure the overall variation between and within treatments (Minitab, 2010). The grouping information among the different treatments was determined using Tukey and Fisher methods. Tukey test analyses pair wise comparison of means. The results from Tukey's simultaneous test indicate which mean level is significantly different from the other. Treatments with similar mean are denoted by same letter indicating no significant

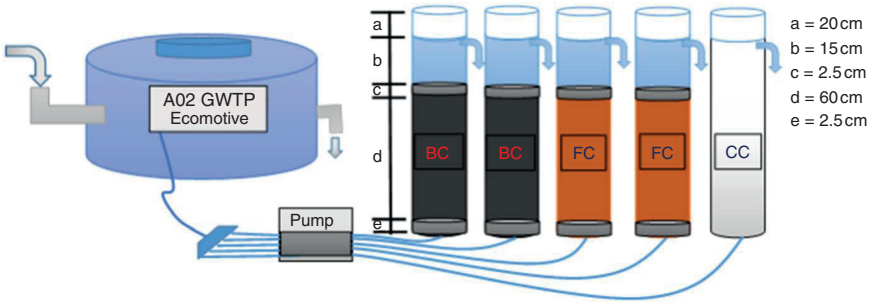

Figure 1.
Schematic diagram of the experimental set-up

Notes: A02 GWTP, A02 greywater treatment plant; BC, biochar colomun; FC, filtralite column; CC, control/unfilled column

difference. Means that does not share the same letter belongs to different group and have *p*-values less than 0.05 indicate significant difference among treatments.

3. Results and discussion

The results of this study elucidate two important observations: on the performance of the A02-GWTP and the possibilities of increasing the efficiency of the system to produce a more acceptable effluent using simple polishing set up. The biofilter effluent from the A02-GWTP unit showed a significant reduction in organic and inorganic constituents of the greywater. The biofilter effluent further passed through a biochar and Filtralit filter media and unfilled column, as a polishing step, for further purification. The COD*t*, P_{tot}, N_{tot}, turbidity, TCB and *E. coli* removal efficiency of biochar, fine filtralite and unfilled column was studied to evaluate the performance and feasibility of the system. Table I shows average values for six months operational period with standard deviation and median of the physico-chemical and biological characteristics of the raw greywater, biofilter effluent and column effluents. The data showed significant reduction ($p < 0.001$) in the effluent concentration for all parameters, except for EC, pH and nitrate, as compared to the raw greywater. The biochar effluent had higher EC and pH throughout the operation period compared to the other treatments and the raw greywater (Table I). Physico-chemical and biological characteristics of influent and effluent greywater: mean ±STDV (median).

As shown in Table I, the polishing step significantly improved the effluent quality. Biochar contributed to a better effluent quality in terms of turbidity, organic matter and nitrogen. filtralite, on the other hand, showed significant contribution on *P* removal (up to the 200 percent nominal loading rate in the A02 system) and in the removal of indicator microorganisms. Higher values of total *P* were obtained in the filtralite effluent in the last two weeks of operation period when the loading in the main system increased to 300 percent. This is the reason for the large variation in total *P* values for this treatment. The concentrations of indicator microorganisms were significantly lower in the filtralite effluent compared to the biochar and unfilled column. The unfilled column used as a tertiary clarifier. The agglomerates formed as a result of microbial biomass at the bottom of this column serve as a biofilter. Therefore, the contribution of this column is significant and results are comparable to biochar and filtralite treatments.

3.1 Turbidity and TSS

In case of local discharge or reuse, treated greywater should not be a source of odor and nuisance, and therefore it should be almost free from suspended solids and color (Nolde, 2005). Results from turbidity measurements show that the A02-GWTP unit significantly reduced the turbidity ($p = 0.000$). The biofilter effluent has on average 5.55 NTU of turbidity as compared to the raw greywater, which has an average of 102.6 (Figure 2). The polishing columns further reduced the turbidity to a more acceptable level. The filtralite column had, however, higher turbidity in the last two weeks of operation due to the increase in the inlet nominal loading of the A02-GWTP to 300 percent, which resulted in an increase in organic load in the biofilter effluent. The turbidity of the effluent from biochar was below 2 NTU throughout the operation period and did not change significantly by a change in organic matter concentration from the biofilter effluent. It was also noted that the TSS in the biochar treated effluent was very low (< 5 mg/L) compared to the other treatments, and was almost free from color and odor. Thus, the effluent that passes through the biochar filter media fulfills the physical quality requirements and has good aesthetic quality.

Table I.
Physico-chemical and biological characteristics of influent and effluent greywater: mean ±SD (median)

Parameter	Observation	Raw greywater	Biofilter effluent	Biochar effluent	Filtralite effluent	Unfilled column effluent
pH	11	6.95 ± 0.158 (6.99)	7.28 ± 0.19 (7.22)	7.66 ± 0.127 (7.71)	7.46 ± 0.18 (7.48)	7.27 ± 0.25 (7.24)
EC in µS/cm	11	276 ± 32.09 (260)	269 ± 28 (270.5)	311 ± 43.76 (323)	279 ± 31.97 (279)	269.5 ± 24.08 (271)
Turbidity in NTU	9	102.61 ± 55.43 (75.70)	5.55 ± 2.54 (5.07)	0.87 ± 0.23 (0.86)	1.76 ± 2.67 (0.86)	2.94 ± 3.43 (1.26)
CODt (mg/L)	11	309.91 ± 38.88 (320)	55.13 ± 20.55 (49.60)	10.99 ± 5.51 (11.90)	20.63 ± 5.79 (19.40)	24.75 ± 5.26 (23.40)
Part-PO_4 (mg/L)	11	0.55 ± 0.11 (0.55)	0.23 ± 0.13 (0.22)	0.18 ± 0.03 (0.18)	0.09 ± 0.14 (0.05)	0.19 ± 0.10 (0.15)
P_{tot} (mg/L)	10	1.50 ± 0.14 (1.49)	0,53 ± 0.18 (0.48)	0.22 ± 0.03 (0.21)	0.15 ± 0.17 (0.09)	0.30 ± 0.08 (0.29)
NH_4-N (mg/L)	5	7.61 ± 1.59 (8.52)	3.81 ± 0.68 (3.81)	2.17 ± 0.72 (2.07)	2.91 ± 0.79 (2.84)	1.54 ± 0.58 (1.55)
NO_3-N (mg/L)	5	0.16 ± 0.02 (0.16)	1.44 ± 1.21 (1.27)	0.32 ± 0.19 (0.30)	0.86 ± 0.63 (0.95)	3.19 ± 1.90 (4.06)
N_{tot} (mg/L)	9	17.76 ± 2.18 (17.90)	10.03 ± 2.26 (9.60)	3.41 ± 0.92 (3.16)	6.38 ± 1.26 (6.33)	8.41 ± 2.29 (7.91)
TBC (MPN/100mL)[a]	21	2.97E + 06	8.30E + 04	6.40E + 02	5.29E + 02	1.56E + 03
E. coli (MPN/100mL)[a]	21	8.51E + 05	2.64E + 04	1.46E + 02	5.29E + 01	2.60E + 02

Note: [a]Geometric mean

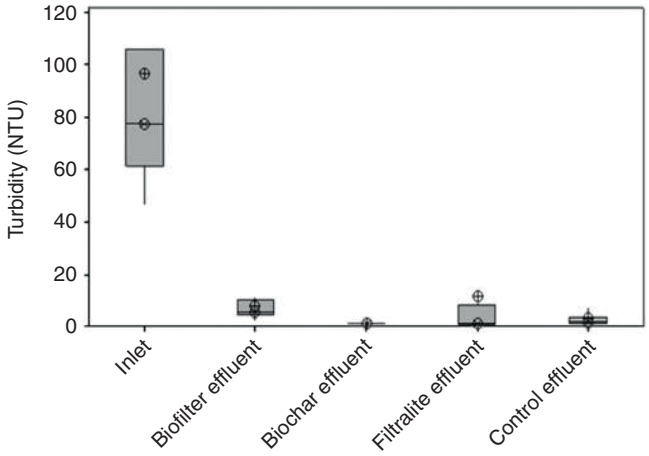

Figure 2. Mean and interquartile range (first and third) of Turbidity for inlet and treated effluents

In the unfilled column, a thin layer of biofilm was developed at the bottom surface. This biofilm is formed as a result of the sedimentation of particles, growth and attachment of different microorganisms and the extracellular polymer they excrete (Melo, 2003). Such attachment and growth of microorganisms is responsible for the formation of porous flocs which act as a biofilter for the unfilled column and removed a considerable amount of organic and inorganic constituents. The effluent quality from this column is comparable with the biochar and filtralite effluents. The slightly higher TSS and turbidity values for the unfilled column were partly caused by occasional detachment and floatation of deposited particulate and biomass matter. The reason may be gas formation because of microbial processes under partial anoxic conditions (Gerardi, 2002) and/or due to the aging of the biomass.

filtralite effluent had significantly low turbidity, less than 2 NTU for most of the samples analyzed compared to the biofilter effluent. However, the turbidity of the effluent from the filtralite column was influenced by the increase in the organic load of the biofilter which was increased due to an increase in the nominal loading rate to 300 percent in the A02-GWTP. TSS in the column effluents showed a considerable reduction in values as compared to the inlet raw greywater. The TSS of the inlet ranged from 51 mg/L to 278 mg/L with an average of 101.25 mg/L. The biochar effluent had the lowest concentration of TSS with an average of 2.0 mg/L. The filtralite and control column had an average TSS value of 6.0 mg/L and 13.0 mg/L, respectively.

3.2 Organic matter removal

Organic matter, in terms of BOD and COD, is the main concern in the treatment of greywater. Total COD was taken throughout the operation period and is used to indicate the concentration of organic pollutants. The variation of total COD in the inlet raw greywater and effluents from the different columns are indicated in Figure 3. The results in Table I showed 12 to 25-fold reductions in COD in the effluents as compared with the raw greywater, which averaged 309.91 mg/L. The biofilter resulted an average reduction of CODt to 55.13 mg/L. The polishing step further reduced this concentration to an average of 10.99, 20.63 and 24.75 mg/L with biochar, filtralite and

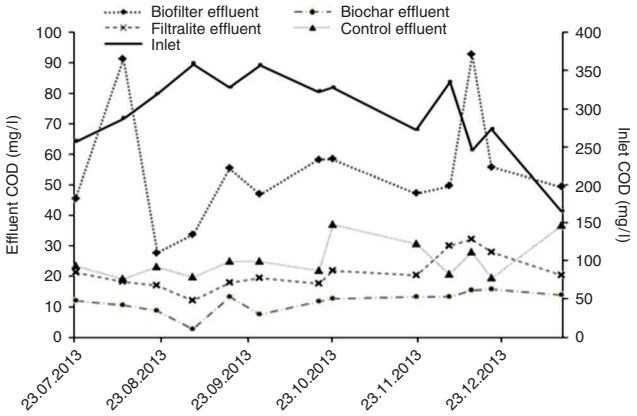

Figure 3.
COD concentration in the inlet greywater and treated effluents

unfilled column, respectively. The highest reduction was obtained in the biochar column effluent with an average CODt of 10.99 mg/L. The biochar was significantly more ($p < 0.001$) effective than the filtralite and the unfilled column in reducing CODt because biochar has a high affinity and capacity for sorbing organic compounds (Smernik, 2009).

The biochar was similarly effective and stable in removing the organic matter (CODt) when the A02-GWTP system was exposed to higher hydraulic loading. On the other hand, an increase in the total COD concentration occurred in the filtralite effluent as the loading in the A02 system increased. The increase in the COD, N and P in the column effluents are related to an increase in the concentration of organic constituents in the biofilter effluent.

3.3 Nutrient removal

Phosphorus removal. The removal of P from wastewater protects the aquatic ecosystem from eutrophication and with novel retention filter material P can be recovered, recycled and reused. Phosphorus sorption efficiency from aqueous solutions is, however, governed by various factors. Surface functional group, specific surface area of the adsorbent, metal-ion complex formation (Zeng *et al.*, 2013), filter material grain size and distribution, pH, and the concentration and valance of Al, Fe, or Ca and Mg elements (Khadhraoui *et al.*, 2002, Adam *et al.*, 2007a, b) are the most important factors controlling the sorption efficiency. The surface of the biochar is often negatively charged (Eberhardt *et al.*, 2006, Yao *et al.*, 2011). Most biochar have carboxylic functional groups which contribute to high cation exchange capacity, and suggesting sorption of phosphate via surface chemistry is minimal (Zeng *et al.*, 2013) as compared to filtralite, which is composed of Si, Al and Fe oxides.

The biofilter *P* removal efficiency of the A02-GWTP was 65.2 percent. The polishing step substantially improved the *P* removal efficiency of the system from 65.2 percent up to 90.0 percent (Table II). *P* removal from the biofilter effluent of the GWTP by the biochar, filtralite and unfilled column was 58.3, 71.41 and 43.38 percent, respectively. The P removal efficiency of A02-GWTP thus improved to 85, 90 and 80 percent by introducing a biochar, filtralite and unfilled polishing columns, respectively.

Table II.
Removal efficiency of the biofilter (A02 GWTP), the removal efficiency of the polishing treatment from biofilter effluent and cumulative removal efficiency for the different parameters

Parameter	Removal efficiency in % Biofilter	Biochar	Cumulative efficiency due to addition of Biochar filter	Contribution of Biochar filter to the total efficiency	Filtralite	Cumulative efficiency due to addition of Filtralite filter	Contribution of Filtralite filter to the total efficiency	Unfilled column	Cumulative efficiency due to Unfilled Column	Contribution of Unfilled column to the total efficiency
Turbidity	94.39	84.37	99.15	*4.56*	68.24[a]	98.28	*3.69*	60.57	96.97	*2.58*
COD*t*	82.21	80.01	96.45	*14.24*	61.55	93.34	*11.13*	55.11	92.01	*9.80*
P_{tot}	65.02	58.30	85.41	*20.39*	71.41[a]	90.00	*24.98*	43.38	80.19	*15.17*
Par-PO_4	43.77	19.72	54.86	*11.09*	37.53	64.87	*21.10*	20.21	55.14	*11.37*
N_{tot}	43.51	66.07	80.83	*37.32*	36.45	64.10	*20.59*	16.18	52.65	*9.14*
NH_4-N	49.95	43.07	71.50	*21.55*	23.58	61.75	*11.8*	59.56[b]	79.76[b]	*29.81*[b]
NO_3-N	−823.82[c]	77.47			40.67			−121.33[c]		

Notes: [a] Higher turbidity and P values in the filtralite effluent taken from the last two weeks of operation were not included in the above calculation as the nominal loading rate in the A02 GWTP unit was increased to 300 percent. This increase in loading significantly affected the turbidity and P in the filtralite effluent. [b]This high value is due to the conversion of NH_4 into NO_3 not due to removal of the NH_4 in the unfilled column. [c]NH_4-N is reduced into NO_3-N. This indicate that nitrification process occurred in the biofilter compartment of the A02 GWTP unit and unfilled column where biological activity is most active. The significant reduction in NH_4-N in the biochar effluent suggests adsorption.

The filtralite performed best in the removal of phosphorous up to 200 percent nominal loading in the GWTP system. For the six months operation period, the concentration of total *P* in the filtralite effluent ranged from 0.063 mg/L to 0.16 mg/L with an average of 0.097 mg/L (Figure 4) which corresponds to the removal of about 80 percent of total P coming from the biofilter effluent.

The biochar effluent had a total P concentration ranging from 0.179 mg/L to 0.281 mg/L with an average value of 0.216 mg/L that corresponds to the removal of about 57 percent of total P from biofilter effluent. The colloidal nature of the biochar surface may have contributed to the removal of P in the biochar column.

Further sedimentation and the biofilm developed at the bottom surface of the unfilled column removed a considerable amount of P. The total P concentration in this column effluent ranged from 0.192 mg/L to 0.411 mg/L with a mean value of 0.306 mg/L and removal efficiency of 37 percent. The microorganisms that form the biofilm may also have assimilated parts of the P.

From December 3 to December 20 2013, the A02-GWTP system was supplied with a nominal loading rate of 300 percent. During this period, the filtralite showed a dramatic decrease in the removal efficiency of total and particulate P. The particulate orthophosphate concentration in the effluent increased from 0.028 mg/L to 0.816 mg/L. Total P concentration in the effluent increased from less than 0.16 mg/L to over 1.99 mg/L. TSS also increased from 0.3-8 mg/L to over 90 mg/L. Similarly, the turbidity increased from 0.55-8.84 NTU to 26-93 NTU. Odor and color of the filtralite effluent also increased considerably.

Yellowish colored suspended solids dominated the TSS in the filtralite effluents. The suspended solids, which were filtered through a 45 μm Whatman Glass filter paper and dried at 105°C, were put in a furnace from 500 to 700 °C in order to see if the color is due to humic substances or from inorganic material. The material kept its color and particle size that clearly indicate the mineral nature of the suspended particles. The presence of this mineral matter in the effluent could result from structural degradation of the filtralite and dissociation and release of iron compound from the filtralite itself. However, after three weeks of 100 percent dosing, the P concentration in the filtralite effluent decreased to 0.21 mg/L and the combined removal efficiency reached 82 percent. On the other hand, the biochar and the unfilled column maintained their removal effectiveness with a very slight increment (4). Increased loading in the A02-GWTP system did not affect the biochar performance.

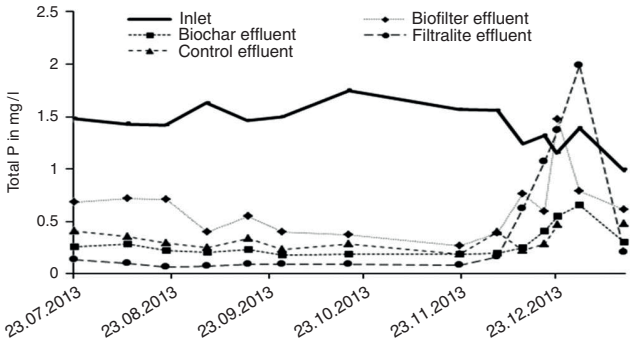

Figure 4.
Total P concentration in the inlet greywater and treated effluents

Nitrogen removal. Nitrogen concentration in greywater varies widely, depending on household uses. In general, greywater has very low concentration of N and P as compared to combined household wastewater. Similarly, total nitrogen concentrations in the raw greywater from Kaja student dormitory were very low (ranging from 13.2 mg/L to 21.1 mg/L). However, this concentration is enough to cause eutrophication in the aquatic environment if released untreated. Figure 5 shows the effect of A02 treatments and polishing effect on effluent N concentration.

The A02-GWTP had an average total N removal efficiency of 43 percent in the six months operation period. The total N concentration in the effluent treated by biochar was below 5.0 mg/L, with an average concentration of 3.53 mg/L. The biochar resulted to more than a five-fold reduction in N from the raw greywater and about three-fold reduction from the biofilter effluent. The nitrogen removal efficiency of biochar was significant ($p < 0.001$), and it increased the total N removal efficiency of the system from about from 43 to 80 percent. The filtralite effluent had an average total N concentration of 6.51 mg/L. The polishing effect due to fine filtralite is 20.6 percent, raising the efficiency of the system from 43 to 64 percent. The unfilled column had the least performance as compared to the other treatments. The average total N effluent concentration in this column was of 8.85 mg/L, contributing about 9 percent to the total N reduction efficiency. The maximum permitted level of effluent N concentration in several EU countries is 8 mg/L (Daims and Wagner, 2010). The N concentration in the effluents from the biochar and filtralite are, therefore, far below from this maximum allowed level.

Ammonium in the inlet greywater, with an average value of 7.61 mg/L, constitutes about 44 percent of the total nitrogen. Ion exchange and adsorption are, therefore, the main mechanisms for NH_4-N removal. While percolating through the aerobic biofilter, part of the nitrogen may be immobilized by the microbial biomass, but a portion of the nitrogen can also be transformed into the nitrate form by nitrifying bacteria. Decreases in ammonium concentration in the effluent does not necessary indicate nitrification as the ammonium could be assimilated and or sorbed. However, nitrification is verified by the production of nitrite and nitrate in the effluent.

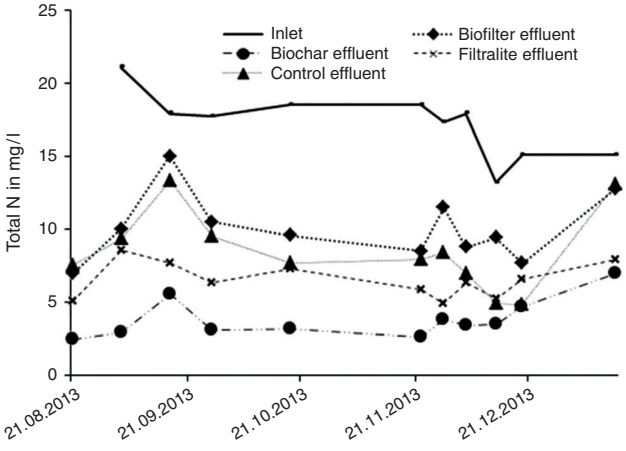

Figure 5.
Total N concentrations in the inlet greywater and treated effluents

The nitrate concentration in the influent was very low throughout the operation time, with an average of 0.16 mg/L. However, the nitrate concentration in the effluents increased five to 30 fold as compared to raw greywater. Nitrate values were slightly higher in the effluent from the biofilter reflecting slight nitrification in the A02 system. Mean NO_3 concentration for the biochar effluent was 0.32 mg/L (ranging from 0.14 to 0.584 mg/L) and 0.86 mg/L (ranging from 0.13 to 1.61 mg/L for filtralite. Whereas, significant increase occurred in the unfilled column from 0.87 to −5.28 mg/L.

The higher nitrate concentration could be due to the biofilm developed at the bottom of the column which favors nitrification process. The low concentration of ammonium nitrogen in this column resulted from its conversion into nitrate. In addition, sporadic presence of clumps of floating sludge in this column also indicates the nitrification process. The simultaneous presence of nitrifying and anoxic denitrifying bacteria in the same biofilm may allow for the conversion of ammonium to nitrogen gas (Melo, 2003). The floating sludge may, therefore, be produced when large numbers of insoluble molecular nitrogen, produced as a result of denitrification, is trapped in the sludge (Gerardi, 2002). The formation of gas can responsible for the detachment and floating of clumps of sludge. Another important factor for the detachment of clumps could be the age of the biofilm.

The nitrification process, however, was influenced by the high loading rate in the A02 system. Nitrification decreases with an increase in loading from 150 percent nominal loading (980 L/day) to 300 percent nominal loading (1,967 L/day) in the A02 system. However, it increased again with decreasing the nominal loading to 100 percent. Thus, the hydraulic and organic load has significantly affected the nitrification process in the biofilter. This was also reflected in the unfilled column. Nitrification was very low in the filtralite treated effluent and did not occur at all in the biochar treatment (Figure 6).

3.4 Removal of indicator bacteria

Microbial analysis during the test period showed a variation in TCB concentration in the raw greywater ranging from 5.47 to 7.38 $\log_{10}$/100 mL and *E. coli* from 5.04 to 6.61 $\log_{10}$/100 mL. These concentrations are higher than normally expected. These microorganisms can be introduced into grey wastewater by hand washing after toilet use, washing of babies, as well as from uncooked vegetables and raw meat (Eriksson *et al.*, 2002).

The TCB is within the range in comparison with reported in literatures. For example, Gerba *et al.* (1995) reported 7.2-8.8 $\log_{10}$/100 mL. However, the *E. coli* concentrations in this particular greywater are higher than reported in other Scandinavian studies which ranged from 3.2-5.4 $\log_{10}$/100 mL (Ottoson and Stenstrom, 2003). However, 4.3-6.8 $\log_{10}$/100 ml *E. coli* was detected at the collection point of CW in Vibyåsen greywater (Ottoson and Stenstrom, 2003). Eriksson *et al.* (2002) also observed an *E. coli* concentration in the range 1.3×10^5-2.5×10^8 MPN/100 mL, which is even higher than found in this study. The higher concentration of the fecal indicator bacteria may be due to the high load of easily degradable organic compounds, which may favor the growth of coliform bacteria (Ottoson and Stenstrom, 2003).

This high concentration of indicator organisms in the raw greywater indicates a potential health risks associated with direct contact or use of the untreated

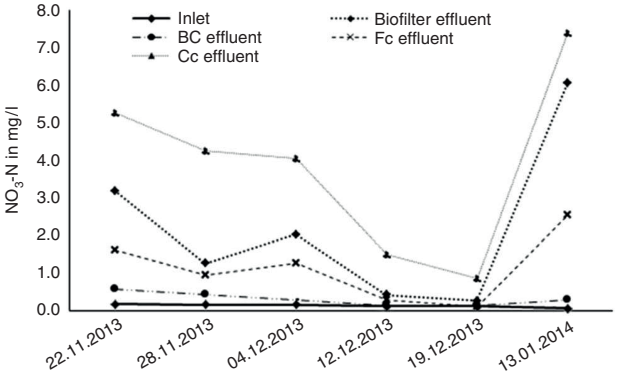

Figure 6.
The variation in NO_3-N between the different treatments and inlet greywater

greywater. This demands the necessity of treatment to reduce contamination and health risk if greywater is to be reused or safely discharged into the ecosystem. Biochar, fine filtralite and unfilled column significantly ($p < 0.001$) reduced the concentration of both TCB (Figure 7) and *E. coli* from the raw greywater (Figure 8). Significant difference ($p < 0.05$) was also observed between the concentrations of TCB and *E. coli* in the biofilter effluent and the polishing column effluents. However, no significant difference was observed between the biochar and fine filtralite, and between the biochar and unfilled column treatments in TCB reduction, while there was a significant difference ($p < 0.05$) between the filtralite and unfilled column in *E. coli* reduction.

The concentrations for TCB and *E. coli* in the effluent from the filtralite are in agreement with the revised EU-Guidelines for bathing waters which sets *E. coli* < 500 MPN/100 mL as excellent quality for inland water (see Directive 2006/7/EC (EU, 2006) and European-Commission (1975). The biochar effluent also met the requirements for most of the samples analyzed except for three samples that had *E. coli* concentrations greater than 1,000 MPN/100 mL.

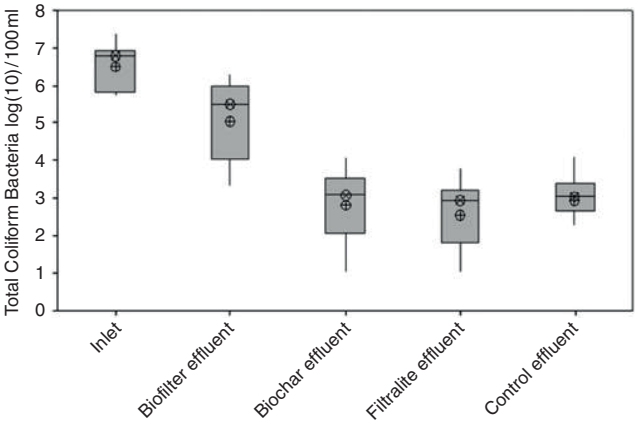

Figure 7.
Mean and interquartile range (first and third quartile)of TCB concentrations for the inlet and treated effluents

An average of 1.49 $\log_{10}$ in TCB and 1.51 $\log_{10}$ in *E. coli* reductions was obtained as a result of A02 system. Further reductions of 2.18, 2.26 and 1.81 $\log_{10}$ in TCB and 2.26, 2.7 and 2.01 log reductions in *E. coli* was obtained as a result of biochar, filtralite and unfilled column, respectively. Polishing, therefore, reduced the total concentration of indicator organisms considerably and contributed to raise the removal of TCB by the A02 system from 1.49 $\log_{10}$ TCB up to 3.75 $\log_{10}$ and from 1.51 $\log_{10}$ *E coli* to 4.21 $\log_{10}$.

3.5 Reuse potential of treated greywater

To evaluate the feasibility of reusing treated effluent, results were compared with WHO guidelines for safe use of wastewater (WHO, 2006). The physico-chemical characteristics of the effluents, especially from the biochar, were indicators of the aesthetic value which describe how user-friendly is the effluent. TCB and *E. coil* in the treated greywater are also in agreement with the limit in the quality parameters defined in the WHO document. Therefore, the effluents from the filtralite and biochar are suitable for unrestricted irrigation for leaf crops and landscape irrigation. However, regulations regarding wastewater reuse are site specific, and local offices should be consulted and treated greywater must be used in accordance with local regulations. Norway has an abundance of fresh water and the issue of wastewater reuse is hardly deliberated, but it could be practiced for sanitation and environmental protection purposes.

4. Conclusion

The present study showed the importance of polishing step in improving the effluent quality of treated greywater from a decentralized and compact GWTP. Filtralite showed superior effect in reducing phosphorus and indicator microorganisms. Biochar treated effluent retained good physical appearance throughout the operation period than the filtralite and unfilled column. Biochar was also very effective in reducing organic matter, N and TSS. According to the findings of this study, biochar can be used as a stable and efficient polishing material for decentralized and compact GWTP. Biochar and filtralite treated effluents can provide additional economic benefit, for example, through sustainable hydroponic production of fodder crops. At the same time the,

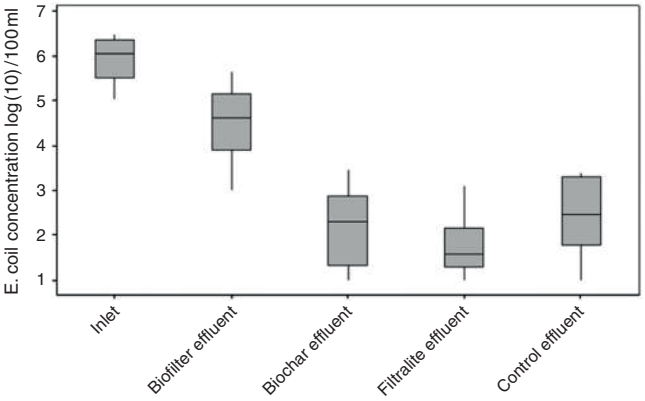

Figure 8.
Mean and interquartile range (first and third) of *E. coli* concentrations for the inlet and treated effluents

ammonium and phosphate-laden biochar can be used as a slow-release fertilizer to enhance soil fertility without any pre-desorption process. The values added from reclamation and utilization of treated wastewater in terms of social, economic, aesthetic and environmental aspects are significant.

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Appendix

The following figures demonstrate the results of the Tukey's test in terms of Tukey Simultaneous 95 percent CIs difference of means. The reference line at 0 shows how the wider Tukey confidence intervals can change your conclusions. Confidence intervals that contain zero indicate no difference. If an interval does not contain zero, the corresponding means are significantly different.

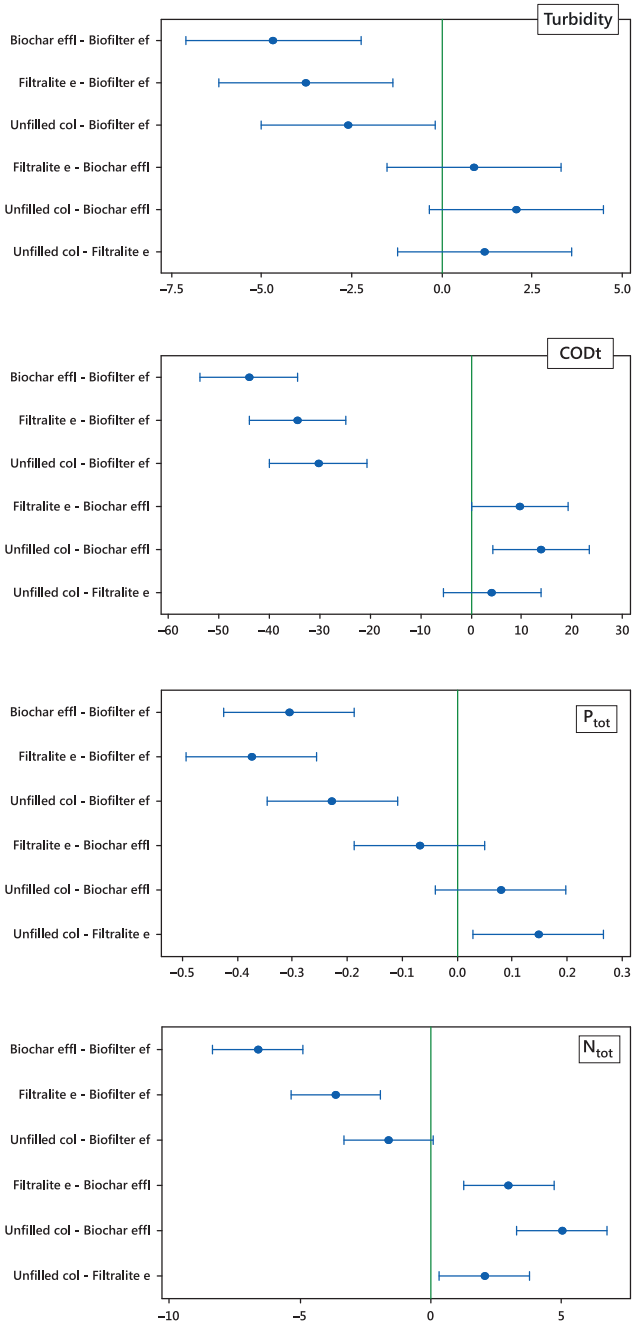

Figure A1.
Tukey test analysis results for the different parameters

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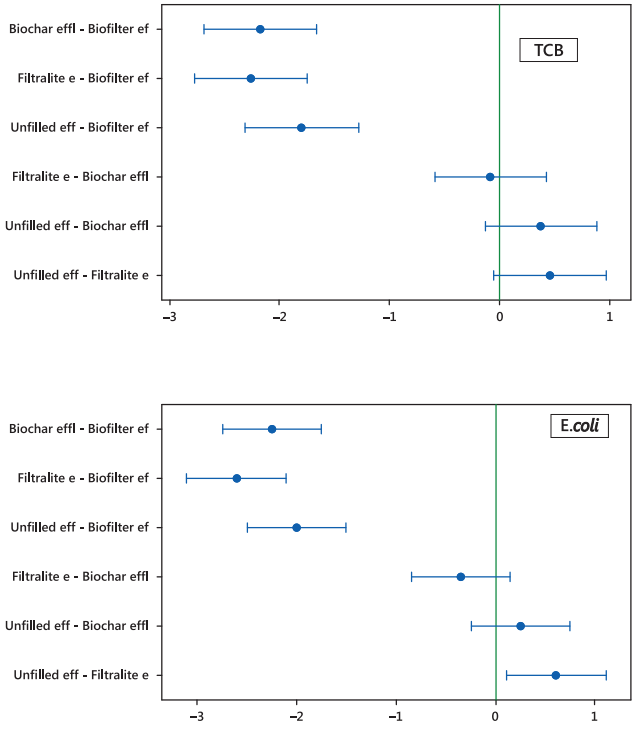

Figure A1.

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Paper III

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Sludge blanket anaerobic baffled reactor for source-separated blackwater treatment

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ABSTRACT

The performance of a sludge blanket anaerobic baffled reactor was tested as an integrated treatment system for source-separated blackwater. The system consists of a stirred equalization tank, a buffer inlet tank, and two identical reactors, each with a working volume of 16.4 L, operated in parallel. Both reactors run at 3-days hydraulic retention time with different intermittent pulse feeding. Pulse lengths of 12 and 24 seconds per feed were set with respective rates of 114 L h^{-1} and 52 L h^{-1} for the short-pulse fed reactor (RI) and the long-pulse fed reactor (RII). Stable performance of the reactors was attained after 120 and 90 days, for RI and RII, respectively. After stable conditions attained, total chemical oxygen demand (COD) removal efficiency stabilized above 78%. Biogas production ranged from 0.52 to 1.16 L d^{-1} L^{-1} reactor volume, with 67–82% methane concentration and an average conversion of 0.69 ± 0.2 and 0.73 ± 0.2 g CH_4-COD $g^{-1}COD_{in}$ for RI and RII, respectively. The results imply that source-separated blackwater can be treated effectively in an anaerobic sludge blanket process on average loading rate of 2.3 ± 0.5 g COD d^{-1} L^{-1} reactor volume with high methane production potential and more than 80% removal of organic and particulate matter.

Key words | anaerobic digestion, blackwater, resource-recovery, sludge blanket, source-separation

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INTRODUCTION

Considering the increasing concerns of water scarcity and environmental pollution, a new trend has emerged for decentralized and source-separated approaches to processing wastewater as a resource. Source-separation of wastewater involves separate collection and treatment of the different domestic wastewater streams. About 70% of organic matter (chemical oxygen demand (COD)) and 80% of nutrients discharged by a household into the wastewater originate from toilets (Langergraber & Muellegger 2005; Kujawa-Roeleveld & Zeeman 2006; Todt 2015), which constitute only 1% by volume of the total domestic wastewater.

Recent studies on separate collection and treatment of blackwater (BW) fraction show that anaerobic upflow reactors have the potential for energy and nutrient recovery (Kujawa-Roeleveld & Zeeman 2006; Zeeman & Kujawa-Roeleveld 2011). The key feature of anaerobic upflow reactors is the formation of sludge blankets in which biomass and particulate organic matter are retained in the reactor. The upflow mode provides sufficient contact between anaerobic sludge and incoming substrate of the wastewater, thereby increasing the physical removal of suspended solids and biological conversion of dissolved organic compounds (Luostarinen & Rintala 2005). Understanding factors that influence those key features of upflow reactors will help to develop robust and effective treatment processes. The feasibility of sludge bed anaerobic processes for blackwater, therefore, depends primarily on: (i) the nature of the organic components in the blackwater, (ii) the operational conditions, particularly the organic loading rate (OLR), hydraulic loading rate (HLR), pH and temperature, and (iii) the reactor configuration, especially its capacity to retain biomass in the sludge bed.

The suspended solids content of blackwater is higher than what is considered suitable for upflow anaerobic sludge blanket (UASB) reactors so an anaerobic baffled

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reactor (ABR) was applied. Studies with animal manure as feed have shown that feeds with high suspended solids content can be treated in sludge blanket ABR reactor at organic loading rates up to 400 g COD L^{-1} reactor d^{-1} at hydraulic retention time of 1.7 h (Bergland *et al.* 2015), which makes it potentially attractive for blackwater treatment. The performance of such a reactor principle, adapted for an integrated treatment system for source-separated blackwater, was tested here. The aim of this study was to evaluate effects of load and feed pulses on the system performance in terms of initial adaptation, stability, effluent quality, the removal efficiency of organic and suspended particulate matter, biogas production and methane yield.

METHODS

Collection and characterization of source-separated blackwater

The substrate used in this anaerobic digestion experiment is source-separated blackwater (BW) collected from student dormitories at the Norwegian University of Life Sciences with 48 inhabitants. The dormitory is equipped with vacuum toilets with 1.2-L flushing volume. A vacuum pump with an integrated grinder (Vacuumarator™ 25MB, Jets, Hareid, Norway) delivers the BW to a pumping station from which it is transferred with an impeller pump (40U, Tsurumi Europe GmbH, Düsseldorf, Germany), into a stirred storage tank located in the laboratory facility. Total retention time in the sewer system is 36–48 h. More details are given in Todt *et al.* (2015). Samples were taken from this tank on weekly bases to study and the composition of this BW was analyzed according to standard methods as described in the Liquid analysis section.

Reactor configuration and setup

Figure 1 displays the schematic flow of the experimental set-up. The experimental set up consists of a continuously stirred raw BW storage tank, a buffer tank and two cylinder shaped laboratory-scale two stage sludge blanket anaerobic baffled reactors with a working volume of 16.4 L each. The reactors were constructed from 10 mm thick PVC pipe section with an internal structure to establish two chambers. The first chamber has internal dimensions of 315 mm height and 315 mm diameter. The buffer tank has a working volume of 12 L with a retention time of 8 h. The pH in the buffer tank lowered to an average of 7.4 ± 0.6 from the inlet blackwater pH of 9 ± 0.3. The temperature in the buffer tank ranged from 10 to 15 °C in the winter time and from 18 to 21 °C during the summer time. The feed enters from the buffer tank to the bottom of the first chamber of the reactors using peristaltic pumps. The blackwater flows from the top of this first chamber, directed by a baffle, to the bottom of a smaller chamber of 245 mm height and 135 mm diameter, therefore defined as an ABR. One-third of the second chamber is used for down flow and remaining two-thirds is used for upflow. The reactors were fed intermittently with 16 pulses per day with partially hydrolyzed blackwater from a buffer tank using peristaltic pumps. Two different pulse lengths, 12 and 24 seconds per pulse, were applied for Reactor I and Reactor II, respectively. The hydraulic load was 6 L d^{-1} for both reactors and flow rates were set at 114 L h^{-1} for the short-pulse fed reactor (RI) and 52 L h^{-1} the long-pulse fed reactor (RII). The flow rate was set by adjusting the rotation speed of the peristaltic pumps with help of a frequency converter. The flow velocities in the compartments were calculated based on the pulse volume, pulse length and related cross-section area. A water lock on the outlet was used to separate the produced gas from the effluent liquid. The reactor temperature was adjusted to stay within the 25 and 28 °C range with help of a heated water bath to keep the reactors at a constant temperature. The reactors were inoculated with the same sludge from previous experiment. One-third of the operational volume was filled with inoculum.

Liquid analysis

Inlet raw blackwater and digested effluent samples were taken on a weekly basis in form of 24 h composite samples. Samples were also taken at the bottom of the two chambers in each of the reactors every 2 to 3 weeks to sample and analyze the sludge. Analysis of chemical oxygen demand, both total (CODt) and soluble (CODs), pH, total ammonia nitrogen (TAN), total and soluble phosphorus (P-tot and PO_4-P), total suspended solids (TSS), total solids (TS), volatile solids (VS), volatile suspended solids (VSS), and measurement of the concentration of volatile fatty acids (VFAs) were carried out to determine the characteristics and efficiency of the system. Total COD and total P were measured from the unfiltered sample. Soluble COD, PO_4-P, and TAN were measured from filtered samples using 1.2 μm glass fiber filters. CODt and CODs concentrations were analyzed using spectrophotometric test kits (Hach-Lange, Berlin, Germany) LCK 014 and LCK 514, respectively. Total P, PO_4^{-3}-P, and

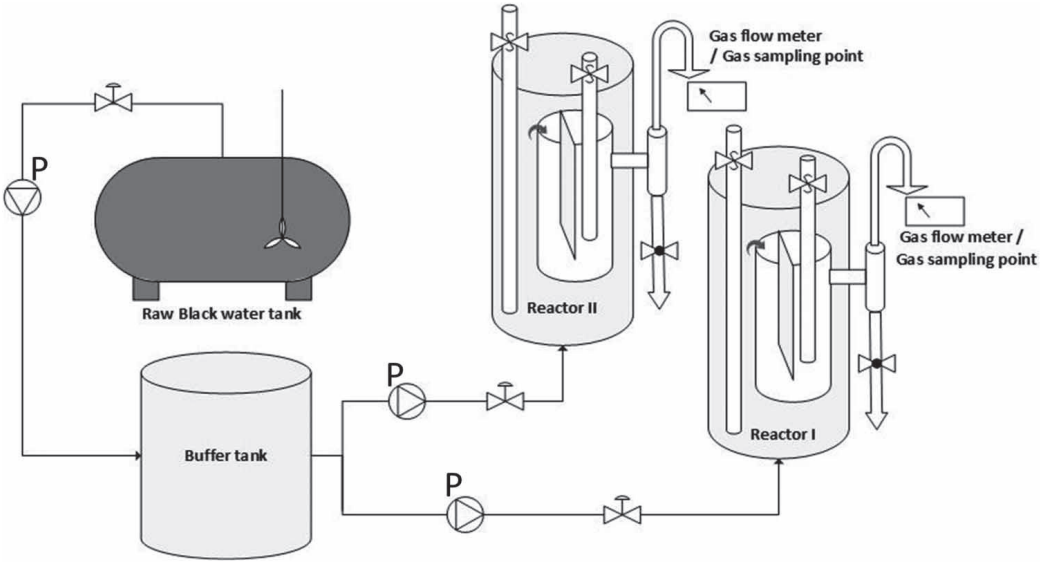

Figure 1 | Flow scheme of the experimental setup. P indicates pumps and the valve signs indicate sampling points.

NH_4-N in the filtered samples were diluted (with a dilution factor of 10^3) and analyzed using Hach-Lange test kits of LCK 349 and LCK 304, respectively.

TSS and VSS retained on the 1.2 μm glass fiber filters (Whatman GF-C, GE Healthcare, Little Chalfont, UK) and TS and VS were determined using standard methods (American Public Health Association (APHA) 2005). Settling rate of effluent sludge was measured as volume of settled sludge per L effluent sample both after 5 min and 30 min (standard for the sludge volume index (SVI)) to obtain more information about settling rate than SVI alone. For VFA analysis, samples were centrifuged at 6,000 rpm for 10 min and the supernatant was filtered through 0.45 μm membrane filter prior to analysis. VFA was analyzed using gas chromatography (HP 6890 serial C) with a flame ionization detector and a capillary column DB-FFAP 30 m long, inner diameter 0.25 mm and 0.25 μm film. Helium was used as the carrier gas, with flow velocity of 23 mL/min. The detector gases were hydrogen and air. The injector and the detector temperatures were set to 200 °C and 250 °C, respectively. The oven was programmed to hold at 80 °C for 1 min, go to 100 °C at a rate of 15 °C/min, and then to 230 °C at a rate of 100 °C/min (Bergland *et al.* 2015).

Biogas monitoring

Biogas production, from both reactors, was monitored daily. The gas volume was measured continuously using Ritter® MilliGas counter (Dr.-Ing. Ritter Apparatebau GmbH & Co. KG). Gas samples were collected using 1 L collection bag (7˝x7˝ multi-layer RESTEK, Bellefonte, USA) for CH_4 and CO_2 determination. Biogas composition, as methane (CH_4) and carbon dioxide (CO_2), was measured using Agilent Technology 3000A Micro Gas Chromatograph (Agilent Technologies Inc., Germany). The gas chromatograph comprised of a micro injector, thermal conductivity detector and a high-resolution capillary column. Helium was used as the carrier gas at a flow rate of 17 mL min^{-1}.

Methane production as COD mass load in the biogas (COD_{CH4}) was calculated from the average measured methane CH_4 fraction (partial pressure of methane) in the biogas (fCH_4 in *Pa*), the daily cumulative gas flow rate (*Qgas* m^3/d), and the theoretical oxygen demand for CH_4 *TOD(CH4)* (64 g COD_{CH4} mol^{-1}). R is the universal gas constant (8.3145 m^3 Pa mol^{-1} K^{-1}) and T is operational reactor temperature (°C)).

$$CODCH4 = \frac{fCH4 * \mathrm{Qgas}}{R * (T + 273)} * \mathrm{TOD}(CH4) \quad (1)$$

Mass balance calculation

A Microsoft Excel COD mass balance sheet was created to calculate the COD mass balance with the COD mass loading (g O_2 d^{-1}) determined for the inlet (COD_{in}), effluent (COD_{out}), excess sludge (COD_{sludge}, removed infrequently to sample the sludge) and gas (COD_{CH4}). OLR is expressed as the daily load of organic matter determined as COD normalized per reactor volume unit (g O_2 $L^{-1}d^{-1}$) where Q is the hydraulic load in L d^{-1}; Ccod is the COD concentration in g L^{-1} at the particular sampling point and a working volume of the reactor in L (Vr):

$$OLR = \frac{Q*\mathrm{Ccod}}{Vr} \quad (2)$$

COD accumulated in the reactor in form of biomass/sludge (COD_{acc}) was then calculated from the OLR at COD_{in}, COD_{out}, $COD_{sludge,}$ and COD_{CH4}. All of the given mass balance figures are normalized per reactor volume unit (g O_2 d^{-1} L^{-1} working volume).

$$CODacc = COD(in - out) - CODCH4 - CODsludge \quad (3)$$

Statistical analysis

Analysis of variance (ANOVA) test (using Minitab 17 Statistical Software, Minitab, 2017) was performed to test whether the two feed pulse lengths of treatment and variation on organic loading have any significant effect on the performance of the process. Before ANOVA analysis, the data were checked to see whether they satisfied the conditions of normality and equality of variance required for ANOVA. The distribution of residuals was very similar at all levels and the normality plot showed that the residuals lie close to the diagonal line, which represent the ideal normal distribution. The distribution of the residuals further tested using Anderson-Darling Test for Normality. Test for equal variance was also performed using Leven's Test. Both the conditions of normality and equality of variance were satisfied to perform ANOVA.

RESULTS AND DISCUSSION

The raw blackwater (BW) composition used in this research is presented in Table 1 and is characterized by organic matter concentration measured as CODt, CODs, TSS, TS, VS, pH, volatile fatty acid, ammonium nitrogen and phosphorus. The total COD concentration in the influent ranged between 1,900 and 7,600 mg/L, and the corresponding soluble COD concentrations were in the range between 400 and 2,300 mg/L. The average of the influent particulate COD ratio ((CODt-CODs)/CODt) ratio remained relatively high (0.8 on average) throughout the operation. The influent COD is therefore mainly particulate and constitutes about 77% of the total COD. Similar blackwater composition results are also reported (Murat Hocaoglu *et al.* 2010; Todt *et al.* 2015). The COD of the filtered sample, defined as the soluble fraction, constitute only about 23% of total COD.

Table 1 | The composition of BW used as feed for the reactors during the experimental period

Parameter	Unit	Average
pH		9 ± 0.3
CODt	mg/L	5,500 ± 1,300
CODf	mg/L	1,200 ± 330
TSS	mg/L	3,000 ± 900
TS	mg/L	6,300 ± 700
VFA	mg/L	400 ± 200
VS	mg/L	4,800 ± 600
NH_4-N	mg/L	900 ± 180
Tot P	mg/L	120 ± 20
PO_4^-P	mg/L	60 ± 20

The ± shows the standard deviation.

The influent TSS concentration ranged from 1,000–5,900 mg/L. The high standard deviation of COD and TSS indicates the significant temporal variability of raw BW composition during the study period. The variations in BW composition could arise from several factors including the diet of the inhabitants, toilet paper consumption and numbers of flushing events per toilet visit.

COD removal efficiency

During the start-up phase that lasted about 5 months, the removal efficiency of total COD varied from 24 to 67% with an average of 48% in RI and from −4 to 74% with an average of 36% in RII (Figure 2 top). Suspended particulate COD fraction removal during this stage of the operation was on average 68 and 76% for RI and RII, respectively. The filtered COD fraction (CODs) removal was negative for the first 3 months (Figure 2 bottom), implying a greater hydrolysis rate of accumulated organic matter compared to the methane production rate during the first 120 days of operation.

The surplus dissolved organics in the effluent compared to influent dissolved organics diminished with time and

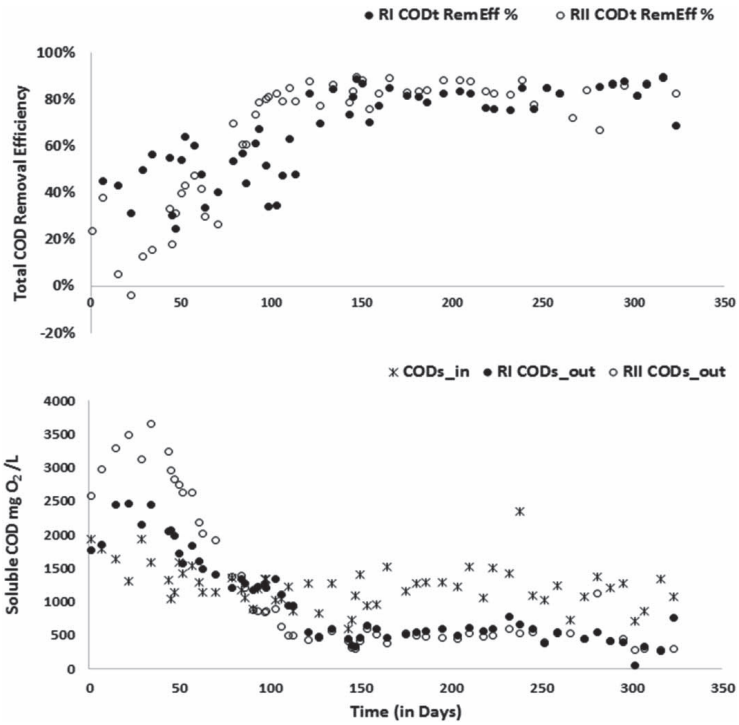

Figure 2 | Total COD removal efficiency (CODt RemEff %) in RI and RII (top), and measured soluble COD (CODs) in and out of the reactors (bottom) during the start-up and after stable performance.

reached stable condition after 120 and 90 days for RI and RII, respectively (Figure 2 bottom). During this period, both particulate and soluble organic fraction removal stabilized with an average removal efficiency of 86 and 90% for particulate COD and 55 and 54% for the soluble fraction in RI and RII, respectively. This implies that the sludge blanket–ABR reactor configuration achieved efficient retention and degradation of particulate organic matter.

Effect of organic loading rates

During the stable condition period, the two reactors received on average an organic load of 38 ± 7 g O_2 d^{-1} and $28. \pm 10$ g O_2 d^{-1} COD for RI and RII, respectively. This translates into an OLR normalized per reactor volume of 2.3 ± 0.5 and 1.6 ± 0.6 g O_2 d^{-1} L^{-1}, respectively. The variability of the organic load was more pronounced in RII than RI (Figure 3) and likely a result of different flow velocities out of the buffer tank during feeding, which were 610 m/h and 320 m/h for RI and RII, respectively. However, this difference did not influence the effluent quality at stable conditions. Both reactors achieved similar COD removal efficiencies ($p = 0.197$) and had comparable ($p = 0.588$) methane conversion rates of 0.69 and 0.73 g CH_4-COD g^{-1}CODin L^{-1} reactor volume for RI and RII, respectively.

Effects of feed pulse length

It can be seen from Figure 4 that effluent sludge settling rate at 5 min and 30 min of sedimentation for both RI (top) and RII (bottom) were similar. Most of the effluent sludge from both reactors settled within 5 min. Hence, the change in the volume of effluent sludge between 5 and 30 min sedimentation time was insignificant ($p = 0.81$ for RI and $p = 0.66$ for RII). The settled effluent sludge volume was higher for RI than in RII except for the first few days. However, after a stable condition was reached, the effluent sludge volume in both reactors were close to zero.

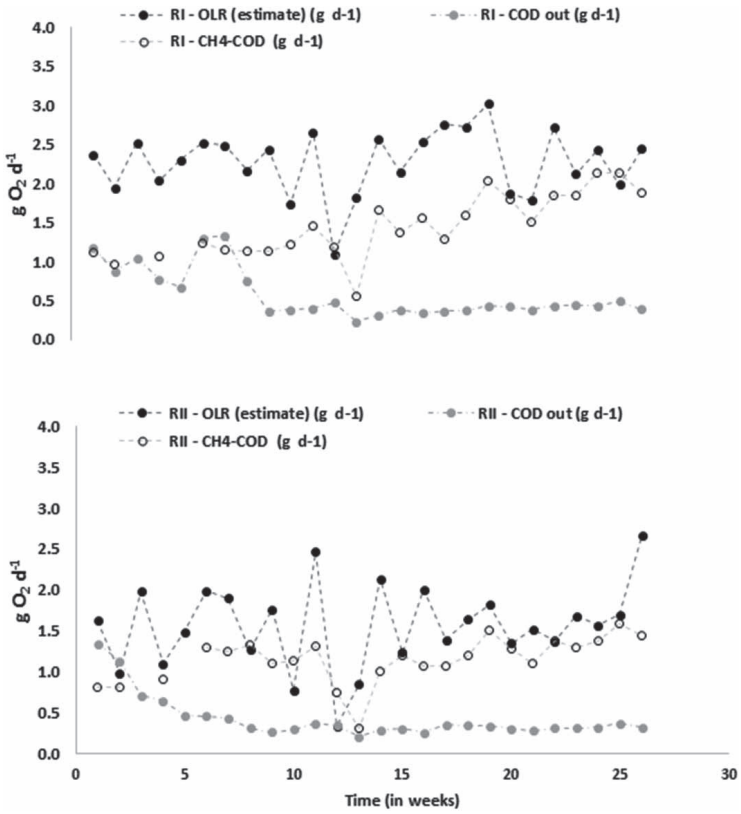

Figure 3 | COD mass loading rates, normalized per liter reactor volume for inlet, gas (CH_4) and effluent for RI (top) and RII (bottom).

The upflow velocity plays an important role in determining the behavior of sludge development in sludge beds and sludge blanket expansion (Wiegant 2001; Mahmoud 2002; van Lier *et al.* 2008). In our reactors, the upflow velocity is determined by the actual flow rate during pulse feedings of 114 L h^{-1} and 52 L h^{-1} resulting in an upflow velocity of 1.5 and 0.7 m h^{-1} for RI and RII, respectively. The up-flow water velocity usually ranges between 0.1 and 1.4 m h^{-1} in UASB reactors (Korsak 2008). The high rate of flow in this study lasts, however, only for a very short time for 12 and 24 seconds per pulse with 90 min long pulse intervals. The average upflow velocity was therefore much less than this actual pulse upflow velocity. It is calculated that the high flow rate, during pulse feed, lifts the sludge blanket by about 6 mm but it slowly sinks between the pulses. In unmatured reactors, this may cause instability and removal of more biomass to the effluent, which is especially the case at the startup stage in RI, requiring a longer time to reach steady. Stable condition was reached sooner for the less intense feed pulse (RII) than for the high flow pulse (RI). Studies on the effect of upflow velocity on suspended solid removal indicated deterioration of effluent quality as upflow velocity increases from 0.7 to 0.9 m/h to 3.2 m/h (GonÇalves *et al.* 1994). However, no differences in residual sludge volume were observed in the effluents of the two reactors (RI vs RII) after a stable condition was achieved (Figure 4) where, in both cases, effluent sludge volume was close to zero. Both reactors showed further a comparable COD removal efficiency (Figure 2), implying that the reactors had sufficient sludge expansion volume, solid separation and mass transfer capacity for both feed pulses tested.

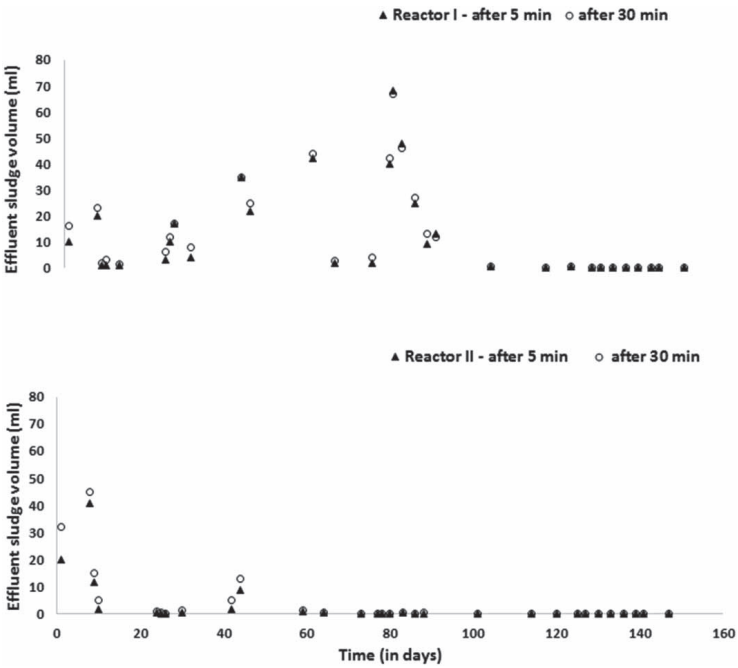

Figure 4 | Effluent sludge volume of RI (top) and RII (bottom) at 5 and 30 min of sedimentation time during the study.

Production and influence of volatile fatty acid

Start-up period

The organic substrates present in the blackwater were subjected to simultaneous hydrolysis and acidification by hydrolytic and acidogenic bacteria in the feed buffer tank, reflected in low pH at the bottom of the buffer tank and formation of VFA. Acetate was the prime VFA constituent in the buffer tank, as well as in the different parts of the reactors and effluents. The ratio of acetate to total VFAs reached up to 93% with an average of 71 ± 15%, which shows high efficiency of acidogenic and acetogenic bacteria. Acetate is produced in anaerobic biodegradation of carbohydrates, protein, and fats (Narkis *et al.* 1980). During this start-up phase, total VFA concentrations in the reactor effluent were higher (with an average of 893 ± 473 mg/L for R I, and 1,700 ± 561 mg/L for RII) than the feed blackwater (440 ± 234 mg/L) and reached peak value after 2 months in both RI than RII (Figure 5). This demonstrates that the establishment of methanogenesis was lagging behind acidogenesis due to the slow growth rate of methanogenic archaea. Effluent VFA decreased sharply towards the end of the start-up period and all the acetate produced was converted into methane after stable condition attained. The concentration of VFA in the effluent also corresponds with the aforementioned filtered COD (CODs) concentrations of the effluent (Figure 2 bottom). Propionic acid concentration was also relatively high in the blackwater but lower in the reactor effluents, implying that methanogenesis was the overall rate-limiting step until the stable condition reached.

Stable performance period

The methane production progressively increased when the reactors matured and 60–70% of the feed COD was converted to methane. Effluent VFA concentrations decreased and the COD and TSS removal reached up to 89 and 90%, respectively. Figure 6 shows the average VFA concentration after a stable condition is attained from the inlet tank, buffer tank and the two chambers of the two reactors.

The concentration of VFA in the buffer tank reached a peak value of 4,750 mg/L and had higher values than the

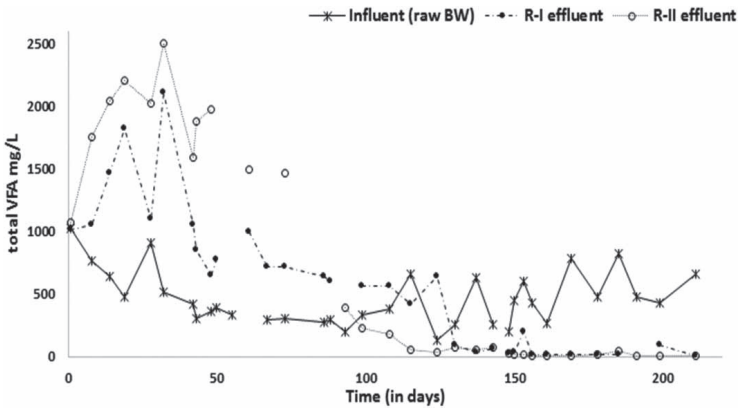

Figure 5 | Total VFA in the influent (raw blackwater) and effluents of RI and RII during the start-up period and after a stable condition was attained.

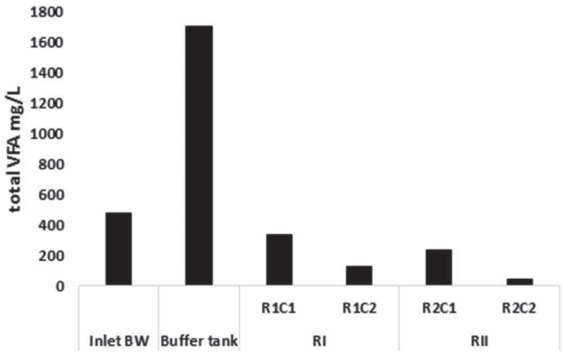

Figure 6 | Average total VFA with standard deviation of 238, 204, 125, 42, 109, and 27 in the inlet blackwater, buffer tank, and different compartments of RI and RII, respectively (where R1C1 = reactor 1 compartment 1, R1C2 = reactor 1 compartment 2, R2C1 = reactor 2 compartment 1, R2C2 = reactor 2 compartment 2).

raw blackwater throughout the operation period, but degraded very rapidly in the reactors. The buffer tank, therefore, serves as a pre-hydrolysis and fermentative step. Most of the VFA was removed in the first reactor compartment and it was almost completely removed in the effluent (compartment 2). Such VFA concentration levels indicate the stability of the reactors (de Mes *et al.* 2003; Colón *et al.* 2015). VFAs can be considered reliable for process monitoring (Murto *et al.* 2004).

pH

Overall, in both reactors pH remained stable for most of the time both in the influent and in the effluent during the operation period. This is mainly due to the high buffer capacity (alkalinity of 560 ± 58 mg/L CaCO3), as well as high ammonium concentration (851 ± 174 mg/L NH4-N) in the influent. The average pH of the influent was 9.1 ± 0.3 and the corresponding pH for the effluent of RI and RII was 8.4 ± 0.2 and 8.1 ± 0.3, respectively. In AD, pH is a key factor in the formation and characterization of VFA and the ammonium/free ammonia equilibrium (Ortiz *et al.* 2014).The pH influences bacterial and archaeal growth rates (Espinoza-Escalante *et al.* 2009). Acetate was the main product of acidogenic degradation in the buffer tank and was also the main VFA component in the different reactor compartments and effluents. In such highly buffered systems, pH changes were small even if VFA varied considerably.

Effluent quality

To investigate the influence of feed pulse length on the effluent quality of the sludge blanket ABR, the reactor performance and effluent quality of the two reactors were compared. The effect of differences in feed pulse length was observed at the start-up period. However, the removal efficiencies of the two reactors demonstrated no significant effects on effluent quality after a stable condition was attained. The results of TSS, CODt, CODs, and VFA removal efficiencies were similar in both reactors at a confidence interval of 95% with *p*-values of 0.241 and 0.197 for TSS and COD, respectively. Likewise, the effluent concentrations of NH_4-N (926 ± 113 mg/L for RI and 959 ± 188 mg/L for RII), and PO_4-P (84 ± 12 and 87 ± 17 mg/L for RI and RII, respectively) in both reactors were comparable but much higher than the concentrations in the raw blackwater (851 ± 174 mg/L NH_4-N and 60 ± 17 mg/L PO_4-P). Hence, the system produced excess soluble N and P in the effluent, which opens up the opportunity to recover these valuable resources with novel post-treatment steps.

Mass balance and potential methane recovery

Biogas production

Biogas production and methane content were measured and compared between the two reactors. Biogas production ranged from 8.6 to 19 L d^{-1} in RI and 6 to 10 L d^{-1} for RII, with an average methane content of 70 ± 6% and 74 ± 8%, respectively. The biogas production variations were attributed to organic loading fluctuation. High biogas yield and methane content in the present study can be attributed to a combination of reactor configuration, feed composition and significant pre-hydrolysis in the buffer tank. The methane content in this sludge blanket anaerobic baffle reactor was higher compared to some other systems such as conventional UASB with biogas methane content fluctuating between 40 and 60% (Yu *et al.* 2002), but comparable to reported biogas yield in co-digestion of blackwater (Elmitwalli *et al.* 2002) and in a 'MIX-UASB reactor' (Tervahauta *et al.* 2014). The study shows that biogas with high methane content can be recovered from source-separated blackwater under conditions tested here.

COD mass balance

Figure 7 presents steady state COD mass balance for the two reactors. The cumulative organic load after stable condition was achieved 0.30 and 0.21 kg COD with an average daily normalized OLR of 2.3 and 1.6 g O_2 d^{-1} L^{-1} reactor volume and a hydraulic loading of 681 and 718 L for RI and RII, respectively. The amount of COD retained or accumulated as biomass in the reactors were 14% for RI and 5% for RII implying slow build-up of the sludge bed. In the 18 weeks of stable performance period, only 1.1 and 1 L of sludge was removed from RI and RII, respectively, This is beneficial from the operational point of view, as it demonstrates that the process requires little withdrawal of excess sludge. Lower retained COD in RII is attributed to the higher conversion of COD to methane and more effluent COD. Residual COD fractions in the effluents were 17% and 20% in RI and RII, respectively.

During the stable condition period, an average of 1.60 ± 0.06 g O_2 COD d^{-1} L^{-1} reactor volume and 1.20 ± 0.02 g O_2 COD d^{-1} L^{-1} reactor volume was converted to CH_4 in RI and RII, respectively. This translates into a methane conversion rate of 69% and 73% relative to the inlet COD load. This is high compared to other studies on concentrated blackwater where only 40% of the incoming COD load converted to biogas, while 40 to 50% was accumulated as non- or slowly-degradable matter and 10 to 20% washed out from the system (Verstraete *et al.* 2009). The high biogas yield in the present study can be attributed to a combination of reactor configuration, feed composition, pulse feeding and significant pre-hydrolysis in the buffer tank. The study shows the potential of methane recovery from the source-separated blackwater with 3 days of hydraulic retention time.

CONCLUSIONS

In this study, source-separated blackwater was anaerobically treated with a sludge bed anaerobic reactor at controlled temperature (i.e. 25 to 28 °C) for several months, going from variable efficiency to steady-state in less than half a year. The results revealed that concentrated source-separated blackwater was treated efficiently at 3 d hydraulic retention time (HRT) with total COD removal efficiency stabilized above 78% at steady state. Biogas production ranged from 6 to 19 L d^{-1} and an average conversion of 0.69 and 0.73 g CH_4-COD $g^{-1}COD_{in}$ at steady-state for the two reactors operated with different feed pulses. Feed pulse length influenced significantly the early phase of the AD process. Short and strong feed pulse resulted in a more unstable performance at start-up phase and longer time to reach stable condition compared to the longer pulse feeds with lower flow rate, but similar steady-state

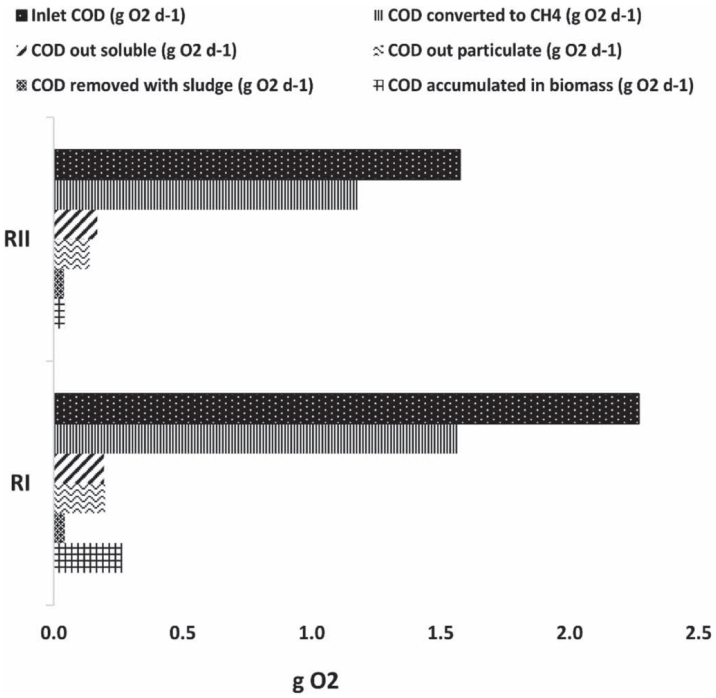

Figure 7 | The COD mass balance of RI and RII at steady state.

performances were observed for the two feed pulses. Although gas production was mainly influenced by the uncontrolled change in the influent composition, the biogas methane concentration was quite stable. The results imply that source-separated blackwater can be treated effectively in an anaerobic sludge blanket process at an average loading rate of 2.3 g COD d^{-1} L^{-1} reactor volume with high methane production and removal of organic particulate matter. It also revealed that the reactors had sufficient sludge expansion volume, solid separation and mass transfer capacity for both feed pulses tested.

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Paper IV

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Article

Treatment of Source-Separated Blackwater: A Decentralized Strategy for Nutrient Recovery towards a Circular Economy

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Abstract: Using a filter medium for organic matter removal and nutrient recovery from blackwater treatment is a novel concept and has not been investigated sufficiently to date. This paper demonstrates a combined blackwater treatment and nutrient-recovery strategy and establishes mechanisms for a more dependable source of plant nutrients aiming at a circular economy. Source-separated blackwater from a student dormitory was used as feedstock for a sludge blanket anaerobic-baffled reactor. The effluent from the reactor, with 710 mg L^{-1} NH_4–N and 63 mg L^{-1} PO_4–P, was treated in a sequence of upflow and downflow filtration columns using granular activated carbon, Cocos char and polonite as filter media at a flow rate of 600 L m^{-2} day^{-1} and organic loading rate of 430 g chemical oxygen demand (COD) m^{-2} day^{-1}. Filtration treatment of the anaerobic effluent with carbon adsorbents removed 80% of the residual organic matter, more than 90% of suspended solids, and turbidity while releasing more than 76% NH_4–N and 85% of PO_4–P in the liquid phase. The treatment train also removed total coliform bacteria and *E. coli* in the effluent, achieving concentrations below detection limit after the integration of ultraviolet (UV) light. These integrated technological pathways ensure simultaneous nutrient recovery as a nutrient solution, pathogen inactivation, and reduction of active organic substances. The treated nutrient-rich water can be applied as a source of value creation for various end-use options.

Keywords: source-separation; blackwater treatment; nutrient-recovery; on-site wastewater treatment

1. Introduction

Population growth and rapid urbanization during the early 1900s led to the development of synthetic fertilizers to supplement crop production and meet the world's food demand [1,2]. Harnessing P from phosphate rock reserves, K from potash reserves and N fixed from the atmosphere by the Haber–Bosch process helped to spawn the Green Revolution, and resulted in a rapid intensification of anthropogenic flows of N, P and K over the last century [3]. Phosphorus and nitrogen from agricultural food products are transported into cities and eventually end up in domestic wastewater streams. Globally, nearly 20% of manufactured nitrogen and phosphorous is contained in domestic wastewater [4,5]. The majority of these nutrients and organic matter in domestic wastewater come from a small fraction of the wastewater stream—human urine and feces, hereafter called blackwater [6,7]. Most modern cities established centralized sewer systems with a network of collection pipes for transporting domestic wastewater to a municipal wastewater treatment plant, and with that improved public health and environmental quality [8,9]. In these systems, enormous volumes of freshwater are required to transport the small volume of human excreta from the toilet to the

wastewater treatment plant [10,11]. Moreover, the nutrients from the toilet are highly diluted by wastewater from other sources including storm water and groundwater intrusion. Hence, high levels of energy and large amounts of chemicals are needed for processing in order to recover these resources, and significant amounts of nutrients are lost before reaching the treatment plant through leakages and overflows.

Increased demand for water, energy and food by the growing population and the necessity for a simultaneous reduction of the environmental impact of wastewater has increased the need for an innovative solution. Protecting water bodies from eutrophication, ensuring long-term food security and shifting to a circular economy represent compelling objectives for water-, energy- and nutrient-management strategies [12]. In this regard, domestic wastewater can be seen as a resource rich in water, energy and plant nutrients [13–15] with potential to contribute to the circular economy. Using the principles of source-separation, the perception of wastewater treatment is now advancing from end-of-pipe removal of pollutants to the recovery of resources [13,16–18] with the realization of the value of treated water, energy and nutrients. However, many of the energy-recovery technologies and most of the resource-recovery approaches require large-scale operations to be economically viable [19]. Research should focus, therefore, on technologies that could be economically realistic in smaller treatment plants and suitable for small-scale and decentralized systems.

Source-separating and on-site treatment systems allow targeted treatment of source-separated wastewater streams, recovery and reuse of resources and control of pollutants in areas close to the sources [20,21]. By source-separating concentrated blackwater and co-digesting it with wet organic wastes (such as food waste), approximately 90% of the nitrogen, 74% of the phosphorus and 79% of the potassium can be reclaimed and recycled [22,23]. One potential approach to improve the effectiveness of recovery and recycling of nutrients is to couple it with biogas production through anaerobic digestion (AD). Anaerobic digestion alone, however, does not provide the necessary requirement in terms of nutrient recycling. Developing mechanisms for the removal of residual organic contaminants including micropollutants from anaerobically treated blackwater, while keeping essential plant nutrients in the liquid-phase, is vital as a source of value creation and for reducing both health-related and environmental risks. The principal advantages of this proposed system are, therefore, to couple energy recovery from the anaerobic digestion of source-separated blackwater with the production of a high-quality nutrient solution in an economic and environmentally friendly way. This further requires coupling the nutrient-recovery methods with the removal of pathogens and micropollutants such as pharmaceutical residues and personal care products (PPCP). On-site treatment of this untapped valuable resource using the appropriate level of technology and subsequent resource recovery will make source-separation an attractive domestic wastewater management option and a source of value creation.

The aim of this study is twofold: firstly, to develop and demonstrate a combined treatment and resource recovery approach for processing source-separated blackwater; and secondly, to promote closed-loop flows of resources and nutrients within the area close to the source of generation. Thereby, processing units remove organic substances and suspended solids while selectively recovering P and N in the liquid-phase as a nutrient solution. When integrated with a disinfection unit, the sanitized water can be applied for different end-use options, and/or safe discharge.

2. Materials and Methods

2.1. Composition of Anaerobically Digested Blackwater Effluent

Anaerobically treated blackwater effluent from a lab-scale anaerobic sludge blanket reactor [24] was collected in a 12 L storage tank and used as an inlet for a column filtration study. The composition of the effluent with respect to organic matter (both total and dissolved), total suspended solids (TSS), pH, and dissolved nutrients (mainly NH_4–N and PO_4–P) and *E. coli* were analyzed using standard methods as described in Section 2.3.

2.2. Filtration Column Study

For this study, three treatments of a sequential upflow and downflow filtration system with granular activated carbon (GAC), Cocos char (CCCH), and coarse polonite (C-Pol) was designed, each with two replications. Thus, 12 columns of 4.2 cm internal diameter and 41 cm bed height, filled with GAC, CCCH and C-Pol, were prepared. GAC was obtained from Chemviron Carbon, Calgon Carbon Corporation, Feluy, Belgium. Cocus char was obtained from Haaland A/S Stavanger, Norway and polonite from FANN Miljøteknikk AS, Drøbak, Norway. The operational parameters of this column experiment are shown in Table 1.

Table 1. Operational parameters of the column experimental setup.

Influent	Anaerobically Digested Blackwater Effluent
Column height (cm)	50
Internal diameter (cm)	4.2
Area of column (cm^2)	13.85
Column material	Plexi Glass
Bed height (cm)	41 of which 2 cm is filled with 3 mm glass beads at the bottom and on the top of the filter media
Bed volume (cm^3)	553.5
Filter media (adsorbents)	Granular activated carbon, Cocos char (CCCH) and polonite
Mass of adsorbent (g)	200, 200, 741, respectively
Particle size range (mm)	0.5–1.4, 0.35–1.18, and 2.8–4 for GAC, CCCH and polonite, respectively
Flow rate (mL/h)	35 (corresponds to—600 L m^{-2} day^{-1})
Retention time (h)	5 h for GAC and CCCH and 7 h for polonite
Mode of flow	Continuous upflow mode for saturated condition followed by downflow mode for unsaturated step (without effluent recycling)
Organic loading rate (g COD m^{-2} day^{-1})	430

Experimental Setup

Six columns, represented by CCCH_1, GAC_1 and C-Pol_1 in duplicates for the Cocos char column, granular activated carbon and coarse polonite, respectively, were operated in upflow. The other six, which also represent the final effluent, indicated as CCCH_2, GAC_2 and C-Pol_2 were operated in downflow mode for the three adsorbents in two replications. The particle sizes of the adsorbents range from 0.5–1.4, 0.35–1.18, 2.8–4 mm for GAC, CCCH and C-Pol, respectively. Before packing into the columns, adsorbents were thoroughly washed with tap water to remove fine particles and dried at 105 °C for 24 h. At the bottom of the filter column, a 1 mm diameter mesh and 2 cm of 3 mm diameter glass beads was placed for uniform distribution of the influent and as supporting material to prevent the particles from entering the inlet tube. Another 2 cm glass bead of 3 mm diameter was placed on top of each column to prevent floating of the carbon filter particles in the upflow mode and to allow uniform distribution in the unsaturated column. Figure 1 shows the flow scheme of the sequential upflow and downflow filtration system in two replicates for the three treatments.

The anaerobically digested blackwater was pumped into the first set of six columns in upward flow mode using a multi-channel peristaltic pump from a 12 L storage tank at a rate of about 600 L m^{-2} day^{-1}. The effluent of the first set of columns then flowed by gravity to the second set of columns in unsaturated flow mode (Figure 1). The final effluent passed through an ultraviolet (UV) light chamber 290 mm long and 55 mm diameter with an 11 Watt UV lamp inside a quartz sleeve of 200 mm length and 40 mm diameter with a working volume of 0.9 L. The retention time of treated effluent in the UV chamber was about 3 h. Samples were taken from the upflow and downflow effluents for analysis.

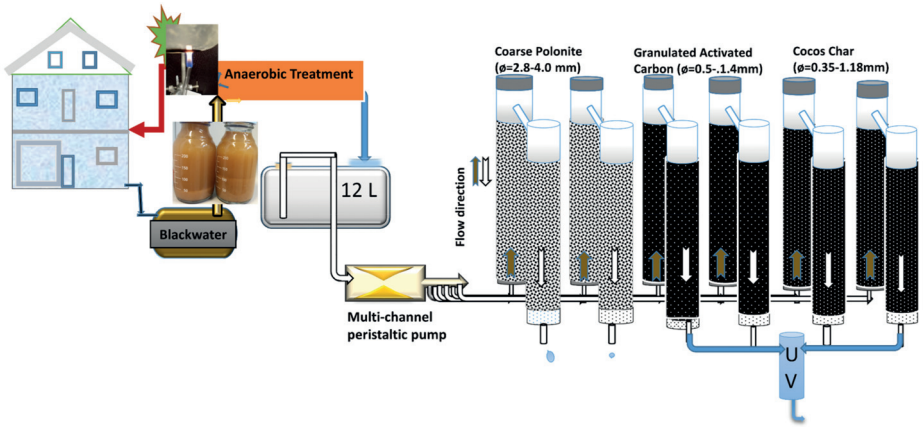

Figure 1. Flow scheme of sequential upflow and downflow filtration system in two replicates for the three treatments: coarse polonite (ø = 2.8–4.0 mm), granulated activated carbon (ø = 0.5–1.4 mm) and Cocos char (ø = 0.35–1.18 mm).

2.3. *Effluent Analysis*

For the first four months, samples of the anaerobically digested blackwater effluent and final filtrate were taken on a weekly basis and analysis for chemical oxygen demand (total CODt and filtered CODf), pH, ammonium nitrogen (NH_4–N), soluble phosphorous (PO_4–P), and total suspended solids (TSS) were carried out to determine the characteristics and efficiency of the filtration system. After four months, sampling was carried out every two weeks. A limited number of samples were also analyzed for turbidity, BOD_5, UV_{254}, NO_3–N, and NO_2–N.

Total COD was measured from the unfiltered sample. Filtered COD, PO_4–P, and NH_4–N were measured from filtered samples using 1.2 µm glass fiber filters. CODt and CODf concentrations were analyzed using spectrophotometric test kits (Hach-Lange, Berlin, Germany) LCK 014 and LCK 514, respectively. Soluble phosphate and NH_4–N in the filtered samples were diluted (with a dilution factor of 103) and analyzed using Hack Lange test kits of LCK 349 and LCK 304, respectively. Total suspended solids (TSS) retained on the 1.2 µm glass fiber filters (Whatman GF-C, GE Healthcare, Little Chalfont, UK) were determined using standard methods [25]. Samples were also taken, once a month, for *E. coli* and total coliform bacteria analysis from the effluent of the reactor and the filtrates to investigate the effects of the different filter media and the flow setup on pathogen reduction, following the standard analytical methods [25] using Colilert 18 test kits (IDEXX Laboratories Inc., Westbrook, ME, USA). In addition, macronutrients, K, Ca, Mg, S and Na, and selected heavy metals were analyzed from three samples of each sampling points using inductively coupled plasma mass spectrometry (ICP–MS, Oban, UK).

The amounts of organic matter, nitrogen and phosphorus that were retained in the filter column systems were calculated as the concentration difference between the influent and effluent COD, NH_4-N and PO_4-P, respectively. The retention/removal (R, %) of total COD, filtered COD and nitrogen and phosphorus within the filter columns, was calculated according to the following equation:

$$R = (1 - Ce/Ci) \times 100 \quad (1)$$

where Ce is the effluent concentration and Ci is the influent concentration.

3. Results and Discussions

3.1. Composition of Anaerobically Digested Blackwater Effluent

The raw blackwater contains more particulate organic and particulate phosphate fractions. The dissolved organic fraction in the raw blackwater constituted only 24%. Similar values are also reported in literature [23]. Likewise, the soluble phosphate fraction was 42% of the total P. Hydrolysis and fermentation in the anaerobic process resulted in the breakdown and solubilization of particulate organic matter and proteinaceous biomass. Subsequently, the effluent of anaerobically digested blackwater contained more than 60% dissolved organic fractions, which was 36% more than in the raw blackwater. Moreover, the anaerobically digested effluent comprised a higher concentration of soluble fractions of NH_4–N and PO_4–P. The increase in concentration of NH_4–N in the digestate resulted from mineralization of organic nitrogen and reduction of N-fixing carbon compounds [26]. Up to 86% of the total P was recorded in the effluent of the anaerobic digestion, of which 82% was in the soluble fraction. This provides an opportunity to recover and recycle the nutrients from blackwater as a source of value creation for value-added agricultural and other biomass production such as algal production.

3.2. Removal of Organic Matter and Suspended Solids

As shown from Figure 2, both Cocos char and granulated activated carbon filters columns had similar effects ($p > 0.05$) and removed on average about 80% of residual total COD and 73% of the residual filtered COD. The removal efficiency of the carbon filters for both total and filtered COD were also stable during the eight-month operation. Most of the removal of COD in both treatments occurred in the upward flow mode. The contribution of the downward unsaturated flow mode was, however, insignificant ($p = 0.32$). The average removal efficiency of polonite for both total and filtered COD was not substantial. During the eight-month operation period, the removal efficiency in the polonite column was only 43% and 12% for the total and filtered COD, respectively. Figure 3 shows the total and filtered COD concentrations during the filtration study period. The filtered organic matter in the polonite columns' effluent reached saturation after 50 days (Figure 3 (right)). It was also observed that the removal efficiency for filtered COD became negative after 100 days, probably due to dissociation of soluble organics form the biofilm that developed over time.

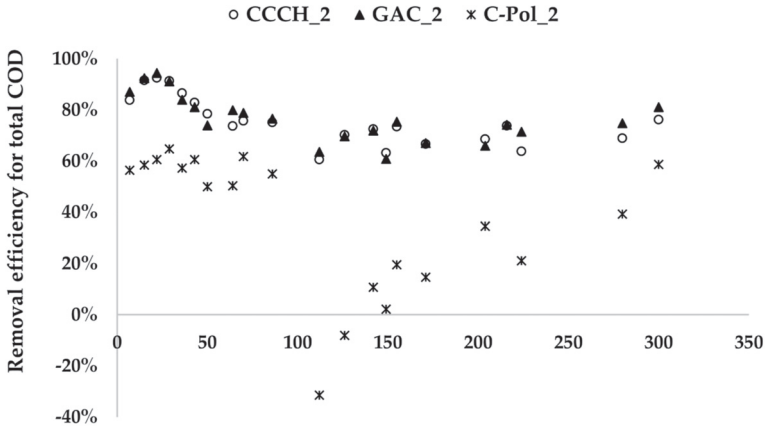

Figure 2. *Cont.*

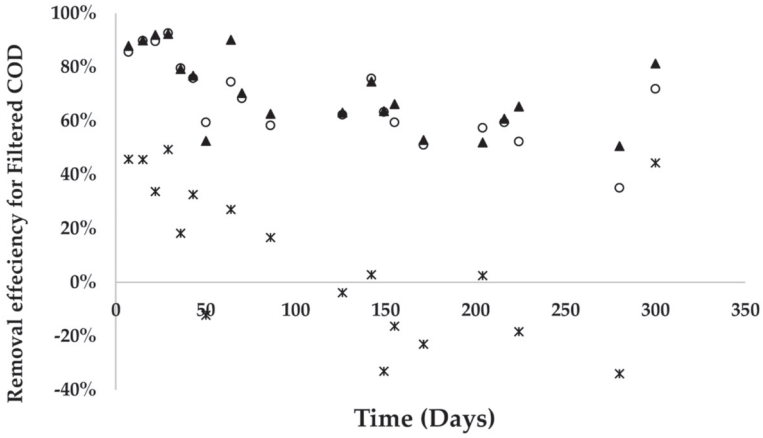

Figure 2. Total chemical oxygen demand (COD) (**top**) and filtered COD (**bottom**) removal efficiency of Cocos char (CCCH_2), granular activated carbon (GAC_2), and coarse polonite (C-Pol_2).

The filtration step removed more than 90% of the residual suspended solids from the anaerobically digested effluent. Although the contribution from the downflow filtration step to the removal of COD, both total and filtrated, was insignificant ($p = 0.32$) in all cases compared to the upflow column, this step significantly contributed to the removal of TSS (Figure 4). As an overall combined treatment chain, the anaerobic reactor and the carbon-filled filter columns achieved a removal efficiency of more than to 99% for TSS, i.e., from an average of 2700 mg/L in the raw blackwater to less than 10 mg/L in the effluents of the downflow columns. This also corresponds to a substantial reduction in turbidity and UV_{254} absorbance resulting <10 NTU and 93%, respectively. The reduction in turbidity and UV absorbance is a prerequisite for pathogen disinfection.

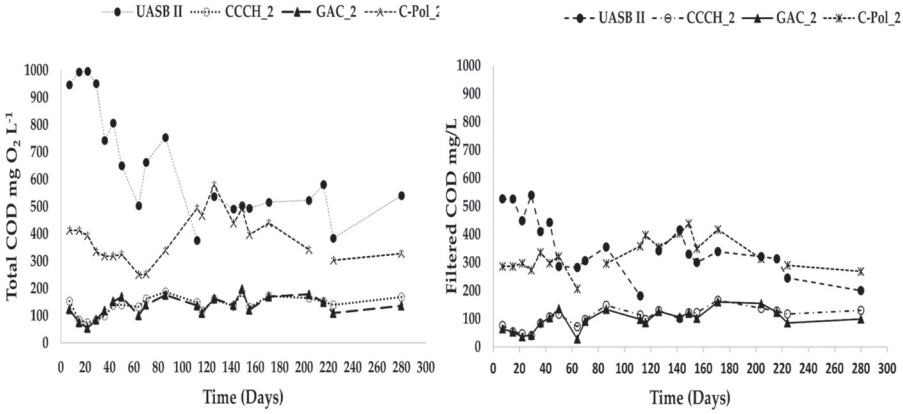

Figure 3. Total (**left**) and filtered (**right**) COD for the effluents of the anaerobic reactor (UASB II) and post-filter column effluents—Cocos char (CCCH_2), granular activated carbon (GAC_2), and coarse polonite (C-Pol_2).

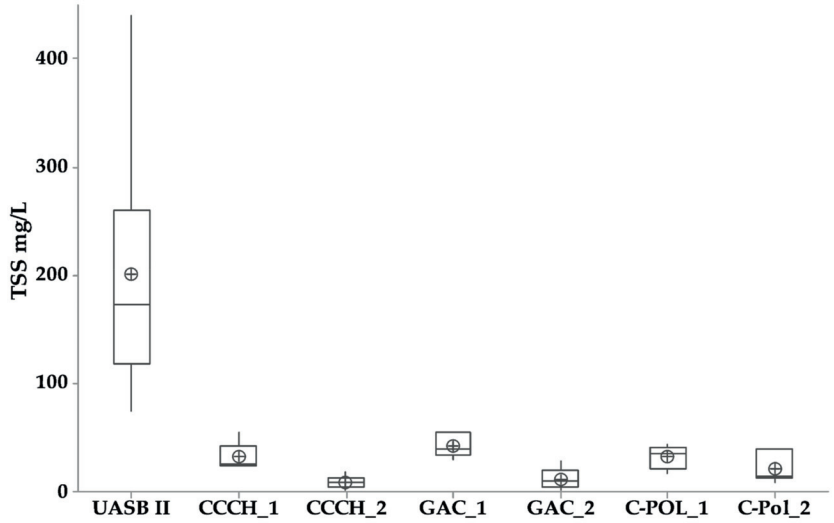

Figure 4. Total suspended solids (TSS) concentration of effluents of the UASB anaerobic rector (UASB II), upflow Cocos char column (CCCH_1) downflow Cocos char (CCCH_2), upflow granular activated carbon (GAC_1) downward granular activated carbon (GAC_2), upflow coarse polonite (C-Pol_1) and downflow coarse polonite (C-Pol_2).

3.3. Nutrient Recovery from Anaerobically Digested Blackwater Effluent

During anaerobic treatment, the soluble fraction of phosphate concentration increased from on average 42 mg PO_4–P L^{-1} in the raw blackwater to 63 mg PO_4–P L^{-1}. Similarly, the average NH_4–N concentration increased from 610 mg L^{-1} in raw blackwater to 720 mg L^{-1} in the anaerobic effluent. This could be mainly due to hydrolysis of organically bound particulate phosphorus and a decrease in pH during anaerobic treatment (from about 9 in BW to 8.2 in the anaerobically digested effluent) which leads to the solubilizing of inorganic phosphates [27]. The filtration of nutrient-rich anaerobic effluent through adsorbent filters is a cost-effective way of recovering these nutrients. The system described here could offer nutrient recovery in two ways. First, preserving the nutrients adsorbed at the surface of the filter materials for later use as a slow-release fertilizer. The second and most important way was through selective removal of organic matter and TSS and recovering N and P as a nutrient solution in a liquid phase.

3.3.1. Phosphorus Recovery

Figure 5 indicates the effect of the different filter materials on PO_4–P concentrations in the liquid-phase. Polonite showed high affinity to phosphate ions and higher phosphorus-retention efficiency has been observed in the polonite filtration column. Polonite exhibited a complete retention (100%) of phosphate in the first two weeks of the experiment and then stabilized between 70% and 92% retention until the fourth month. On the other hand, more than 85% of the PO_4–P is released in the liquid phase in the case of the carbon-based filter columns. This PO_4–P can be harvested as nutrient solution together with other plant nutrients or can be recovered in the form of struvite or Ca–PO_4–P.

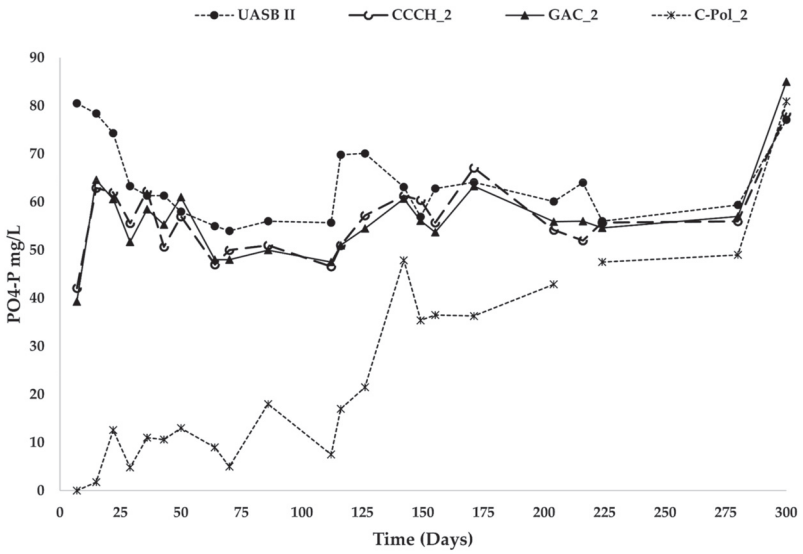

Figure 5. PO_4–P concentrations in the liquid-phase for effluents of the anaerobic reactor (UASB II) and filtration columns—Cocos char (CCCH_2), granular activated carbon (GAC_2), and coarse polonite (C-Pol_2).

The complete removal of phosphate by the polonite filter column in the first two weeks could be attributed to the high pH (above 10.6), the increased adsorption at the calcium–silicate surfaces, and precipitation by dissolved calcium [28]. However, the PO_4–P removal efficiency of the polonite column dropped to 24–42% after 140 days and to 15–18% after 240 days. Studies indicated that sorption of organic compounds to the mineral surface increases the negative charge or decreases the point of zero charge (PZC) [29]. Furthermore, the formation of biofilm at the surface of the polonite particles and simultaneous sorption of organic ions by the mineral surfaces could also alter the surface charge and cause phosphate ions to be electrostatically repelled. This inhibition of P sorption due to increased repulsion from the negatively charged surface might be the cause for the sharp increase of phosphate concentration in the effluent from the polonite filter column. The reduction of phosphate removal after 140 days could also be associated with the drop in pH to less than 8. The high organic load has also been found to reduce the lifetime of polonite considerably by preventing access to P on the adsorbed site of the filter material [30]. It was also observed that the presence of organic ligands could inhibit the precipitation of calcium phosphates, one of the principal mechanisms for P removal in polonite [31]. Consequently, when the polonite is saturated with the soluble organic matter, the polonite surface affinity to PO_4–P decreases and more phosphate ions will be released into the liquid phase.

Saturation of phosphate in the carbon filter column was reached in about 30 days compared to the polonite, which took 280 days. The low phosphorus-retention capacity observed in the carbon-based adsorbent could be attributed to its surface characteristics and the presence of a large amount of soluble organic ions. Previous studies have revealed that the surface of the biochar is often negatively charged, making it repel negatively charged ions such as phosphate [32,33]. Carbon-based adsorbents have a high affinity for organic ions [34] suggesting that soluble organic substances derived from hydrolysis and degradation in the anaerobic digestion are adsorbed first and occupy the sorption sites, thereby limiting P sorption. Moreover, the existence of high concentrations of bicarbonate in the solution reduced the phosphate adsorption [35]. High pH (higher than 7.8) of the anaerobically digested blackwater effluents can result in competitive reactions taking place

between hydroxyl and phosphate ions [36], thereby occupying the available adsorption sites on the carbon-based adsorbents.

3.3.2. Ammonium–Nitrogen Recovery

Adsorption of NH_4–N on the polonite column was significantly higher than on the two carbon columns ($p = 0.035$ for the Cocos char column and $p = 0.024$ for the granular activated carbon column). On average, only 22% NH_4–N was retained in the carbon filter column. The high initial pH measured from polonite effluent at an early stage may suggest the loss of NH_4–N in the first week due to the stripping of NH_3. The decrease in effluent NH_4–N concentration observed in the polonite column at the later stage could also result from microbial immobilization and nitrification due to the biofilm's development [37]. This is explained by the higher nitrite and nitrate concentrations in the polonite effluent compared to that in the other columns.

More than 75% of the NH_4–N from the anaerobic effluent, on average 570 mg L^{-1}, was released into the liquid phase from the carbon filtration column (Figure 6). Recovery of this valuable nutrient as liquid N fertilizer together with P and K, therefore, adds value to the circular economy and at the same time reduces its impact on environmental pollution. This demonstrates the potential of domestic wastewater to supply readily available liquid N fertilizer for local biomass production and reduce the use of chemical nitrogen fertilizer produced using a high-energy intensive Haber–Bosch process [29]. Substituting just 5% of the existing global nitrogen fertilizer production with N from domestic wastewater would save more than 50 terawatt-hours of energy [26]. Utilizing this untapped resource with the less-energy intensive method is, therefore, fundamental. Moreover, the nitrification–denitrification process for removal of N from wastewater requires more energy and at the same time releases nitric oxide (NO) and nitrous oxide (N_2O) to the atmosphere, which has harmful effects on the environment (greenhouse gases and acidification). Selecting filter materials for nutrient recovery involves analysis of the adsorption capacity of the filter materials for the different substances in blackwater. For N and P to be in the liquid phase, choosing filter materials that can selectively remove particulate and dissolved organic fractions including micropollutants, suspended solids, heavy metals and pathogens, is a necessity.

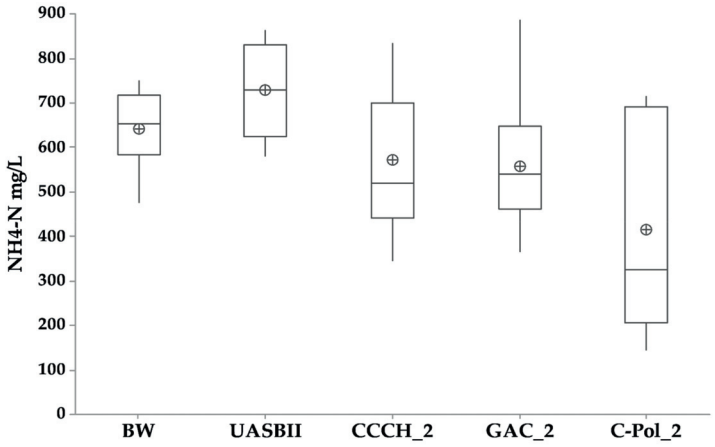

Figure 6. NH_4–N concentrations in the liquid phase for raw blackwater (BW), effluents of the anaerobic reactor (UASB II) and filtration columns—Cocos char (CCCH_2), granular activated carbon (AGC_2), and coarse polonite (C-Pol_2).

3.3.3. Other Essential Macronutrient Concentrations in the Raw Blackwater and Effluents

Figure 7 presents the average concentrations of the other macronutrients (K, Ca, Mg, S, Na) from three grab samples from each sampling point. It was revealed that in addition to the N and P, the K concentration in the liquid phase was higher in the carbon-based filtered effluents than the polonite. The concentration of K in these effluents reaches up to 190 mg/L compared to 67 mg/L for the raw blackwater.

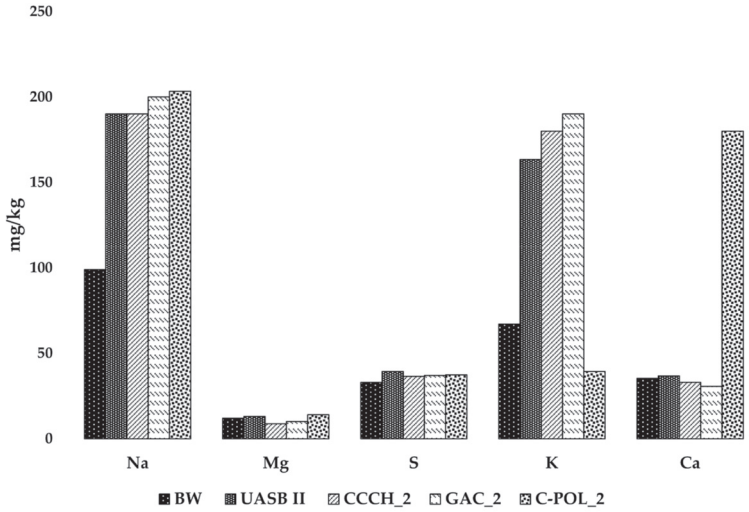

Figure 7. Macronutrient concentrations in raw blackwater (BW), anaerobic effluents (UASB I and UASB II), and filtration columns—Cocos char (CCCH_2), granular activated carbon (GAC_2), and coarse polonite (C-POL_2).

However, Ca was found in higher concentration in the polonite-filtered effluent than in the carbon-filtered effluent and in the raw blackwater and anaerobically treated blackwater effluents (Figure 7). This could be due to the solubilization of the Ca from the Ca-rich aluminosilicate polonite. This was also indicated from the release of Fe and Al in this column as a result of the dissolution of the aluminosilicate (Figure 8). The Mg concentration in the raw blackwater, anaerobic effluent, and filtration effluent was very low compared to the other macronutrients.

3.3.4. Micronutrients and Heavy Metals

Figure 8 shows the concentration of selected micronutrients and heavy metals in the raw blackwater, anaerobically treated effluent and filtration effluent. The heavy metal concentrations particularly As (<10 µg/L), Cd, Cr, Co., and Ni in the raw blackwater as well as in the effluents were lower than even the threshold level in drinking water stated in the guidelines for drinking-water quality [38] suggesting low heavy metal risk if sanitized blackwater is used as source of plant nutrients. The heavy metal concentration in blackwater was by far lower than their presence in sewage sludge, livestock manure and artificial fertilizer, and comparable results were reported in the Netherlands [39]. As shown from Figure 8, the carbon filter columns substantially reduced Al, Fe, Mn, Cu and Zn minimizing. On the other hand, the concentrations of Al, Fe, Mn and Zn in the polonite effluent are higher than the corresponding values in the anaerobically digested effluent. This might be due to the fact that polonite contains Ca, Al and Fe silicate as the main components and small fraction of Mn. The relatively high concentration of these heavy metals in polonite-filtered effluent could result from dissolution of these elements from the polonite.

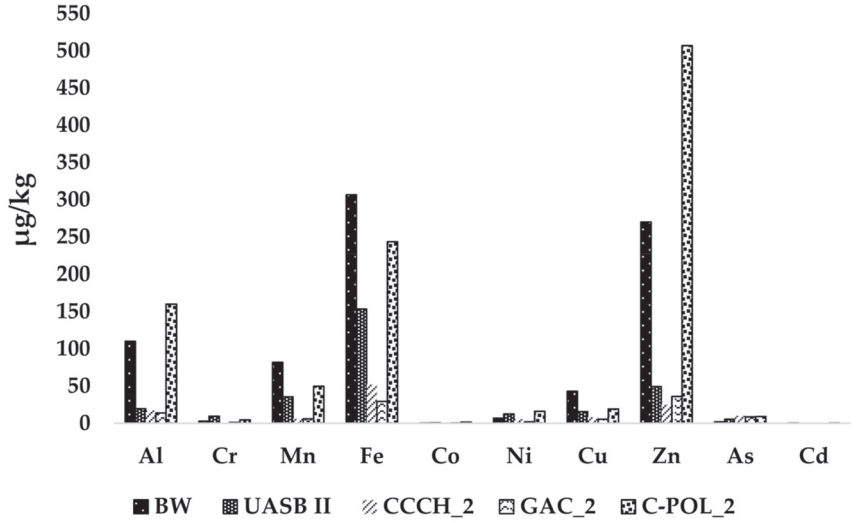

Figure 8. Micronutrient and heavy metal concentrations in raw blackwater (BW), anaerobic effluents (UASB I and UASB II), and filtration columns—Cocos char (CCCH_2), granular activated carbon (GAC_2), and coarse polonite (C-POL_2).

This study revealed the potential of safe recovery of N, P and K from source-separated blackwater as a nutrient solution in a liquid phase and can be used locally for various purposes with low risk of heavy metals, pathogens and micropollutants. Additionally, the system overcomes the challenges of source-separated blackwater including many of the unpleasant aspects, mainly to do with smell and aesthetics, the need for long-term storage for disinfection, and the risks of unwanted precipitation of phosphorus compounds and ammonia volatilization. Along these lines, it is possible to enhance recycling to better close the nutrient loop, contribute to the green circular economy, and protect the environment. For large-scale applications, this nutrient solution could be used as a raw material for the production of concentrated nutrients. Moreover, if blackwater is collected and treated separately, not only are resources recovered, but also energy, chemicals and resources are saved in conventional wastewater treatment plants that are normally required for removal of the high organic matter, nitrogen and phosphorous emanating from toilets. This approach also allows a more specific treatment and selective removal systems for the control of pathogens and micro-pollutants at the source.

3.4. Pathogen Removal in the Treatment Chain

The *E. coli* concentration in the raw blackwater was on average 1.275×10^7 MPN/100 mL. The log reduction of *E. coli* in the anaerobic digestion stage ranged from 1.3 log to 2.4 log with an average reduction of about 1.86 log. Anaerobic treatment systems are not designed to remove pathogens to a level that meet the required regulations [40] but greatly contribute to post-treatment of the effluent by reducing the particulate organic matter and total suspended solids. The major concern in the treatment and direct reuse of anaerobically digested blackwater is, therefore, the associated health risk from pathogens [26]. In order to comply with local regulations for reuse or discharge and control of the health risk from pathogens, disinfection mechanisms need to be integrated. Figures 9 and 10 show the effects of anaerobic digestion, filtration and UV light on *E. coli* removal and cumulative *E. coli* log reduction, respectively.

The polonite filtration column completely removed *E. coli* in the first two weeks. This was mainly attributed to the high pH 10.6. At this pH, microorganisms are inactivated and their regrowth

is restricted. Over time, the pH in the column decreased and, consequently, the efficiency of pathogen removal was reduced. The pathogen-removal efficiency of the carbon columns was lower than expected. However, efficient removal of organic matter, TSS, and correspondingly turbidity and UV_{254} absorbance by the carbon filter columns made it feasible for UV disinfection. The treated water from the carbon column that passed through a 11 Watt UV lamp had *E. coli* concentrations below the detection limit. The cumulative log reduction of *E. coli* thus reached 7 log after the application of UV, achieving the sanitizing potential of the system and potential reduction of health-related risks from reuse or discharge perspectives.

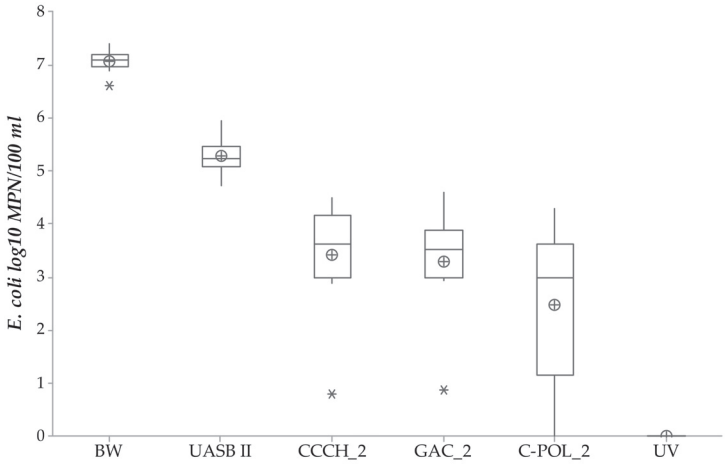

Figure 9. *E. coli* concentration of the raw blackwater (BW), reactor effluent (UASB II), in the effluents of filtration columns—Cocos char (CCCH_2), granular activated carbon (GAC_2), and coarse Polonite (C-POL_2) at different levels of treatments, and after ultraviolet light treatment (UV).

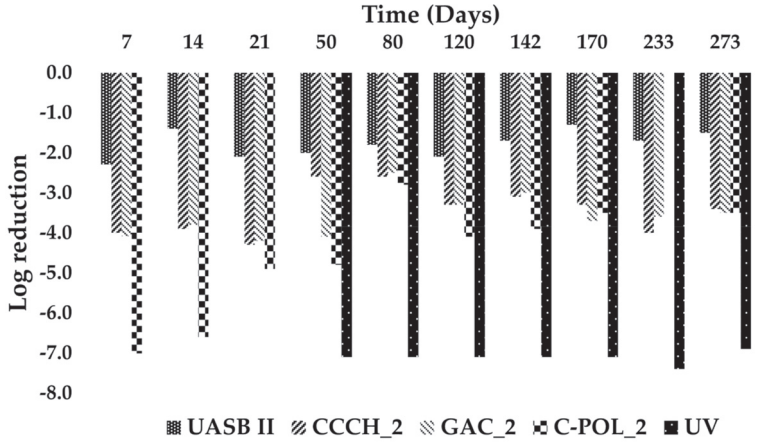

Figure 10. Cumulative log reduction *E. coli* of by the different treatments (reactor effluent (UASB II), in the effluents of filtration columns—Cocos char (CCCH_2), granular activated carbon (GAC_2), and coarse polonite (C-POL_2) at different levels of treatments, and after UV treatment (UV)) at different sampling times during the study period.

4. Conclusions

This study demonstrated opportunities for a combined treatment and sustainable resource recovery from source-separated blackwater through systematic integration of a sludge blanket anaerobic-baffled reactor and a novel compacted filtration system. The system achieved a high effluent quality in terms of organic matter, TSS, turbidity and indicator bacteria. Both carbon-based filters removed 80% of the residual organic matter, more than 90% of residual TSS, and 93% of the turbidity and UV_{254} absorbance from the effluent of the anaerobic reactor. Efficient removal of organic matter, TSS, turbidity and an increase in UV transmission makes the system further feasible for UV disinfection.

With carbon filtration, the majority of nutrients, on average about 570 mg/L NH_4–N, 56 mg/L of PO_4–P and 190 mg/L of K, remained in the liquid phase. Hence, the liquid effluent consisted of a highly valuable fertilizer which can be reintroduced as a nutrient solution in the production system aiming towards a circular economy. Phosphorus and ammonium recovery from blackwater in this way, in turn, reduces the unwanted enrichment of surface water, thereby reducing the associated environmental impact. Particular heavy metals still present in digested BW are reduced notably in the filter columns so that an effluent quality well within the present guidelines for organic fertilizers can be ensured. Moreover, the system overcomes the challenges of source-separated blackwater including many of the unpleasant aspects to do with smell and aesthetics, the need for long-term storage for disinfection, and the risks of unwanted precipitation of phosphorus compounds.

Integration of anaerobic digestion of source-separated blackwater with filtration and UV as novel post-treatment steps resulted in effluent quality that meets levels demanded and opens up the opportunity of reusing the valuable resources from blackwater for a range of end-use options without compromising public health and the environment.

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Author Contributions: The corresponding author, Melesse Eshetu Moges, conceived, designed and performed the experiment, collected and analyzed the data, and drafted the manuscript. Arve Heistad and Daniel Todt provided the required support in the experimental design and critically reviewed and commented on the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Paper V

Melesse Eshetu Moges, Arve Heistad and Thorsten Heidorn. *Optimization of Nutrient Recovery from anaerobically treated blackwater and improving effluent quality through Microalgae biomass production.* (*Manuscript in preparation*)

Optimisation of nutrient recovery and improving effluent quality of anaerobically treated blackwater through microalgae biomass production

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Abstract

Opportunities and challenges of nutrient recovery from treated source-separated blackwater by microalgae are presented. The blackwater stream of domestic wastewater contains the majority of nutrients that can contribute to the circular economy. This, however, requires a sustainable means of recovering to provide alternative and effective nutrient sources. Integration of microalgae into a treated source-separated blackwater (BW), can effectively assimilate and recover phosphorus (P) and nitrogen (N), as well as another macro- and micronutrients, and convert these nutrients into valuable by-products (e.g., biofertilizer) and hence closing the nutrient loop. With this objective, a flat photobioreactor was used to cultivate unicellular green microalgae species *Chlorella sorokiniana* strain (CHL176) obtained from NIVA. The growth of *C. sorokiniana* on treated source-separated blackwater as a substrate was monitored by measuring optical density (OD) and dry biomass weight (DBW) at a substrate flow rate of 1.8 L d^{-1}, the temperature of 37 ^{o}C and pH of 7. The results indicate that *C. sorokiniana* can assimilate and recover N and P from a treated source-separated blackwater. The N and P removal rates were 99 mg N $L^{-1}d^{-1}$ and 8 mg P $L^{-1}d^{-1}$ for 10% treated BW and reached 212 mg N $L^{-1}d^{-1}$ and 35 mg P $L^{-1}d^{-1}$, respectively when using 20% treated BW as a substrate. The corresponding biomass yield on light, biomass yield on N and P on the 20% treated BW substrate were 0.37 g (mol photon)$^{-1}$, 9.1 g g^{-1} and 54.1 g g^{-1}, respectively.

Keywords: blackwater, chemostat, microalgae biomass, nutrient recovery, effluent quality

1. Introduction

There is an increasing interest in biogas production from organic wastes using anaerobic digestion (AD) for treating wastewater sludge and animal waste, such as in pig raising. Recent studies have also shown the use of AD for the treatment of source-separated domestic blackwater (Kujawa-Roeleveld and Zeeman 2006, de Graaff et al. 2010, De Graaff et al. 2011, Moges et al. 2018, Zeeman et al. 2008). The effluent of an AD reactor is rich in nutrients, particularly N and P. Without proper treatment, excess nitrogen and phosphorus in discharged AD effluents can lead to downstream eutrophication and ecosystem damage.

The increasing amount of AD effluent and the need for its appropriate disposal presents a challenge for biogas plants. This would be a serious problem if the direct use of AD effluents as fertilizer in agriculture is not permitted, or even if permitted, the large agricultural area required may be not available in the near vicinity of biogas plants and often result in long transportation distances (Fuchs and Drosg 2013). It should also be noted that, as compared to the typical agricultural, municipal, and industrial wastewater, AD effluents have relatively low levels of carbon. Most of the organic carbon is converted to methane and microbial biomass during the anaerobic digestion (Wang et al. 2010a) but high concentrations of dissolved nutrients mainly ammonium nitrogen and orthophosphate retains in the effluent. Hence, the removal of nitrogen from such effluent, with very low carbon/nitrogen (C/N) ratio, can often be limited in conventional wastewater plants (WWTPs) because organic carbon is a limiting factor for denitrification (Vazquez-Padin et al. 2009). Innovative bacterial nitrogen removal pathways such as shortcut nitrification/denitrification (Ruiz et al. 2006, Gao et al. 2009, Gao et al. 2010), simultaneous nitrification/denitrification (Helmer and Kunst 1998, Yilmaz et al. 2008, Virdis et al. 2010), and the nitritation-anammox process (Fux et al. 2002, Vazquez-Padin et al. 2009, Lackner et al. 2014) can remove nitrogen with low or zero dosage of organic carbon sources (Sun et al. 2010). These processes, however, do not allow N recovery as a resource.

The core principles of the circular (bio)economy are based on the recycling and re-use of resources towards sustainable approaches, which require a holistic resource utilization and protection of the ecosystems. Using AD effluents as a resource and combining AD effluent treatment with the production of microalgae-based bioproducts can overcome several of the major challenges. The need for nutrients for the production of bioproducts from microalgae on the one hand and the threats from the release of AD effluents, on the other hand, open up opportunities for combined solutions. For a sustainable feed

production from algae, the value of anaerobically digested effluent as a low-cost nutrient supplement has been evaluated in a number of studies (Kebede-Westhead et al. 2004, Chinnasamy et al. 2010a) and was found to be promising. At the same time, converting the nutrients from AD effluents into microalgae biomass results in an improved effluent quality fulfilling the permitted levels for the safe discharge into the environment. The recovery of nitrogen through microalgae biomass could also enhance the environmental quality by reducing the nitrous oxide (N_2O) emission. Nitrous oxide is a major greenhouse gas (GHG) with a global warming potential of about 300 times that of CO_2 over a 100-y time period (IPCC 2007).

Similarly, the challenges in domestic wastewater treatment can be addressed through the integration of a source-separation sanitation system with AD and nutrient recovery technologies. Through this integration the local recovery of N and P can be optimized, the need for mineral fertilizer and the associated or indirect energy and transportation costs reduced, and the risk of environmental impact minimized. Although locally treated and the hygienized nutrient solution recovered from the source-separated blackwater can be used as liquid fertilizer (Eshetu Moges et al. 2018), the storage and transportation could be a challenge. A concentration of the treated nutrient solution into a smaller volume would, therefore, be advantageous.

The use of a wide range of microalgae such as *Chlorella*, *Scenedesmus, Phormidium, Botryococcus, Chlamydomonas* and *Spirulina* for treating domestic wastewater has been reported and effectiveness of this method was found to be encouraging (Olguín 2003, Chinnasamy et al. 2010b, Kong et al. 2010, Wang et al. 2010b). Only very few studies have shown the feasibility of using microalgae to recover nutrients from concentrated urine or source-separated blackwater (Tuantet et al. 2014b, Vasconcelos Fernandes et al. 2015). Moreover, the nitrogen in the AD effluent is mainly in the form of ammonium (Singh et al. 2011). Dilution of the AD effluent is usually needed before feeding to algae in order to avoid the potential ammonium inhibition of algal growth (Wang et al. 2010c). In addition, as there is a significant amount of bacteria in the AD effluent, proper pretreatment, such as filtration and autoclaving, may be necessary to prevent the contamination of algae production systems (Wang et al. 2010a).

Chlorella sorokiniana is used in this study to evaluate the opportunities and challenges of using microalgae as an option for nutrient recovery from source-separated and anaerobically treated blackwater. This study also aims to assess the nutrient removal efficiency of *C. sorokiniana* and its potential to improve effluent quality. Biomass

concentration, productivity, and biomass yield on light, N and P were studied in a chemostat mode of operation.

2. Material and Methods

2.1. Culture media and strain

Anaerobically digested, pre-treated and hygienized blackwater (Eshetu Moges et al. 2018) was used as a substrate for a continuous microalgae culture. The nutrient concentrations of the treated blackwater and a defined medium used for microalgae cultivation are shown in Table 1.

The defined medium contained the following ingredients: $NaNO_3$ (1.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.1 g/L), KH_2PO_4 (0.05 g/L), K_2HPO_4 (0.1 g/L), $NaHCO_3$ (0.084 g/L), $CaCl_2 \cdot 2H_2O$ (0.05 g/L) and 1 mL/L Hutner's Trace Elements (Hutner et al. 1950).

Ammonium nitrogen was the main form of N in the treated blackwater (Table 1). Nitrite and nitrate concentrations in the treated blackwater were negligible at the start of this experiment. However, increased nitrite and nitrate concentrations have been registered later due to the installation of a membrane bioreactor (MBR) after the sludge blanket anaerobic baffled reactor to remove dissolved methane. But the ammonium nitrogen still represented more than 80 % of the inorganic N. Orthophosphate was the main form of available P and the concentrations of Mg and other micronutrients were low (Eshetu Moges et al. 2018) so that the supplementation of these nutrients was found to be necessary for the growth of *C. sorokiniana*. The N:P ratio in the treated blackwater rages from 11 to 14 which is close to the required the Redfield ratio of 16:1 N:P ratio usually reported for phytoplankton (Redfield 1958).

Chlorella sorokiniana strain NIVA CHL 176, obtained from NIVA (NIVA) is a eukaryotic, unicellular microalga with spherical cells of 5–10 μm diameter (Becker 2007). *Chlorella sorokiniana* was selected because of its high maximal specific growth rate of 0.27 h^{-1} and its tolerance to high irradiance, high temperature and high CO_2 concentrations (Matsukawa et al., 2000).

Table 1. Nutrient concentration of anaerobically digested blackwater effluent, effluent after filtration and UV treatment and defined medium

Nutrient		Anaerobically digested blackwater	After filtration and UV treatment	10% diluted	Defined medium
Ntot	mg/L	1140 - 2360	1100 - 1980		247
NH_4-N	mg/L	580 - 1390	580 - 1200	58-120	
NO_3-N	mg/L	0 - 4.05	6 – 93.6	0.6-9.36	247
NO_2-N	mg/L	0 – 0.6	0.2 - 130	0.02-13	
Ptot	mg/L	100 - 140	76 – 92.4		26.7
PO_4-P	mg/L	46 - 92.5	54 – 85.3	5.4-8.53	29.2
Mg	mg/L*	11.3	9.4	0.94	9.8
K	mg/L*	165.0	185.0	18.5	59.3
Ca	mg/L*	38.8	31.8	3.18	13.6
Na	mg/L*	191.7	195	19.5	411
Al	µg/L*	22.0	15.5	1.55	0.0
Fe	µg/L*	143.3	40.7	4.07	1.0
Cu	µg/L*	30.7	6.9	0.69	0.4
Mn	µg/L*	37.5	6.0	0.60	1.4
Ni	µg/L*	11.1	3.7	0.37	0.0
Zn	µg/L*	62.0	30.5	3.05	5.0
Co	µg/L*	0.43	0.17	0.017	0.4
Turbidity	NTU	80 - 160	0.15 - 2		
E. coli	MPN/100ml	10^5	< 1		

* For blackwater based media: Averages of three samples taken at earlier points of time before the algae experiment started

2.2. Photobioreactor (PBR) set-up and culture conditions

Two flat panel photobioreactors (PBR) with outer dimensions of 240×360×40 mm (W×H×D) and respective inner dimensions of 180×300×30 mm, resulting in culture chamber volumes of 1.6 L and light paths of 30 mm (Skjånes et al. 2016) were continuously illuminated by LED panels on illuminated surface areas of 0.054 m^2 with an average light intensity of 1450 µmol photons m^{-2} s^{-1}. The culture volumes were kept at 1.3 L. The photobioreactor systems were equipped with a control system for controlling and logging of multiple parameters such as temperature, pH, optical density (OD), and dissolved oxygen (DO) as described in Skjånes et al. 2016 (fig. 1).

The continuous culture was operated as a chemostat with the temperature controlled at 37 ± 0.1 °C and the pH at 7.0 ± 0.5 by intermittent addition of CO_2 (0.1 L/min). The culture was mixed by aeration with compressed air at a flow rate of 2 L min^{-1}.

After the chemostat reached a steady-state with the defined medium at a dilution rate of 1.38 d^{-1} the medium was replaced by a 1:10 or 1:5 dilution, respectively, of a treated and hygienized nutrient solution from anaerobically digested blackwater effluent (Eshetu Moges et al. 2018) and operated with the same dilution rate.

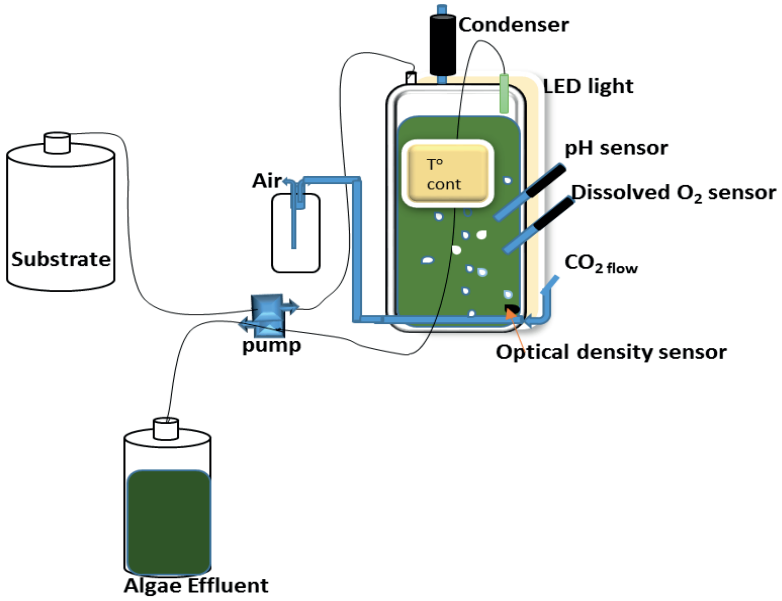

Figure 1. Schematic experimental set-up of the chemostat

158 ***2.3. Nutrient analysis and Algae Growth Determination***

159 Samples of the substrate and the culture were taken once a day. NH_4-N, NO_2-N, NO_3-N,
160 and PO_4-P were analyzed using the Hach-Lange kits LCK 303, LCK 339, LCK 342, and
161 LCK 348/350, respectively, on a DR 3900 spectrophotometer. The growth of *Chlorella*
162 *sorokiniana* was monitored by optical density (OD) and dry biomass weight (DW). The
163 OD was measured using a spectrophotometer (Spectroquant® Pharo 100 MERCK) at 750
164 nm. To reduce error in measurements above 1.0 absorbance, a well-mixed sample was
165 diluted with deionized water. For dry biomass determination, two ml of well mixed
166 undiluted samples were taken in 2 ml tubes and centrifuged at 15.000 rpm for 2 min. The
167 supernatant decanted and the biomass was dried at 105 ˚C overnight.

168 The volumetric P_v (g L^{-1} d^{-1}) and areal P_A (g m^{-2} d^{-1}) biomass productivity refer to the
169 amount of biomass produced at a defined flow rate per unit volume (area) per day and
170 was calculated from equation 1, respectively.

171 $$P_V = \frac{X*Q}{V} \quad \text{and} \quad P_A = \frac{X*Q}{A} \qquad 1$$

172 X= dry biomass (g L^{-1}), Q= flow rate (L/d), V= culture volume in photobioreactor (L), and
173 A is illuminated surface area (m^2).

174 Nitrogen removal efficiency N_{reff} and Phosphorous removal efficiency P_{reff} from the
175 substrate were calculated as follows

176 $$N_{reff} = \frac{(Ni-Ne)*100}{Ni} \qquad 2$$

177 and

178 $$P_{reff} = \frac{(Pi-Pe)*100}{Pi} \qquad 3$$

179 Where ***Ni*** is the influent nitrogen concentration of the substrate (mg N L^{-1}), ***Ne*** is effluent
180 nitrogen concentration (mg N L^{-1}), ***Pi*** is influent phosphorus concentration of the
181 substrate (mg P L^{-1}), ***Pe*** is effluent phosphorus concentration (mg P L^{-1}). (Ni-Ne) and (Pi-
182 Pe) is the change in influent and effluent N (ΔN) and P (ΔP) concentrations, respectively.

183 The biomass yield on substrate $Y_{x/S}$ is defined as the ratio of the amount of biomass
184 produced to the amount of substrate consumed (g biomass/g substrate). The biomass
185 yield on the substrate for N $\mathbf{Y}_{X/N}$ (g g^{-1}) and for P $\mathbf{Y}_{X/P}$ (g g^{-1}) was then calculated as

186 $$Y_{\frac{X}{N}} = \frac{X}{(Ni-Ne)} \qquad 4$$

187 and

$$Y_{\frac{X}{P}} = \frac{X}{(Pi-Pe)} \quad (5)$$

The nitrogen removal rate N_r (mg N L^{-1} d^{-1}, as the sum of NH_4-N, NO_2-N and NO_3-N) and the phosphorus removal rate P_r (mg P L^{-1} d^{-1}, as PO_4-P) were calculated according to equations 6 and 7 (González-Camejo et al. 2018).

$$N_r = \frac{(Ni-Ne)*Q}{V} \quad (6)$$

and

$$P_r = \frac{(Pi-Pe)*Q}{V} \quad (7)$$

The efficiency of light utilization $Y_{X/Ph}$ (g/mol photons) of *C. sorokiniana* expressed as biomass yield on light energy in grams of dry matter per mol of photosynthetically active radiation (**PAR**) photons supplied during steady state was calculated using equation 8 by dividing the total amount of biomass produced per day by the total amount of light irradiated to the photobioreactor, as described by Cuaresma et al. (Cuaresma et al. 2011).

$$Y_{\frac{X}{Ph}} = \frac{Ch*Xc*10\hat{}6}{PFD*A*24*3600} \quad (8)$$

Where ***Ch*** is the culture volume harvested during one day (L d^{-1}), ***X*** is dry biomass concentration measured (g L^{-1}), ***PFD*** is the photon flux density (mol photons m^{-2} s^{-1}) and ***A*** is illuminated surface area (m^2).

The nutrient removal yield in relation to the used amount of light $Y_{Nr/Ph}$ (mg N (mol photons)$^{-1}$ and $Y_{Pr/Ph}$ (mg P (mol photons)$^{-1}$ by the 24 h average PAR LED light illuminated at the surface area of the PBR is calculated according to equation 9 and 10 (González-Camejo et al. 2018)

$$Y_{\frac{N_r}{Ph}} = \frac{Ch*(Ni-Ne)*10\hat{}6}{PFD*A*24*3600} \quad (9)$$

and

$$Y_{\frac{P_r}{Ph}} = \frac{Ch*(Pi-Pe)*10\hat{}6}{PFD*A*24*3600} \quad (10)$$

3. Results and Discussion

3.1. Biomass productivity and nutrient removal with a defined medium

Figure 2 displays the concentrations of the biomass, NO_3–N, and PO_4–P. The N:P ratio of the defined medium was 8.7 which is by far less than the Redfield ratio of 16:1 for planktons (Redfield 1958). The results indicate that the growth of *C. sorokiniana* started immediately without clear lagging phase and showed a high specific growth rate in the exponential phase. The culture reached a steady-state in about 30 h. The average biomass concentration during steady state was 1.58 ± 0.21. The average biomass production rate or volumetric productivity at steady state was on average 2.17±0.245 g L^{-1} d^{-1} which corresponds to an aerial productivity of 52.2 g m^{-2} d^{-1}. The average biomass yield of *C. sorokiniana* on light during this period was 0.42 ± 0.05 g dry weight (mol photons)$^{-1}$. Comparable results have been reported in other studies as well (Vasconcelos Fernandes et al. 2015).

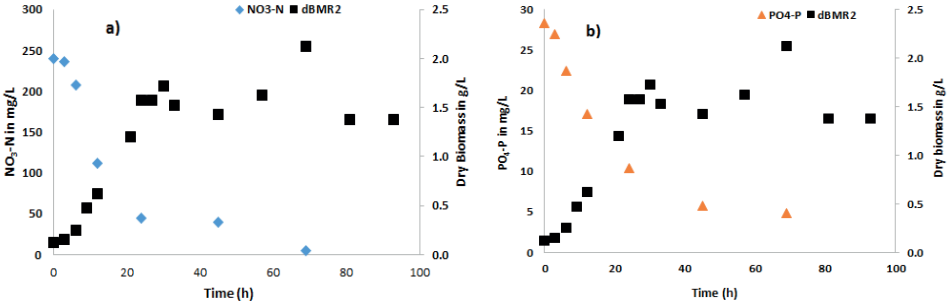

Figure 2. *Chlorella sorokiniana* biomass and the concentrations of a) NO_3–N and b) PO_4–P in the effluent.

The change in N and P concentration between the influent and effluent was assumed to be taken up by the algae for their biomass production. Within 24 h 81 % of the N and 63 % of P is converted into biomass and at the 70th h, 98 % of NO_3–N and 83 % of P was removed (Fig. 3). At steady-state, the average NO_3–N removal rate N_r was 291.14 ± 29.8 mg L^{-1} d^{-1} and the N removal yield in relation to the used amount of light $Y_{N/Ph}$ was 55.95 mg NO_3–N mol photons^{-1}. Similarly, the average PO_4–P removal rate P_r over this same period was 29.5±4.1 mg L^{-1} d^{-1} and the P removal yield in relation to the used amount of light $Y_{P/Ph}$ was 5.67 mg P mol photons^{-1}.

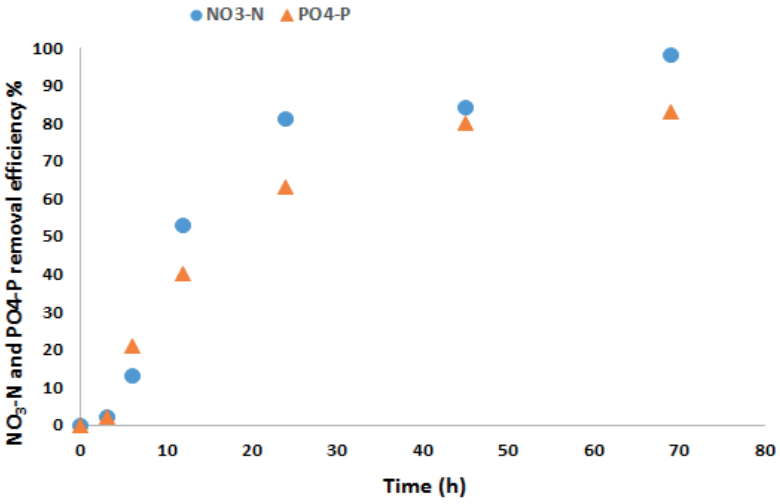

Figure 3. NO_3–N and PO_4–P removal efficiency of *C. sorokiniana.*

3.2. Biomass productivity and nutrient removal with treated blackwater as a substrate

Chlorella sorokiniana grew fast with ammonium as a nitrogen source. The growth of *C. sorokiniana* on 10 % treated blackwater without additional Mg and trace elements increased and reached a maximum within 36 h. However, it sharply decreased to about 90 % of the maximum biomass concentration after 90 h. This suggests that the culture growth was supported by nutrients from the inoculum during the first 36 hours but later the 10% treated blackwater substrate could not supply enough nutrients to sustain the growth of *C. sorokiniana*. As presented in table 1, P, Mg, and most of the trace elements (except Fe and Cu) in the 10% diluted treated blackwater are very low. At the 96th h $MgSO_4$ and Hutner´s trace element solution was added to the substrate at the same concentration as it was used in the defined medium.

Addition of Mg and trace elements improved the growth of *C. Sorokiniana,* reached its maximum and sustained steady-state growth (Fig. 4). This was also reflected in the increased removal of ammonium. Enhanced growth of microalgae with supplementation of Mg and micronutrients was also reported in literature (Tuantet et al. 2014a). As shown in the figure the ammonium concentration in the effluent was 0.42 mg L^{-1} within 12 h and 0.03 mg L^{-1} after 24 h from initial influent substrate concentration of 81 mg/L. However, with a decline in biomass productivity, the removal of ammonium by the microalgae decreased sharply and the concentration in the effluent reached the substrate concentration after 70 h. With the addition of Mg and trace elements, which improved

biomass productivity, the ammonium concentration in the effluent decreased again and reached close to zero (Fig. 4).

Moreover, the volumetric and areal biomass productivity of *C. sorokiniana* with the 10 % diluted treated blackwater measured at steady-state were found to be influenced by P. The P concentration in the 10% treated water was about 5 mg L^{-1}. Addition of 29 mg L^{-1} of extra P together with Mg and trace elements at the same concentration as defined medium as a supplement improved biomass productivity. The volumetric and areal productivity with extra P was 2.1 g $L^{-1}d^{-1}$ and 50.4 g $m^{-2}d^{-1}$, respectively. However, a significant reduced volumetric productivity of 1.5 g $L^{-1}d^{-1}$ and areal productivity of 36.48 g $m^{-2}d^{-1}$ (*p=0.006)* was observed when the culture was grown without extra P (Fig 4). Similar effects were observed in Tuantet et al, (Tuantet et al. 2014b). Although this does not affect the N removal, it is important to note that the effluent P concentration does not increase above the discharge limit. Adding an extra 29 mg P L^{-1} resulted in an effluent P concentration ranging from 3.6 to 8.3 mg L^{-1}, which is well above the permitted P discharge limit (1 mg L^{-1}). On the other hand, the 10% treated blackwater without adding extra P resulted in an effluent P concentration far below the discharge limit. The N: P ratio should be considered not only for optimal biomass production but also to achieve the desired effluent quality.

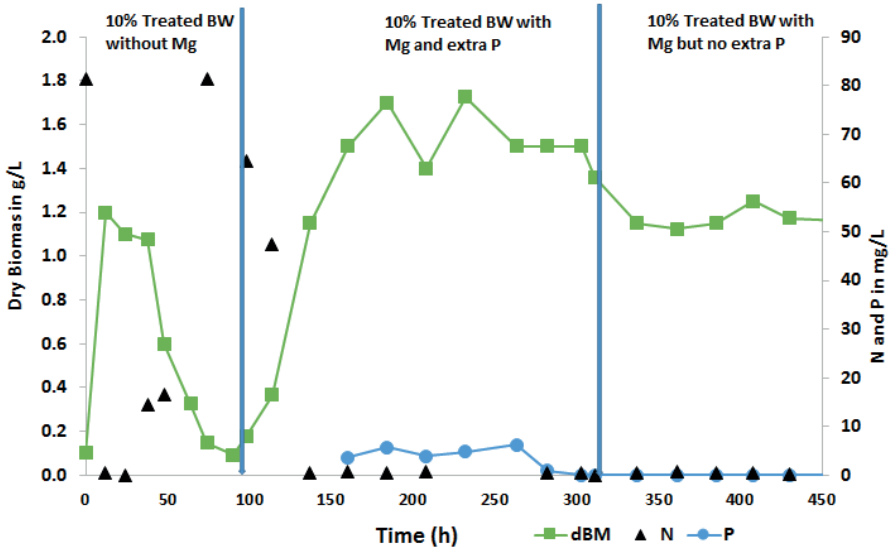

Figure 4. Dry biomass in g L^{-1} without Mg and without extra P, with Mg and extra P and with Mg but not extra P (▪), N concentration in the effluent (▲), and P concentration in the effluent (●) using 10% treated source-separated blackwater as a substrate.

Chlorella sorokiniana was able to actively remove ammonium and phosphate from the treated blackwater for growth. The average N and P removal rate at steady state with 10% treated blackwater with extra P were 110.5±2.2 and 44.4 mg $L^{-1}d^{-1}$, respectively. The corresponding N and P removal rates for the 10% treated blackwater substrate solution without extra P were 99.2±0.4 and 8.3±0.4 mg $L^{-1}d^{-1}$, respectively. The higher N and P removal rates in the defined medium than in 10% treated blackwater may be due to the high initial influent concentrations of N and P in the defined medium compared to the initial concentration in the treated blackwater. However, both N and P were completely taken up for the 10% treated blackwater without excess influent P.

The N and P removal yield in relation to light for the treatment with extra P were, 20.9±0.4 mg $(mol\ photons)^{-1}$ and 8±0.1 mg $(mol\ photons)^{-1}$, respectively, and 19.1±0.1 and 1.59 mg $(mol\ photons)^{-1}$, respectively, for the treatment without extra P. Similarly, the biomass yield on light 290 mg dry biomass $(mol\ photons)^{-1}$ of algae with 10% treated blackwater without the addition of extra P. The corresponding value of biomass yield on light when extra P was added to the 10% treated blackwater was 400 mg $(mole\ photons)^{-1}$ which is comparable to the 420 ± 0.05 mg dry biomass $(mole\ photons)^{-1}$ with the standard medium. Thus, a proportional amount of P is required for increased biomass production. In contrast to the results reported from other studies using urine and blackwater as substrate (Tuantet et al. 2014b, Fernandes et al. 2017), our results show lower values. However, the biomass yield on light, N and P removal rates were comparably high.

3.3. Effect of NO_2-N on Chlorella sorokiniana

The productivity of *C. sorokiniana* with a 20% treated blackwater, with increased ammonium concentration was challenged by nitrification in the substrate storage tank. The biomass concentration of *C. sorokiniana* increased from 350 mg L^{-1} of the first day to 1200 mg L^{-1} in the 4th day with 20% treated blackwater. However, the biomass concentration declined and reached 170 mg L^{-1} on the 6th day where the culture becomes pale yellow in colour. This was assumed to be associated with an increased nitrite concentration in the stored substrate. The long retention time of the substrate, while stirring to supply a homogenized medium to the culture, results in oxidation of the NH_4-N and thus the nitrite concentration in the substrate gradually increases (Fig. 5). A nitrite concentration of 72 mg L^{-1} resulted in bleaching out of the culture and higher effluent concentrations of NH_4-N, NO_2-N, and NO_3-N (Fig.6).

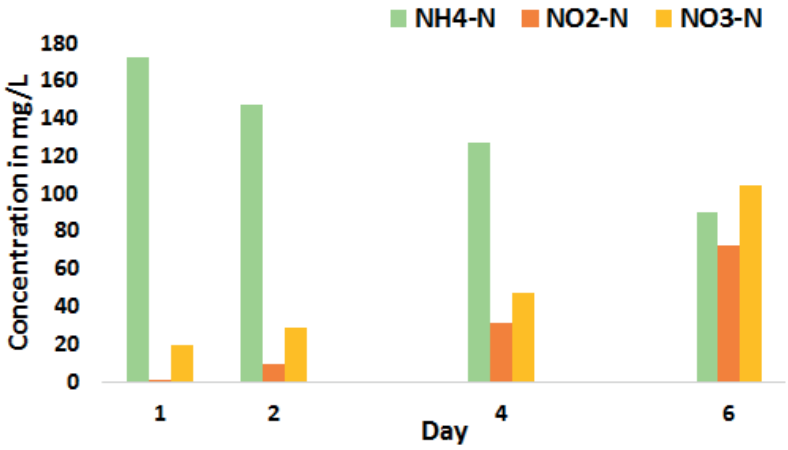

Figure 5. Change in NH_4-N, NO_2-N and NO_3-N concentrations in the substrate during the 6 day feeding period.

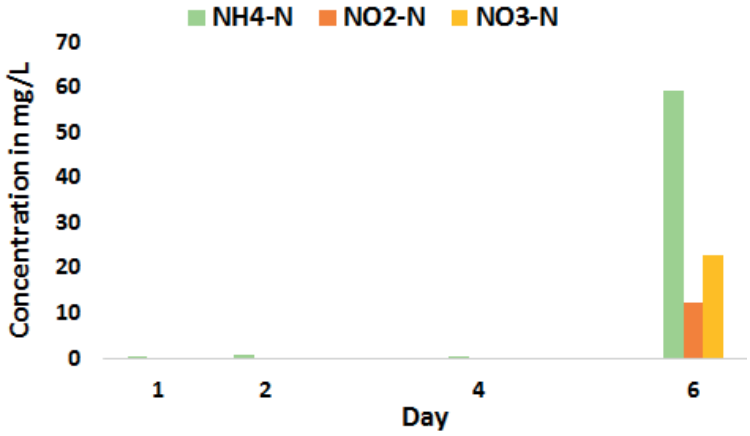

Figure 6. NH_4-N, NO_2-N, and NO_3-N concentrations in the effluent culture during the 6 day feeding period.

To prevent *C. sorokiniana* from toxic effects of nitrite the following approaches are possible: (1) complete nitrification of ammonium (NH_4-N) to nitrate (NO_3-N) without accumulation of nitrite (NO_2-N), (2) a short substrate retention time without further oxygen supply (stirring), or (3) anaerobic conditions in the feeding tank to prevent oxidation of NH_4-N. In the following experiment, the substrate bottle of one bioreactor (PBR I) was flushed with molecular nitrogen for oxygen removal and then connected to a bag with N_2 to keep the substrate anaerobic. The substrate bottle of the second

bioreactor (PBR II) was first kept aerobic for the first 5 days and on day 5 anaerobic and connected to a bag with N_2.

Figure 7 shows the effect of the anaerobised substrate on the biomass production of *C. sorokiniana.* The anaerobic substrate supply in PBR I resulted in an increased biomass concentration and reached a steady state. In contrast, the reactor with the aerobic substrate showed a decreasing biomass concentration in the first five days. When the substrate of this reactor (PBR II) was anaerobised on day 5, the biomass concentration of *C. sorokiniana* increased and reached almost the same level as PBR I on day 10 (Fig. 7). The corresponding NO_2-N concentrations in the substrate of both PBR is given in figure 8. The NO_2-N concentration in the bioreactor with the aerobic substrate (PBR II) was increased to more than 50 mg/L when at the same time the biomass concentration in this bioreactor was decreasing. This confirmed that the decline in biomass concentration was caused by a too high NO_2-N concentration.

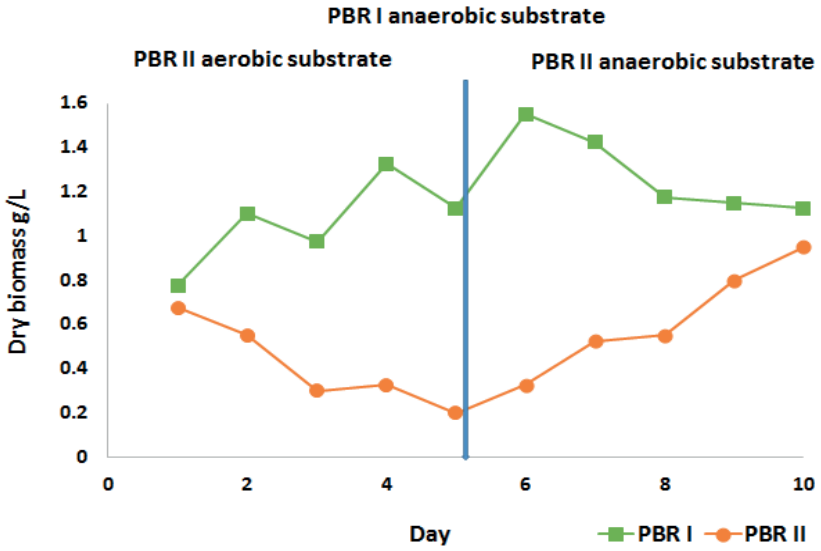

Figure 7. The response of *Chlorella sorokiniana* to anaerobic substrate condition. PBR I substrate was kept anaerobic during the entire 10 days of the experiment, while in PBR II the substrate was kept aerobic in the first 5 days and anaerobised then after.

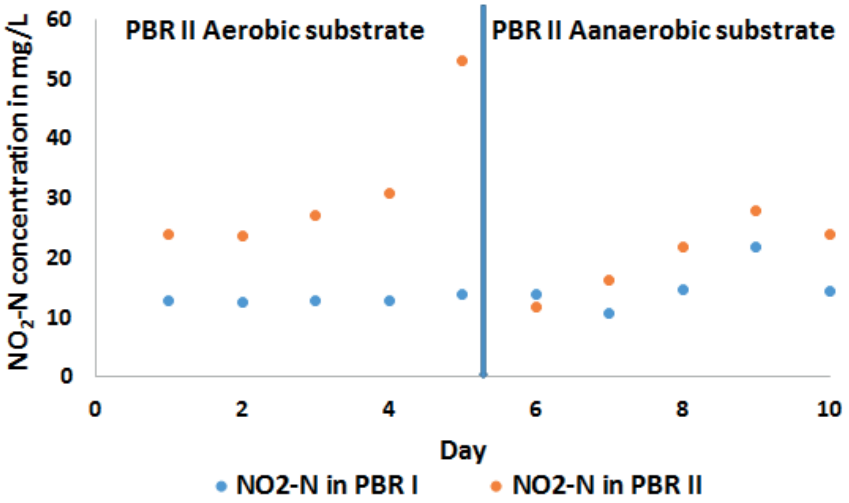

Figure in 8. Nitrite concentrations in the anaerobic substrate PBR I, and aerobic and anaerobic substrate in PBR II.

3.4. Effects of reducing substrate retention time, stopping stirring and removing $CaCl_2$ as a supplement

As an alternative solution to the anaerobisation of the substrate, the effect of a reduced substrate retention time in the medium bottle and a reduced oxygen supply by switching off the stirring on the performance of *C. sorokiniana* was evaluated. When renewing the substrate every 3 days instead of 5 to 6 days without stirring the growth of *C. sorokiniana* was stable as the NO_2-N concentration did not increase to an inhibiting level. In addition, colourless crystals shaped like blunt ended needles or prisms were observed in the substrate through a microscope. A possible precipitation of $Ca_3(PO_4)_2$, which can limit P availability, was suspected and the addition of supplementing $CaCl_2$ stopped. The combination of reducing the substrate retention time, stopping the stirring and not supplementing with $CaCl_2$ resulted in a steady-state of the *C. sorokiniana* culture growing on a medium with 20% treated blackwater.

Figure 9 presents the average influent and effluent concentration of NH_4-N, NO_2-N and NO_3-N from the 20% treated blackwater substrate after changing the operational conditions stated above. The results show that the NO_2-N concentration increased from 5 mgL^{-1} in the influent to 23 mgL^{-1} in PBR effluent. Similarly, the NO_3-N concentration showed a slight increase from 9 mg L^{-1} influents to 12 mgL^{-1} in the effluent. The increase of the NO_2-N concentration in the effluent but not much of NO_3-N indicates the presence of only ammonium oxidizing bacteria (AOB) but not nitrite oxidizing bacteria (NOB)

which may be the case for the accumulation of NO_2-N. Only 4% of the influent N left the reactor in the form of NH_4-N.

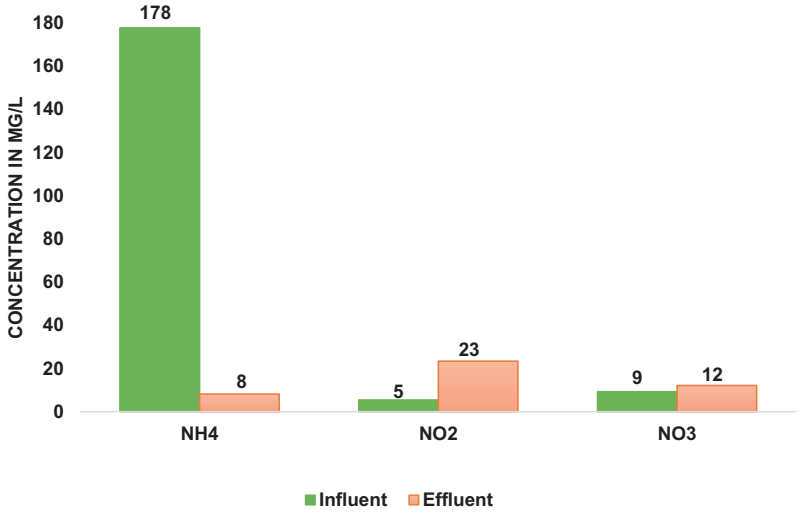

Figure 9. Average influent and effluent concentrations of NH_4-N, NO_2-N and NO_3-N

The higher concentrations of NO_2-N and NO_3-N than NH_4-N in the effluent shows the fact that NH_4-N is preferably taken up by *C. sorokiniana* when other forms of N compounds are also available to the microalgae (Maguer et al. 2007). Several studies have also indicated that nitrate consumption by microalgae does not occur until the ammonium is nearly completely removed (Maguer et al. 2007, Hii et al. 2011, Gonzalez-Camejo et al. 2018). Ammonium is the preferred form of nitrogen because a redox reaction is not involved in its assimilation; thus, it requires less energy (Maguer et al. 2007, Cai et al. 2013). On the other hand, nitrate must be converted to ammonium inside the cell and requires energy (Maguer et al. 2007, Podevin et al. 2015).

The average N removal efficiency of *C. sorokiniana* with the 20% treated blackwater was 78%, which was lower than observed at 10% treated blackwater. The P removal efficiency remains, however, high with an average of 99.95% (Fig.10). The nitrogen and phosphorus removal efficiency with 5 times dilution are similar with the results obtained from 5 times diluted urine at a light intensity of 1500 µmole photons m^{-2} s^{-1} (Tuantet et al. 2014a). Similarly, high recovery of nitrogen (75%) and phosphorus (100%) was also reported when growing *Chlorella sorokiniana* from anaerobically treated BW (Fernandes et al. 2017). The lower N removal efficiency could be either due to P limitation with higher N

availability or due to light limitation. Increasing the light intensity may improve N uptake and thus biomass yield.

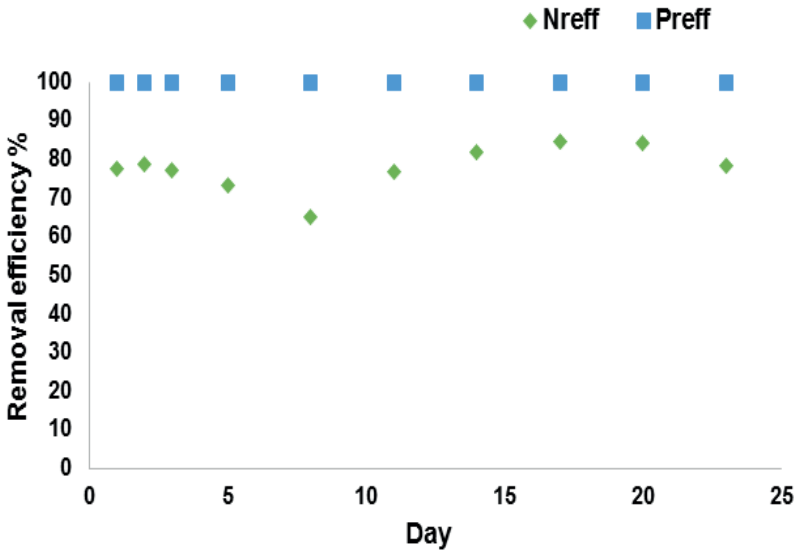

Figure 10. N and P removal efficiency of *C. sorokiniana*

The amount of N and P that is assumed to be taken up by *C. sorokiniana* at the 20 % treated blackwater as the substrate is given as a change in concentration of N and P in the influent and effluent i.e. ΔN=(Ni-Ne) and ΔP=(Pi-Pe), respectively. This is also related to the efficiency of *C. sorokiniana* in removing N and P. The N and P removal rates under this condition were on average 212.65 ± 23 mg L^{-1} d^{-1} and 35.28 ± 0.65 mg L^{-1} d^{-1}, respectively. Both N and P removal rates are higher for 20% treated BW compared to the 10% treated BW substrate. The N and P removal rate obtained in this study was relatively higher than reported in anaerobically treated source-separated blackwater in the Netherlands (Vasconcelos Fernandes et al. 2015) but much lower than from undiluted urine (Tuantet et al. 2014a).

The volumetric biomass production for 20% treated BW substrate was 1.91 g L^{-1} d^{-1} with a corresponding areal biomass production of 46 g m^{-2} d^{-1}. The biomass yield on light and biomass yield on substrate N and P were on average 370 mg of dry microalgae biomass per mole photons, 9.1 g g^{-1} and 54.1 g g^{-1}, respectively. The N removal efficiency is largely related to the initial influent N concentration, which is higher at higher initial N concentration. The biomass yield in this study was low compared to results reported in the literature (Cuaresma et al. 2009, Tuantet et al. 2014a, Vasconcelos Fernandes et al. 2015). The high biomass yield obtained by Tuantet et al. (2014a) and others may be due

to the high surface to volume ratio of the photobioreactor used. Moreover, the photobioreactor used in those studies had a smaller light path (5 and 10 mm (Tuantet et al. 2014a)) which is 3 to 6 times smaller than the light path used in the present study. This could be the main factor for differences in biomass yield. Table 2. Summarizes the nutrient removal rates, the nutrient yield on light, biomass yield on light, and biomass yield on a substrate (N and P) of the 10% and 20% treated blackwater as compared to the yields on defined medium.

Table 2. The nutrient removal rates, the nutrient yield on light, biomass yield on light, and biomass yield on a substrate (N and P) on the defined medium as compared to the treated blackwater.

	unit	Defined medium	10% treated BW Without extra P	10% treated BW With extra P	20% treated BW With extra P
N	mg/L	247	54 - 85	54 - 85	177 - 212
P (PO4-P)	mg/L	29.2	4.5 - 6	33.7 - 35.2	18.8 - 21.3
N : P ratio		8.7	12-14	3.5	10
Volumetric biomass productivity	g L^{-1} d^{-1}	2.17	1.5	2.1	1.91
Areal biomass productivity	g m^{-2} d-1	52.21	36.18	50.43	46.04
N removal efficiency	%	80-98	99.8	99.7	77.8
P removal efficiency	%	63-83	99.2	86.1	99.5
N removal rate Nr	mg N $L^{-1}d^{-1}$	291.1±29.8	99.17±0.3	110.46±0.4	212.7±23
P removal rate Pr	mg P $L^{-1}d^{-1}$	29.5±4.2	8.3±.04	42.7±2.6	35.3±0.6
N removal yield on light $Y_{N/Ph}$	mg/mol photons	55.9±5.7	19.1±0.3	20.9±0.1	41.0±4.1
P removal yield on light $Y_{P/Ph}$	mg/mol photons	5.7±0.8	1.6±0.01	8±0.9	6.8±0.1
Biomass yield on light $Y_{X/Ph}$	mg/mol photons	420	290	400	370
Biomass yield on N $Y_{X/N}$	g/g	7.9±1.3	15.2±0.2	18.9±1.5	9.1±1.1
Biomass yield on P $Y_{X/P}$	g/g	78.4±9.0	187.9±9.5	50.2±4.5	54.1±5.2

In relation to effluent quality, the treatment with 10% treated BW without the addition of extra P (with an influent PO_4-P concentration of ca. 5 mg L^{-1}) achieved complete removal of both N and P and comply with the discharge limit. However, the microalgae biomass production in this treatment was very low compared to the other treatments. Addition of 29 mg L^{-1} extra P to the 10% treated BW substrate improved the biomass productivity and maintain the high N removal efficiency (99.7%). However, the effluent P concentration (ranged from 3.6 to 8.3 mg L^{-1}) surpassed the discharge limit of 1 mg L^{-1}. In the case of the 20% treated BW substrate, complete removal of P was achieved with initial influent PO_4-P concentration of up to 25 mg L^{-1}, but the N removal efficiency decreased to 78%. The decrease in N removal efficiency at high initial influent N may be related to light limitation. The high initial NH_4-N concentration in the influent is also followed by transformation of a fraction of NH_4-N into NO_2-N/NO_3-N which may result in the

preference uptake of NH_4-N. The NO_2-N and NO_3-N will, therefore, remain in the effluent.

The nutrient removal yield on light $Y_{Nr/Ph}$ (mg N(mol photons)$^{-1}$ and $Y_{Pr/Ph}$ (mg P(mol photons)$^{-1}$ were calculated in order to estimate the amount of light energy required to remove N and P from the substrate at a given photon flux density (PFD). The N removal yield on light with the defined medium was higher than the N removal yield on the light in the 10% and 20% treated blackwater substrate. This is related to the removal rate which is less in the later cases. The P yield on light, on the other hand, was lower in the case of the 10% treated blackwater. The yield on the substrate is higher for P than for N in all cases indicating the role of P in biomass production.

Conclusion

This study demonstrates the growth of *Chlorella sorokiniana* strain CHL176 in diluted treated blackwater. An average of up to 2.1 g biomass $L^{-1}d^{-1}$ or 50.4 g dry matter m^{-2} d^{-1} was produced in a short light path of 30 mm panel photobioreactors with a continuous irradiance of an average of 1450 µmol photons m^{-2} s^{-1}. *Chlorella sorokiniana* removed more than 99% of NH_4-N and PO_4-P on the 10% treated blackwater. The N and P removal rates reached on average up to 110.46±0.4 mg L^{-1} d^{-1} and 43±2.6 mg L^{-1} d^{-1}, respectively. Magnesium and trace elements in the treated blackwater were a limiting factor. The supplementation of these nutrients is necessary for optimal biomass productivity and subsequent removal of N and P. Increased substrate P resulted in increased biomass but effluent P was above the discharge limit. Nitrification of the ammonium in the substrate caused accumulated nitrite which inhibit the growth of *C. sorokiniana* and resulted in a declined biomass productivity when 20% treated blackwater was used. Although nitrite toxicity to *Chlorella sorokiniana* is not well understood, the results revealed its inhibiting effect on growth above 50 mg L^{-1}. Anaerobic substrate supply or reduced substrate retention time during feeding can help to minimize nitrite accumulation and its effect on growth and biomass productivity of *Chlorella sorokiniana*. At 10% treated blackwater and low nitrite concentrations, complete removal of NH_4-N and PO_4-P were achieved. This implies that N and P can be stored in the form of microalgae biomass and the effluent from microalgae reactor meets the discharge permit limit.

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