Norwegian University of Life Sciences
Faculty of Veterinary Medicine and Biosciences
Department of Animal and Aquacultural Sciences

Master Thesis 2016
30 credits

Unicellular Algae from the Genus Chlorella Grown Under Various Conditions-Potential for Use as Feed

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Dejan Knezevic

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Contents

Introduction

Diverse morphological and physiological characteristics of microalgae are enabling their use in the production of protein, vitamins, antioxidants, drugs, immunostimulants, biofuels and food supplements (Lum et al., 2013). These physiological characteristic was the main reason for intensive research on microalgae cells in recent years. Being singe celled gives them the opportunity to spread on the much wider surface maximizing use of sunlight contrary to plants that has limited surface and position. Their simple cell structure will ensure the rapid and successful growth of under various conditions. This characteristic enables them to be present in the most diverse ecosystems (sea, rivers, lakes, lagoons) and the habitats with unfavorable environmental conditions for other species (Chu, 2012). Algae can grow in places that are unsuited for agriculture, such as desert, wastelands or unfertile coastal areas (Kova\vc et al., 2013). Microalgae have evolved in the hostile environment of the primordial earth where there was not so much oxygen in the atmosphere.

C. vulgaris is a spherical, unicellular microalga, with a diameter of 2-10 μm that grows in fresh water conditions (Safi et al., 2014). It shows rapid growth during favorable conditions, and it is resistant to invaders and harsh environment. The minimal conditions necessary for algae growth, in the water medium, are light and CO_2. By changing the medium and modifying conditions, their growth is accelerated and target the production of the particular set of compounds. Rapid growth rates can be achieved with the optimal light exposure (intensity and wavelength), pH, temperature, mixing speed, change of substrate composition, and the ratio of the concentration of dissolved oxygen and CO_2 in the medium (Cheah et al., 2015). These conditions will favor algal growth (production of biomass), and protein production.

During unfavorable growth conditions, algae will start to store energy in the form of lipids and carbohydrates. To achieve this, we need to stress algae by limiting nitrogen and\or phosphorus sources, their primary nutrient sources. Other stress factors include temperature increase, excessive exposure to light, and high iron content(Singh & Singh, 2015). To achieve desired biomass composition (lipid, proteins, carbohydrates and pigment content), development of various growth techniques is necessary.

Light as a substrate for growth of C.vulgaris

Light is not a simple “substrate” as phosphorus or nitrogen, but it is an essential for algae growth. The intensity and type of light significantly affect yield and composition of the biomass. In addition, the wavelength spectrum that algae can utilize determines the efficiency of photosynthesis(Blair et al., 2014).

Generally, light sources are divided into natural (sunlight) or artificial (classical lamps, LED). Sunlight is the cheapest energy source for photosynthesis, but it is limiting regarding exposure and amount that can be obtained. The amount of available light for production is dependent mostly on geographical position, and change of climate conditions(Janssen, 2002).

Algae growth is affected by the light in ways of quality, strength and intensity of light. The quality of light is its wavelength, and it determines the color of light if it is monochromatic or white light. The ultraviolet rays (small wavelength and high energy) have chemical effects and infrared (greater wavelength and lower energy) thermal effect. One part of the sun's rays reflected from the atmosphere, a part that penetrates the atmosphere is weekend by absorption and dispersion. The atmosphere completely absorbs the rays of small wavelength and a large part of the ultraviolet rays. The strength of the sunlight can be affected by diffusion of the ozone layer in the upper parts the atmosphere, annual changes and the pollution in the air. Sunrays of small wavelength are lethal to living organisms, and the atmosphere filters them. The rest of the ultraviolet rays, visible part of the spectrum are reaching the Earth with the wavelengths from 320 to 1100 nm. Part of the visible spectrum, which is important for photosynthesis, have a wavelength of 280-760 nm (Blair et al., 2014).

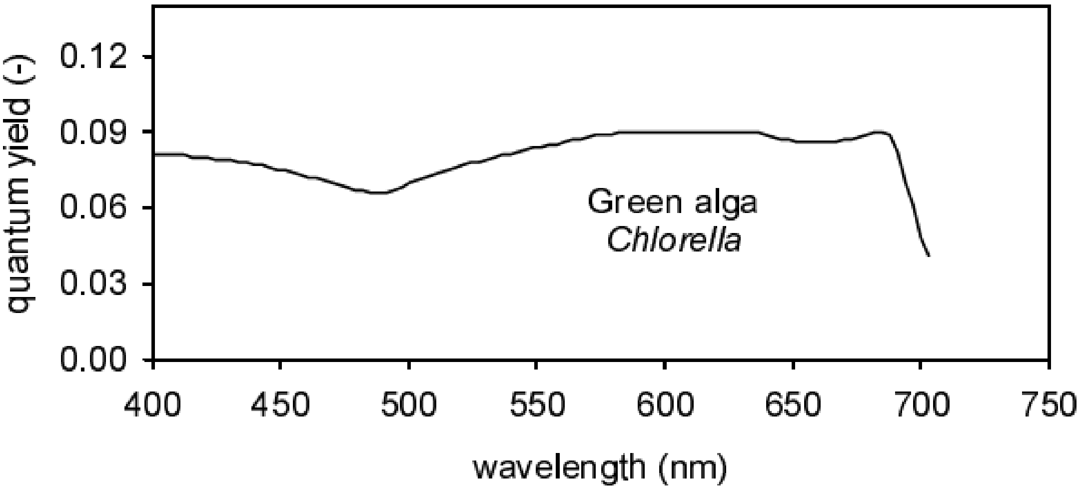


Figure 1. Quantum yield as a function of wavelength in *C.vulgaris* (Janssen, 2002).

Under the intensity of light, we consider the total energy of photosynthetically active radiation that reaches the surface. As an intensity of light increases, photosynthetic pigments are becoming more efficient until a saturation point is reached. The excessive amount of light or the increase in light intensity can cause degradation of chlorophyll or even cellular damages (Béchet et al., 2013).

The duration of illumination of photosynthetic organs is vital for the efficiency of photosynthesis. Exposure to an intense light in a shorter period is tolerable while in the extended periods of time can be harmful. The total number of hours of daylight is an important aspect of the algae production. Since sunlight is an unreliable source of energy, researchers have relied more on artificial sources that can provide more homogenous illumination.

In the experiments on microalgae, the wide range of artificial illumination have been used so far. Fluorescent tubes are the most readily available solution, but they do not provide homogenous illumination. The tungsten-halogen lamps have higher intensity at wavelengths higher than 700 nm, but the lover intensity in the lower wavelength of 400-500 nm (blue spectrum). Light emitting diodes (LED) are more versatile regarding control of emitting frequencies (Zhao et al., 2013). According to only wavelengths between 400 and 700 nm are effective in algae growth.

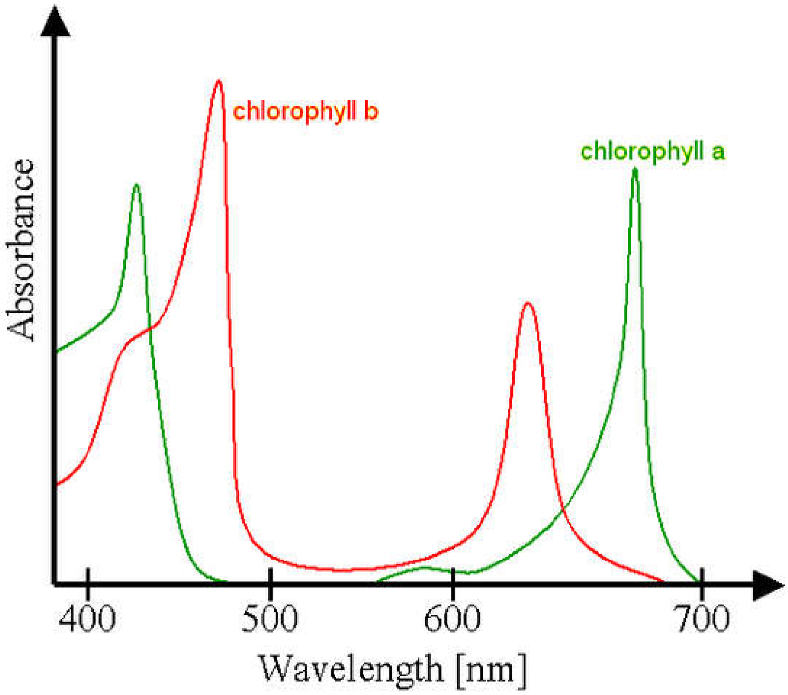


Figure 2. Chlorophyll absorption in the spectrum of visible light.

Wastewater as a potential growth medium

Urban development, industrialization, and an increase in population leads to worldwide environmental challenges that have a severe effect on the availability and supply of drinking water. The problem of wastewater is a serious obstacle to health and development of people. In order to protect ecosystem as a whole, it is necessary to treat wastewater before discharging it into the waterways. Wastewater is defined as water that is in any way contaminated or degraded during use, and it cannot be reused or released into nature before previous treatment (Wang et al., 2010). The composition of urban water can vary due to different sources of contamination, the social structure of the community and industrial levels of technology.

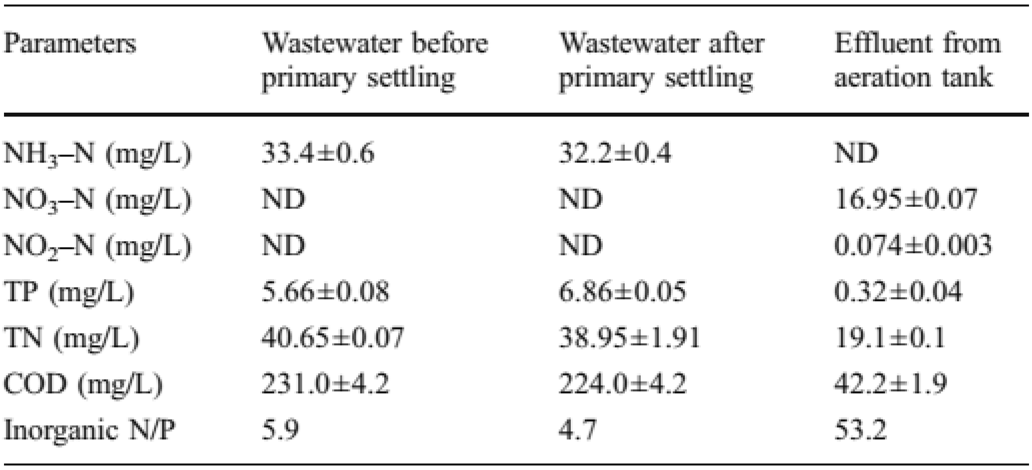
After tertiary treatment, industrial and municipal wastewaters can be used as a source of nutrients for algae production. Algae have a high capacity to remove inorganic nutrients efficiently from the water and to reduce amounts of nitrogen and phosphorus more than 90%

(Scheper et al., 2008). Effluent waters are an ideal medium for algal growth since they contain high amounts of nitrogen and phosphorus.

Urban wastewater

Municipal wastewater includes domestic sewage, water coming from public institutions and industrial effluents that are being drained through the sewage system. Industrial wastewater is usually the primary cause of pollution of water resources. The degree of contamination depends on the type of industry, methods of production, raw materials and reagents which are used in the technological process. Municipal wastewater is a mixture of household and industrial wastewater and rainwater. Wastewater is the result of the use of water in rural and urban households, health care, education, service and other productive sectors.

Physical, chemical and biochemical composition of the waste water is dependent on many factors, including consumption of water per person in the household, way life, topographical and geographical conditions of the sewage system, available quantities of water, types of industrial wastewater and other uncontrolled factors that can enter the sewage system (Wang et al., 2010).



Parameters	Wastewater before primary settling	Wastewater after primary settling	Effluent from aeration tank
NH_3–N (mg/L)	33.4±0.6	32.2±0.4	ND
NO_3–N (mg/L)	ND	ND	16.95±0.07
NO_2–N (mg/L)	ND	ND	0.074±0.003
TP (mg/L)	5.66±0.08	6.86±0.05	0.32±0.04
TN (mg/L)	40.65±0.07	38.95±1.91	19.1±0.1
COD (mg/L)	231.0±4.2	224.0±4.2	42.2±1.9
Inorganic N/P	5.9	4.7	53.2

Table 1. Characteristics of the four wastewaters sampled from the St. Paul Metropolitan Wastewater Treatment Plant (Wang et al., 2010).

Wastewater treatment is the process of reducing contamination in the discharged water to the amount or concentration in that will not have an adverse effect on the environment or human health. In the water processing plants, there are usually two stages of retention of harmful substances. The first step involves the adsorption of pollutants, precipitation, and removal of solids. The second phase includes aerobic and anaerobic biodegradation of the activated sludge (Cho et al., 2013). In some plants, the wastewater is disinfected, usually by chlorination or ultraviolet radiation (Priyadarshani & Rath, 2012).

Ways of Production of Microalgae in the wastewater medium

Autotrophic growth of algae

Autotrophic growth is characteristic for open ponds and photobioreactors. It requires only sunlight energy and carbon dioxide for photosynthesis in microalgae. Depending on the cultivation conditions, there are significant differences in yield and production of lipids in the biomass (from 5% to 68%). If targeted production is biodiesel, it is necessary to achieve high yields of biomass with a high lipid content. To achieve this goal, cultivation is carried out in two stages. The first step ensures the efficient growth of biomass while the second phase is completed under limiting conditions (nitrogen depletion) to provide the higher synthesis of lipids in the cells (Rawat et al., 2013).

Open pond systems

Open ponds are usually shallow pools with constant nutrient input. They are the cheapest method of production. These systems are typically built next to municipal water plants, thermal power plants or other sources of high CO2 discharge. The main advantage of this system is that it uses CO2, as carbons source, ensures cheap production, and simultaneously removes it from the atmosphere decreasing pollution effect. Large surface area, constant mixing and pond depth of 15–30 cm allow penetration of sunlight to all layers of stratification. This is important at the end of the first growth stage, where the primary goal is maximizing biomass production (Slade & Bauen, 2013).

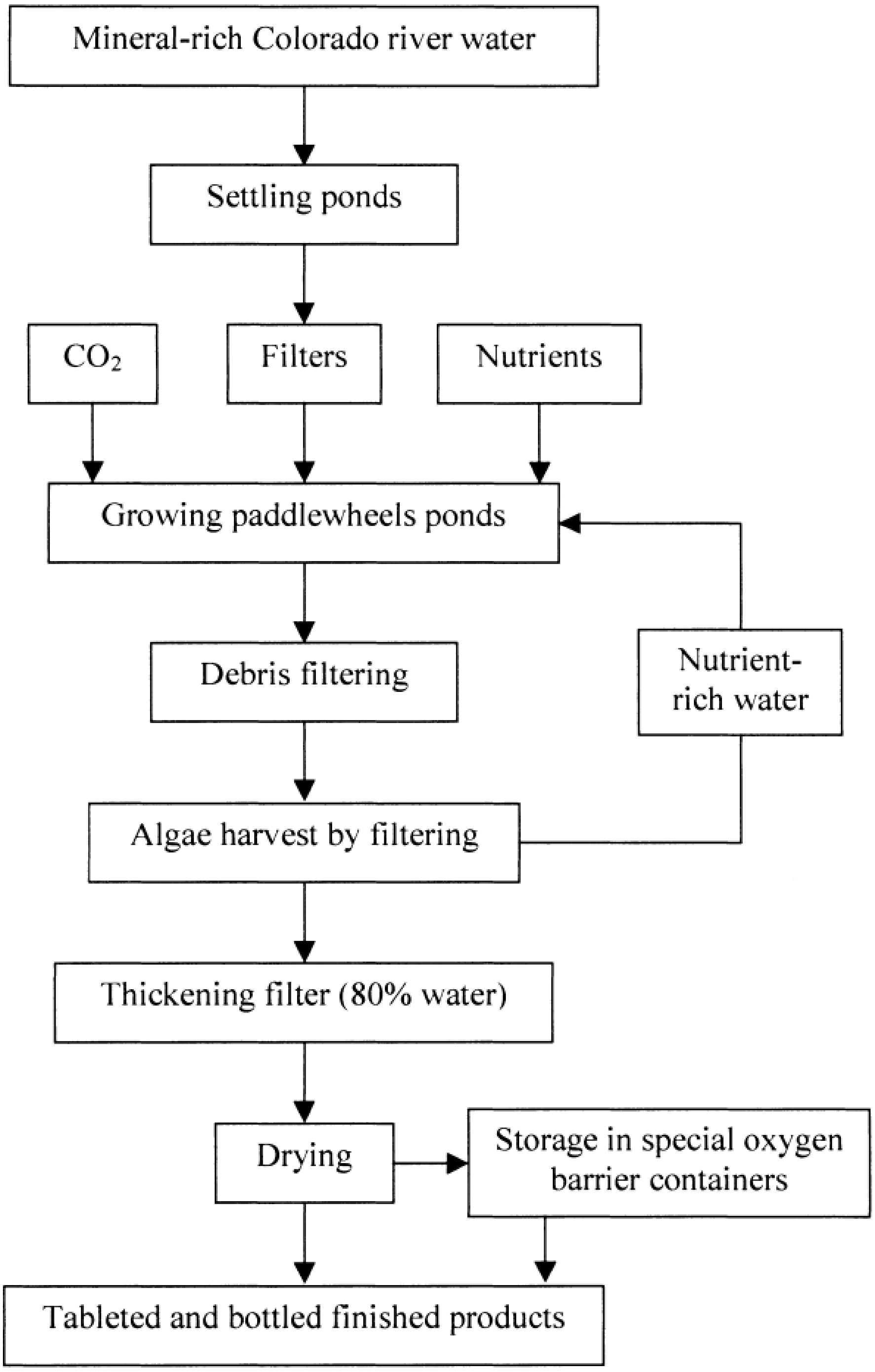


Figure 3. Earthrise Farms microalgal production process (Spolaore et al., 2006).

Open pond systems may use the land unsuited for agricultural purposes. However they are limited geographically due to temperature requirements for algal growth. Also, an open structure is exposed to environmental pollutions, the risk of contamination with other species of algae and bacteria and water evaporation (Slade & Bauen, 2013). Exposure to sunlight is based on seasonal changes and weather conditions while the concentration of CO_2 is hard to control. To ensure stable and controlled production some engineering issues must be addressed, especially in decreasing risks of contamination and providing better control of growth conditions.

Growth in the photo-bioreactor

This technology has been developed in order to achieve high yields and to increase the productivity of microalgae biomass production. It is overcoming limitations in the open pond system by ensuring optimal conditions for the cultivation. With the ability to control pH, light intensity, temperature and carbon dioxide concentration microalgae can significantly increase the production. The quality of final product is high, and it can be used for a production of human supplements, cosmetics, and pharmaceutical products (Bowles, 2007).

Better control of growing conditions has enabled the use of various strains of algae that are usually limited by harsh environmental conditions. Also, this system is not restricted by the availability of land and shape of a terrain; it can be developed in any part of the word in any form. Various designs of photo-bioreactors have been tested, such as tubular reactors, where algae are continuously circulating through the transparent pipes allowing for maximal light

Picture 1. Courtesy of *LA JOLLA, Calif.*

Picture 2. *Photo courtesy of Heliae Inc., USA*

absorption; circular photo-bioreactors, plate panels, in the forms of bags and much more (Ugwu et al., 2008).

The use of chemically defined culture media composed of pure chemicals is easy to control and provide optimal conditions for growth but significantly increases the price of the production process. For large-scale production, this is economically unsustainable. An alternative to the industrial scale use is growth media prepared from cheap raw materials such as by-products of the meat industry, milk, cereal and alcohol (Trivedi et al., 2015).

Although closed system bioreactors provide stable production without contamination, the main disadvantage is the cost of the construction. Initial investments in infrastructure are much higher than with open system. The production relies on the artificial light source that uses a significant amount of energy. Providing sufficient light source, which is economically justified, is the biggest obstacle for upscaling of bioreactors and wider industrial use. One of the more technical issues is overcoming friction in pipes, which slows down algae flow in a bioreactor and increases energy used for pumping. The slower flow makes algae more prone to stick to the surface. This surface layer will significantly decrease light penetration, and it will increase the cost of cleaning and sanitizing the pipes (Demirbas, 2010).

In order to overcome these problems, it is necessary to find more efficient ways of delivering equal amounts light to the individual cells and more innovation in the new, more, efficient light sources will significantly increase productivity.

Cultivation methods

Heterotrophic growth

During heterotrophic cultivation, algae are grown in the bioreactor without a light source, using an organic source of carbon for growth. This production is characterized by relatively high biomass production with limited or no light input into the suspension in bioreactors, and it is mainly used for the production of lipids due to high production of biomass, which lowers the cost of harvesting and extraction of oil (Ugwu et al., 2008).

This technique of metabolic control was used to produce high-quality biodiesel from *C. protothecoids* with a high heating value of 41 MJ\kg, a density of 0.8 kg\L, and a viscosity of 5.2 × 10−4 Pa s (at 40 °C) (Xu et al., 2006). The quality of produced biodiesel was comparable to petrol fuels. In this experiment corn powder hydrolysate was used as a carbon source instead of glucose in order to increase biomass production and reduce the price of the final product. Compared to autotrophic growth, the structure of the algae was significantly changing to adapt to the new environment. Observed under differential interference microscopy chlorophyll and carotenoid cells have disappeared, and heterotrophic cells were mainly composed of lipid vesicles (Xu et al., 2006).

The advantages of this cultivation compared to the autotrophic production are a production of algae during periods of low light and darkness. Heterotrophic cultivation can achieve significantly greater production of biomass compared to autotrophic cultivation. *C.protothecoides* that were produced in this way had a crude lipid content of 55%, which is four times higher than when it was grown under photoautotrophic conditions (Chen et al., 2011). In some cases, the productivity could be increased up to 20 times compared to autotrophic cultivation methods (Brennan & Owende, 2010). For growth and lipid, production different organic carbon sources can be used such as glucose, acetate, glycerol, fructose, sucrose, lactose, galactose, and mannose (Sun et al., 2008).

The main disadvantage of heterotrophic cultivation is a price and availability of carbon sources necessary for growth. Sugars are efficient growth medium. However, they provide a high possibility of growth for various contaminants, and their use will directly compete with feed and food production resources.

Mixotrophic growth

Mixotrophic cultivation of *C.vulgaris* combines the use of inorganic (CO_2) and organic carbon sources with photosynthesis. Cultivation can be carried out in open or closed systems and various conditions because *C.vulgaris* will not be constricted to the use of one medium for growth (light or organic substrate). This capability of combining both autotrophic and heterotrophic growth is reducing the amount of sugars added to the system, and growth can continue during dark periods, maximizing the growth of biomass (Amin, 2009). The wider implementation of the mixotrophic system is also constricted by economic limitations in upscaling and problems with contamination.

Harvesting technique's

Harvesting the biomass from microalgae represent additional technological and economic challenge in addition to the previous process of production and expenses of energy consumption. The primary technical operations for separation of biomass from microalgae culture are centrifugation, flocculation, filtration, pressing, sedimentation and flotation (Kurniawati et al., 2014). The conventional process is carried out in two steps. The first step is used for removal of water from the suspension, and concentration of algae while a second phase removes the remaining water by filtration or centrifugation (Bilad et al., 2012).

Centrifugation

This method extracts microalgae from the culture medium by using centrifugal force. The cost of extraction increases manufacturing costs of dry matter biomass by 20 to 30% (Bowles, 2007). Key advantages of this method are relatively short processing time (5-15min), during which 90-95% of algae can be extracted and on an industrial scale, larger volumes can be processed. Also, it is easy to change machine parameters to adapt to current algae density and criteriums of the final product. The disadvantage of this process is the high-energy consumption. Most microalgae are damaged during the process of centrifugation while

C.vulgaris rigid structure allows it to tolerate greater stress without structural damage (Lam & Lee, 2012).

Flocculation and coagulation

Flocculation is the process of gathering destabilized or "coagulated" particles to create larger clusters or flocs. It is used as a pre-harvest technique in order to aggregate dispersed algae in the medium and accelerate harvesting process. This method can reduce energy demand for harvesting by increasing cell concentration. Flocculation may be carried out chemically by an addition of inorganic flocculants such as sodium hydroxide, aluminum or iron salts. New technologies are using magnetic nanoparticles to aggregate algae cells (Vandamme et al., 2013). Main reason for algal dispersion is their negative charge on the cell surface coming from the carboxylic (-COOH) and amine (-NH2) groups. High pH values contribute to the negative charge, while anime groups are not affected. Upon adsorption of aluminum or iron ions on the surface, the negative charge is removed allowing the creation of clusters (Barros et al., 2015).

The efficiency of flocculation is determined by the type of flocculants used (size of molecules), functional groups on the surface of algae cells (surface charge of the cell wall is determined by the type and amount of functional groups on the wall), pH and composition (density) of the suspension. Aluminum coagulants are used for the treatment of drinking water, and their dosage is determined by correlation to total organic carbon that is present in the treated water (Chen et al., 2011).

Flocculation and sedimentation can be carried out by increasing the pH of the suspension by the addition of sodium hydroxide. Although effective, this treatment is expensive for the large-scale production, so the industrial purposes use of lime is more common. During phototropic growth, auto-flocculation can occur due to increase in pH and high utilization of CO2 from the culture medium (nitrate and phosphate absorption). Other useful flocculant agents are magnesium hydroxide, chitosan, and bio-flocculants like Paenibacillus sp (Wan et al., 2015).Disadvantages of this method are the collection of large amounts of sludge due to a use of inorganic coagulants, sensitivity to the changes in pH, and separated biomass contains significant quantities of iron or aluminum ions, so it is necessary to implement additional purification processes.

Sedimentation is combined with flocculation in order to ensure higher speed of processing when effluent waters are used as the growth medium. Sedimentation depends on the particular weight of microalgae cells where cells with larger specific weight are more easily extracted than cells with lower specific mass (Leite et al., 2013).

Flotation

During the flotation, air is added to the water in order to adhere to the particles and create gas formations, which have a smaller density than water and will rise to the surface, where they can be collected. Flotation is most often used in combination with flocculation. Both processes are based on the difference in specific weight of solid components and water. Floccules have a large surface, which improves the adhesion to air bubbles and their transfer to the surface (Gui et al., 2014).

The speed and efficiency of flotation depend on pressure and retention time of air bubbles in the medium, ratio of suspended air in the medium, size and affinity of algae cells for air adhesion. In the laboratory conditions, a combination of flotation and flocculation was significantly more efficient than relying only on sedimentation of floccules (Barros et al., 2015).

Filtration

Filtration process involves repeated passing of biomass through the filters of appropriate structure and pore size. This method will increase the concentration of algae, and it will simplify the drying process. Due to small size (2-10 microns) of *C.vulgaris*, it is necessary to use microfilters for extraction. The most commonly used are rotary filters with a system for flushing the filter. Factors that affect filtration are a type of filters, working pressure, type and the velocity of flow. Accumulation of biomass can quickly clog the pores of the filter that slows down the process. In order to prevent this, screen need frequent washing (flushing). Using vacuum filtration will increase the efficiency, and the speed clogging of the filter pores (Bilad et al., 2013).

Major limitations of this process are the presence of cross-contaminates (larger species of algae and other particles larger than 10 microns) that can cause the blockage and stop the filtration process. Frequent replacement of membranes can become a limiting economic factor for the process of filtration on an industrial scale. Application of membrane microfiltration is limited to the small volume production while centrifugation and rotary vacuum filters are more appropriate for large-scale production (Bilad et al., 2012). Using filtration in combination with flotation or flocculation can improve harvesting process.

In the study of untreated dairy farm wastewaters, as a resource for biodiesel (Hena et al., 2015) have shown that *C. vulgaris* can successfully use this medium for the production of oil. The research was focusing on utilizing total N concentration of 35.6–47.8 mg\L and total P concentration of 46.7–54.7mg\L, for algae growth. With supplementation of 10% CO2 after ten days of cultivation, algae have shown an increase of 58% in chlorophyll a concentration, showing stable growth response (Hena et al., 2015). The study is suggesting that *Chlorella sp.* can be used for the treatment of primarily settled dairy farm wastewaters.

Another potential use for *C. vulgaris* is the treatment of swine wastewater that has high concentrations of ammonia nitrogen. In the experiment (Wang et al., 2015) under mixotrophic and heterotrophic conditions, have used several dilution of swine wastewater. Original concentration of ammonia was 1434 mg/L and it was diluted 5-fold (264 mg/L) 10-fold (153 mg/L) and 20-fold (86 mg/L). *C. vulgaris* was efficiently utilizing nitrogen up to 91% in 20-fold dilution and with highest biomass production (3.9 g/L) under 5 –fold dilution. Due to high nitrogen content in the medium, there were not enough stress factors to induce oil production, and the resulting algal composition had high carbohydrate composition of 46% - 58% respectively. Glucose was dominate sugar with 90% of total monosaccharides. The content of arabinose and xylose were relatively low 1.6% - 2.4% respectively. Research has shown that *C.vulgaris* can be efficiently used to remove ammonia from swine wastewater and that carbohydrate-rich biomass provides benefit for conversion into other high-value products (Wang et al., 2015).

With the development of industry, a major problem in environmental protection is the increase in the concentration of synthetic organic substances in surface water, especially in rivers. Organic contaminants such as personal care products and pharmaceutical products, flame retardant and pesticides are often overlooked in the wastewater treatment (WWT) and can significantly disrupt the normal functioning of the endocrine glands, which can lead to a variety of systemic disorders (Matamoros et al., 2015). Pharmaceutical products can be present in the water supply system through the unnecessary disposal of medications in the toilet, overconsumption of medicines, or excretion of drugs through urine or feces. Microalgae treatment in symbiosis with bacteria can provide a possible solution for degradation of organic pollutants.

Due to the high water solubility, polarity, and exceptional resistance to degradation, majority of pharmaceutical compounds are difficult to absorb and can easily pass through the process of purification, but also through processes natural filtration. These characteristics enable them to avoid sedimentation and biological treatment process. Coagulation, flocculation, and sedimentation processes are not effective in removing dissolved organic pollutants. Biological processes are very effective to reduce the concentration of biodegradable compounds while chlorination and ozone treatment are considered the most efficient way removing a substantial number of medicines (Pittman et al., 2011) .

The research by (Matamoros et al., 2015) revealed that some drugs remain in water even after treatment of wastewater and filtration in water purification plants and that most effective ways of removal are photodegradation a biodegradation in high-rate algal ponds (HRAP). Depending on the season, they have used different composition of algae for the experiment. During the warm season, HRAP was primarily consisted of *Stigeoclonium sp., Chlorella sp.* and *Monoraphidium sp.* while during cold season they have used *Chlorella sp*. and *Stigeoclonium* sp.

"They have found that system had high efficiency of removal (>90%: caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate and hydrocinnamic acid), moderate- to-high removal (from 60% to 90%: oxybenzone, ketoprofen, 5-methyl/benzotriazole, naproxen, galaxolide, tonalide, tributyl phosphate, triclosan, bisphenol A and octylphenol), moderate- to low removal (from 40 to 60%: diclofenac, benzotriazole, OH- enzothiazole, triphenylphosphate, cashmeran, diazinon, ben-zothiazole, celestolide, 2,4-D and atrazine) and reduced or no removal (<40%, carbamazepine, methylparaben, tris (2-chloroethyl) phosphate)."

Microalgae treatment of wastewaters has shown the capability to remove a wide range of organic contaminants. The efficiency of removal was higher in the summer than in the cold season, although removal of caffeine, acetaminophen and ibuprofen had high efficiency of removal (90%) from effluents during the whole year and it was not affected by seasonal fluctuations (Matamoros et al., 2015).

There is a lot of information about growing algae in the source waters, but there are not many strategies available for algae growth in water treatment plants (WTP). In praxis, this has caused several operational issues, and questionable water quality does to development that is based on a trial-and-error. These techniques need to be more available to the water industry.

Applications and potential interests for algal development

Biofuel production

Renewable energy is considered one of the key factors for future development strategies. Fossil fuels are still available and with their currently, competitive cost demand is not likely to change in the near future. However, constant depletion of fossil fuels, increased demand for energy and adverse effects on the environment are the main reasons for the steady increase in the production of energy from renewable resources, and one of them is biodiesel (Kligerman & Bouwer, 2015).

Biomass derived from algae is a renewable energy source that is an alternative to fossil energy sources. Depending on the type of biomass, algae can be utilized as energy source trough processes of biochemical conversion to produce bioethanol and methane, thermochemical conversion to produce gas or lipid-based biofuels (Bharathiraja et al., 2015). Another potential use for algae biomass is in the production of feed, human nutrition, pharmaceutical products, fertilizer or they can be directly combusted to produce energy.

The most important factors for growth of *C.vulgaris* are water quality and climate. Within water quality, it is necessary to provide basic nutrients for growth (nitrogen and phosphorus), an adequate amount of carbon dioxide (CO2), micro and macronutrients such as Iron (Fe), are necessary for photosynthesis. The climate is another key factor for algae growth. Sunlight provides conditions for photosynthesis, and temperature causes thermal stratifications of reservoirs and basins, which promotes algae growth (Villicaña-Ortiz et al., 2015).

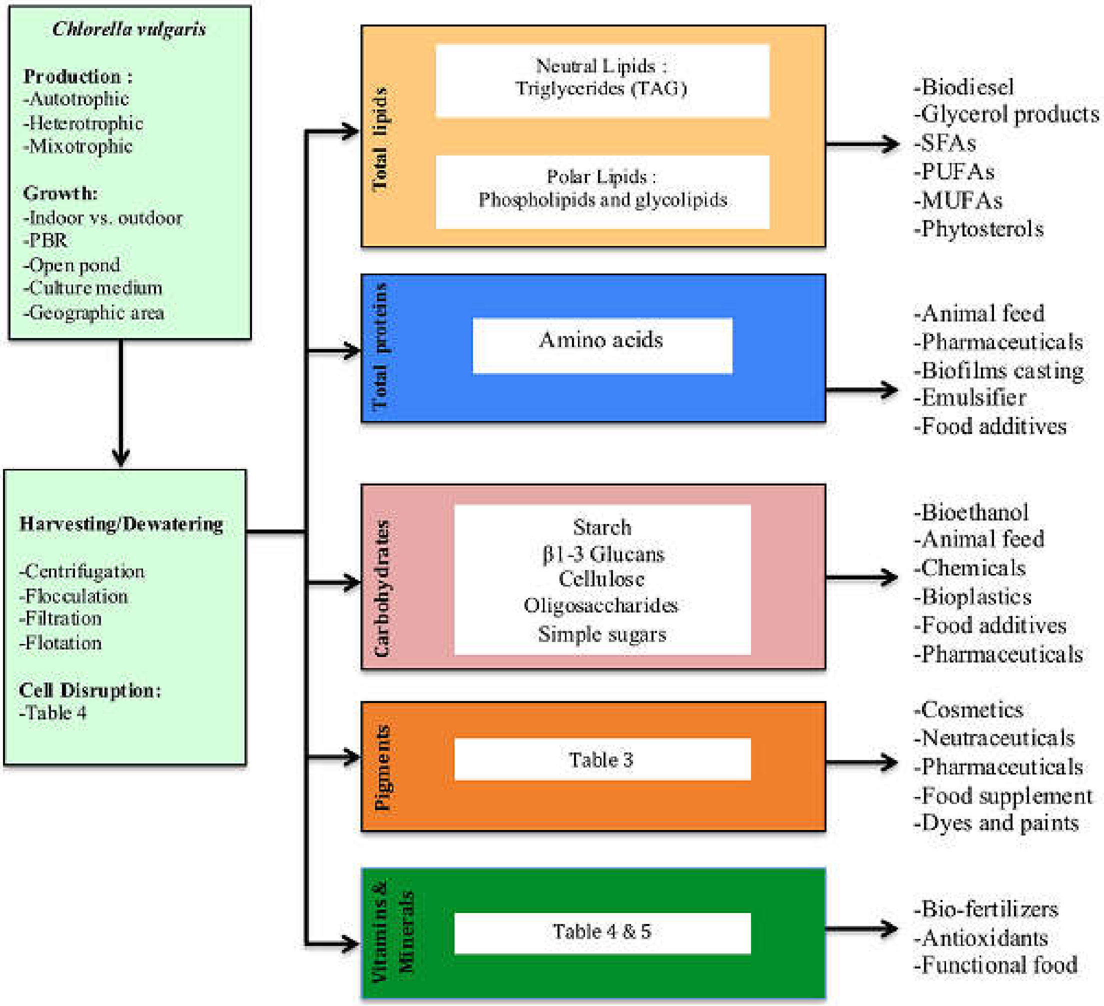


Figure 4. Algo-refinery concept from production to valorization (Safi et al., 2014).

The interest for algae as a biofuel comes from increased demand for the green technologies development. Algae production represents original green technology that is renewable, sustainable, and environmentally friendly. Despite these advantages, bioprocesses for the production of lipids from algae are not yet sufficiently developed. Existing technology does not provide a commercially acceptable production of biodiesel (Chisti, 2007). It is, therefore, necessary to develop new methods of cultivation of microalgae biomass with a high lipid content in the biomass and to ensure efficient extraction and purification of lipids from the biomass. Development of new types of bioreactors for economically viable production will contribute to use and optimization of new and more efficient procedures.

According to (Rawat et al., 2013) after oil extraction with n-hexane and gaseous chromatography analysis, fatty acid profile of *C.vulgaris* is composed of 52% unsaturated fatty acids and 29% of saturated fatty acid in maximum biomass concentration of 3 g\L. This fatty acid composition present most adequate raw material for quality biodiesel production especially regarding content of linolenic acid (C18:3) of 19,05%and other polyunsaturated fatty acids (Rawat et al., 2013).

Drying of algal biomass

Drying is a necessary step before the extraction of lipids and biodiesel production. The presence of water slows down the process of extraction and biodiesel transesterification. Drying is carried out in closed system dryers. This process can significantly increase consumption of energy. By some reports, this increase can reach 69% of total energy consumption for the production of biodiesel, and contributes to reducing the commercial viability of the production (Chen et al., 2011). If climatic conditions are favorable, drying of biomass can be carried out in the open.

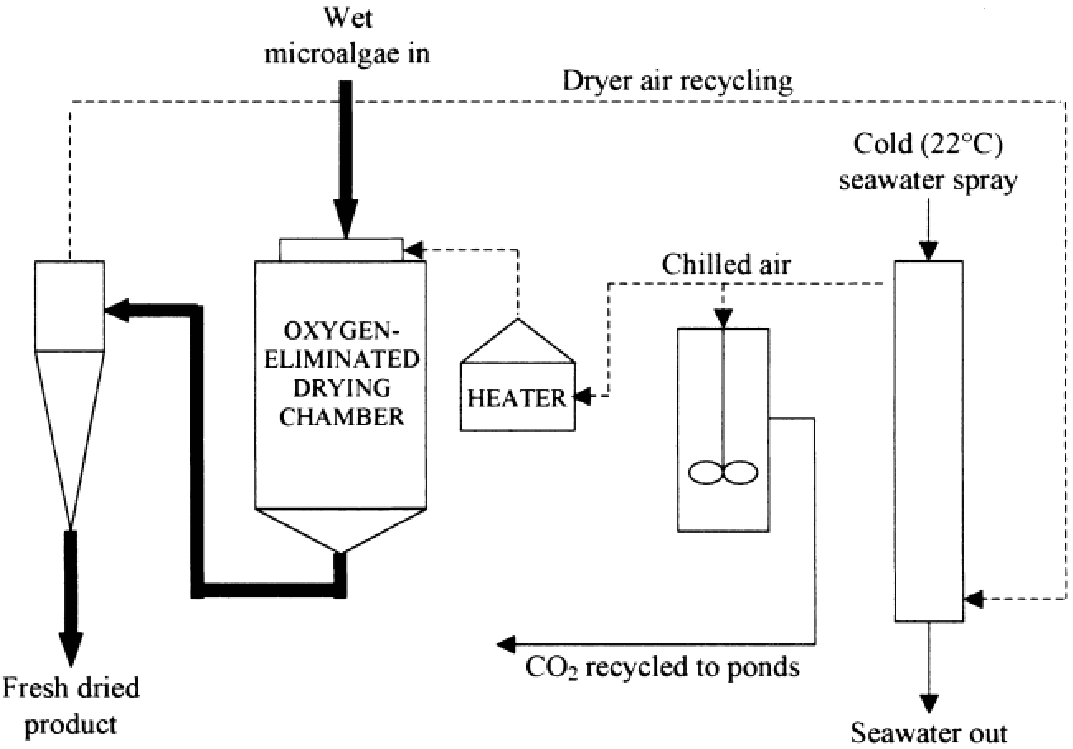
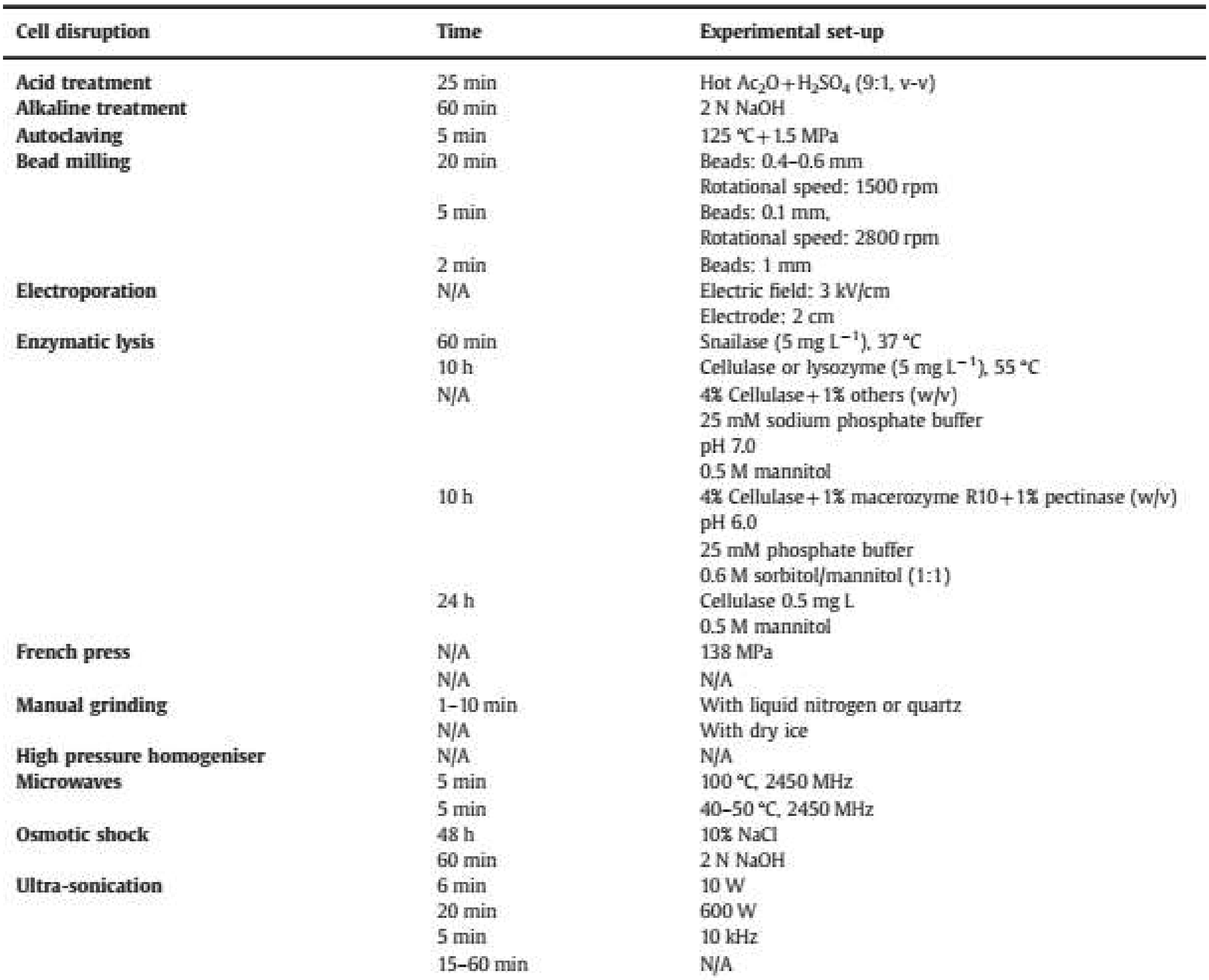


Figure 5. Process of drying microalgae biomass (Spolaore et al., 2006).

Methods for cell disruption

After drying, it is necessary to break up the cells of microalgae, isolate and purify the desired product. Microalgae cells can be successfully broken using the homogenizer, bead mills, ultrasound and thermal processes. The use of thermal processes and ultrasound energy is inefficient, and the quality of lipids after the treatment is relatively low. Using mills and microwaves gave a satisfactory quality of fats for further processing (Rodriguez et al., 2015).



Cell disruption	**Time**	**Experimental set-up**
Acid treatment	25 min	Hot $Ac_2O + H_2SO_4$ (9:1, v-v)
Alkaline treatment	60 min	2 N NaOH
Autoclaving	5 min	125 °C + 1.5 MPa
Bead milling	20 min	Beads: 0.4–0.6 mm Rotational speed: 1500 rpm
	5 min	Beads: 0.1 mm, Rotational speed: 2800 rpm
	2 min	Beads: 1 mm
Electroporation	N/A	Electric field: 3 kV/cm Electrode: 2 cm
Enzymatic lysis	60 min	Snailase (5 mg L^{-1}), 37 °C
	10 h	Cellulase or lysozyme (5 mg L^{-1}), 55 °C
	N/A	4% Cellulase + 1% others (w/v) 25 mM sodium phosphate buffer pH 7.0 0.5 M mannitol
	10 h	4% Cellulase + 1% macerozyme R10 + 1% pectinase (w/v) pH 6.0 25 mM phosphate buffer 0.6 M sorbitol/mannitol (1:1)
	24 h	Cellulase 0.5 mg L 0.5 M mannitol
French press	N/A	138 MPa
	N/A	N/A
Manual grinding	1–10 min	With liquid nitrogen or quartz
	N/A	With dry ice
High pressure homogeniser	N/A	N/A
Microwaves	5 min	100 °C, 2450 MHz
	5 min	40–50 °C, 2450 MHz
Osmotic shock	48 h	10% NaCl
	60 min	2 N NaOH
Ultra-sonication	6 min	10 W
	20 min	600 W
	5 min	10 kHz
	15–60 min	N/A

Table 2. Different cell disruption techniques carried out on *C.vulgaris* (Safi et al., 2014).

Lipid extraction from the biomass

Processing continues with lipid extraction from the biomass of microalgae. Efficient extraction of lipids from the biomass is significant for the extraction from cells with small amounts of fats. Using mechanical presses is difficult due to the strength of cell walls of microalgae. Therefore, the most often used method is an extraction with organic solvents such as hexane, chloroform, isopropanol, methanol, ethanol (96%), the mixture of hexane and ethanol. The disadvantage of organic solvents in this stage is their toxicity, and the necessity to dispose of used solvents after the extraction (Lam & Lee, 2012).

Ethanol is the most used extracting agent for *C.vulgaris*. It provides the advantage of less toxicity than n-hexane and has the possibility of production from renewable sources. Although the n-hexane can be successfully used for the extraction of lipids from the oil seeds high proportion of unsaturated fatty acids in the cells of microalgae and nonpolar nature of n-hexane makes extraction of lipids difficult. The downside of ethanol use is binding of water (5% water), which reduces the efficiency of extraction (Bharathiraja et al., 2015). To Increase the speed of the processing; extraction can be combined with a method for disrupting cells walls. However, the energy consumption during the disruption of cells is high. That is why using only the extraction can be more energy-efficient.

Extraction of lipids by using microwaves was, comparing the previous method increased 50% and the extraction time was shorter. The research was conducted at frequencies of 19, 25, 40 and 300 kHz, and in combination during the extraction of microalgae *Crypthecodinium cohnii*. Results have shown the increase in efficiency of extraction up to 25.9% (Cravotta et al., 2008). However, this method did not find the wider industrial applications due to significant energy expenditures.

Ethanol from corn vs. algae fuel

Algae biofuel is a fungible fuel- it is the direct replacement for the fuels we are using now. Fuels derive from algae will be processed into diesel, gasoline, and jet fuel. Ethanol from corn is ethyl alcohol that is currently added to petroleum fuel of approximately 5%. When 5% addition is exceeded, unwanted characteristics decrease the efficiency of the fuel because ethanol brings water with it into the mixture. This increase completely changes the way motor vehicles behave, makes them more prone to malfunctions, and it will eventually increase the maintenance costs. Fuels made from algae are exact replacements for the ones we use today (Pate et al., 2011).

Algae can produce the same sugars that we get from the corn, and we can process them into ethanol. However, lipids that they produce during photosynthesis are a most efficient way of storing energy than sugars or protein. This natural way of saving energy can easily be converted into gasoline or other products. Biologically we can produce good quality lipids in high concentration, but the economical aspect and increasing scale of the production can be problematic (Rawat et al., 2013).

Carbon neutral fuels: the amount of carbon that we release into the atmosphere should be equal to the amount of carbon that is taken from the environment during growth. To get the exact calculations, we need to measure all the energy inputs into the system. With corn-based ethanol, is not just the energy used for growth it is also energy used for agriculture, transport, fermentation processes, and then distilling ethanol uses much more energy inputs than algae production. When we add up all these data, we can see the real carbon impact of the production. The ethanol production requires almost same energy inputs as the energy that we get from burning the fuel, and it is almost the same as using petroleum. Also, it competes with feed production, arable soil, and uses large amounts of water.

All energy that algae produce comes from photosynthesis. Carbon dioxide if being fixed into the form of sugars. Furthermore, CO_2 can be much more concentrated in the water than in the air. We can use CO_2 coming from the thermal plant, and pump it directly into the ponds, significantly increasing productivity. Being single-celled gives them an opportunity to spread on the much wider surface maximizing use of sunlight contrary to a leaf that has limited surface and position. Algae can be grown in places that are unsuited for agriculture, such as desert, wastelands or unfertile coastal areas. Algae has adapted to these hostile conditions during billions of years of evolution, and these characteristics make them much more versatile and efficient than plants (Krienitz et al., 2015).

When conditions are unfavorable, algae will accumulate storage lipids to use them for reestablishing growth and rebuilding organism in more favorable conditions. Another reason for this storing process can be protection from ultraviolet light (UV) and protection from dehydration. We are taking advantage of their ability to protect themselves, and that protection that they have is anti-oxidant, so when we consume it we will get the oxidant benefit from astaxanthin and beta-carotene. That is why algae are produced as a nutraceutical.

Estimation is that there are 300-500 thousand of different algae species, and we have considered only a small fraction of that. In the laboratory conditions, less than hundreds species has been investigated. The major part of the future research will be looking for the new species in the environment so that we can identify the best ones to be used for fuel. Algae with characteristics needed will be the ones that can grow fast, produce large quantities of oil and are resistant to predators living in the water. Constant improvement on the strains that we have now and the discovery of new types and species is necessary to obtain stable and functionally production.

We cannot only observe biological functions of algae and making improvements in that field, but also we need to interact with engineers. Algae need to be grown in ponds, harvested, oil needs to be extracted from them, and that oil needs to be converted into gasoline or diesel. To get maximal benefits of the system, we need to combine both biology and engineering. Chemical engineers need to cooperate with agricultural engineers, biologist and ultimately with the farmers, who are going to grow algae. The combination of all of these new technologies and experiences that we had so far will certainly result in the creation of a new industry.

Energy from a floating algae pods

Most WTP are embedded into the cities and releases they wastewater in the local water systems. Offshore membrane enclosures, uses wastewater (nutrients&CO_2) wave's water provide mixing and temperature (controlled by a surrounding temperature). By utilizing solar energy, we will obtain products such as biofuels, fertilizers, feed, food, and oxygen. Moreover, the system is modular and contained. In the case of catastrophically failure, freshwater algae are leaking into saltwater where they will be biodegraded. Efficient methods for recovering wastewater are also necessary in order to maximize the cleaning potential of algae.

The structure itself will take a lot surface in the ocean, but it can provide an artificial habitat and increase biological diversity. It is important to develop tools that will allow us more precise insight into algal living cycle. To monitor and maintain their growing conditions not only for them to survive but to grow and thrive.

The most important feature are photobioreactors (PBR) containers that will take wastewater with algae and circulate it through the structure, maximizing algal exposure to the sunlight and increasing the efficiency of the photosynthesis. Oxygen that algae produce will become problematic at one point, and it needs to be removed with the simultaneous increase of CO_2. This can be achieved by diverting flow into side system and circulating them through the column of water where CO_2 will be added. This column can also help with sedimenting the algae and making them more readily available for harvesting.

Interaction of these systems with wildlife is also vital and, as well as environmental influence, has to be more researched in the future.

Economical aspect currently does not seem favorable for such projects. Unless we observe this problem as an opportunity for integrating several other existing systems. With the current solutions for WWT (water quality, CO_2 reduction, O_2 increase), we can integrate wave energy, photovoltaic panels, wind power or even aquaculture, can make this project economically feasible in the future. Providing the market with a high-value product can be the driver for further development and system scaling up. This approach can give more value than focusing only on biofuels. There is no limit to what we can achieve if we are open to new ideas, and we do not care who is going to get credit. Sustainable solutions for the future must be diverse and many.

Microalgae as animal feed

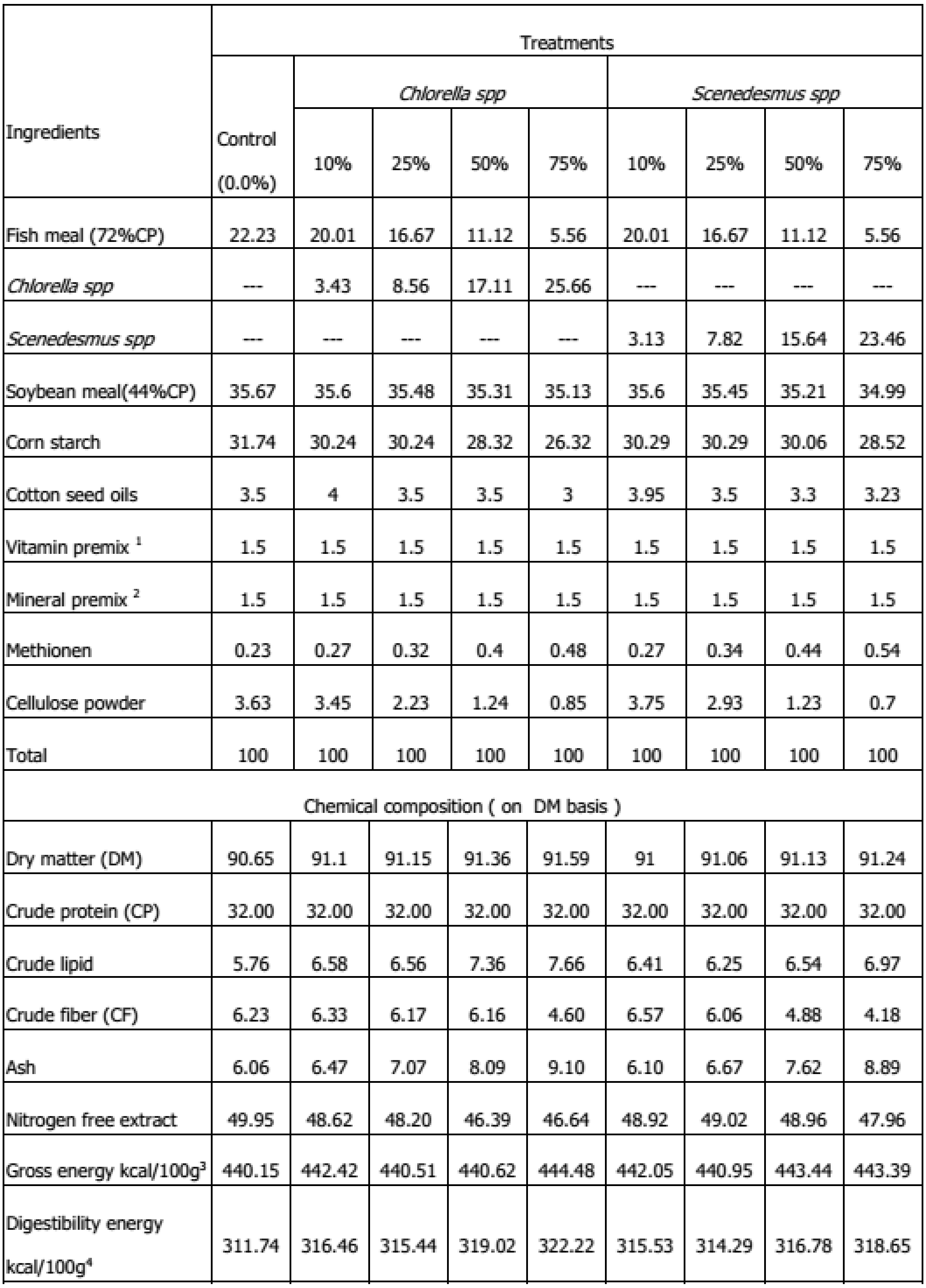
The increase in the world population and the challenges in agricultural production has led to the exploration of new energy and food sources. Because animal feed production, directly competes with crops for human food it is necessary to develop a viable replacement for animal feed ingredients that are more efficient and sustainable. Modern animal production is characterized by a tendency to intensify production of meat, milk and eggs. This type of production is mainly based on the increase of protein content in the meal (Tolkamp et al., 2010). However, there are few nutrients, especially of plant origin, which are rich in protein of high biological value. Therefore, the protein is the primary limiting factor for the livestock production in the world.

These problems can be solved by composing a meal, which is a mixture of proteins of animal and vegetable origin of high biological value. Due to high prices and the deficit of plant and animal protein, it is necessary to find a sustainable substitute. Algae production presents a potential nutrient rich source for aquaculture production, pig, and broiler feed. The basic advantage of algae production over traditional agriculture is the speed of growth of microorganisms, high conversion of substrate, low cost of production, which is not using vast areas of land and does not depend on weather changes, pest infestation, diseases and water shortages.

Aquaculture is a fast growing industry that produces a high-quality source of protein, and demand to produce more food is constantly increasing. Providing a sustainable source of feed with high-quality ingredients is the main condition for successful growth and future intensification of aquaculture. The cost of feed takes up to 60% of total production cost (Kyntäjä et al., 2014).

Tilapia (*Oreochromis niloticus*) is one of the most significant commercial species with the constant increase in production. It is an herbivores/omnivorous species that utilizes a wide range of low-quality feed ingredients. In the natural environment, tilapia mainly feeds on phytoplankton, zooplankton, larval fish, periphyton, detritus and higher plants. In commercial production, they are fed with a well-balanced pelleted feed containing proteins and lipids of animal or plant origin to provide fast growth of the fish. The optimal inclusion of protein is estimated from 30% to 50%, lipid inclusion to 5-12% and dietary starch 22-46% respectively (Appler, 1982). These ratios of inclusion may vary depending on fish age, size, and environmental conditions.

Fishmeal is widely used in aqua feed, and it is the most expensive ingredient that is also unsustainable. There is a high interest in replacing fishmeal with an alternative protein source. To reduce the cost of feed production algae are being researched as a non-conventional source of protein. Partial replacement of fishmeal with dried microalgal biomass of *Chlorella sp.* and *Scenedesmus sp.* was investigated by (BADWY et al., n.d.). In their experiment on Nile tilapia fingerlings, they have followed changes in their growth performance, feed efficiency and body composition during 90 days. In the formulated diets fishmeal was gradually substituted from zero (control diet) to 10, 25, 50 and 75%.



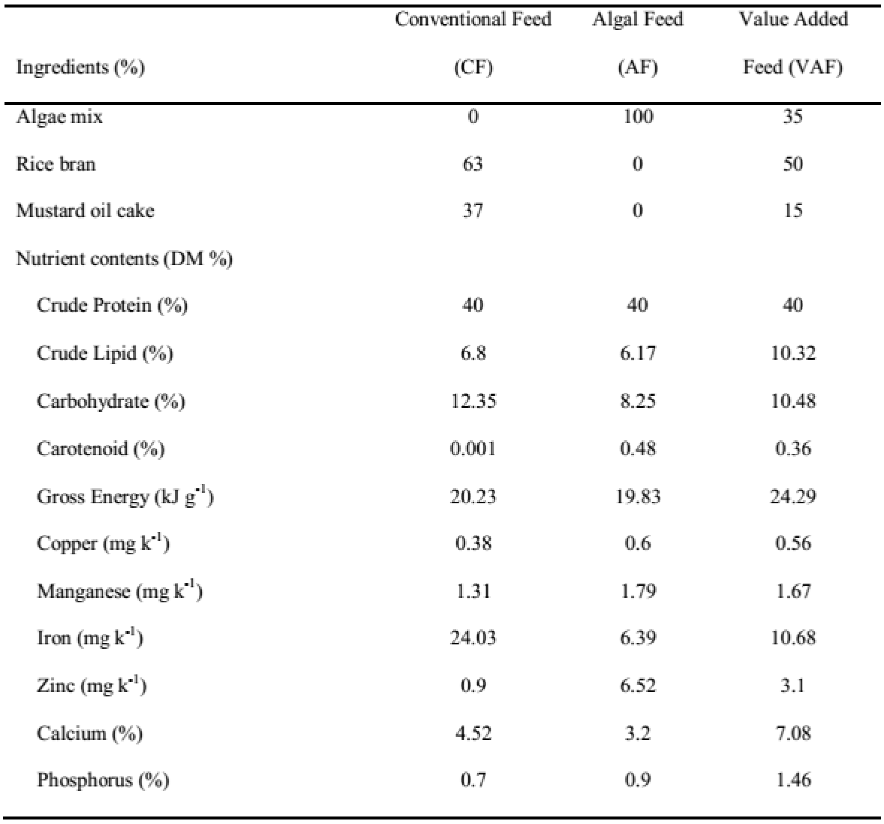
Ingredients	Treatments								
		Chlorella spp				*Scenedesmus spp*			
	Control (0.0%)	10%	25%	50%	75%	10%	25%	50%	75%
Fish meal (72%CP)	22.23	20.01	16.67	11.12	5.56	20.01	16.67	11.12	5.56
Chlorella spp	---	3.43	8.56	17.11	25.66	---	---	---	---
Scenedesmus spp	---	---	---	---	---	3.13	7.82	15.64	23.46
Soybean meal(44%CP)	35.67	35.6	35.48	35.31	35.13	35.6	35.45	35.21	34.99
Corn starch	31.74	30.24	30.24	28.32	26.32	30.29	30.29	30.06	28.52
Cotton seed oils	3.5	4	3.5	3.5	3	3.95	3.5	3.3	3.23
Vitamin premix [1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix [2]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Methionen	0.23	0.27	0.32	0.4	0.48	0.27	0.34	0.44	0.54
Cellulose powder	3.63	3.45	2.23	1.24	0.85	3.75	2.93	1.23	0.7
Total	100	100	100	100	100	100	100	100	100
Chemical composition (on DM basis)									
Dry matter (DM)	90.65	91.1	91.15	91.36	91.59	91	91.06	91.13	91.24
Crude protein (CP)	32.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00
Crude lipid	5.76	6.58	6.56	7.36	7.66	6.41	6.25	6.54	6.97
Crude fiber (CF)	6.23	6.33	6.17	6.16	4.60	6.57	6.06	4.88	4.18
Ash	6.06	6.47	7.07	8.09	9.10	6.10	6.67	7.62	8.89
Nitrogen free extract	49.95	48.62	48.20	46.39	46.64	48.92	49.02	48.96	47.96
Gross energy kcal/100g[3]	440.15	442.42	440.51	440.62	444.48	442.05	440.95	443.44	443.39
Digestibility energy kcal/100g[4]	311.74	316.46	315.44	319.02	322.22	315.53	314.29	316.78	318.65

Table 3: The formulation and chemical composition of the experimental diets (BADWY et al., n.d.).

The Tilapia fingerlings have shown increase in growth performance for both *Chlorella sp.* and *Scenedesmus sp.* and the replacement diets from zero to 50% inclusion, with maximum growth at 50% replacement. Inclusion levels of 75% in the fish feed have shown lower values for growth performance in both algae species. Diets with replacement from zero to 50% have shown significantly ($P<0.05$) higher feed conversion ratio and protein productive value than diets containing 75% algal biomass.

The results did not show any negative effects of dried *Chlorella sp.* and *Scenedesmus sp*. in Nile tilapia diets. The inclusion of this algae meal up to 50% substitution level was able to replace the same amount of fishmeal in the diet and simultaneously improve growth performance, FCR, fish body dry matter and protein content in Nile tilapia fingerlings (BADWY et al., n.d.). Fully substituting fishmeal with *Chlorella* based meal shows poor growth responses. However, total replacement of soybean meal is possible with use of *C. pyrenoidosa* due to higher crude protein and lipid content and better-balanced amino acid profile (Maisashvili et al., 2015).

Algal supplementation in the nutrition of juvenile Tilapia (Oreochromis mossambicus) was tested by (Roy et al., 2011). They have compared commercial formulation with the diet made from a composite algal mix (*Phormidium valderianum, Spirulina subsalsa, Navicula minima, Chlorococcum infusionum and Rhizoclonium ripariumof-* with a ratio of 35:35:12:12:6), with the combination of conventional feed ingredients. The two replacement diets were made to meet the 40% protein requirement, with the first diet counting for 35% replacement of the commercial diet composition and second diet counting for 100% replacement with algae meal. They have followed effect on growth performance, feed efficiency, nutrient utilization, and body composition during 12 weeks of the experiment.



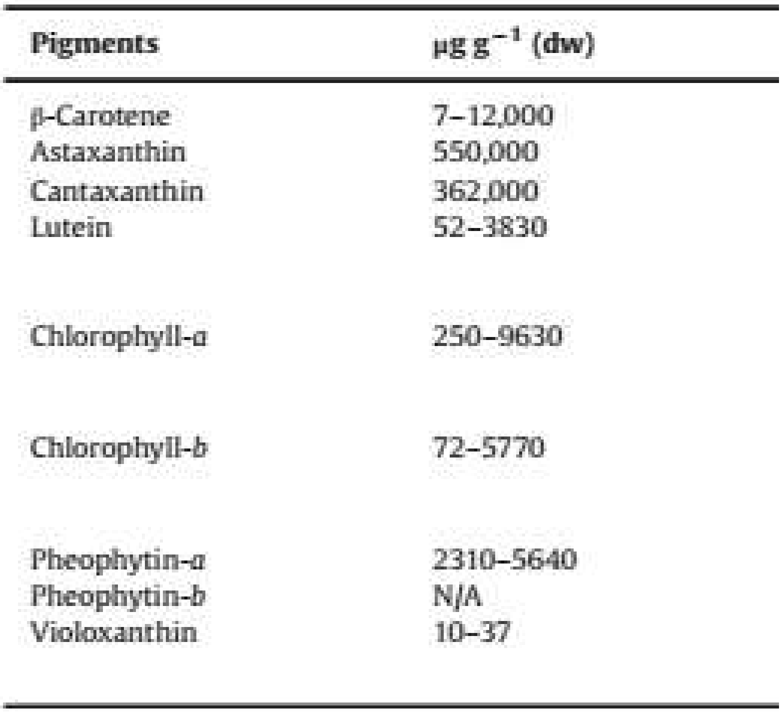
Ingredients (%)	Conventional Feed (CF)	Algal Feed (AF)	Value Added Feed (VAF)
Algae mix	0	100	35
Rice bran	63	0	50
Mustard oil cake	37	0	15
Nutrient contents (DM %)			
Crude Protein (%)	40	40	40
Crude Lipid (%)	6.8	6.17	10.32
Carbohydrate (%)	12.35	8.25	10.48
Carotenoid (%)	0.001	0.48	0.36
Gross Energy (kJ g^{-1})	20.23	19.83	24.29
Copper (mg k^{-1})	0.38	0.6	0.56
Manganese (mg k^{-1})	1.31	1.79	1.67
Iron (mg k^{-1})	24.03	6.39	10.68
Zinc (mg k^{-1})	0.9	6.52	3.1
Calcium (%)	4.52	3.2	7.08
Phosphorus (%)	0.7	0.9	1.46

Table 4: Percentage and proximate composition of experimental diets (dry weight basis) (Roy et al., 2011)

They have found that growth performance and feed conversion ratio (FCR) was highest with inclusion level of 35% and it has significantly surpassed other two diets. Compared to a commercial feed both algae-based diets have shown an increase in the protein level of the fish carcass. Furthermore, high levels of vitamin C from algal biomass helped with the increase in protein levels by promoting lipid metabolism and reducing lipid levels in the fish. The result suggested that 100% supplementation did not show significant improvement over commercial feed regarding weight gain and FCR while the diet with 35% supplementation has shown that can be used as a cheaper alternative to conventional feed. Therefore, algal biomass has the possibility to promote the body weight, FCR and nutritious value of the fish, simultaneously reducing the production cost of the feed by using open pond algae production in mixed culture (Roy et al., 2011).

Chlorella sp. as a source of pigments in aquaculture

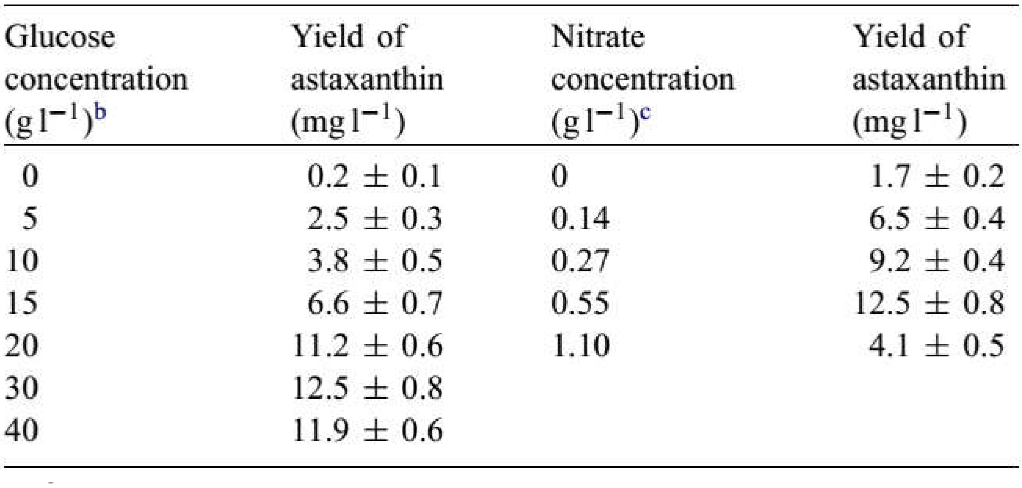
Microalgae are a natural source of carotenoid pigments mainly comprised of β-carotene, lutein, and astaxanthin (Liu et al., 2014). Astaxanthin is used in salmonid production as a strong pigmentation supplement. It provides higher color intensity and better absorption than canthaxanthin and in addition to pigmentation it's a potent antioxidant, superior to the vitamin E, increases protection against ultraviolet radiation and has benefits to the survival of larval fish and shrimp (Ip & Chen, 2005).



Pigments	**μg g^{-1} (dw)**
β-Carotene	7–12,000
Astaxanthin	550,000
Cantaxanthin	362,000
Lutein	52–3830
Chlorophyll-*a*	250–9630
Chlorophyll-*b*	72–5770
Pheophytin-*a*	2310–5640
Pheophytin-*b*	N/A
Violoxanthin	10–37

Table 5. Potential pigments content in C. vulgaris under different growth condition (Safi et al., 2014).

The mixotrophic growth of *Chlorella zofingiensis* shows high pigment formation when it is supplemented with glucose and nitrate in the culture medium.



Glucose concentration (g l^{-1})[b]	Yield of astaxanthin (mg l^{-1})	Nitrate concentration (g l^{-1})[c]	Yield of astaxanthin (mg l^{-1})
0	0.2 ± 0.1	0	1.7 ± 0.2
5	2.5 ± 0.3	0.14	6.5 ± 0.4
10	3.8 ± 0.5	0.27	9.2 ± 0.4
15	6.6 ± 0.7	0.55	12.5 ± 0.8
20	11.2 ± 0.6	1.10	4.1 ± 0.5
30	12.5 ± 0.8		
40	11.9 ± 0.6		

Table 6: Astaxanthin production by *C. zofingiensis* at different glucose and nitrate concentrations (Ip & Chen, 2005).

In the rainbow trout diets (*Oncorhynchus mykiss*)(Gouveia et al., 1998) have compared pigments derived from *Chlorella vulgaris* against commercially available pigments of canthaxanthin and astaxanthin. The comparison included tests at two different lipid contents (15% and 20%). The use of high fat diets has significantly higher carotenoid concentrations and retention in the muscle the Rainbow trout (deposition increase of 30% at week 6). Supplementation with dry *C. vulgaris* biomass has shown 1.5 times higher retention of astaxanthin in muscle than a diet with commercial astaxanthin. Due to high demand for pigments in aquaculture, supplementation with *C. vulgaris* may contribute to rainbow trout culture regarding higher carotenoid absorption and retention in the muscle while decreasing production cost (Gouveia et al., 1998).

Efficiency of C.vulgaris in poultry nutrition

Dried *Chlorella* biomass is a rich source of carotenoids (1.2-1.3% of DM), essential amino acids, vitamins, minerals, and antioxidants. It also contains many bioactive substances such as glycoproteins, peptides, polysaccharides and the fiber fraction that can affect immunity, intestinal microflora and can be used as growth promotors in poultry.

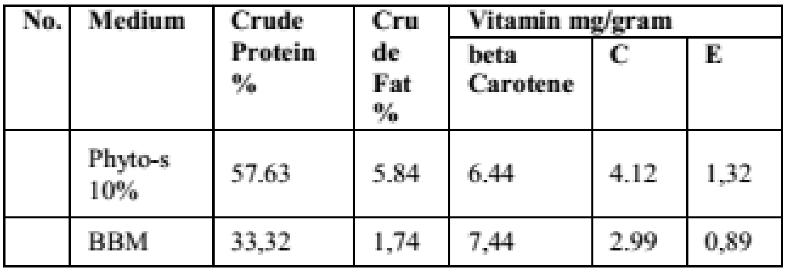
Amino acids	***C. vulgaris***[b]
Aspartic acid	9.30
Threonine	5.30
Serine	5.80
Glutamic acid	13.70
Glycine	6.30
Alanine	9.40
Cysteine	n.d
Valine	7.00
Methionine	1.30
Isoleucine	3.20
Leucine	9.5
Tyrosine	2.80
Phenylalanine	5.50
Histidine	2.00
Lysine	6.40
Arginine	6.90
Tryptophan	n.d
Ornithine	n.d
Proline	5.00

Table 7. Amino acid profile of *Chlorella vulgaris* (Safi et al., 2014).

Positive effect on laying hens performance was reported by (Halle *et al.* 2009). They have shown that improved laying performance with higher shell weight, improved yolk color, as well as an increase in hatchability. *C.vulgaris* was added to the basal diet in the form of a spray (2.5 g, 5.0 g or 7.5 g per kg of basal diet). Algae addition did not show any effect on the FCR, laying intensity or the nitrogen balance.

In broiler chickens, supplementation of *C.vulgaris* was conducted by (Kang et al., 2013) in order to investigate possible replacement of antibiotics in the diets. They have used liquid *Chlorella* and dried powder to replace 1% addition of antibiotic growth promoter in young broiler chicken diet. Fresh liquid *Chlorella* had a positive effect on intestinal bacteria population by significantly increasing *Lactobacillus* concentration while did not have any effect on the concentration of *Escherichia coli* and *Salmonella.* They believe that increase in beneficial bacterial communities in the digestive tract of young chickens has a tendency to improve the development of intestines, and it could increase growth performance in later stage. However, this can also be contributed to the high amount and high quality of protein in *C.vulgaris* (Kang et al., 2013).

In order to produce a high-quality supplement for broiler chickens (Salvia et al., 2014) have researched optimal conditions for growth of *C.vulgaris* that will produce “tailor-made”, biomass with specific composition and quality necessary for intensive broiler production. Their growth medium (Phyto-s) consisted of urea, ZA, SP36, sugar, vitamin B1 and B12 was used at four different concentrations (5 %, 10%, 15% and 20%). For optimal protein production and growth of *C.vulgaris,* they have found that 10% Phyto-s medium will provide optimal biomass for broiler production (crude protein 57.63%, lipid 5.84%, beta-carotene 6.44 mg/gram, Vitamin C 4.12 mg/gram and Vitamin E 1.32 mg/gram). The composition of growth medium is the main factor that determines the quality of algal biomass and with optimization of its composition; *C.vulgaris* can achieve rapid growth and high quality necessary to be a supplement in broiler feed (Salvia et al., 2014).



No.	**Medium**	**Crude Protein %**	**Cru de Fat %**	**Vitamin mg/gram**		
				beta Carotene	**C**	**E**
	Phyto-s 10%	57.63	5.84	6.44	4.12	1,32
	BBM	33,32	1,74	7,44	2.99	0,89

Table 8. The level of protein, fat, and vitamins of *Chlorella vulgaris* grown in the 10% Phyto-s medium (Salvia et al., 2014).

Conclusion

In recent years, there is an increase in demand for food with high nutritional value. Meat and egg industry is keeping with these demands by using feed ingredients that are rich in polyunsaturated fatty acids. Algae have been already successfully used to increase meet and egg quality. Key benefits of microalgae cultivation over land-based crops for biodiesel production are their simple structure and high photosynthetic efficiency that enables their rapid growth in adverse conditions, ability to accumulate up to 50% of lipids in dry matter biomass, faster growth rate than terrestrial plants and high productivity. In addition, algae production does not compete with terrestrial plants for arable land, water sources or herbicide needed for conventional agriculture. Algae can be grown in areas unsuited for any other crop, such as deserts, coastal areas or near industrial facilities and they are not seasonal. Their production can be connected to the industry and municipal wastewaters that are saturated with nitrogen and phosphorus that can be absorbed by the algae and cleaning the water before releasing into the oceans. This process will also provide added benefit of not using artificial fertilizers for algae growth because nitrogen and phosphorus from wastewaters provide sufficient concentrations for growth. With the above advantages, the production of biomass microalgae provides removal of CO_2 from the atmosphere and reducing the impact of greenhouse gasses on climate change.

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