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SUSTAINABILITY OF *CHLORELLA PYRENOIDOSA* FOR THE SIMULTANEOUS TREATMENT OF WASTE WATER AND BIOFUEL PRODUCTION: A REVIEW

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ABSTRACT
Continued use of fossil fuels is now widely recognized as unsustainable because of diminishing supplies and the contribution of these fuels to the increased carbon dioxide concentration in the environment. Microalgae are considered as a most capable feedstock for the generation of biofuels due to its several advantages, compared to the feedstock of first- (food feedstock) and second- (non-food feedstock) generation biofuels. It is a photosynthetic organism that converts carbon dioxide to potential biofuels, foods, feeds and high-values bioactives. The integration of microalgae growth with anaerobic digestion could significantly improve the economics and energy balance of biofuels production. Nutrient removal, in particular nitrogen and phosphorus; and COD reduction from wastewater is a developing regulatory need and the use of algae cultivation could create a unique integration between waste treatment and biofuel production. Therefore, the study discusses about the design, development and integration of microalgal production using anaerobic digestion under outdoor conditions. This decreases the production cost of microalgae as well as biofuel.

KEYWORDS: Chlorella pyrenoidosa, Waste water, Biofuels production, Remediation.

INTRODUCTION
The worldwide usage of the fossil fuels cannot stay at its current rate, because of accumulation of carbon dioxide in the atmosphere drastically increases global temperature. Man must explore clean and renewable sources of energy that could minimize dependence on fossil fuels. To minimize the requirement for purchasing fossil fuels, a lot of processes being developed. These process developed the first, second and third generation biofuels.

This has two advantages; it improves the lifecycle greenhouse gas emissions for the process and lowers the operating costs by avoiding fossil fuel purchases. First generation biofuels, which are generally accepted to be ethanol produced from sugar or starch crops and biodiesel (methyl esters) made from vegetable oils and animal fats, have been introduced and used commercially as transportation fuels in a number of countries around the world. These first generation biofuels do provide some environmental benefits, have supported agriculture and rural economic development, and have diversified the transportation fuel supply system in many countries. These fuels have generally required some financial support from governments, adjustments to the fuel distribution system to allow their introduction, and in many regions there has been some resistance from the existing market participants to adopt these fuels.

There are other biofuel productions processes that are being developed and promoted that may over some advantages over the existing biofuels. These fuels have been called second generation biofuels. The second generation biofuels are the biofuels that are produced from lignocellulosic feedstocks such as straw, wood and grass through either a biochemical production process or a thermochemical production process. It is claimed that these 2nd generation biofuels may over even greater benefits in terms of environmental performance, better overall energy efficiency, the ability to use lower cost and more widely available feedstocks and be more easily integrated into the existing fuel supply and distribution system. The main drawbacks of the second generation biofuel production are the demand of large area for cultivation and that woody part of plants that do not compete with the food production. Moreover, According to International Energy Agency (IEA) 2006, many of the 2nd generation biofuels are facing the same market barriers as the first generation biofuel.

The disadvantages related with first and second generation biofuels can be overcome with the use of microalgae. This can produce large volumes of biomass, and subsequently biofuels, on much smaller areas, as viable alternative energy resource. Moreover, microalgae involves in different and diverse activity of ecological balance for pollution control in the environment.[1] They act not only as remover of greenhouse gases from the atmosphere but can also be used for wastewater treatment (NH^{4+}, NO^{3-}, PO_4^{3-}) and environmental pollution control.

One of the main concerns in algal-derived biofuels is the microalgae harvesting process and high amount of chemical fertilizers that are required.[2] [3] This increases the final production cost of biofuel and the discharge of unconverted nutrients that can cause the eutrophication and greenhouse gases emissions. The utilization of wastewater as nutrient source and the reuse of the spent biomass as well as water could help to solve that problem, reducing also the fresh water consumption. Lim et al successfully used synthetic urban organic waste streams to culture microalgae (C. sorokiniana), obtaining consistent algal productivities and very high nutrient removal.[4] Other studies have proven that agricultural wastewaters can also support the growth of algae for biofuel production, proving also the potential of algae for tertiary or quaternary wastewater treatment.[5] Moreover, several authors have studied the utilization of the residual algal biomass after the oil extraction as a way to reduce the environmental impact and to improve the economy of the process. Gao et al concluded that algal biomass residue after oil extraction is also a potential source for biofuel production.[6] Models and experiments linking biodiesel production with simultaneous biogas yielded from oil-spent algal biomass have been studied.[7] Moreover, it has been proved that large-scale growth of algae can be potentially used to fix CO_2 rich flue industrial gases by direct injection on airlift photobioreactors (PBRs).[8]

Bioremediation of waste water as well as CO_2 removal by microalgae

The potential of microalgae due to its various applications has made them the subject of considerable research effort in the past.[9] These applications involves renewable energy source, source of high value chemicals for the pharmaceutical industry, source of proteins for animal feedstock and fertilizer. Cultivation of Chlorella seems to be one of the feasible methods to reduce the amount of nitrogen and phosphorus entering the nearby coastal water, thus preventing the eutrophication problem.[10] As algae started to grow and multiply, both nitrogen and phosphorus content in wastewater decreased significantly.[10] Similarly, growth of Chlorella on wastewaters sampled from four different points of the treatment process ow of a local municipal wastewater treatment plant (MWTP) was done by Wang et al.[11] They investigate how well the algal growth removed nitrogen, phosphorus, chemical oxygen demand (COD), and metal ions from the waste waters. Moreover, domestic wastewater samples from sewage wastewater treatment plant of Bopodi, Pune city was used to study the role of microalgae in wastewater treatment. Chlorella species showed the best removal capacity of nitrate and phosphate reduction.[12]

CO_2, a green-house gas is a part of medium for the culturing of microalgae. CO_2 is also a major component of biogas from anaerobic digestion. Algal systems are capable of utilizing the CO_2 from biogas. Emissions of CO_2 to the atmosphere can be reduced through biological CO_2 mitigation which can further lead to the extensive uses of biofuel.[13] Algal systems can also utilize the waste or toxic contaminants like nitrate nitrogen, nitrite content present in biogas digester outlet slurry as a substrate for its growth. Number of marine microalgae species has been tested for CO_2 sequestration applications got positive results.[14] They showed that algae contain the pigment chlorophyll and use the Calvin Cycle to fix carbon auto-tropically.

The purification and treatment of the biogas remains an area for further improvement. Since, traditional biogas purification technologies rely on chemical and physical processes (e.g.: PSA) for the purification of biogas generated during fermentation. Furthermore, large amount of CO_2 generation during fermentation is another critical issue associated with it. However, biogas purification could be effectively combined with biogas production to reduce both the CO_2 emission and economic of algae cultivation.

This may be possible as the algae, especially micro algae, have eminently desirable composition of bio-molecules such as polysaccharides, lipids, proteins etc., in their cell organizations, which can easily be converted into methane rich biogas under anaerobic digestion. The produced biogas can be utilized as a CO_2 source for growth of algal cell. This will enable both the purification of the biogas and improved economics for the algae growth and also the anaerobic digestion of that algae biomass for biogas production. The advantages of using microalgae over conventional methods as summarized are[15]:

a) Nutrients can be removed more efficiently;
b) No generation of toxic by-product (sludge);
c) Biofuels can be produced from biomass harvested (energy efficient);
d) Cost-effective;

Several microalgae species have been studied as useful for nutrient removal including *Botryococcus braunii*, *Chlamydomonas*, *Scenedesmus* and *Chlorella*. Ambati et al successfully cultured *Botryococcus braunii* in secondary effluent in both batch and continuous experiments, with the removal efficiency of 99% for nitrate and 93% for phosphate.[16] Tam and Wong reported removal of nitrate nitrogen by cultivating *Chlorella vulgaris* in wastewater. The removal efficiency increased corresponding to decreased initial nitrogen concentration: 100% nitrogen removal was achieved with initial nitrogen concentration lower than 20 mg/L and 95% nitrogen removal corresponding to 40-80 mg/L initial concentration and 50% removal with initial concentration higher than 80 mg/L.[17]

Moreover, Algae have been proposed as a method to fix atmospheric carbon dioxide. Vunjak-Novakovic et al used a pilot-scale microalgae photo-bioreactor and found that CO_2 removal efficiency was 50.1% on cloudy days and 82.3% on sunny days from flue gas with a CO_2 concentration of 8%.[18] Processes that produce CO_2 can use algal biomass to fix carbon and to avoid air pollution. The carbon fixation occurs by the accumulation of fatty acids and hydrocarbons in algae biomass, which can be converted to bio-oil or biogas. Atmospheric air contains 0.03% of carbon dioxide, which can sustain algae growth, but below the maximum potential growth rate. Therefore, additional carbon dioxide can be supplied to increase the algae growth rate if sufficient light and nutrients is available.[19]

Microalgae, Chlorella vulgaris consumed 38.7% of an enriched CO_2 stream (6-8% by volume) and produced 1 kg of algae biomass from 1.74 kg of CO_2 Doucha et al. (2005). The algae fixed 4.4 g CO_2 in 24 h with the enriched air stream compared to 3.0 g for atmospheric air. This microalgae is one example of an algae that can shift between an organic and inorganic carbon source according to the light availability.[19] The presence of organic carbon is an alternative resource to the algae that may reduce the biomass loss during the dark period. Organic carbon could take the form of sugars that are supplied to algae during heterotrophic fermentation to increase the biomass and oil yield. Using animal manure as a nutrient source could also provide an organic carbon source to limit respiration losses during dark periods.

Biomethane production potential of microalgae

In order to get an idea of microalgae bio-methane potential, it is advisable to look at their biochemical composition. A study conducted by Brown et al. for the nutritive characteristics of microalgae. The overall composition of microalgae differs between the species. In their study, they report protein content of 6-52%, carbohydrates 5-23% and lipids 7-23%.[20] Angelidaki and Sanders compiled specific methane yields for carbohydrates, lipids and proteins shown in Table 1.[21] According to them, when the composition of the organic matter is known, it is possible to evaluate the theoretical methane and ammonium yields that can be expected from the anaerobic digestion.

Table 1: Specific methane yield from three types of organic compounds.

Substrate	Composition	L CH_4 gmV/S
Proteins	$C_6H_{13}ON_{0.6}$	0.851
Lipids	$C_{57}H_{104}O_6$	1.014
Carbohydrates	$(C_6H_{10}O_5)n$	0.415

Sialve et al studied the algae's theoretical methane potentials by using organic compounds as a basis of their calculations.[22] Table 2.2 shows the composition and theoretical methane potential of different microalgae species calculated. These yields were calculated by using the following formula modified by Symons and Buswell.[23] It shows that the methane yield varies from 0.09 to 0.95 L gmV/S depending on the species and culture conditions. Based on the analysis given in the table, microalgae *Chlorella pyrenoidosa* could produce more biomethane as compare to other species and also having high carbohydrate content as compared to microalgae Euglena gracilis which is also having high biomethane potential.

Table 2.2: Certain algae species composition and theoretical methane potential.[22]

Species	Proteins (%)	Lipids (%)	Carbohydrates (%)	CH_4 (ml CH4/g VS)
Euglena gracilis	61	20	18	800
Chlamydomonas reinhardtil	48	21	17	690
Chlorella pyrenoidosa	57	2	26	800
Chlorella vulgaris	58	22	17	630
Dunallella salina	57	6	32	680
Spirulina maxima	71	7	16	749
Spirulina platensis	63	9	14	690
Scenedesmus obliquus	56	14	17	690

Brune and Yen & Kaosol and Sohgrathok[24] [25] have studied the effects of the carbon nitrogen ratio in microalgae to efficacy anaerobic digestion. Algal biomass normally having C:N ratio of 6:1, which means too high nitrogen content. When the biomass has high nitrogen content, it leads to an increase in ammonia in anaerobic digestion by ammonification and can become an inhibitory factor in methane output. The studies have concluded that optimal ratio between carbon and nitrogen varies around 12-20:1. Caporgno et al have written that the functional ratio is somewhere between 16:1 and 25:1.[26] To improve the C/N ratio extra cellulose, or other carbon sources, needs to be added. This is called as co-digestion process. In addition, the biomass can be mixed with wastes having higher carbon content, such as animal manures, sludges or waste paper.[24]

Ehimen et al concluded that in semi-continuous reactors, Hydro Retention Time is a major single factor when digesting algal residues from biodiesel production.[27]

Methane yields improved when microalgae were digested for periods over 5 days. They suggested that C/N ratio of 12.44:1 was optimum for bio gasification of algal residues when HRT was 15 days. Initially, C/N ratio was 5.4 and the methane yields were significantly low. They co-digested residual algal biomass with different amounts of glycerol and temperature being at 35°C. Mussgnug et al studied the bio-methane potential of six different microalgae species by digesting them anaerobically for a period of 32 days. They concluded that the yield of biogas was noticeably dependent on the species and should be tested separately. The methane yields of those species that had a robust cell wall structure were lower than of those that had an easily degradable cell wall or no cell wall at all.[28]

Integrated processes for biofuel production

With the integration of microalgae cultivation and application of Anaerobic Digestion (AD), several advantages can be identified. First of all, biogas is produced, which represents a source of renewable energy. Furthermore, during AD, nutrients such as phosphorus and nitrogen are released, in the form of phosphates and ammonia respectively. These can then be recovered and reused as substrate for microalgae cultivation, contributing to the economical balance of the process.[29] Sialve et al have already pointed out the real need of anaerobically digesting algal residues to make microalgal biodiesel a feasible alternative.[22]

Most researches have also concentrated on the suspended microalgae growing in suspension termed high rate algal pond. The result of such effort is that some commercial technologies and processes are already available in the market such as the Advanced Integrated Wastewater Pond Systems (AIWPS) technology commercialized by Oswald and Green, LLC, in the United States.[30] One of the main limitations of this technology is that it is difficult to harvest or separate the suspended microalgae biomass from the treated water discharge. None of the harvesting approaches have proved to be simple, inexpensive and suitable enough for a large-scale outdoor treatment.[31]

Sialve et al reviewed and concluded that explored about fifty years ago, the promising integration process coupling anaerobic digestion and microalgal culture deserves sustained research and development efforts.[22] This will probably re-emerge in the coming years either as a mandatory step to support large scale microalgal cultures or as a separate bioenergy producing process.

Similarly, the integration of microalgae growth with anaerobic digestion can significantly improve the economic and energy balance of such a promising platform technology.[29] The mass (carbon, nitrogen and phosphorus) and energy balances in the integrated process of *Chlorella sorokiniana* cultivation (under photoautotrophic and mixotrophic conditions) coupled with anaerobic digestion in batch mode was done. This designed process reduced the overall microalgae cultivation costs was done Alcantara et al.[29] Similarly, Ichsan et al. used Palm Mill Oil Effluent (POME) to cultivate microalgae, Spirulina species and Chlorella species. The high COD value in POME can be converted into biogas production. Based on the findings, it was also possible to integrate the biogas system with microalgae cultivation. The microalgae could grow in different POME concentrations. This integration of biogas microalgae from POME had given additional benefits for the palm industry, local community and environment.[32]

Role of Chlorella pyrenoidosa for various waste water treatment and biogas production

Chlorella pyrenoidosa was found to have the highest value of theoretical and stoichiometric methane potential.[33] In a study for the biogas production potential through BMP protocols using Chlorella pyrenoidosa as feedstock, relatively higher biogas yield was found 0.464 0.066 m^3 biogas kg^{-1} VS with 57% (v/v) CH_4 during 30 day digestion.[33] Summary of waste water treatment from different sources by *Chlorella pyrenoidosa* is shown in Table 1.2.

Table 1.2: Examples of different wastes.

Source of waste water	Culture time (h)	% Removal			Biomass Yield (g/L)	Ref.
		NH_4^+	TP	COD		
Soybean Processing Wastewater	120	89.1	70.3	77.8	0.64	[34]
Settled Sewage	136	81.4	57	-	0.5	[35]
Activated Sewage	136	83.5	66	-	0.29	[35]
Anaerobic sludge for starch processing	624	83.06	96.97	65.99	0.37	[8]
Piggery wastewater	240	91.2	77.7	55.4	0.04	[36]

CONCLUSION

The present study indicates microalgae *Chlorella pyrenoidosa* to be a potential substrate for anaerobic fermentation which can be utilized for production of biogas with high methane content. Beside this, C. *pyrenoidosa* can also be a source for wastewater treatment as it consumes nitrates, phosphates, etc. This results in improving the energy balance of an integrated microalgae wastewater treatment with anaerobic digestion in outdoor conditions.

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