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Review

바이오디젤 생산원료로써 미세조류의 배양을 위한 대체 영양원 사용 기술

당낫민 · 이기세†

명지대학교

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Recent Trends of Using Alternative Nutrient Sources for Microalgae Cultivation as a Feedstock of Biodiesel Production

Nhat Minh Dang and Kisay Lee†

Department of Environmental Engineering and Energy, Myongji University, Yongin 17058, Korea

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초 록

미세조류는 바이오연료 생산을 위한 가장 지속가능하고 장래성이 좋은 생산 원료로 여겨지고 있다. 하지만 최근의 몇몇 전과정평가 연구에 의하면 미세조류 바이오디젤 생산, 특히 배양 단계에 많은 에너지가 소요된다는 단점이 있다. 유기탄소, 질소 및 인과 같은 영양분, 그리고 배양에 필요한 용수 비용이 전체 배양 단계의 80%까지 이를 수 있다. 본 총설에서는 최근 미세조류 배양에 필요한 인공배지의 대체용으로 사용 가능성이 높은 하폐수, 유기비료 연소배가스, 유기성 폐기물 등에 대한 최근의 활용 경향과 사용 전략에 대하여 문한 조사를 통해 요약 및 고찰하였다.

Abstract

Microalgae is considered as one of environmentally sustainable and potential feedstocks to produce biodiesels. However, recent studies on life cycle assessments (LCA) of microalgal buidiesels have shown that energy requirement is not small to produce biodiesel from microalgae, especially during cultivation stage. The costs for carbon sources, nutrients like nitrogen or phosphorous, and water for cultivation can contribute up to 80% of the total medium costs. In the present article, recent trends on the utilization of several promising nutrient sources such as municipal wastewaters, organic fertilizers, combustion exhaust emissions and organic solid wastes were reviewed, and the potential strategies to be used as substitutes of artificial culture media, especially for the biodiesel production, were discussed.

Keywords: *Microalgae, biodiesel, cultivation, medium substitutes, nutrient sources*

1. Introduction

The growth of world population and industrial activity during last decades has been based on fossil fuels. Fossil fuels are considered as unsustainable energy sources because they release greenhouse gas into atmosphere. The increasing concerns over the cost and availability of crude oil reserves have prompted researchers all over the world to look for alternative sources of energy. Global issues such as fossil fuel depletion, global climate change and ethic problem of using food crops as energy production increased the demand of renewable biofuels these days.

First generation of biofuel feedstocks are mostly food crops like sugar or starch and raised the issue of competition between food and energy production[1,2]. The production technologies of biofuels from lignocellulosic biomass, such as agricultural and forest residues, are demanding massive amount of energy and thus still not economically feasible[3]. Therefore, finding an inexpensive source of feedstock that does not use farmland is indispensable for biofuel to compete economically with traditional fossil fuel. Simultaneously, finding a profitability of the production process is also necessary[4].

Microalgae are considered as one of prospective renewable sources for biofuels (both liquid and gaseous). They also represent an environmentally friendly alternative option for biological carbon capture and storage (CCS) technology. Microalgae with high lipid content and fast growth rate are going to be potential biofuel feedstock[5]. Some of them are capable to survive in harsh environments such as non-potable wastewater or high salinity water (brackish water or sea water), without competing with terrestrial crops for fresh water and arable land[6].

Unfortunately, some of recent life cycle assessment (LCA) studies on microalgae biofuels have shown that energy requirement for microalgal biodiesel is pretty high during cultivation and refinery stages. And the costs for carbon sources, nutrients like nitrogen or phos-

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† Corresponding Author: Myongji University,
Department of Environmental Engineering and Energy, Yongin 17058, Korea
Tel: +82-31-330-6689 e-mail: kisay@mju.ac.kr

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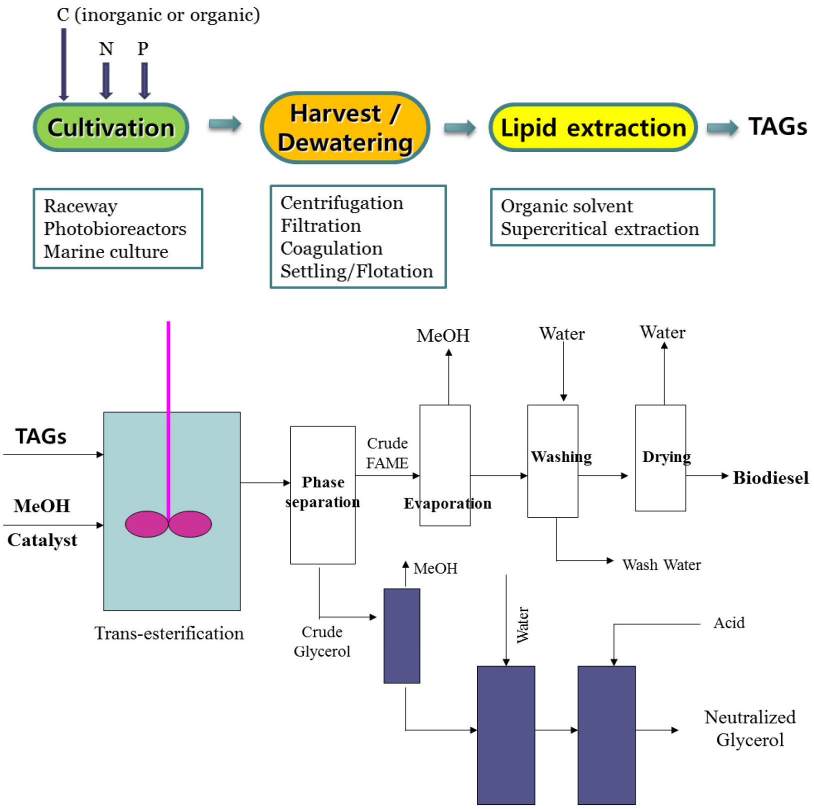

Figure 1. General scheme of biodiesel production from microalgae. Cultivation, harvesting, extraction, and the conversion of extracted TAGs to FAME biodiesel through trans-esterification and separation/purification.

phorous, and also water supply for cells growth can contribute up to 80% of the total medium costs[7]. Therefore, despite numerous advantages, biodiesel production from microalgae should hurdle many obstacles to go to commercialization. Those LCA studies resulted in a negative energy balance, indicating that further renovations are necessary for microalgal biodiesel production to enhance the sustainability in terms of energy balance and chemical consumption[8].

In order to improve economic feasibility and environmental sustainability of microalgal biodiesel production, a variety of non-conventional nutrient sources has been considered as potential sources of substitutes for traditional nutrition to reduce the costs in cultivation process. A lot of studies were reported on the performance of using non-conventional nutrient sources for microalgae growth and its lipid synthesis. In the present paper, recent trends of using several promising nutrient sources such as municipal wastewater, organic fertilizer, exhaust emissions or solid waste were reviewed, and their potential strategies for microalgal biodiesel production were discussed.

2. Overview of Biodiesel Production from Microalgae

Microalgae are one of the potential sources to produce biofuels such as biodiesel, bioalcohols and hydrocarbons. Microalgae can utilize various materials in waste streams (wastewater, combustion flue gas, organic solid waste, composted fertilizer, etc.) and produce variety of products. Lipids, mostly neutral lipids in the form of triacylglycerides (TAGs), can be processed into biodiesel through trans-esterification. If carbohydrates are major products, they are processed into ethanol through saccharification and fermentation. Biodiesel is produced after trans-esterification of TAGs as fatty acid methyl ester (FAME) uaually and fatty acid ethyl ester sometimes, depending upon the involved alcohol. Neutral lipids like TAGs are stored in cytoplasm as lipid bodies. Lipid bodies are responsible for lipid transport, protein storage and degradation, as well as the reservoir of lipid storage. If the purpose of cultivation is to produce biodiesel, a microalgae strain with high lipid content is preferred. If the desired product is ethanol, a strain that can store a large amount of starch is advantageous.

Starch, as the primary energy source, is produced in microalgae chloroplasts in the presence of light. Secondary energy sources, such as triacylglycerol and fatty acids, are often located in the microalgal cytoplasm too. These molecules act as electron sinks as well as energy reserves for microalgae metabolism[9]. In order to convert oils from various sources into biodiesel with acceptable quality as a fuel, a variety of methods was developed and applied, including trans-esterification, blending, cracking, micro-emulsification and pyrolysis. Trans-esterification is the most widely used method by which neutral lipids like TAGs are converted to FAMEs as biodiesel, liberating glycerol as a byproduct[10].

Most microalgae can utilize one or more of the three metabolic pathways depending on carbon and energy sources: photoautotrophy, heterotrophy, and mixotrophy[11]. Under light conditions, microalgae are usually capable of photoautotrophic growth. Photoautotrophic cultivation requires elaborated reactor design and process control to overcome the limitation of light transmission, and also a high water quality as a culture medium, especially in terms of turbidity or color, is necessary[12]. Some microalgae may utilize organic carbons as carbon and energy sources for propagation via heterotrophic metabolism. Microalgae usually show higher growth rates in heterotrophic cultivation than those in photoautotrophic cultivation, and thus heterotrophic cultivation is considered as the promising way to increase the productivity[13-15]. Microalgae can adapt to different organic carbon sources such as glucose, sucrose, organic acids, glycerol or a wide range of wastewater BOD constituents after appropriate acclimatization[16].

Figure 1 shows the general production scheme of microalgal biodiesel: cultivation, cell harvesting and dewatering, lipid extraction, transe-esterification, and a series of separation and purification. Microalgae can be cultivated through several different cultivation systems. Open ponds have been used as a traditional method of wastewater treatment by microalgae. This inexpensive system has the disadvantage of low cell densities. There would be no problem if treatment needs a long hydraulic retention time. However, low productivity will be an important problem when production matters. Another important limitation of using open ponds as a production reactor is that they are exposed to contamination because sterilization is not possible. Also, due to high ratio of open surface to volume, the rate of water evaporation is pretty high. For the purpose of microalgal biomass production, open raceway pond systems are widely used.

Photobioreactors present several fundamental advantages compared with open pond systems. Photobioreactors have versatile reactor geometry such as tubes, plates or columns and allow accurate process control (light intensity, temperature, mixing, and the concentrations of components, etc.), high degree of protection against contamination and high volumetric productivity. Transparent materials (glass, acryl, plastic, etc.) are usually used as reactor construction. Due to the high ratio of transparent surface to volume compared with open ponds, more light can penetrate deep into the system.

After a desired cell density is reached in cultivation reactor, microalgae cells are harvested usually by centrifugation or a series of coagulation-flocculation-settling. If specific gravity of cells is relatively small compared to culture medium, flotation or coagulant-assisted flotation can be used. For further dewatering and concentrating cells, self-discharged semi-continuous disc-stack centrifuge can be used. If dried biomass is necessary, air drying or thermal drying can be used. Although thermal drying can reduce moisture content down to 12 wt% without biomass loss, a judicious selection of drying methods is important considering which degree of dewatering is necessary, because the moisture content governs the extractability of lipids by the organic solvent[69].

Various methods of total lipid extraction are available to extract the lipid for biodiesel synthesis: mechanical methods (including oil expeller or press, ultrasound assisted or microwave assisted) and chemical methods (including solvent extraction, supercritical CO_2 or ionic liquid extraction). Methods of Floch[17] and Bligh and Dyer[18] have been popularly used due to the simplicity and economic advantages. Although organic solvents and supercritical fluids are technically available to lipid extraction, n-hexane is normally used in large scale lipid extraction from microalgal biomass. After separating the lipid-extracted cell residues, lipid-containing solvent undergoes evaporation to recover lipids. After recovering the extracted lipid, the spent solvent needs to be recycled through distillation.

The extracted lipids are mostly in the form of TAGs and free fatty acids (FAs) in minor. TAGs are converted to FAME biodiesel through trans-esterification, in which TAGs and FAs are reacted with a low molecular weight alcohol in the presence of acid or base catalyst. Methanol is generally used and thus biodiesel is produced in the form of FAMEs although any kind of low molecular weight alcohols can be used for trans-esterification. Potassium hydroxide, sodium hydroxide, sodium methoxides and $CaCO_3$ are used as base catalysts. For acid catalysts, sulfuric acids, phosphoric acid, p-toluenesulfonic acids and methylsulfonic acid are used.

In industrial scale trans-esterification for biodiesel production, base catalysts are popularly used because the rate of base-catalyzed trans-esterification is faster than acid-catalyzed one. However, saponification problem exists in base-catalyzed esterification when the free fatty acid content is high in extracted lipid. Free fatty acids interfere catalyst activity and reduce trans-esterification performance. Therefore, base trans-esterification is not recommended if the free fatty acid content is high. Saponification of free fatty acids by alkali materials can be avoided by using acid catalysts which convert free fatty acids, as well as TAGs, to FAMEs[69].

Trans-esterification of TAGs produces not only FAME biodiesel, but also glycerol as a byproduct and unreacted alcohol. Glycerol can be separated by phase separation or centrifugation utilizing density difference. The unreacted alcohol mixed with glycerol can be recovered through vacuum evaporation. Produced crude biodiesel should undergo further cleaning and dehydration to be used as fuel. Phosphoric acid and water are used for cleaning impurities and then final moisture should be removed as complete as possible by molecular sieve technique or drying.

3. Potential Medium Substitutes as Nutrient Source

3.1. Wastewaters

Industrial scale microalgae cultivation requires large amounts of water and nutrients, and therefore many options have been developed to reduce the costs in cultivation process[19]. The use of municipal or industrial wastewaters has been considered as a plausible option for the achievement of microalgae biomass production and wastewater treatment at the same time[20-25]. It is because the existence of nutrients and minerals in wastewater is beneficial to the cultivation of microalgae as an alternative and economical resource for biodiesel production. Besides, microalgae are also able to remove heavy metals from wastewater[26,27]. Using wastewaters to cultivate microalgae can improve the economics of microalgal biodiesel production, as well as improve the water quality. Microalgae species which have high tolerance to contaminants, such as *Chlorella* and *Dunaliella*, are selected to grow in wastewater. Using municipal wastewaters as nutrient source to cultivate microalgae is gaining a good prospective so far.

The compositions of wastewaters vary significantly depending on local characteristics and activities. The typical constituents of wastewater include organics (BOD or COD), nutrients (N and P), minerals and various microorganisms[28]. Municipal wastewaters usually contain high quantities of nutrients such as ammonium (NH_4^+) and phosphate (PO_4^{-3}), and other components which are essential for microalgae[29]. Li et al.[30] analyzed the feasibility of *Chlorella* sp. to grow in municipal wastewater and showed that the microalgae could remove ammonium nitrogen, phosphorus and chemical oxygen demand (COD) with efficiencies of 94, 89% and 81%, respectively. Besides, Aslan and Kapdan[31] used *Chlorella vulgaris* to remove nitrogen and phosphorus in the municipal wastewater with removal efficiencies of 72% and 28%, respectively. All the above research works indicated that microalgae could be cultivated in municipal wastewater which is rich in nutrients and subsequently promotes the growth of microalgae[32].

Mennaa et al.[33] evaluated the cultivation of seven microalgae species in urban wastewater, and showed that microalgae were able to remove 80-87% of the total dissolved N and P, concurrently achieving 108 to 118 $mgL^{-1}d^{-1}$ of biomass productivity, depending on the species.

Caporgno et al.[34] also used urban wastewater to cultivate two fresh water *Chlorella* species and one marine microalgae *Nannochloropsis oculata*. Those fresh water species showed higher biomass production in urban wastewater (2.7-2.9 gL^{-1}) than the marine species.

Lam et al.[35] reported that *Chlorella vulgaris* was favored to grow in domestic wastewater, showing cellular lipid content of 32.7% under the conditions of 2% of wastewater ratio with 3% of initial amount of microalgae seeding. They also analyzed the fatty acid composition of produced FAME biodiesel and demonstrated that the produced biodiesel from microalgae cultured in the domestic wastewater was suitable for biodiesel quality.

In study of Chang et al.[36], three types of representative wastewater were investigated to evaluate the growth of *C. vulgaris* with ion-exchange-membrane photobioreactor. When cultivated with wastewater containing excess nutrients and heavy metals, microalgae biomass concentrations were significantly improved from 2.34, 2.15 and 0 g L^{-1} in the traditional photobioreactor to 4.24, 3.13 and 2.04 g L^{-1} in the ion-exchange-membrane photobioreactor.

Kuo et al.[37] also estimated the ability of using piggery wastewater for *Chlorella sp.* GD microalgal culture. The results showed that this *Chlorella* strain grew efficiently in piggery wastewater, for long-term microalgal cultivation in a semi-continuous mode. The highest specific growth rate and biomass productivity of the microalga obtained in 100% piggery wastewater were 0.839 d^{-1} and 0.681 g L^{-1} d^{-1}, respectively, after 10 days of batch cultivation. The lipid content and lipid productivity were 29.3% and 0.155 g L^{-1} d^{-1} under 5% wastewater ratio.

Mujtaba et al.[38,68,70] used a system of *Chlorella vulgaris-Pseudomonas putida* symbiotic association to consume the nutrient (ammonium and phosphate) and organics from municipal wastewater. The improved performance of simultaneous consumption of nitrogen, phosphorous and organic carbon was observed and the growth of *C. vulgaris-P. putida* was enhanced mutually.

3.2. Organic fertilizers

Synthetic fertilizers have been used in cultivating microalgae commercially. An alternative of inorganic fertilizers is to use surplus livestock manures or agricultural residues in their raw state, compost state or byproducts of anaerobic digestion. Organic fertilizer is produced by a series of composting and refining processes from organic materials such as food waste, sewage sludge, biomass and animal manure. Composted organic fertilizer contains a high level of ammonium and phosphate and can be prepared as liquid solution or dried pellet which allows transportation and storage easier. Compost contains an abundance of organic material which can serve carbon and energy sources for microalgae, heterotrophically or mixotrophycally[39,40].

The concentrations of P and N which are required to microalgae growth are less than 0.2 μmol L^{-1} as P and greater than 0.2 μmol L^{-1} as N, respectively[41]. These requirements are generally satisfied by livestock manure, most municipal wastewaters and organic solid wastes such as food and agricultural residues. Incorporating microalgae cultivation is the addition of a sort of advanced treatment process (specialized in N and P removal) as up-grading of the existing water treatment process[42]. Also the composted product of livestock waste can be a prospective substitute for artificial medium or inorganic fertilizer which is added to commercial-scale microalgal cultivation[43].

One of the studies reported by Kumaran et al.[44] showed that 30% of the lipid can be extracted from *C. vulgaris* when the microalgae were cultivated using waste-based compost under the following condition: 50 mL of compost, pH of 9, and illuminated continuously for 15 days using fluorescent light. Lam and Lee[45] reported a high adaptability of *C. vulgaris* towards the surrounding environment and suitability to be grown and to be able to reproduce under outdoor conditions. Total 18.1% of lipid from the *C. vulgaris* biomass was successfully extracted and the fatty acids profile was proven to be suitable for making biodiesel.

Dang and Lee[46] applied advanced oxidation (UV + hydrogen peroxide) to liquid fertilizer PAL-1 in order to remove the dark color of the liquid fertilizer. Using the decolorized liquid fertilizer PAL-1 as a culture medium, they achieved higher concentration and lipid accumulation of *C. vulgaris* biomass compared to the culturing in original liquid fertilizer. The fraction of polyunsaturated fatty acids was increased too (Figure 2 and Table 1).

In a study by Banerjee et al.[47], NPK fertilizer-based culture medium was formulated for the cultivation of *Nannochloropsis* sp., and a significant improvement in biomass, lipid and eicosapentaenoic acid (EPA) productivity was obtained using optimally formulated fertilizer medium. Generally, it is possible to use organic fertilizer to cultivate microalgae for biodiesel production and the utilization of such cheap nutrient sources will be beneficial to large scale, typically in terms of cost saving and better environmental protection.

In addition, large quantities of animal wastes are left over in large-scale livestock farms and such wastes threaten livestock's hygiene. However, those livestock wastes are rich in nutrients such as nitrogen and phosphorus. Many studies have shown that manures containing high levels of nutrients, such as livestock manures from pig

Table 1. Fatty Acids Composition of Biodiesel Produced from *C. vulgaris* Grown 14 Days in Artificial Medium (BG-11) and AOP-treated Liquid Fertilizer (PAL-1) Medium[46]

FAME (%)	Artificial medium (BG-11)	AOP-treated liquid fertilizer (PAL-1)
C10 : 0	0.8	1.2
C12 : 0	2.0	1.2
C14 : 0	0.3	0.5
C16 : 0	6.1	13.0
C16 : 1	11.1	20.5
C18 : 1	46.0	44.4
C18 : 2	8.0	8.7
C20 : 0	25.6	12.4
SFA	34.8	26.3
UFA	65.1	73.6
PUFA	8.0	8.7
MUFA	57.1	64.9

and poultry or byproducts from anaerobic digestion, are potential sources of nutrients to use in microalgae cultivation.

Zhu et al.[48] investigated the cultivation of microalgae *Chlorella* sp. with livestock waste compost as an alternative nutrient source. The livestock waste compost medium they used contained a sufficient level of COD (2000 mg L^{-1}), by which they reported the biomass production (288.84 mg L^{-1} day^{-1}) and cellular lipid content (104.89 mg L^{-1} day^{-1}). The specific growth rate of *Chlorella* sp. ranged from 0.275 to 0.375 day^{-1} with the doubling time from 2.52 to 1.85 days. Lipid content ranged from 33.90% to 44.30% depending upon the initial nutrient concentration in the culture medium.

Zhu and Hiltunen[43] also suggested that the compost from livestock wastes could be a plausible nutrient resource to cultivate microalgae. Livestock wastes are a gratuitous and renewable resource because they are generated as long as human beings exist. The utilization of livestock waste compost to microalgae cultivation is beneficial to both valuables production and waste management. Microalgae can produce not only low-value commodity products like biofuels but also high-value products that can be parts of cosmetics and pharmaceuticals.

Besides, nitrogen from livestock slurries contributes largely to environmental pollution, through ammonia and nitrogen oxides emissions to the atmosphere and nitrate leaching to ground and surface water bodies[49]. Therefore, the researches on the recovery of nutrients (especially N and P) from variety of waste streams, converting or fixing to different forms and recycling to valuable purposes, became important. Concerning recovery of nutrients, microalgae are photosynthetic microorganisms capable of taking up inorganic N and P and transforming them into valuable organic compounds using solar energy.

Ledda et al.[50] integrated microalgae cultivation and anaerobic digestion of dairy cattle manure. Anaerobic digestion is usually focused to the organics reduction and thus the resulting digestate contains high levels of nutrients and alkalinity. Microalgae was utilized to digestate treatment, and it was found that this integrated system was helpful to reduce the treatment cost and net energy consumption in the process. The untreated ultra-filtered digestate of manure was used as the growth medium for the cultivation of *Scenedesmus* sp. The tolerance of this strain to digestate showed that over 10% of digestate inhibited the growth of this microalga, but that the productivity was obtained up to 124 mg L^{-1} d^{-1} below this value. They suggested that it is possible to produce 166-190 t y^{-1} of microalgae biomass if microalgae production is integrating with anaerobic digestion. By integrating anaerobic digestion and microalgae cultivation, biomass production and nutrients removal became technically feasible, and also the energy and mass balances of the overall process could be improved.

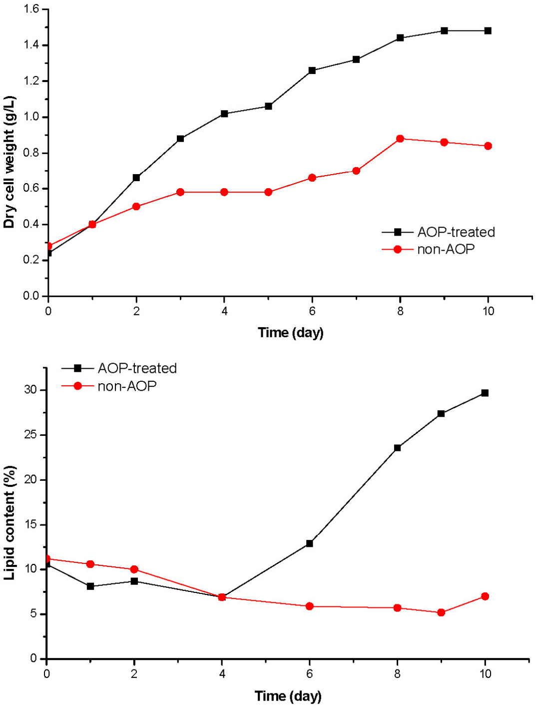

Figure 2. Changes in biomass growth and lipid contentof *Chlorella vulgaris* in AOP-treated and non-AOP of PAL-1 liquid fertilizer. Cells were cultured using 2.5% CO_2 enriched air under white light irradiation[46].

The review of Fenton and Uallacháin[41] also confirmed that the speciation and content of nutrients in agricultural fertilizers have a good potential to facilitate the growth of microalgal biomass. Surplus manures from livestock industries (e.g., pig and poultry) and the byproducts from anaerobic digestion are viable sources of nutrients that microalgae can use. However, some issues should be considered prior to their use as a source of nutrients for algal growth. Firstly, nutrient contents in such resources are not consistent depending upon sources, regions or seasons. Another issue is the turbidity problem since liquors which are related to manure or anaerobic digestion are usually dark colored. Also there could be an issue of nutrient loss during the storage period due to N volatilization and P precipitation. These issues should be properly coped prior to use in microalgae cultivation through appropriate agitation, equalization of liquor reservoir with accurate nutrient testing, and enhancement of transparency by reducing turbidity or color.

3.3. Combustion exhaust emissions (Flue gases)

Instead of purchasing high purity CO_2, utilization of lower quality CO_2 from waste sources has been well documented in the literature as effective in supporting the growth of microalgae. Also, studies show that low levels of NO_x and SO_x, such as in the natural gas combustion exhausts, do not inhibit the growth of microalgae, hence such flue gases can be injected directly into algal cultures[51,52]. CO_2 fixation by microalgae is an option to mitigate CO_2 emissions. Compared to other physico-chemical CCS processes, microalgal biofixaiton could be both economically feasible and environmentally sustainable, producing biomass itself, biofuels and other chemicals. At least 1.83 tons of CO_2 are fixed for 1 ton of algal biomass production[53]. Therefore, the coupling of the cultivation of microalgae with the supply of CO_2 in flue gas from power plants or other combustion sources can reduce carbon emissions as well as the cost of microalgae production[54,55].

Coal is the most abundant fossil fuel on earth, with global reserves estimated at one trillion tons that can meet current energy demands for 114 years[56]. Despite the economic benefits generated by coal, this fossil fuel is the largest contributor of CO_2 emissions and particulate matters (PMs), main cause of global warming and climate changes[57]. Due to this issue, Duarte et al.[58] utilized *Chlorella fusca* and *Spirulina* sp. to mitigate CO_2 from coal power plant. Their results demonstrated that those microlagae species were potential to fix CO_2 from coal flue gas, and sequential biomass production was possible for several different purposes. The intermittent injection of flue gas to the cultures enabled cells to grow through CO_2 fixation. The amount of CO_2 fixed by *C. fusca* isolated from a coal power plant was 2.6 times larger than that by *Spirulina* sp. The maximum CO_2 fixation rate was 360.12 mg L^{-1} d^{-1} with *C. fusca*, showing specific growth rate of 0.17 d^{-1}.

Duarte et al.[59] also reported that, by using a *Chlorella* species isolated from a power with intermittent flue gas injection, cells grew well under variety of conditions and microalgal CO_2 fixation was not interfered in the presence of ash (40 ppm), SO_2 and NO (brlow 400 ppm). The best condition for CO_2 fixation was found as 10% CO_2, 200 ppm SO_2 and NO and 40 ppm ash (50.0%), showing a specific growth rate of 0.18 d^{-1}.

Hosseini et al.[60] reported that a one-meter deep, top-lit gas-lift open bioreactor using air with up to 6% CO_2 resulted in comparable lipid productivity to traditional raceways. They suggested that the footprint of an algal plant utilizing waste industrial off-gas could be substantially reduced..

Kao et al.[61] examined three types of flue gases, for the purpose of utilizing of CO_2, NO_X and SO_2, in the cultivation of *Chlorella* sp. MTF-15. This strain grew successfully and accumulated lipids with a suitable composition for biodiesel production. Outdoor cultivation of the microalgae by utilizing the flue gases from the steel plant was also successful, demonstrated the potential of using microalgae to mitigate CO_2 emission and produce biomass as a biodiesel feedstock.

In Jiang et al.[62] research, *Scenedesmus dimorphus* showed a good tolerance to flue gas containing high concentrations of CO_2 up to 20% and NO 500 ppm, and the maximum SO_2 concentration *S. dimorphus* could tolerate was 100 ppm. By intermittent supplying of flue gas under pH control, the biomass concentration and CO_2 utilization efficiency were reached up to 3.63 g $L^{-1}$and 75.61%, respectively.

3.4. Organic solid wastes

Solid waste such as waste activated sludge has seen as promising nutrient sources for microalgae cultivation. Waste activated sludge (WAS) from the municipal wastewater treatment process is an inevitable by-product. The sludge is generated in large amounts during biological wastewater treatment, and consists of microorganisms, various organic/inorganic substances and a high water content (over 90%). In order to reduce the volume of WAS in wastewater treatment plants (WWTPs), the excessively produced WAS is mechanically dewatered by gravity settling, centrifugation or filtration/compression using a filter (or belt) press.

However, the sludge treatment cost (for dewatering and disposal) accounts for up to 60% of total operating cost of wastewater treatment processes[63]. Whereas, there have been few reports on the utilization of municipal WAS as a nutrient source for microalgal biomass production although it has favorable nutrient composition especially after stabilization through a composting process.

According to Wang et al.[64], WAS can be an effective co-substrate with microalgae in anaerobic digestion process. Digestion of *Chlorella* sp. mixed with WAS (59-96% in mass of VS-content) enhanced biogas yield, 73-79% larger than the gas yield from the digestion of pure microalgae alone. Krustok et al.[65] also reported that the biogas production was improved through anaerobic digestion with municipal food waste mixed with harvested microalgae which was cultivated in lake water.

Ramsunda et al.[66] proposed coupling of *Chlorella sorokiniana* cul ture with excessed activated sludge and final effluent from municipal wastewater treatment to reduce the use of synthetic nutrients and fresh water. Their results showed that waste activated sludge released nutrients into final effluent to support microalgal growth. Mixotrophic mode of nutrition was found to be most suitable for achieving high biomass productivity. It can replace the synthetic nutrients and fresh water with waste activated sludge and final effluent for economically feasible and sustainable microalgal biomass production. Microalgal biomass grown by their proposed strategy has shown a promising potential for its application to biofuel production through decent lipid, protein and carbohydrate productivities.

Wang et al.[67] used a locally screened *Chlorella* strain in wastewater treatment through the combination of activated sludge and the microalgae. *Chlorella* species cultured with activated sludge showed a good performance of growth and nutrient removal in the presence of light. The mixed culture system showed 87.3, 99.2% and 83.9% removal efficiencies of COD, NH_3-N and TP, respectively, for 1 day.

4. Conclusions

Microalgal biodiesel is the current research niche that addresses the concerns of energy and environmental sustainability. Developing a large-scale microalgae cultivation system is required to explore the economic feasibility of this new feedstock for alternative energy.

Microalgae cultivation requires large amount of water, nutrients and energy input, which makes initial cost high. There are several options of using various wastewaters, flue gases, fertilizers or organic solid wastes to substitute artificial media and to support the growth of microalgae for biofuel production and other applicaitons, although technical challenges exist to overcome to make this substitution successful. In order to achieve economic feasibility and environmental sustainability, further investigations are required to search other alternative nutrient sources, without sacrificing the potential of microalgal cell growth and intended application of resulting biomass.

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