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Integration of Algal Biofuel Production with Municipal Wastewater Treatment: a Review

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Abstract. The integration of algae-based wastewater treatment and biofuel production has been of growing research interest in recent years. This article reviewed recent researches in algae biofuel production and the integration of algal wastewater treatment and biofuel production. The methods to algal biofuel production, biofuel productivity, and removal rate of treated municipal wastewater were summarized in this review. Chlorella is largely used, especially in biodiesel and bioethanol production. Spirulina is more often used to produce biogas. The removal rate of NH4+-N can be high in this integration. The most common biofuel products in this integration are biomethane and lipid. Traces of the integration are numerous on laboratory basis and limited in commercial use for now. The barrier to apply it from lab to commercial use may include a high cost during algae harvesting as well as land use and availability. Possible solutions and research gaps are outlined in this article.

1. Introduction

Energy shortage has been one of worldwide issues in decades. It is even on the list of top 50 world issues in the next century. However, energy demand is still increasing and predicted to increase by 40% before 2030 [1]. Thus, renewable energy, especially bioenergy, is key to ease this shortage. There is a 10% minimum binding target to replace fossil fuel with biofuels for each EU member state before 2020, according to European Commission [2]. The first and second generation of biofuels used food crops (e.g. corns) and non-edible materials to produce biofuel, but have their limitations and challenges (concerns of food consumption, complexity in production process, etc.) [3]. Cultivating algae to recover energy is the third generation of biofuel production [4]. The first assumption on algal energy production was made by Oswald in 1960 [5]. The most common biofuels produced by microalgae are biodiesel, bioethanol, bio-oil, biogas, etc. However, simply cultivating algae to recover energy is not economically sound, as huge investment is needed in the cultivation process.

Water safety and water shortage have become serious. Municipal wastewater took up the largest market size in terms of wastewater treatment [6], and can be high in pollutant components. Therefore, taking pollutants in wastewater as nutrients to microalgae, biofuels can be produced simultaneously with microalgae wastewater treatment. This can be cost efficient, and might help alleviating energy shortage and water safety.

This article aims to give an overview of recent researches on algal biofuel production integrated with municipal wastewater treatment. The advantages, disadvantages, challenges, and possible solutions will be discussed.

2. Algal biofuel production

Biofuels normally include biodiesel, bioethanol, bio-oil, and biogas. Biodiesel consists of long chain alkyl esters, and can be made from vegetable oil or animal fats [7]. Biogas, mostly consisted with methane and CO_2 , is often produced by the decomposition of organic matters, and thus is largely considered to be one of the sustainable energies [8]. Most parts of microalgal cell can be used for further ethanol production or transferred to butanol [9]. Effective bio-crude oil production from algae via hydrothermal liquefaction (HTL) were reported [10].

For microalgae biodiesel production, oil needs to be extracted from dried microalgae cells before converted into biodiesel. Usually, oil is extracted in three popular methods: expeller/press, solvent extraction with hexane, and supercritical fluid extraction [11]. Then, the extracted oil is converted into biodiesel in one of the following methods: direct use and blending, thermal cracking (pyrolysis), and transesterification (alcoholysis) [12]. The intracellular lipids of microalgae can also be used to produce biodiesel after the transesterification process with the help of methanol or ethanol [13].

Bio-crude oil is commonly produced by HTL. This is to convert algae biomass into bio-crude oil, but the algae should be low in lipid content. Low-lipid algae can be cultivated fast, which could be an advantage of this approach of algal biofuel production [10]. Kuo's study showed that 39.8% of bio-crude oil can be converted from algae biomass under HTL, attached with a rotating wheel for algae harvesting during algal cultivation [14].

Bioethanol can be produced via yeast fermentation, using the carbohydrates in microalgae biomass, with mechanical pre-processing and pretreatment before fermentation [15]. Thus, intercellular sugars or carbohydrates must be exposed to extracellular solution, which can be achieved by five methods to cell wall destroy: thermic shock, manual grinding, acid treatment, basic treatment, and enzymatic digestion [16]. Yeast species can affect the fermentation process, thus influence the concentration and yield of produced bioethanol.

Biogas is often produced in anaerobic digestion process. Microalgae, as an organic matter, has been used to produce biogas. Biogas productivity could be improved by thermal pre-treatment, anaerobic co-digestion, etc. The research of Dingnan [17] showed that the biogas production can be increased by four times when microalgae were digested with proper cosubstrate, which is septic sludge in that study. Apart from producing biogas, microalgae could also be used to upgrade biogas to biomethane by removing CO_2 from raw biogas. In addition, the solar energy absorbed by microalgae cells can be directly transformed into biofuels (e.g. biomethane) through anaerobic digestion [5].

Recent studies on microalgae biofuel production were listed in **Table 1**. *Chlorella* is largely used, especially in biodiesel and bioethanol production. *Spirulina* is more often used to produce biogas.

Table1. Recent researches on algal biofuel production

Biofuel	Species of Algae	Method	Productivity	Reference
Biodiesel	*Chlorella vulgaris* and *Leptolyngbya* sp.	Extract using a soxhlet apparatus with n-hexane	66-70 mL/L and 62-71 mL/L, respectively	[18]
Biodiesel-lipid	*Chlorella sp*	Extract with glass microparticles added as cell disruptor	Lipid extraction efficiency raised from 23.19% to 36.01%	[19]
	Aurantiochytrium sp	Assisting lipid extraction with a high shear mixer applied at downstream process	Lipid extraction yield: 90%, with a low energy consumption (4.83 $kg^{-1}MJ^{-1}$) and a low solvent usage (5.9 $g^{-1}mL^{-1}$ dry cell).	[20]
	Chlorella sp. and *Scenedesmus sp.*	HTL with Algaewheel®	39.8% VS bio-crude oil after 1 day of HRT	[14]
Bioethanol	*Chrysophyte*	Destroying cell wall: basic treatment, enzymatic digestion, and acid treatment; Fermentation: with *Saccharomyces cereviseae*	43, 40 and 38 % with the three cell-wall destroying methods, respectively	[16]
	Chlorella sp	Combined pretreatment and fermentation	At 100 g/L biomass loading, bioethanol concentration achieved 9.579 g/L with *S. cerevisiae*, and 10.31 g/L with *P. stipitis*	[7]
Biogas	*Spirulina platensis*	Anaerobic digestion with food waste and sludge	Co-digesting with food waste: methane productivity raised 37.5% compared to solo microalgae digesting; also raised 8.74% compared to solo food waste digesting	[21]

Biofuel	Species of Algae	Method	Productivity	Reference
	Microalgae consortium*	CO_2 removed in an outdoor PBR, with agricultural wastewater as microalgae nutrient medium	94.1–98.9% methane after upgrading (raised 7.9-12.7%)	[22]
	Eustigmatos magnus	Anaerobic digestion with 120°C thermal pre-treatment	CH_4 productivity increased 25.36% at 120°C (430 L^{-1} kg^{-1}VS) compared with that at 90°C (343 L^{-1} kg^{-1}VS)	[23]

*Microalgae consortium includes *Chlorella vulgaris, Stigeoclonium tenue, Nitzschia closteirum* and *Navicula amphora*.

3. Microalgae wastewater treatment

Microalgae wastewater treatment has served for wastewater treatment long time ago and recommended by many researchers in 1950s [24]. The most common species of microalgae used in wastewater treatment are *Chlorella, Spirulina, Scenedesmus, Dunaliella,* etc. [25] [10]. They are normally used in the secondary treatment, while some were used in tertiary treatment for nutrient or directly used from the beginning of the process.

The container for different species of microalgae can be different, normally including open pond and closed photobioreactor (PBR). Open pond system includes stirred vessel, natural water, raceway pond, and inclined surface device. The closed PBR can be divided into four types according to the shape: tubular PBR, flat plate reactors, bag systems bioreactors, and annular reactor [26] [27]. Closed PBR can adjust the cultivation environment and process, but costs highly and faces many operational issues such as overheating, fouling, and limitation on gas exchange, the last of which makes it impossible to be scaled up. Open pond system might be lower on capital investment, but could be high on operational cost if scaling it up, and is highly affected by the environment (e.g. temperature, humidity, invasion of bacteria, other algae species, etc.) as it is open [28]. It is beneficial to adjust the type of the bioreactor according to certain microalgae species. Similarly, the microalgae's growth features should also match with the type and the scale of wastewater.

The component in any types of wastewater can be complex and various. Thus, it is necessary to analyse the possible pollutants and its variation with microalgae before the start of microalgae wastewater treatment.

3.1. Feature of algae for wastewater treatment

Microalgal growth needs all kinds of nutrients, which can be found as pollutants in wastewater. As long as the microalgae are alive with normal metabolism in wastewater, they can absorb certain pollutants as nutrients. Microalgae are normally classified as photoautotrophic, heterotrophic, and mixotrophic in terms of carbon utilization ways.

For photoautotrophic microalgae, light is utilized as energy source and CO_2 (inorganic carbon) is a major carbon source [9]. High S to V ratio is thus important for bioreactor to provide light to microalgal anabolic reactions, and to place it vertically could allow diffuse light to reach the bioreactor, enhancing the strength of light. The microalgae biomass productivity rate in a vertical bioreactor can be twice the rate of non-vertical bioreactor [29]. Inserting waste gas with CO_2 (e.g. flue gas) into wastewater treatment process could provide microalgae with necessary inorganic carbon, and could improve the growth rate [30]. Adapting flue gas into microalgae wastewater treatment system is possible and recommended, although the cost of gas transport can be high if without a close source of polluted air.

For heterotrophic microalgae, organic carbon is the main source of carbon for metabolism but light is not necessary [9], allowing the reactors for heterotrophic microalgae wastewater treatment to be designed for increasing the density of microalgae cells and other functional factors. Therefore, the reactor for heterotrophic microalgae can be built deep to accommodate more cells to achieve better treatment result. In addition, heterotrophic culture system can lead to high lipid productivity. According to Nzayisenga et.al, lipid contents achieved the highest (39.5%) in heterotrophic condition with glycerol, compared to that in autotrophic and mixotrophic (10.5%) condition [31].

Several researchers argued that a promising culturing condition for microalgae wastewater treatment should be mixotrophic cultures that present reduction on photo-inhibition while improve microalgae growth rate [9]. By using both organic and inorganic carbon, some species of microalgae can grow autotrophically during daytime and heterotrophically at night or in insufficient sunlight due to high cell density. Compared with ethanol and acetate, glycerol turned out to be a better source of organic carbon added to the mixotrophic cultivation system, bringing about enhancement in microalgae growth, especially in *N. salina* and *C. protothecoides* [32].

The complexity of wastewater components and various conditions in different wastewater treatment require it to select microalgae species for targeted wastewater treatment. The selected microalgae should at least be able to: grow fast, absorb large amounts of nutrients, and utilize low concentration of nutrients. In addition, microalgae should also be easy to harvest in the end, tolerant to the changing environment, and resistant to other microorganism's contamination [33].

3.2. Feasibility of microalgae wastewater treatment

Some pollutants in wastewater can be nutrients for microalgal growth and can be easily absorbed or transferred into more environmental-friendly forms. For example, Kothari et al. found that after 10 days treatment with *C. pyrenoidosa*, the concentration of nitrate, nitrite, fluoride, chloride, and Fe ions in diary wastewater was reduced by 42.10%, 87.14%, 58.3%, 61.03% and 32%, respectively [34]. Therefore, microalgae wastewater treatment is a win-win.

Microalgae treatment can be applied to different types of wastewater, including industrial, municipal, agricultural, and agro-industrial wastewater. Industrial and municipal wastewater have broadly adopted this method for better treatment, although they may contain toxic substances. For example, combining pig biogas slurry with municipal wastewater can improve the growth of microalgae as it introduced Mn, Fe, Zn, and Cu into the system, but the treatment performance could be compromised due to high concentration of the heavy metals [35].

Researches on agricultural and agro-industrial wastewater treated by microalgae are few and lack of practice in real situation. These types of wastewater are difficult to treat because they are non-linear. They are normally high in organic carbon, nitrogen and phosphorus, and low in heavy metals. Thus, microalgae can grow efficiently when applied into the downstream of agricultural and agro-industrial wastewater. For example, a combination of *C. protothecoides, S. obliquus* and *C. vulgaris* were used in a study for beef packaging wastewater treatment, and achieved 91% of COD, 67% of total nitrogen, and 69% of TP- PO_4^{3-} in terms of removal rates [36].

Appropriate wastewater treatment stage, suitable bioreactor, and advisable scale of wastewater are also crucial to the feasibility of algal wastewater treatment. Clarens applied secondary municipal effluent with and without nitrification to microalgae for further treatment (tertiary treatment), which showed a reduction in energy consumption at 22% and 3%, respectively [37].

4. Integrated algal biofuel production and wastewater treatment

The integration of algal wastewater treatment and biofuel production turned out to be energy and cost efficient [38]. In recent studies, the removal rate of all forms of N ($NH_4^+ - N$, TN, ammonium, etc.), P, COD, and some other indicators are widely investigated. Most of them showed high removal rates (more than 50%), with some higher than 80% and a few over 95%. The most common biofuel products are biomethane and lipid, but the productivity or yields may vary. Partially adapting wastewater treatment could benefit microalgae biofuel productivity. The biomethane productivity of microalgae with wastewater primary treatment was higher than microalgae mono-digestion (238-258 vs. 189-225 mL$_{CH_4}$/g VS). This could be due to the co-digestion of microalgae and primary sludge. The mixture of algae and bacteria, or different algae species also presented good performance in wastewater nutrient removal. The efficiency in pollutants removal, however, was not greatly improved with primary treatment, with a 2% drop in ammonium removal and a 3% increase in COD removal [39]. Examples of related studies are listed in **Table 2.**

Table 2. The effect of selected integrated algal biofuel production and wastewater treatment

Microalgae species	Type of wastewater	Wastewater treatment effects	Type of biofuels	Productivity	Reference

Chlorella sp. and *Stigeoclonium sp.*	Urban wastewater	Removal rate: NH_4^+-N: 93-91%; COD: 62-65%	Bio-methane	Highest: CH_4 238-258 L^{-1} kg^{-1}VS	[39]
A mixture of *Chlamydomonas subcaudata* (92%), *Anabaena sp*. (7.5%), and *Nitzschia sp*. (0.5%)	Slaughterhouse wastewater	With HRT of 10 days, the removal rates: Total COD: 86%-92%; NH_4^+-N: 79%-80%; Soluble Phosphorus: 71%-91%	Bio-methane	Highest CH_4: 195 mL CH_4/g VSS	[40]
Spirulina with bacteria	Municipal wastewater-filtered	After 200 hours the removal rates are: Total soluble nitrogen: 86%; Ammonium nitrogen: 100%; Total soluble phosphorus: 95%; Organics: 63%	Bio-crude oil	Yields of bio-crude oil differ from reactors: 52.2% from carboy; 51.3% from PBR; 37.9% from open pond.	[41]
Benthic pennate diatoms	Urban wastewater	Highest rate of N and P in biomass: 5.79% and 3.02%, respectively	Lipid	9.3-22%	[42]
Chlorella pyrenoidosa with a bacteria strain belonged to *Klebsiella sp*	Municipal wastewater	The bacteria strain showed P accumulation at 9.48 L^{-1} mg^{-1} in 48h	Lipid	Lipid content at 26.2% and yield at 0.197 g/L, raised 90.1% compared to control group	[43]
Scenedesmus sp. ASK22	Urban wastewater and diary effluent	After 12 days, COD 90.50%, removal rate 292.85 mg/d; Nitrate 100.00%, removal rate 13.56mg/d; Phosphorus 91.24%, removal rate 6.30 mg/d.	Lipid	31.16 mg L^{-1} d^{-1}	[44]
P. kessleri-1	Lake sewage	Removal rate after 10 days: Total nitrogen: 81%; Total phosphate: 98%; Magnesium: 84%; COD: 69%; BOD: 68%; TOC: 48%.	Lipid	Total lipid yields 96.4 mg L^{-1}, increased by 115%	[45]
Chlorella minutissima	Urban wastewater	Removal rates: N: 100%; P: 91%; Carbon: 85%	Lipid	Lipid content increased from 25% to 58%; lipid productivity was 1.93 mg L^{-1} d^{-1}	[46]

The integration, however, should notice the possible toxic pollutants contained in microalgal cells. Researchers discovered that some microalgae species produce a variety of toxic substances [47]. It is essential to monitor the concentration of these substances during the process. The harvesting process should also not be toxic to algae in order to recycle the nutrients and reduce harvesting cost [47].

Current case studies on commercial use of this integration are limited. This could be because of the uncertainty of benefits from laboratory-based studies to a real situation. The scale with profitable result can be hard to predict. The investment of this developing technology and its maintenance can be high, while the benefit may not be as expected.

5. Challenges and solutions

Challenges to algal wastewater treatment can include: 1) wastewater components can inhibit or even be toxic to algae growth. Wastewater with low N and P, or with heavy metals such as Cd, Zn, Hg, and Cu, inhibits the photosynthesis and growth of microalgae [48]; 2) the concentration of BOD is likely to rise after microalgae treatment due to the accumulation of soluble algal products. Shortening the hydraulic retention time may ease this problem [33].

Algal biofuel production is also facing challenges: 1) microalgae productivity varies from seasons and climates—the warmer the weather, the higher the productivity [49]; 2) drying algae cells can be energy-consuming but is necessary in algal lipid extraction [14].

The above challenges to algal wastewater treatment and algal biofuel production made the integration of the two technologies more challenging. Major challenges to the integration include:

1) Algal harvesting cost made the integration less cost-effective. The harvesting cost of suspended algae could contribute to 30% of the total cost of the system [50]. A possible solution could be attaching a rotating wheel into the algae wastewater treatment and biofuel production system. In Kuo's study [14], with the attached Algaewheel®, the treatment of swine wastewater and production of bio-crude oil system achieved the standard for COD effluent and 39.8% bio-oil from algae biomass with only I day HRT.

2) Land use and availability is another key challenge to the integration. Enough light should be provided by using large S:V containers, which requires large lands. However, simply increasing the dimension of algae treatment container can lead to a huge rise in the total cost. In addition, integrating wastewater treatment plant with algae biofuel production plant will not be energy-efficient in water pumping, if the algae facility is located more than 10km from the wastewater plant.

To address land use issue, it is possible to 1) apply additional equipment for better treatment effect by promoting algae growth. A study showed that the application of underwater light could result in an increase in wastewater N and P removal efficiency by 11% and 12.3%, respectively [51]; 2) upgrade the PBR to increase light-to-land efficiency, such as a PBR with large S:V ratio. For example, a four-layer PBR reduced land use, making land use the lowest capital investment of the project (0.58%) [38].

6. Conclusions

The integrated algal wastewater treatment and biofuel production is highly promising and widely available, although most of them are still on lab scale. It can cut down the cost of operating the system due to the win-win of treating wastewater and producing energy using the same algae. Wastewater nutrient removal rate can be high in such integration, especially removal rate of $NH_4^+ - N$. The most common biofuel products in this integration are biomethane and lipid. Challenges to the integration is especially in the high cost of harvesting algae, and the cost and feasibility of land use. Although there still exists certain difficulties when utilizing it in real situations, the future for the integration of algal wastewater treatment and biofuel production is promising.

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