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Review

Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource

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HIGHLIGHTS

- Agricultural wastewater shows a great potential for microalgal *Chlorella* growth.
- NH_4^+-N in wastewater is the key factor for microalgal biomass and lipid production.
- Optimal N and P concentrations in wastewater for *Chlorella* cultivation were indicated.
- Limitations of microalgal biomass productivity by wastewater were detail discussed.

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ABSTRACT

Using wastewater for microalgal cultures is beneficial for minimizing the use of freshwater, reducing the cost of nutrient addition, removing nitrogen and phosphorus from wastewater and producing microalgal biomass as bioresources for biofuel or high-value by-products. There are three main sources of wastewater, municipal (domestic), agricultural and industrial wastewater, which contain a variety of ingredients. Some components in the wastewater, such as nitrogen and phosphorus, are useful ingredients for microalgal cultures. In this review, the effects on the biomass and lipid production of microalgal *Chlorella* cultures using different kinds of wastewater were summarized. The use of the nutrients resource in wastewater for microalgal cultures was also reviewed. The effect of ammonium in wastewater on microalgal *Chlorella* growth was intensively discussed. In the end, limitations of wastewater-based of microalgal culture were commented in this review article.

1. Introduction

As the fast growing world population and economic developments, the demand for energy use of fossil fuel will continue to rise. But, the fossil fuels are not sustainable energy resource in the long term. The burning of fossil fuels can lead to increase in greenhouse gas (GHG) emissions and the environmental impact on global warming (Hill et al., 2006). Renewable energy resources offer clean alternatives to fossil fuels. They produce little pollution or GHG, and they will never run out. Currently, the main of commercially available biofuel, kinds of renewable energy, are bioethanol and biodiesel. Bioethanol is almost derived from sugar cane or corn starch and biodiesel is derived from oil crops including oilseed rape and soybean. However, these crop-based biofuels are economically competition with the production and the price of food (Hill et al., 2006).

Biofuels derived from microalgae have been proposed as an alternative approach that does not impact on agriculture. Microalgae have been estimated to produce higher biomass productivity than plant crops in terms of land area required for cultivation, are predicted to have lower cost per yield, and have the potential to reduce GHG emissions through the replacement of fossil fuels. The main inputs required for microalgae growth are sunlight, water, carbon dioxide (CO_2), and nutrients. Water and inorganic nutrients are identified as important limiting resources for microalgae culture. The nutrients for microalgae cultivation (mainly nitrogen and phosphorus) can be obtained from liquid effluent wastewater; therefore, besides providing microalgae growth environment, there is the potential possibility of waste effluents treatment. This could be explored by microalgae farms as a source

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of income in a way of wastewater treatment and obtain the nutrients for microalgae growth (Cantrell et al., 2008).

1.1. Microalgae for CO_2 mitigation

Microalgae are one of the earth's most important natural resources. They contribute to approximately 50% of global photosynthetic activity. For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2 absorbed by chloroplasts and used in respiration to produce energy to support growth.

During the recent decades, a number of post-combustion CO_2 capture methods have been developed using chemical, physical and biological methods (Kumar et al., 2011). In recent years, the bio-regenerative methods using microalgae via photosynthesis have been significant potential made to reduce the atmospheric CO_2 to ensure a safe and reliable living environment. In biological methods, particularly microalgal photosynthesis, have several merits, such as higher CO_2 fixation rates than terrestrial plants and no requirement for further disposal of the trapped CO_2. Microalgae-based CO_2 biological fixation is regarded as a potential way to not only mitigates CO_2 emissions but also shows the potential to produce lipid-rich microalgal biomass as a regenerative energy-source (Ho et al., 2011). One of the most understudied methods of CO_2 reduction is the use of microalgae that convert CO_2 from a point source into biomass. Microalgae use CO_2 efficiently because they can grow rapidly and can be readily incorporated into engineered systems, such as photobioreactors. The CO_2 reduction by microalgal photosynthesis and biomass conversion into health food, food additives, feed supplements, and biofuel is considered a simple and appropriate process for CO_2 circulation on Earth (Ho et al., 2011). The incorporation of CO_2 into energy-reserve components in biomass, such as carbohydrates and lipids, by photosynthesis-driven microalgal fixation of CO_2 is the most promising route for CO_2 sequestration from flue gas (Chiu et al., 2011; Kao et al., 2014; Kumar et al., 2014) and biogas (Kao et al., 2012a,b).

1.2. Microalgae for biofuel production

Many microalgae at or near optimal conditions potentially providing the benefits of well-controlling are exceedingly rich in oil (Chisti, 2007; Ho et al., 2010), which can be converted to many products such as renewable fuels, such as biodiesel, by transesterification (Chen et al., 2011). The biodiesel produced from algal oil has physical and chemical properties similar to diesel from petroleum, to biodiesel produced from crops of 1st generation and compares favorably with the International Biodiesel Standard for Vehicles (EN14214) (Brennan and Owende, 2010). Biodiesel from microalgae seems to be the only renewable biofuel that has the potential to completely displace petroleum-derived transport fuels without adversely affecting the food supply and other crop products. According to some estimates, the yield of oil from algae is over 200 times the yield from the best performing plant/vegetable oils (Singh and Gu, 2010). The production of microalgae to harvest oil for biodiesel has not been undertaken on a commercial scale, but working feasibility studies have been conducted to arrive.

Despite microalgae inherent potential as a biofuel resource, many challenges have impeded the development of algal biofuel technology to commercial viability that could allow for sustainable production and utilization. They majorly include: (1) species selection must balance requirements for biofuel production and extraction of valuable co-products; (2) attaining higher photosynthetic efficiencies through the continued development of production systems (Chen et al., 2011); (3) potential for negative energy balance after accounting for requirements in water pumping, CO_2 transfer, harvesting and extraction; (4) few commercial plants in operation. Therefore, there is a lack of data for large scale plants.

1.3. Microalgal biorefinery

Microalgal biomass is one of the most potential feedstock for biorefinery, because it can be converted into biofuels and various co-products (Vanthoor-Koopmans et al., 2013). Microalgal biomass can used to produce biodiesel by extracting lipids to transesterification into fatty acid methyl ester (FAME) as biodiesel. Some oleaginous microalgae as nonedible biodiesel feedstock shows great potential due to its high oil yield (5,000–100,000 L ha^{-1} $year^{-1}$). The non-lipid fraction of the algal biomass, consisting mainly of protein and carbohydrate, can also be processed to various biofuels, including methane and alcoholic fuels (McGinn et al., 2011). Some microalgal strains are rich in carbohydrates which is a potential feedstock for bioethanol production. The carbohydrates in microalgae mainly come from starch in chloroplasts and cellulose/polysaccharides on cell walls. The cellulose/polysaccharides of microalgae should be hydrolyzed to fermentable sugar for bioethanol production. From the point of biofuels, the microalgal cultivation integrated with biorefinery can increase the energy productivity and recycle the CO_2 for fuel production to achieve the sustainable development. To achieve high biomass productivity per unit area, higher biomass densities would be needed. Though high biomass density could be achieved in thin-plate photobioreactors and fermenters, the downstream processes, such as dewatering and dying facilities, remain cost and energy intensive processes. Thus, the new cost-effective downstream process should be developed and introduced for biofuel production.

In addition to produce energy by microalgal biomass, microalgal biomass can be applied in high valuable products such as food supplements, cosmetics and animal feeds, mainly containing polyunsaturated fatty acids (e.g., DHA and EPA), chlorophylls, carotenoids and phycobilins, among other (Ho et al., 2013; Yen et al., 2013). However, many developed processes focused on obtaining one specific product from microalgal biomass without complete utilization. In other words, the product extraction and conversion processes were developed for one specific product. This mostly means that the other available and valuable components in the microalgae were lost as waste. Thus, integrated processes for complete utilization of microalgal biomass are still need to be developed for enhancement of microalgal biomass utilization sustainability.

1.4. Microalgae for wastewater treatment

Inorganic nitrogen and phosphorus are is particularly difficult to remove from wastewater. Due to the ability of microalgae to use both wastewater pollutions for their growth, microalgae are particularly useful to reduce the concentration of inorganic nitrogen and phosphorus in wastewater (Ahluwalia and Goyal, 2007). Many species of microalgae are able to effectively grow in wastewater conditions through their ability to utilize abundant inorganic nitrogen and phosphorus in the wastewater. Therefore, the mass culture of microalgae can be potentially used for wastewater treatment as a tertiary process (Martin et al., 1985).

A complete tertiary process in the wastewater treatment aimed at removing nitrogen and phosphorus is estimated to be about four times more expensive than primary treatment (de la Noüe et al., 1992). It is identical that microalgal cultures offer an elegant solution to tertiary treatment due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth (Oswald, 1988a; Tam and Wong, 1996). And also, their capacity to remove heavy metals, as well as some toxic organic compounds, therefore, does not lead to secondary pollution. In conclusion, microalgae