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Chlorella pyrenoidosa cultivation in outdoors using the diluted anaerobically digested activated sludge

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HIGHLIGHTS

- Integration of algal biomass production and wastewater treatment.
- Successful culture of *C. pyrenoidosa* in diluted ADAS (using STE) in outdoors.
- Seasonal changes significantly affected the algal growth and pollutants removal.

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ABSTRACT

A freshwater green algae *Chlorella pyrenoidosa* (*C. pyrenoidosa*) was cultured in outdoors using the diluted anaerobically digested activated sludge (ADAS). The outdoors batch culture in every season showed that *C. pyrenoidosa* can grow normally under natural conditions in the diluted ADAS (STE/ADAS = 1.5/1, 3/1 and 5/1, v/v). Seasonal changes of environmental conditions significantly affected biomass growth and nutrient removal. Optimal biomass growth and nutrient removal was achieved at STE/ADAS = 1.5/1 during summer culture, harvesting a maximum biomass concentration of 1.97 ± 0.21 g/L, average biomass productivity of 291.52 ± 33.74 g/m^3/day (maximum value of 573.10 ± 41.82) and average lipids productivity of 37.49 ± 5.26 g/m^3/day (maximum value of 73.70 ± 9.75); simultaneously, the microalgae growth effectively removed nutrients from the wastewater, including 105.6 ± 17.1 mg COD_{Cr}/L/day, 36.8 ± 6.1 mg N/L/day and 6.1 ± 1.1 mg P/L/day.

1. Introduction

Biofuels derived from algae are prospectively considered as the third-generation biofuels. These microscopic plant-like organisms are able to generate complex lipids biomolecules, such as phospholipids, glycolipids, triacylglycerols (TAG), saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in their cells, by autotrophically utilizing CO_2 and sunlight or heterotrophically assimilating organic matters as carbon resource (Bellou et al., 2014). Compared with traditional oil terrestrial plants, algae have a much shorter growth cycle, high oil productivities per unit area and less impact on land-use for food production (Amaro et al., 2011). However, despite a number of compelling characteristics, algal biofuels production encounters many challenging technical, economic and regulatory barriers. A high cost of biomass production is the key issue for sustainable algal biofuel production. Algae cultivation usually requires large amounts of freshwater resources and fertilizers supplementation such as nitrogen (N) and phosphorus (P). Scaling up microalgae biomass production will be difficult due to the limited availability of water resources and chemical fertilizers. Therefore, seeking these low-cost nutrients and freshwater sources can bring advantages for sustainable large-scale algal biofuels production.

Integration of microalgae cultivation and wastewater treatment may be an optimal strategy (Cai et al., 2013). The excessive discharge of nutrients (e.g., organic carbon, N, P and heavy metal) from inadequately treated wastewater has actually leaded to lots of environmental crisis, such as eutrophication, degeneration of aquatic ecosystems. Furthermore, traditional processes for nutrients removal are costly due to large amounts of energy inputs for aeration and chemical addictives. Alternatively, these wasted nutrients and freshwater resources can be directly utilized for microalgae growth to generate biomass feedstock. Moreover, this process for nutrients removal doesn't require energy consumption for aeration; rather, microalgae photosynthetic growth can release

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considerable dissolved oxygen for enhancing biological activity. Thus, such an energy-saving and environmentally friendly strategy reduces the costs of both algal biofuels production and wastewater treatment.

At present, various waste streams have been successfully used for culturing microalgae, such as several effluents from wastewater treatment plants (WWTPs) (Caporgno et al., 2015; Cho et al., 2011), food processing wastewater (Shin et al., 2015), soybean processing wastewater (Su et al., 2011), carpet mill wastewater (Chinnasamy et al., 2010), anaerobic digested dairy manure (Wang et al., 2010), piggery wastewater (Wang et al., 2012), etc. These reports have demonstrated that these waste streams are good mediums for culturing several microalgae, such as *Chlorella* and *Scenedesmus* genera. Among these streams, the volume of wastewater in WWTPs is largest and more easily to be collected, representing a good medium for sustainable large-scale culture. The wastewater streams in the WWTPs mainly include raw wastewater, primary effluent, secondary-treated effluent (STE), sludge centrated wastewater and ADAS. Microalgae have been widely used for advanced treatment of STE, such as high rate algal ponds (HRAPs) (Sutherland et al., 2014). However, the biomass concentrations in STE or municipal wastewaters in laboratory culture are generally below 0.2–0.8 g/L due to their low nutrients (Bohutskyi et al., 2015; Cho et al., 2011). Such low biomass concentrations can significantly increase harvesting costs per unit of biomass. In contrast, the effluents from anaerobic digesters generally contain considerable nutrients and may be a better medium, such as ADAS.

The traditional treatment of ADAS is trouble due to its high ammonia concentration. The reflux of ADAS into wastewater stream can increase the ammonia load more than 10–20% in WWTPs. That can increase the energy inputs to meet dissolved oxygen requirement for nitrification and organic carbon addictives for denitrification. Fortunately, microalgae are typically rich in protein (more than 50% of the total dry weight), and their rapid growth in wastewater can directly assimilate large amounts of ammonia, which don't need aeration. Although the ammonia in the ADAS is an important growth substrate, however, the free ammonia is generally toxic to most microalgae (Peccia et al., 2013). Microalgae photosynthesis growth can extract CO_2 from the media, thus medium pH typically increases throughout a growth cycle. With the pH of medium rising near or above 8.0, free ammonia toxicity is a true concern in the systems. Azov and Goldman (1982) have demonstrated that the photosynthesis rates of *Scenedesmus obliquus* and *Dunaliella tertiolecta* are reduced to 50% of their maximum rates when free ammonia reached to 20 mg/L. Such an amount of ammonia over 500 mg/L in the ADAS would likely be inhibitory for most microalgae stains. Dilution is an effective method to reduce the inhibition from high ammonia. The STE with the characteristics of little ammonia (0–5 mg/L) and easily to be collected, might represent a good alternative to ameliorate the ammonia toxicity instead of clean water. In addition, the supplement of CO_2 not only provides sufficient carbon source for algal photosynthesis, and also it balances the increasing pH to ameliorate the negative effects of free ammonia toxicity.

Many previous studies have successfully demonstrated wastewater streams as mediums for culturing algae. However, most of these studies were processed in the laboratory using synthetic wastewater or wastewater sterilized either by autoclaving or by 0.45 μm membrane filtration. However, these strict pretreatments are difficult to be realized in large-scale outdoor culture. Different from the culture in laboratory, the outdoor cultures are generally suffered from extreme environmental conditions and high risk of contamination. For example, the ambient temperature and light intensity varied greatly among seasons. An extremely low or high temperature can cause a sharp decline of the algal growth and even collapse the algal system (Ras et al., 2013). Contamination is another key issue in algal wastewater system, such as rapid breeding of bacteria and microalgae grazers. The rapid growth of bacteria can out-compete the microalgae for essential nutrients (Pittman et al., 2011); the grazers can swallow a large amount of algal cells, and their rapid breeding can collapse the algae culture system within several days. Thus, in this study, microalgae culture in the mixed ADAS and STE were conducted in several photobioreactors in outdoors, aimed to assess the system stability and response to seasonal variations. That is essential to assess the feasibility of the combined processes and large-scale application in the future.

2. Methods

2.1. Wastewater

The wastewater samples used for microalgae culture were collected from a WWTP in Shandong Province, China. The wastewater treatment in the WWTP used traditional activated sludge process. The waste-activated sludge in the plant was stabilized and degraded by anaerobic digesters. The ADAS collected from the effluent of an anaerobic digester. The STE was collected from effluent of the secondary sedimentation pond. The ADAS contained high total suspended solids (TSS between 1200 and 2300 mg/L), and such high TSS in the raw ADAS can hinder the normal growth of microalgae photosynthesis. Thus, the ADAS was first allowed to settle for 4–7 h in a settling tank and filtered using 1250 mesh (10 μm) polyester filters before entering the microalgae photobioreactors. The TSS concentrations in the filtered ADAS were below 90 mg/L. In contrast, the TSS concentrations in the STE were generally below 25 mg/L, and it was directly used for microalgae culture without pretreatment.

2.2. Microorganism

A freshwater green algae *Chlorella pyrenoidosa* (*C. pyrenoidosa*, FACHB-9), was selected as the inoculation candidate due to its high protein content (indicating a large capacity for ammonia assimilation). And more important, *Chlorella* species generally have excellent ability to adapt to environmental variables, especially temperature fluctuations, which is important for microalgae growth in outdoors culture. Algae sample was obtained from the Institute of Hydrobiology (Chinese Academy of Sciences, Wuhan, China). The expansion of cultivation was firstly processed in the laboratory. Inoculation was performed under sterile conditions, and *C. pyrenoidosa* was cultivated in 100 ml of autoclaved selenite enrichment (SE) medium in 250 ml conical flasks and expanded in 500, 1000 and 3000 ml conical flasks. The content of nutrient per liter SE medium was as follows: $NaNO_3$ (250 mg), KH_2PO_4 (175 mg), $K_2HPO_4{\cdot}3H_2O$ (75 mg), NaCl (25 mg), $MgSO_4{\cdot}7H_2O$ (75 mg), $CaCl_2{\cdot}2H_2O$ (25 mg), $FeCl_3{\cdot}6H_2O$ (5 mg), H_3BO_3 (2.86 mg), $MnCl{\cdot}4H_2O$ (1.81 mg), $ZnSO_4{\cdot}7H_2O$ (0.22 mg), $CuSO_4{\cdot}5H_2O$ (0.079 mg), Na_2-$MoO_4{\cdot}2H_2O$ (0.39 mg), $Co(NO_3)_2{\cdot}6H_2O$ (0.049 mg). All of the conical flasks were placed in a light incubator (GZX-300BS-III, CIMO Medical Instrument, China). The cultivation conditions were as follows: light intensity = 127 μmol/m^2/s, light/dark ratio = 12:12, temperature = 25 ± 1 °C and artificial intermittent shaking (four times per day) for six to seven days.

2.3. Outdoor culture in the photobioreactors

Outdoor algae culture was conducted in several rectangle photobioreactors in a starch processing factory near the WWTP. The photobioreactor has a total volume of 175 L (a working volume of 160 L, 0.65 m length × 0.30 m wide × 0.90 m height) and an