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Enhanced lipid and biomass production using alcohol wastewater as carbon source for *Chlorella pyrenoidosa* cultivation in anaerobically digested starch wastewater in outdoors

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ABSTRACT

Alcohol wastewater (AW) as carbon source for enhancing *Chlorella pyrenoidosa* growth and lipid accumulation in anaerobically digested starch wastewater (ADSW) was performed in outdoor cultivation. The biomass and lipid production significantly increased while adding optimal amount of AW (AW/ADSW = 1:15) during exponential phase. In comparison with blank ADSW culture, the optimal AW addition increased the biomass production, lipid content and productivity by 35.29%, 102.68% and 227.91%, respectively. However, AW addition caused severe bacterial contamination and the total bacterial increased by 4.62-fold. Simultaneously, the optimal consortia of microalgae/bacteria effectively removed nutrients from the wastewater, including 405.18 ± 36.47 mg COD_{Cr}/L/day, 49.15 ± 5.54 mg N/L/day and 6.72 ± 1.24 mg P/L/day.

1. Introduction

Biodiesel from microalgae is considered as a promising candidate for the third-generation bioenergy production. Microalgae biomass as an advanced biofuel feedstock has several compelling characteristics to support its development, including the cultivability on nonarable cropland, growth in seawater, freshwater or otherwise nonpotable wastewater, typically high lipid content and per-acre productivity (Chisti, 2007). However, current economical modeling calculates the total cost of microalgal biofuel up to $6.5–8.0 per US gallon, which is about 10 times higher than that of the fossil fuel. Such high cost limits its commercial application. Microalgae cultivation accounts for 70% of the total biodiesel production cost, in which water source and nutrient additives are the core issues (Doe, 2010). Thus, seeking for available water source and cheap nutrients might be an alternative way to reduce the cost.

Wastewater from human activity is very large and many samples contain essential nutrients (e.g., nitrogen, phosphorus, trace elements), potentially representing as available mediums for cultivating microalgae. Such a strategy is viewed favorably because it contributes to biomass production, greenhouse gas capture, pollutants removal and oxygen release for enhancing biological activity in the wastewater (Maity et al., 2014; Pittman et al., 2011). During recent years, various waste streams have been successfully used for cultivating microalgae, including urban municipal, agricultural, industrial wastewater and anaerobically digested wastewater (ADW) (Cai et al., 2013; Caporgno et al., 2015; Chiu et al., 2015). Among these wastewater streams, ADW is considered as a better medium for cultivating microalgae due to its sufficient dissolved nutrients (Bjornsson et al., 2013). It can maintain high-density microalgae cells and significantly reduce harvesting costs per unit of biomass. Additionally, most pathogenic bacteria or viruses in wastewater have already been killed during anaerobic processes, which will reduce the contamination risks during outdoor cultures.

Several microalgae species have been successfully cultivated in diluted or filtered ADW (e.g., anaerobically digested food processing, agro-zootechnical and municipal wastewater) (Shin et al., 2015; Franchino et al., 2013; Halfhide et al., 2015; Veronesiv et al., 2017). The maximal biomass concentrations in these ADW were 4–12 times higher than that in municipal wastewater or secondary effluent. In previous study, a freshwater microalgae *Chlorella pyrenoidosa* (*C. pyrenoidosa*) had been successfully cultivated in outdoors using the anaerobically digested starch processing wastewater (ADSW) in an airlift circulation photobioreactor (Tan et al., 2014). The maximal biomass concentration can reach to 2.0 g/L. However, it is difficult to simultaneously obtain high biomass production and lipid content during outdoor culture, especially during the hot summer months. The lipid content in algal biomass harvested in summer was only in range of 6–10%, leading to low lipid productivity regardless of high biomass

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production.

Many researchers have investigated the mechanism of lipid accumulation in algal cells. It is found that specific stress conditions can trigger lipid accumulation, such as low temperature, nutrients deficiency and high salinity (Ho et al., 2014). However, these stress conditions generally cause low growth rate and biomass production. Thus, it is difficult to achieve high lipid productivity under stress conditions. Fortunately, studies have demonstrated that several algal strains simultaneously promote lipid content and biomass production, by means of mixotrophic growth utilizing organic carbon sources (e.g., glucose, glycerinum). Yeh & Chang (2012) mixotrophically cultured *C. vulgaris* in medium using glucose (10 g/L) and CO_2 (2%, v/v) as carbon sources, and found that lipid content and productivity increased by 1.65 and 2.58 times compared to autotrophic culture. Several studies have investigated the carbon flux distribution in algal cells between mixotrophic and autotrophic growth, and revealed that assimilated CO_2 mainly goes to the synthesis of upstream carbohydrate-based metabolites, while supplementing glucose recalibrates the metabolism towards lipid (saturated fatty acid) accumulation (Ren et al., 2016). However, the sugars and volatile acid residue in ADSW was few because most organics have been degraded and recycled by biogas during anaerobic processes. It limited mixotrophic growth of *C. pyrenoidosa*. Therefore, extra carbon source must be supplemented for sustainable mixotrophic growth.

However, pure chemical additives such as acetate or glucose will significantly increase the cost of microalgal cultivation more than 60–80% (Li et al., 2007). Therefore, using these wastewater containing high sugars or short-chain fatty acids may represent as an alternative way to enhance lipid accumulation(Ma et al., 2016; Yan et al., 2011). Alcohol processing wastewater (AW) contains abundant simple dissolved organics, indicating the possibility as carbon source for mixotrophic growth. However, high-strength organics in algal system may cause severe heterotrophic bacterial contamination during outdoor culture. It may impose significantly negative effect on algal growth and even cause algal system collapse. However, its practical application is so scarcely studied that these risks are still uncertain. Thus, in this study, AW as carbon source for enhancing microalgal growth and lipid accumulation in ADSW was processed in outdoor cultivation, and different modes of AW addition and their effects on bacterial contamination were also evaluated. That is important to assess the feasibility for algal wastewater system using organics from wastewater as carbon source during outdoor culture.

2. Materials and methods

2.1. Culture inoculum

A freshwater green algae *C. pyrenoidosa* (FACHB-9) was selected as the inoculation candidate due to its ability to utilize organic carbon for heterotrophic growth. The microalgal strain was obtained from the Institute of Hydrobiology (Chinese Academy of Sciences, Wuhan, China). Inoculation was performed under sterile conditions, and 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), $FeCI_3{\cdot}6H_2O$ (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 was 127 μmol m^{-2} s^{-1}, light/dark ratio was 12 h: 12 h, temperature was 25 ± 1 °C. Before the start of cultivation in outdoors, the microalgae was adapted in laboratory and expanded in several glass tanks as the inoculation.

2.2. Wastewater samples

All of the wastewater samples for cultivating microalgae were collected from a starch and alcohol processing plant in Shandong Province, China. Starch processing wastewater was firstly settled in several ponds for recovering suspended solids. These available solids were subsequently utilized for producing alcohol in several fermentation tanks. The starch processing wastewater was treated by upflow anaerobic sludge bed (UASB) reactors, followed by anoxia-biological contact oxidation processes. The ADSW was collected from the effluent of a UASB reactor, and AW was from the effluent of alcohol distillation tower. To obtain more dissolved organics, a rapid acidification of the AW was processed. The AW was firstly cooled to 30–32 °C and stored in a closed plastic container (300 L). The temperature in tank was kept in range of 30–32 °C by a temperature control device. The acidification process was lasted for five days, and the acidified AW was collected for cultivating microalgae.

The ADSW and acidified AW contained high suspended solids and hinder microalgae photosynthesis**.** Thus, two wastewater samples were allowed to settle for 5–7 h in several tanks and filtered using polyester filters (1 μm) before entering the tanks. The concentrations of suspended solids in the filtered ADSW and AW were generally below 15 mg/L. The characteristics of the pretreated ADSW and AW for cultivating microalgae are summarized in Table 1. From Table 1, the ADSW contained sufficient dissolved ammonia and phosphorus for *C. pyrenoidosa* growth. Moreover, other nutrients and trace elements necessary for microalgae growth, including potassium, magnesium, iron, copper, zinc, manganese and boron, were all detected in the ADSW. However, the sugars and volatile acids in ADSW was only 29.36 ± 4.27 and 101.76 ± 9.58 mg/L, respectively. Such few sugars and volatile acids cannot maintain mixotrophic growth of *C. pyrenoidosa*. In contrast, total sugars and volatile acids in acidified AW reached to 1792.52 ± 29.63 and 7265.06 ± 117.54 mg/L, respectively. These characteristics suggest that the acidified AW potentially represent as carbon source for *C. pyrenoidosa* mixotrophic growth in ADSW.

2.3. Microalgal culture in outdoors

Outdoor culture was processed in several closed rectangle tanks. The tank has a total volume of 175 L (a working volume of 160 L, 0.65 m long × 0.30 m wide × 0.90 m height) and an illuminated area

Table 1
Characteristics of pretreated anaerobically digested starch wastewater (ADSW) and acidified alcohol wastewater (AW) for cultivating *C. pyrenoidosa*.

Parameters	AW	ADSW
pH	3.2–4.5	7.1–7.3
Chemical oxygen demand (COD) (mg/L)	45,683.06 ± 1760.28	792.28 ± 19.60
Total sugars	1792.52 ± 29.63	29.36 ± 4.27
Total volatile acids	7265.06 ± 117.54	101.76 ± 9.58
Total volatile phenol	43.57 ± 1.51	1.04 ± 0.18
Formaldehyde	119.62 ± 3.36	1.79 ± 0.06
NH_4^+-N(mg/L)	214.56 ± 9.71	273.15 ± 9.05
TN(mg/L)	725.34 ± 31.46	307.64 ± 15.28
PO_4^{3}-P (mg/L)	19.71 ± 0.89	27.38 ± 1.29
TP(mg/L)	64.38 ± 4.15	37.57 ± 2.42
Al(mg/L)	1.04 ± 0.05	0.14 ± 0.02
B(mg/L)	0.43 ± 0.02	0.16 ± 0.01
Ca(mg/L)	16.95 ± 1.35	126.36 ± 4.68
Fe(mg/L)	2.30 ± 0.11	0.94 ± 0.12
K(mg/L)	127.63 ± 3.09	112.34 ± 5.92
Mg(mg/L)	116.94 ± 5.83	49.12 ± 2.06
Mn(mg/L)	1.22 ± 0.04	0.13 ± 0.01
Na(mg/L)	315.21 ± 12.71	226.17 ± 17.48
Zn(mg/L)	0.06 ± 0.00	1.24 ± 0.02