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Nutrients removal and lipids production by *Chlorella pyrenoidosa* cultivation using anaerobic digested starch wastewater and alcohol wastewater

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HIGHLIGHTS

- Microalgae culture used anaerobic starch wastewater and alcohol wastewater.
- Addition of alcohol wastewater obviously improved biomass and lipids production.
- Pollutants in wastewater were efficiently removed.

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ABSTRACT

The cultivation of microalgae *Chlorella pyrenoidosa* (*C. pyrenoidosa*) using anaerobic digested starch wastewater (ADSW) and alcohol wastewater (AW) was evaluated in this study. Different proportions of mixed wastewater (AW/ADSW = 0.176:1, 0.053:1, 0.026:1, v/v) and pure ADSW, AW were used for *C. pyrenoidosa* cultivation. The different proportions between ADSW and AW significantly influenced biomass growth, lipids production and pollutants removal. The best performance was achieved using mixed wastewater (AW/ADSW = 0.053:1, v/v), leading to a maximal total biomass of 3.01 ± 0.15 g/L (dry weight), lipids productivity of 127.71 ± 6.31 mg/L/d and pollutants removal of COD = 75.78 ± 3.76%, TN = 91.64 ± 4.58% and TP = 90.74 ± 4.62%.

1. Introduction

In China, annual starch production has increased to more than 10 million tons, generating about 60 million cubic meters of starch wastewater (SW) by approximately 600 starch production plants (Lu et al., 2009; Xue et al., 2010). SW is generated from extraction processes, and contains abundant nutrients, including organic matters, nitrogen (N) and phosphorus (P). SW treatments generally focus on simple sedimentation for suspended solids recovery to produce alcohol, whereas supernatant treatments use traditional anaerobic–aerobic (A/O) processes, such as an upflow anaerobic sludge bed (UASB), an expanded granular sludge bed (EGSB) and the sequencing contact oxidation process or sequencing batch reactor (SBR) (Chan et al., 2009). Anaerobic processes are appropriate for the treatment of high-strength SW to recover most organic matters using methane. However, it is controversial to achieve nitrogen and phosphorus removal goals using aerobic processes, especially for anaerobic effluents containing large amounts of nutrients (Yang et al., 2011). Aerobic processes for nitrogen and phosphorus removal require high energy costs and generate abundant greenhouse gases and sludge into the environment (Fricke et al., 2005). Thus, an optimal alternative treatment process should be more energy-saving and environmentally friendly to utilize the production of useful organisms and remove pollutants from wastewater.

Integration of wastewater treatment and microalgae cultivation may be an optimal alternative treatment process. Microalgae have

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recently attracted considerable attention as a next generation energy feedstock (Brennan and Owende, 2010; Fenton and Ó hUallacháin, 2012). Compared to other oil crops, microalgae have a number of compelling characteristics to support their development, including high per-acre productivity, short growth cycle, and utilization of non-arable land and a wide variety of water sources (fresh, saline, and wastewater) (Wu et al., 2012). However, the costs of microalgae-based energy are much higher than traditional fossil fuels, in which the supplements of water resources and nutrient elements (i.e., nitrogen, phosphorus and potassium) represent a major obstacle to cost reduction (Behzadi and Farid, 2007; Komolafe et al., 2014). Both stable water resources and nutrient elements in wastewater are considered to be good resources for microalgae cultivation, and simultaneously, microalgae growth can effectively remove pollutants from wastewater associated with little sludge production and carbon emission. Anaerobic digested wastewater generally contains abundant dissolved nutrients that can be directly utilized for microalgae growth. Several researchers have explored the cultivation of microalgae using anaerobic digestion liquids: Marques et al. (2013) tested the potential combination of the anaerobic process and *Chlorella vulgaris* cultivation for vinasse treatment, Park et al. (2010) cultured green algal *Scenedesmus* sp. in an anaerobic digestion effluent of livestock waste for ammonia removal and Bahr et al. (2013) used an alkaliphilic microalgal-bacterial consortium for nutrients removal from anaerobic effluents and flue gas capture. These reports demonstrated that the effluents from anaerobic digesters are good mediums for microalgae culture. Anaerobic digested starch wastewater (ADSW) is rich in dissolved nitrogen phosphorus and other trace elements, which might also be a good medium for microalgae culture.

Microalgae, by means of mixotrophic or heterotrophic growth utilizing organic carbon as the major carbon source, can promote biomass production and lipids content. Reports have demonstrated that simple dissolved organics (i.e., glucose, short-chain fatty acid) were beneficial to microalgae mixotrophic growth (Zhang et al., 2014). However, the content of organic carbon in ADSW was limited; even part of the organic matters were inert after the anaerobic processes (Cai et al., 2013; Levine et al., 2011). Usually pure artificial organics (i.e., glucose) are preferred, but are costly, and their utilization may cause an adverse effect on food production. Therefore cheaper alternatives need to be explored. Alcohol processing is usually associated with starch production (recovering suspended solids from starch processing wastewater), and alcohol wastewater (AW) which contains abundant simple dissolved organics as an appropriate carbon source for microalgae mixotrophic growth. This suggests that combining ADSW and AW with an appropriate ratio may have potential advantages to optimize both biomass production and lipids content.

In this study, *Chlorella pyrenoidosa* (*C. pyrenoidosa*) was chosen as the inoculation candidate to generate complex biomolecules mixotrophically utilizing organic carbon or CO_2 as a carbon source. To investigate the optimal ratio of ADSW and AW for microalgae growth and nutrients removal, three different proportions of mixed wastewater (AW/ADSW = 0.176:1, 0.053:1, 0.026:1, v/v) and single pure ADSW, AW were evaluated for *C. pyrenoidosa* cultivation as well as for biomass production, lipids content and pollutants removal.

2. Methods

2.1. Wastewater used for microalgae cultivation

All of the wastewater samples were collected from a starch processing plant in Shandong Province, China. Starch wastewater produced from the starch processing plant was first settled in the tank. Then the supernatant was treated using a UASB reactor, followed by two-stage aerobic processing (anoxia-biological contact oxidation process); the suspended solids (SS) recovered by sedimentation were utilized for alcohol production through fermentation. Alcohol wastewater (AW) was first settled for three or four days by a rapid acidification process to obtain abundant dissolved organics, especially volatile fatty acids. In addition, both anaerobic digested starch wastewater (ADSW) and pretreated AW still contained high suspended solids that could hinder the growth of microalgae photosynthesis. Thus, all of the wastewater samples were allowed to settle for several hours and were filtered using a 0.45 μm polyester filter. The wastewater after sterilization was stored at 4 °C prior to the experiments.

2.2. Microalgae strain and culture medium

C. pyrenoidosa (*C. pyrenoidosa*, FACHB-9) was obtained from the Institute of Hydrobiology (the Chinese Academy of Sciences, Wuhan, China). Prior to being cultured in wastewater, *C. pyrenoidosa* was cultured under sterile conditions in sterilized SE medium, which consists of $NaNO_3$ (0.25 g/L), K_2HPO_4 (0.075 g/L), $MgSO_4{\cdot}7H_2O$ (0.075 g/L), $CaCl_2{\cdot}2H_2O$ (0.025 g/L), KH_2PO_4 (0.175 g/L), NaCl (0.025 g/L), $FeCl_3{\cdot}6H_2O$ (0.005 g/L), H_3BO_3 (2.86 mg/L), $MnCl_2{\cdot}4H_2O$ (1.86 mg/L), $ZnSO_4{\cdot}7H_2O$ (0.22 mg/L), $Na_2MoO_4{\cdot}2H_2O$ (0.39 mg/L), $CuSO_4{\cdot}5H_2O$ (0.08 mg/L) and Co $(NO_3)_2{\cdot}6H_2O$ (0.05 mg/L). The pH value of the SE medium is approximately 7. The cultivation conditions were as follows: light intensity = 127 μmol m^{-2} s^{-1}, light/dark ratio = 12:12, temperature = 25 ± 1 °C and artificial intermittent shaking four times in a day for 6–8 days.

To ensure normal microalgae growth in ADSW, a two-phase cultivation strategy was adopted for microalgae adaption according to the reports by Tan et al. (2014): (I) *C. pyrenoidosa* was cultured in wastewater at low temperature (15 °C) and low light intensity (60 μmol m^{-2} s^{-1}) for 3–4 days, and (II) the culture was subjected to high temperature (35 °C) and high light intensity (220 μmol m^{-2} s^{-1}) for 6–8 days. Finally, *C. pyrenoidosa* adapted successfully and exhibited a good growth performance in ADSW.

2.3. Experiments design

The samples of AW and ADSW after pretreatment were used for *C. pyrenoidosa* cultivation. To investigate the optimal ratio of AW and ADSW for microalgae growth and nutrients removal, three experimental groups (total volume of 1000 ml) were evaluated, including G1 (AW/ADSW = 0.176:1, v/v), G2 (AW/ADSW = 0.053:1, v/v) and G3 (AW/ADSW = 0.026:1, v/v). Additionally, comparative experiments were also conducted using single pure ADSW (G4) and AW (G5) for *C. pyrenoidosa* cultivation. All of the experiments were performed in triplicate. The initial inoculation concentration of *C. pyrenoidosa* was approximately 0.50 g/L (dry weight). The initial pH was controlled at 6–7 by adding 1 mol/L NaOH solution. *C. pyrenoidosa* were cultivated in 2 L glass conical flasks placed in an illumination incubator (GZX-300BS-III, CIMO Medical Instrument, Shanghai, China). The cultivation conditions were as follows: light intensity = 127 μmol m^{-2} s^{-1}, light/dark ratio = 12: 12, temperature = 25 ± 1 °C and artificial intermittent shaking four times per day for 9 days.

2.4. Analytical procedure

2.4.1. Microalgae biomass

The total biomass was determined based on the relationship between the dry cell weight and Chlorophyll *a*. To determine the chlorophyll *a* concentration, a 5 ml solution was collected each day and centrifuged at 4500 rpm for 10 min. The cell pellets were