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Continuous cultivation of *Chlorella pyrenoidosa* using anaerobic digested starch processing wastewater in the outdoors

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HIGHLIGHTS

- Pilot-scale microalgae cultivation using wastewater in the outdoors.
- *C. pyrenoidosa* grow normally in DSPW in continuous outdoors culture.
- Successful long-term continuous culture of microalgae using wastewater.

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ABSTRACT

Microalgae cultivation using wastewater might be a suitable approach to support sustainable large-scale biomass production. Its compelling characteristics included the recycling of nutrients and water resources, reducing carbon emissions and harvesting available biomass. In outdoor batch and continuous cultures, *Chlorella pyrenoidosa* completely adapted to anaerobic digested starch processing wastewater and was the dominant microorganism in the photobioreactor. However, seasonal changes of environmental conditions significantly influenced biomass growth and lipid production. The long-term outdoor operation demonstrated that the biomass concentration and productivity in continuous operations at different hydraulic retention times (HRTs) can be successfully predicted using the kinetic growth parameters obtained from the batch culture. A moderate HRT (4 days) in the summer provided the best microalgae and lipid production and achieved relatively high biomass concentrations of 1.29–1.62 g/L, biomass productivities of 342.6 ± 12.8 mg/L/d and lipids productivities of 43.37 ± 7.43 mg/L/d.

1. Introduction

Biodiesel has attracted considerable attention as one of the most important new alternative energy sources because it is non-toxic and its combustion causes low greenhouse gas (GHG) emissions. Biodiesel, which is derived from microalgae, has been widely recognized as the feedstock for third-generation biofuels. Several microalgae species (e.g., *Scenedesmus*, *Chlorella*, *Dunuliella*, *Botryococcus* and *Nannochloropsis*) are able to generate complex lipid biomolecules, such as phospholipids, glycolipids, triacylglycerols (TAG), saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in their cells, by autotrophically utilizing CO_2 and sunlight or heterotrophically assimilating organic matters as a carbon resource (Bellou et al., 2014; Chisti, 2007). The lipid content in these microalgae species generally varies between 20% and 50% (to a maximum of 80%) of their dry weight (DW), and higher lipid accumulations can be reached under special culturing conditions. The overall lipid biosynthesis pathway and its regulators have been widely reported (Bellou et al., 2014; Ho et al., 2014b). Compared to traditional oil terrestrial plants, microalgae have numerous advantages, such as being more simple to culture and having higher growth rates and greater biomass and oil productivities per unit area.

However, despite the advances over more than 50 years of algal research, relevant information about large-scale microalgae biomass production is scarce. Several challenging technical, economic and regulatory barriers must be addressed to support the development of a large-scale algal biofuel industry. Among these barriers, nutrient sources and water treatment/recycling represent major technical and economic problems for sustainable microalgae biofuel production (DOE, 2010). Utilizing existing agricultural, municipal or industrial waste streams is an optimal strategy to lower the costs of nutrient additives and water resources. Additionally,

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such strategies are viewed favorably because they reduce wastewater nutrients and carbon releases, contribute to biofuel production and provide oxygen for biological activity.

Several microalgae species are able to grow in wastewater conditions by utilizing the abundant organic carbon (COD), inorganic nitrogen (N) and phosphorus (P) in the wastewater. Some unicellular green microalgae species, such as the *Chlorella* genus, are particularly tolerant to sewage effluent conditions (Pittman et al., 2011). Moreover, *Chlorella* species have the ability to adapt to temperature fluctuations, which is important for microalgae growth in outdoor cultures (Ras et al., 2013). Many studies have demonstrated that the *Chlorella* genus completely adapts to various types of wastewater, including urban municipal wastewater, agricultural manure wastewater, carpet mill wastewater and soybean processing wastewater (Chinnasamy et al., 2010; Mutanda et al., 2011; Su et al., 2011; Wang et al., 2012). The *Chlorella* genus exhibits good growth in these wastewaters, and effectively removes large amounts of COD, N and P from the wastewater. However, the microalgae growth and pollutant removal varied greatly in the different wastewaters due to different concentrations of essential nutrients, including organic carbon, N, P (and the ratios of these constituents) and other trace elements (e.g., calcium, magnesium and iron). Despite being successfully cultured in the laboratory or using synthetic wastewater, scaling cultures of microalgae using wastewater in the outdoor environment still faces several risks. For example, the presence of toxins, such as cadmium or mercury, or organic chemicals is an issue for algal growth in wastewater, particularly in industrial wastewater. Pathogenic bacteria or predatory zooplankton can also have negative influences on microalgae growth, and other microorganisms in wastewater can out-compete the microalgae for essential nutrients (Pittman et al., 2011).

To avoid introducing unacceptable pathogens or other chemical compounds or heavy metals into the biomass stream, food processing wastewater that lacks toxic and hazardous substances represents a good nutrient resource for microalgae growth (Andrade and Costa, 2007; Su et al., 2011). Starch processing wastewater (SPW) is a typical food processing wastewater that contains abundant organics and nutrients. Processing wheat or corn into starch generates approximately 6–10 m^3 per ton of wastewater that is characterized by extremely high quantities of organics (COD_{Cr} of 6000–12,000 mg/L) and nutrients (e.g., nitrogen, phosphorus and potassium). After anaerobic digestion, the effluent is ideal for microalgae growth due to its low organic strength and high dissolved inorganic nutrients. A previous study successfully cultured *Chlorella pyrenoidosa* outdoors using anaerobic starch processing wastewater (DSPW) in an airlift circulation photobioreactor (890 L) (Tan et al., 2014). However, the microalgae were cultured in the photobioreactor at controlled temperatures using a heating device, and there is a lack of long-term continuous cultures at different hydraulic retention times (HRTs) under natural conditions. Different HRTs in continuous cultures can result in different levels of biomass and lipid production as well as pollutant removal. Additionally, it is important to assess the response of system stability in long-term outdoor cultures to seasonal variations. In this study, the microalgae culture in the photobioreactor was first operated under natural conditions (without temperature control except in the winter) in batch mode and then in continuous mode at different HRTs. The key factors that govern the technical feasibility of the system are evaluated and include: (1) biomass growth and nutrients removal in the batch and continuous culture under natural conditions, (2) system stability response to seasonal variations and culture contamination during long-term culturing, (3) prediction of continuous operation at different HRTs using kinetic growth parameters obtained from the batch cultivation, and (4) the influence of different HRTs on the biomass and lipid yields in the continuous culture.

2. Methods

2.1. Microorganism

Freshwater *C. pyrenoidosa* (*C. pyrenoidosa*, FACHB-9) was obtained from the Institute of Hydrobiology (Chinese Academy of Sciences, Wuhan, China). 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 components in the artificial SE medium included the following: $NaNO_3$ (250 mg L^{-1}), KH_2PO_4 (175 mg L^{-1}), $K_2HPO_4{\cdot}3H_2O$ (75 mg L^{-1}), NaCl (25 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (75 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (25 mg L^{-1}), $FeCl_3{\cdot}6H_2O$ (5 mg L^{-1}), H_3BO_3 (2.86 mg L^{-1}), $MnCl{\cdot}4H_2O$ (1.81 mg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.22 mg L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.079 mg L^{-1}), Na_2-$MoO_4{\cdot}2H_2O$ (0.39 mg/L), and $Co(NO_3)_2{\cdot}6H_2O$ (0.049 mg/L). 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^{-1}, light/dark ratio = 12:12, temperature = 25 ± 1 °C and artificial intermittent shaking (four times per day) for six to seven days.

2.2. Outdoor microalgae cultivation

Microalgae cultivation was performed in a wheat starch processing factory in Shandong Province, China, in which starch processing wastewater (SPW) was treated using an upflow anaerobic sludge blanket (UASB) and followed by two-stage aerobic processing (anoxia-biological contact oxidation process). The wastewater for *C. pyrenoidosa* cultivation came from the effluents of the UASB reactor. The wastewater was first allowed to settle for 5–7 h in a settling tank and filtered with 270 mesh (53 μm) polyester filter bags before entering the microalgae reactor. The total suspended solids (TSS) concentrations in the wastewater after pretreatment were less than 70 mg L^{-1}.

Microalgae cultivation was conducted in an airlift photoauto trophic–heterotrophic photobioreactor. The structure of the photobioreactor and the operational process, including the CO_2 supply and contamination control, was detailed in a previous study (Tan et al., 2014). In this study, the microalgae culture was processed under natural conditions without a temperature-control device (except in the winter). In the winter, the temperatures can drop below −7 °C, and the microalgae cannot survive. To ensure normal growth in the winter, a heating device was necessary to maintain the photobioreactor temperatures between 7 and 12 °C. Based on the environmental variables (e.g., temperature, light intensity and illumination time), the microalgae culture was divided into four operational periods, including summer (June–September in 2013), autumn (September–November in 2013), winter (November in 2013 to February in 2014) and spring (March–May, 2014). In each period, the microalgae culture was divided into two successive stages: the first was operated in batch mode until reaching stationary phase, and then the second was operated in continuous mode at different HRTs. The initial inoculation concentration of *C. pyrenoidosa* was 0.15–0.20 g/L (dry weight) and the total volume of inoculum was 820 L. Once the operation was completed in one period, the reactors were washed cleanly to prepare for the next culture period. In each period, samples were collected from the reactor daily to assess the biomass and lipid yields as well as to remove nutrients from the wastewater.