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Two-stage cultivation of two *Chlorella* sp. strains by simultaneous treatment of brewery wastewater and maximizing lipid productivity

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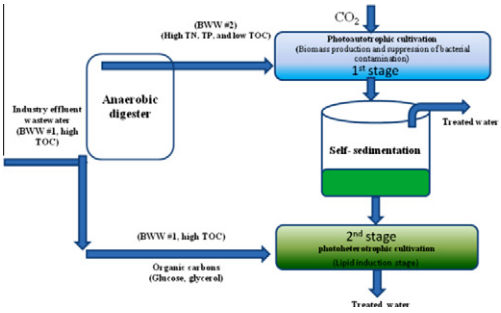
HIGHLIGHTS

- Utilization of wastewater before and after anaerobic digestion as nutrient sources.
- A hybrid system to improve algal biofuel economy with wastewater treatment.
- A new two-stage microalgae cultivation mode to increase lipid productivity.
- Better control on bacterial contamination without any pretreatment.

GRAPHICAL ABSTRACT



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ABSTRACT

A cultivation system in the two-stage photoautotrophic–photoheterotrophic/mixotrophic mode was adapted to maximize lipid productivity of two freshwater strains of *Chlorella* sp. grown in brewery wastewater (BWW). The endogenous *Chlorella* sp. isolated from BWW had a higher growth rate than wild-type *Chlorella vulgaris* (UTEX-265) while *C. vulgaris* (UTEX-265) had a higher maximal biomass and lipid contents than that of endogenous *Chlorella* sp., resulting in more than 90% of the inorganic nutrients in both total nitrogen (TN) and phosphorus (TP) was removed during the first stage in the two-stage photoautotrophic–photoheterotrophic mode in each *Chlorella* sp. The maximal biomass and lipid contents of *C. vulgaris* (UTEX-265) for single stage photoautotrophic cultivation were 1.5 g/L and 18%, respectively. Importantly, during two-stage photoautotrophic–photoheterotrophic cultivation for *C. vulgaris* (UTEX-265), the biomass was increased to 3.5 g/L, and the lipid productivity was increased from 31.1 to 108.0 mg/L day.

1. Introduction

Fossil fuels play an important role in current global economics. However, energy crises due to limited supply on the one hand and environmental problems associated with greenhouse-gas emissions on the other are compelling factors driving the search for renewable and environment-friendly biofuel (Pittman et al., 2011). Microalgal biodiesel is attractive as one such alternative (Chisti, 2007). Microalgae, which are among the oldest organisms in the world, not only produce biofuel but also capture CO_2 from the atmosphere. Nutrient and water supplies for microalgal cultivation are the major cost-contributory factors. Indeed, due to the high cost and energy associated with chemical fertilizer as a source of nutrients, and given the relative unavailability of fresh water, inexpensive and easily accessible alternatives are necessary for

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sustainable microalgal-based biofuels (Lam and Lee, 2012). Microalgae can perform the dual role of wastewater bioremediation and lipid-containing biomass production (Rawat et al., 2011). Thus, the integration of wastewater treatment with microalgal cultivation for biodiesel production is a promising route toward economical production of biofuel (Clarens et al., 2010). Moreover, the self-sedimentation of microalgae grown in wastewater is an additional advantage that can reduce the cost of microalgal harvesting (Park et al., 2011a,b).

There are various microalgal cultivation modes, among which are those involving photoautotrophic, heterotrophic, and mixotrophic growth conditions (Devi et al., 2012; Liang et al., 2009; Xiong et al., 2010). Cultivation of *Chlorella vulgaris* in these ways has a positive influence on both biomass and lipid productivity (Liang et al., 2009). Mixotrophic cultivation has the advantage over the photoautotrophic and heterotrophic modes in its higher biomass and lipid productivity. Two major problems associated with each of single stage mixotrophic and heterotrophic cultivation in wastewater are the high cost of organic substrate and the growth of unwanted bacteria due to the organic carbon sources (Zheng et al., 2012). Thus, applications of these methods are not possible in real open-pond wastewater systems, particularly where microalgal cultures have to compete with foreign species (Perez-Garcia et al., 2011). In fact, in the case of a real wastewater system, where there are a large number of endogenous bacteria and large volume of wastewater to be treated, the wastewater pretreatment step will be another cost-contributing factor impacting on microalgal biodiesel production. Additionally, the economic feasibility of microalgal biofuel with simultaneous treatment of wastewater is more viable if cultivation is carried out in an open system without any additional pretreatment of wastewater (Chisti, 2007). Due to the continuous threat of invasion from undesired microalgae and bacteria, photoautotrophic cultivation of microalgae, rather than mixotrophic or heterotrophic cultivation, usually is employed in raceway ponds (Park et al., 2011a,b).

Different types of wastewater from industrial, municipal, and agricultural sources have been utilized for microalgal cultivation and nutrients removal (Chinnasamy et al., 2010; Wang et al., 2010, 2012). Due to the complex nature of wastewater however, wastewater treatment efficiencies and microalgal biomass production remain low with high lipid contents. Therefore, extensive research is required in order to improve wastewater treatment and enhance microalgal growth rates to levels sufficient for economic and sustainable microalgae biofuel.

Wastewater has been proposed as an alternative to more expensive organic carbon sources (Abreu et al., 2012). In wastewater facilities, the anaerobic digester, wherein anaerobic bacteria consume organic carbon, is an essential component of wastewater treatment systems. Significantly, wastewaters from brewery wastewater (denoted BWW) contain biodegradable organic carbons that are nontoxic to microalgae.

For the purposes of maximizing lipid productivity, various two-stage cultivation modes have been adopted (Das et al., 2011; Mitra et al., 2012; Ogbonna et al., 1997; Xiong et al., 2010; Zheng et al., 2012). However, in most cases, either artificial media were used or wastewater was pretreated by sterilization or membrane filtration. This is first report using wastewater with our proposed system for simultaneous treatment of wastewater and maximizing lipid productivity. In the present study by contrast, a two-stage cultivation mode was applied using BWW as a source of inorganic nutrients and organic carbons, without any additional pretreatment. Currently, the worldwide volume of brewed beer is about 1339×10^6 hetoliters (hL), for every hL of which, 5–6 hL of wastewater is produced (Fillaudeau et al., 2006). BWW contains sufficient concentrations of total nitrogen (TN) and phosphorus (TP) for microalgal cultivation. Traditionally, BWW was treated in an anaerobic digester to reduce the high organic loads that are a potential source of organic carbons. Specifically, microalgae used organic carbons for their metabolic requirement (Wang et al., 2010). Thus, BWW not only has the potential to serve as a source of inorganic nutrients but also has sufficient organic carbons to be useful as an alternative carbon source.

In the two-stage cultivation mode, microalgal strains are first grown in the photoautotrophic mode in anaerobically digested BWW (denoted BWW #2) for efficient utilization of inorganic nutrients, biomass production, and better control of bacterial growth; then, in the late-exponential phase, the microalgae are exposed to organic carbon in the form of glucose or undigested BWW (denoted BWW #1). This microalgal cultivation system efficiently utilizes both inorganic and organic nutrients from wastewater and suppresses bacterial contamination; as such, it is well suited for large-scale cultivation. During the photoautotrophic growth stage, microalgal biomass is produced, while in second stage, lipid productivity is enhanced under nutrient-limited conditions, i.e., beyond the condition of consumption of nutrients, following exposure in high organic carbons such as glucose or glycerol or especially BWW #1.

The objectives of this study were as follows: (1) design of an effective cultivation mode for bioremediation-based microalgal growth in BWW, (2) maximizing the lipid productivity of microalgal culturing in BWW, and (3) investigation of simultaneous treatment and superior control of bacterial contamination using organic carbons. The two-stage cultivation mode was run for thirteen days with batch experiments in order to evaluate its performance.

2. Methods

2.1. Microalgal culture and growth conditions

Two strains of microalgae, *C. vulgaris* (UTEX-265) obtained from UTEX (Algae Cultural Collection Center) at the University of Texas (Austin, TX, USA), and an endogenous *Chlorella* sp. isolated from the BWW using the serial dilution method. Both microalgal species were maintained on standard Tris–Acetate–Phosphate (TAP) agar plates. The cell cultures of both species were maintained in 250 mL-Erlenmeyer flasks containing standard TAP media at 25 °C under 100 μmol/s m^2 illumination in an orbital shaker agitated at 150 rpm. The cell growths of *C. vulgaris* (UTEX-265) and *Chlorella* sp. were optical density (OD)-measured at 682 and 680 nm, respectively. Cell counts were performed under optical microscopy (Leica Microsystem, model DM2500, Switzerland) with hemocytometer (Marienfeld, Germany). The specific growth rates (μ, day^{-1}) of the microalgae in the exponential phase were calculated according to the equation (Liu et al., 2011)

$$\mu = \frac{\ln X_2 - \ln X_1}{t_1 - t_1}$$

where X_2 and X_1 are the OD values at times t_2 and t_1, respectively.

2.2. Brewery wastewater (BWW) collection

BWW was collected from a brewery in Jeonju, Korea. The samples were collected BWW #1 and BWW #2, and subsequently the samples were then characterized according to their physico-chemical and biological compositions and stored at 4.0 °C for further use. BWW #2 was filtered using an ordinary cloth filter, while BWW #1 was centrifuged at 5000 rpm for 10 min to remove suspended solid (SS) and sterilized prior to microalgal cultivation.