Journal of Environmental Management 184 (2016) 585–595

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Enhanced biomass production through optimization of carbon source and utilization of wastewater as a nutrient source

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ARTICLE INFO

Article history:
Received 1 August 2016
Received in revised form
5 October 2016
Accepted 12 October 2016
Available online 24 October 2016

Keywords:
Wastewater
Microalgae
Glucose
Biomass
Mixotrophy

ABSTRACT

The study aimed to utilize the domestic wastewater as nutrient feedstock for mixotrophic cultivation of microalgae by evaluating appropriate carbon source. The microalgae *Chlorella vulgaris* was cultivated in municipal wastewater under various carbon sources (glucose, glycerol, and acetate), followed by optimization of appropriate carbon source concentration to augment the biomass, lipid, and carbohydrate contents. Under optimized conditions, namely of 5 g/L glucose, *C. vulgaris* showed higher increments of biomass with 1.39 g/L dry cell weight achieving biomass productivity of 0.13 g/L/d. The biomass accumulated 19.29 ± 1.83% total lipid, 41.4 ± 1.46% carbohydrate, and 33.06 ± 1.87% proteins. Moreover, the cultivation of *Chlorella* sp. in glucose-supplemented wastewater removed 96.9% chemical oxygen demand, 65.3% total nitrogen, and 71.2% total phosphate. The fatty acid methyl ester obtained showed higher amount (61.94%) of saturated fatty acid methyl esters associated with the improved fuel properties. These results suggest that mixotrophic cultivation using glucose offers great potential in the production of renewable biomass, wastewater treatment, and consequent production of high-value microalgal oil.

1. Introduction

According to an assessment by the International Energy Agency (IEA), global energy consumption will increase up to 53% by 2030 (Ong et al., 2011), and the total energy utilization of developing nations will surpass that of the developed countries in 2030 (Saito, 2010). Because fossil fuel resources are limited and are consumed expeditiously, it is important to focus on the expansion and improvement of alternative sources of renewable energy. In this regard, microalgae have become promising candidates for biofuel feedstock and have gained considerable attention, due to key features such as high growth rate, high cell density, and high biomass productivity for biotechnological exploitation (Gupta et al., 2015). Microalgal wastewater treatment system are possible substitutes for traditional wastewater treatment systems and can provide the sustainable environmental benefits of sequestering CO_2 to mitigate global warming (Abinandan and Shanthakumar 2015; Bilanovic, 2015). Alternatively, microalgal biomass can be employed in the production of animal feed and fertilizers, as well as in various nutraceutical applications (Barba et al., 2014; Taelman et al., 2015). Apart from extensive use in cosmetics, fine chemicals, and value-added products, microalgae can be also used for energy generation, as biodiesel, bioethanol, or bio-hydrogen fuels, and in photosynthetic microbial fuel cells (Wang et al., 2015a; Zhu, 2015).

Microalgae cultivation can be performed in photoautotrophic, heterotrophic, and mixotrophic modes. Photoautotrophy depends on light to carry out photosynthesis whereas heterotrophy requires organic carbons (e.g. simple sugars). In case of mixotrophy, both autotrophic and heterotrophic conditions are involved (Perez-Garcia et al., 2011). Photoautotrophic cultivation is conventionally a long-established technique and has been extensively adopted for microalgae cultivation. However, photoautotrophic cultivation is not feasible option in wastewater treatment operations, owing to large volumes of wastewater in bioreactor there by restricting light penetration and delimiting the growth. Furthermore, photoautotrophic cultivation is often associated with low biomass productivity and several studies have suggested alteration and refinement

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http://dx.doi.org/10.1016/j.jenvman.2016.10.018
0301-4797/

of the cultivation strategy to enhance biomass productivity (Bhatnagar et al., 2011). In this regard, utilizing wastewater by mixotrophic microalgal cultivation is an effective approach towards lowering the cost of microalgal biomass (Abinandan and Shanthakumar 2015; Gupta et al., 2016a, 2016b). Furthermore, along with increase biomass production, mixotrophic cultivation offers extra benefits of low light requirement and production of photosynthetic metabolites such as phycocyanin, chlorophyll and carotenoids, which is impractical in case of heterotrophic cultivation (Perez-Garcia et al., 2011).

Microalgal wastewater treatment offers substantial benefits over conventional wastewater treatment. The O_2 produced during microalgae photosynthesis provides a disinfecting effect and can considerably reduce the cost of mechanical aeration making the wastewater treatment more effective (Wang et al., 2015b). In addition, the auto-settling property of some prominent microalgae allows reduced use of chemicals pertaining to flocculation process. Furthermore, the biomass generated from such algae-based wastewater treatment systems can concurrently provide dual benefits by nutrient reduction and production of value-added products. Since the performance and effectiveness of algal wastewater treatment differs among various microalgae strain types and also influenced by the characteristics of the wastewater used, it is essential to optimize the wastewater treatment process in order to achieve high biomass growth.

In the present study, the microalga *C. vulgaris* was cultivated in different types of organic sugars to identify the prime carbon source. Subsequently, appropriate concentration of suitable organic carbon was determined to augment biomass production in wastewater. Furthermore, effect on removal of total nitrogen, total phosphate, and chemical oxygen demand (COD) was also evaluated under optimized conditions in wastewater. Finally, a scale-up study was performed in a 3L photobioreactor (PBR) under the optimal concentration of organic carbon source in wastewater to determine the effective nutrient removal and biomass production, along with effect on lipid, carbohydrate, and protein contents of the cultivated microalgae.

2. Materials and methods

2.1. Microalgae cultures, medium, and chemicals

The freshwater strain *Chlorella vulgaris* (FC-16) was acquired from the Korea Marine Microalgae Culture Center (KMMCC; Busan, Korea). The *C. vulgaris* was cultivated in Jaworski's medium (Tompkins et al., 1995). All the experiments were performed at a temperature of 25 ± 2 °C with a light intensity of 12:12 h light/dark cycle for 12 d period at 7.10 pH.

2.2. Wastewater collection and characterization

Raw wastewater was obtained from the preliminary sedimentation unit of a sewage plant in Gangeung, Korea. Table 1 represents the characteristics of the raw wastewater used in the experiments. After the analysis, the wastewater was found to be favorable for treatment with microalgae and removal of available nutrients. The ratio of chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TN) (i.e., 100:20:2) was excessive for the wastewater treatment system, but is recommended for nutrient removal in activated sludge plants. Similarly, the biochemical oxygen demand (BOD_5) and total phosphorus (TP) as well as BOD_5 and PO_4 ratios were found to be reasonably high. Also, the PO_4 and TP ratio was at higher range for municipal wastewater system. The calcium, potassium, and manganese levels did not preclude biological wastewater treatment and were found to be favorable for microalgal growth. The high nutrient contents present can be attributed to the fact that the wastewater was collected from a preliminary sedimentation tank and thus did not undergo secondary treatment.

Table 1
Characteristics of the municipal wastewater.

Average concentration (mg/L)		
Parameters	Raw	After Autoclave
BOD_5	159.63	93
TCOD	209.92	201
TP	9.24	12.8
PO_4	8.19	8.9
TN	40.02	45.1
NH_3	31.38	17.19
Ca^{+2}	60	76–80
Mg^{+2}	13	16
Mn^{+2}	<1	<1

2.3. Experimental setup

The wastewater used in this study was filtered with Whatman™ Grade 1 qualitative filter paper (pore size 11 μm) to remove suspended particles. Further, the wastewater was sterilized in autoclave at 15 psi for 30 min. The temperature for sterilization was maintained at 121 ± 2 °C. Experiments were conducted in various batches using 1 L Erlenmeyer conical flasks. In each flask, 500 mL of wastewater was inoculated with 10% of 7-d-old microalgal culture (initial cell density 0.07 g/L). The entire study was carried out in four sections, described as follows.

In the first set of experiments, various organic carbon sources viz. glucose (10 g/L), glycerol (10 g/L), acetate (10 g/L) and their combinations, in ratio 1:1 (5 g/L carbon 1 + 5 g/L carbon 2) were supplemented in wastewater to determine their effects on biomass growth of microalgae *C. vulgaris*. Control flasks were maintained without addition of any carbon source to wastewater. After finding the optimal carbon source among those tested, the effects of different carbon source concentrations (0, 2, 5, 10, 20, and 30 g/L) were determined to find an optimal concentration to yield the highest possible biomass, lipid, carbohydrate, and protein contents. The total nitrogen, total phosphate, and COD removals were also observed. Finally, a scale-up study was performed in a 3L stirred PBR (0.255 m height, 0.115 m length, and 0.115 m width) to study the nutrient removal and biomass productivity under the optimized concentration of the carbon source. The scale-up study was conducted for 10 d at neutral pH (7.1 ± 0.3), constant temperature (25 ± 2 °C), and at constant light intensity (100 μE/m^2/s), under a 12:12 h light/dark cycle. The PBR culture was agitated with by sparging air, injected with nozzle sparger from a pre-filtered gas using air compressor. The pH was maintained by an automated pH controlling device (Autopilot™ APCEPH1) connected to low voltage peristaltic dosing pump to add acid (0.1 M HCl) or base (0.1 M NaOH). Prior to each experiment, the PBRs and associated inner walls of pipeline were thoroughly cleaned to remove debris, salt deposits and were sterilized as described above.

2.4. Analytical procedures

2.4.1. Determination of growth and biomass productivity

Microalgal growth was monitored gravimetrically by measuring the dry cell weight. Culture samples of 50 mL were placed in preweighed 50 mL polypropylene conical tubes and centrifuged at 3000 g for 15 min. The supernatant was discarded and cell pellets were dried in an oven at 80 °C for 24 h. Dry cell weight was