Bioresource Technology 179 (2015) 624–628

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Effect of food wastewater on biomass production by a green microalga *Scenedesmus obliquus* for bioenergy generation

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HIGHLIGHTS

- A dual strategy for wastewater reuse and biofuel feedstock production.
- Food wastewater improved the growth and lipid productivity of *S. obliquus*.
- Food wastewater supported the algal autoflocculation.

ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form 12 December 2014
Accepted 15 December 2014
Available online 20 December 2014

Keywords:
Scenedesmus obliquus
Biomass
Food wastewater
Bioenergy
Autoflocculation

ABSTRACT

Effect of food wastewater (FW) on the biomass, lipid and carbohydrate production by a green microalga *Scenedesmus obliquus* cultivated in Bold's Basal Medium (BBM) was investigated. Different dilution ratios (0.5–10%) of BBM either with FW or salt solution (NaCl) or sea water (SW) were evaluated. *S. obliquus* showed the highest growth (0.41 g L^{-1}), lipid productivity (13.3 mg L^{-1} day L^{-1}), carbohydrate productivity (14.7 mg L^{-1} day L^{-1}) and nutrient removal (38.9 mg TN L^{-1} and 12.1 mg TP L^{-1}) with 1% FW after 6 days of cultivation. The FW promoted algal autoflocculation due to formation of inorganic precipitates at an alkali pH. Fatty acid methyl ester analysis revealed that the palmitic and oleic acid contents were increased up to 8% with FW. Application of FW improved the growth, lipid/carbohydrate productivity and biomass recovery efficiency of *S. obliquus*, which can be exploited for cost effective production of microalgae biomass.

1. Introduction

Microalgal biomass production is significantly influenced by the presence of inorganic and organic carbon sources in the cultivation medium, which affect the biochemical properties of microalgae (Cheirsilp and Torpee, 2012). Autotrophic microalgal growth for biodiesel production has gained much attention, but insufficiency of light at high cell concentrations limits its application for high cell density biomass production (Liang et al., 2009; Hu et al., 2012). Mixotrophic growth occurs when the microalgae are provided with both inorganic (CO_2) and organic carbon (e.g., glucose and acetate) sources (Wang et al., 2012). This can significantly reduce the dependence of microalgae growth on light, which is observed under pure photoautotrophic conditions, and stimulate the algal growth and increase the cell density (Wang et al., 2012). However, the additional cost of organic carbon will make the mixotrophic cultivation economically unfeasible (Bhatnagar et al., 2011), which will further increase the cost of biofuel production and impede its commercialization.

Microalgae cultivation using organic wastewaters for simultaneous biomass production and wastewater treatment has been reported as a cost effective strategy (Bhatnagar et al., 2011; Ji et al., 2014a). Food effluent is one of the most highly produced wastewaters throughout the world, and its treatment has emerged as a social issue by the law of London Dumping Convention in South Korea. Food wastewater (FW) is rich in nutrients including nitrogen, phosphorus, calcium, iron, aluminum and total organic carbon, which might be an efficient wastewater feedstock for mixotrophic microalgal cultivation to achieve a reasonably high biomass yield and lipid productivity for energy generation (Shin et al., 2014).

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http://dx.doi.org/10.1016/j.biortech.2014.12.053
0960-8524/

Several studies have reported the application of municipal and piggery wastewaters with synthetic CO_2 for biomass and bioenergy production (Zhou et al., 2011; Wang et al., 2012). FW has not been investigated extensively for microalgal cultivation. The present study investigated the effect of food wastewater on biomass production, lipid productivity and fatty acid composition of *Scenedesmus obliquus* cultivated in Bold's Basal Medium (BBM) supplemented with CO_2 as flue gas.

2. Methods

2.1. Algal strain, culture conditions and inoculum preparation

S. obliquus was isolated from acid mine drainage (AMD) (Donghae, South Korea) and was registered in GenBank under Accession No. HE861884. The microalga was inoculated in 2 L reactor containing 1.7 L Bold's Basal Medium (BBM) at 10% concentration ($V_{inoculum}/V_{media}$) (Bischoff and Bold, 1963). The microalgal cells were incubated under white fluorescent light illumination at a light intensity of 120 μmol photon m^{-2} s^{-1} at 25 °C for 10 days. During the incubation, each column was aerated with filter-sterilized air at a flow rate of 0.5 L min^{-1} to agitate the suspension and to simultaneously supply CO_2 (0.03%). Three milliliters (OD_{680} = 1.5) of microalgae were used as initial inoculums for further experiments.

2.2. Algae cultivation and growth

The BBM was diluted with each of food wastewater (FW) and NaCl/sea water (SW) (control), to give five different concentrations (0.5%, 1%, 3%, 5% and 10%), i.e., a total of sixteen solutions including undiluted BBM were prepared, and the volume ratio of the BBM to each of the diluents were as follows: 100:0 (undiluted wastewater), 99.5:0.5 (BBM or FW/NaCl/SW), 99:1 (BBM or FW/NaCl/SW), 97:3 (BBM or FW/NaCl/SW), 95:5 (BBM or FW/NaCl/SW) and 90:10 (BBM or FW/NaCl/SW). SW and NaCl were used to compare the effect of total organic carbon from the FW on the microalgae growth based on concentration of same salinity. The batch experiments were conducted using 500 mL aluminum crimp sealed serum bottles containing 300 mL BBM, inoculated with 1.5% ($V_{inoculum}/V_{wastewater}$) of microalga solution and supplemented with 5.1% flue gas CO_2. The bottles were incubated in a shaker incubator at 150 rpm, 27 °C, under white fluorescent light illumination (alternate light/dark periods of 16 h/8 h) at an intensity of 120 μmol photon m^{-2} s^{-1} for 6 days.

2.3. Analytical methods

FW was collected from a food wastewater treatment plant at Dangjin, South Korea and sea water (SW) from gyeongpodae at Gangneung, South Korea. Wastewaters were immediately filtered using 0.2 μm nylon micro filters to remove the microorganisms and suspended solid particles. The physicochemical properties of the FW were analyzed (Table 1). The analytical methods of TN, NH_4-N, TP, anions (i.e., NO_2^-, NO_3^- and PO_4^{3-}), metal ions (i.e., Ca^{2+}, Mg^{2+} and Fe_{total}), total organic carbon (TOC) and pH were described by Ji et al. (2014a). Other analytical methods are detailed in Supplementary Information.

3. Results and discussion

3.1. Growth assessment of S. obliquus

FW supported higher microalga growth than the undiluted BBM and NaCl/SW diluted BBM after 6 days of cultivation (Fig. 1). The highest dry cell weight (0.41 g dwt L^{-1}) was observed for FW 1, which was 2.8 times higher compared to the undiluted BBM due to highest removal of nutrient (38.9 mg L^{-1} T-N and 12.1 mg L^{-1} T-P) (Supplementary Fig. 1). The TOC concentrations (TOC = 75–149 mg L^{-1}) in FW 0.5 and FW 1 were favourable for the microalgal growth. Shin et al. (2014) reported that the TOC (83% acetate, 15% lactate and 2% propionate) from FW (Table 1) could be utilized by some algae species for rapid growth under mixotrophic or photoheterotrophic mode in light. Acetic acid (or acetate) is one of the most commonly used organic carbon substrate by the mixotrophic or heterotrophic cultures of microalgae (Hu et al., 2012; Liu et al., 2013). Shin et al. (2014) reported that optimal microalgal growth was observed in 78 mg L^{-1} of acetate from food wastewater, and acetate above 400 mg L^{-1} might be toxic for microalgal growth as reported in literatures (Chen and Johns, 1996). The mixotrophic microalgal culture can simultaneously assimilate the inorganic and organic substrates through concurrent respiratory and photosynthesis processes (Bhatnagar et al., 2011). The growth rate of mixotrophic culture is the sum of the photoautotrophic and heterotrophic growth (Liang et al., 2009). The algae cultures exhibit a higher growth under CO_2 conditions in the presence of organic carbon substrates (i.e., acetate and glucose) (Liang et al., 2009; Hu et al., 2012). Shin et al. (2014) reported that the highest growth of *Scenedesmus bijuga* was observed in 20 times diluted anaerobically digested food wastewater. But, the biomass production was similar to that observed in synthetic media (BG-11), which might be due to lack of inorganic carbon for mixotrophic cultivation.

Concentrations higher than FW 1 decreased the microalgal growth, which might be due to toxicity of salinity, TOC and aluminium from FW (Liang et al., 2009; Salama et al., 2013). The formation of inorganic precipitate occurred during cultivation in FW 0.5 to FW 10, which might coat the microalgae cell surface and decrease the nutrient uptake at above FW 3 (Ji et al., 2014a). Increase of NaCl and SW concentration in the culture media from 0% to 3% (NaCl/SW) slightly increased the microalga growth. Sodium ion facilitates photosynthesis in microalgae through inorganic nutrients uptake, intracellular pH regulation, and

Table 1
Physico-chemical characteristics of (a) NaCl, (b) sea water and (c) food wastewater.

Parameter	(a) NaCl	(b) SW	(c) FW
pH	6.7	7.83	6.0[a]
T-N (mg L^{-1})	ND[b]	0.27 ± 0.02	1385 ± 1.9
NH_4-N (mg L^{-1})	ND	ND	1197 ± 1.1
NO_3-N (mg L^{-1})	ND	0.25 ± 0.01	105.4 ± 0.9
NO_2-N (mg L^{-1})	ND	ND	45.2 ± 2.2
T-P (mg L^{-1})	ND	0.03 ± 0.01	108 ± 0.6
PO_4-P (mg L^{-1})	ND	0.04 ± 0.01	91.6 ± 0.73
TOC (mg L^{-1})	0.1 ± 0.03	20.3 ± 0.12	14,898 ± 0.54
Salinity (%)	3.7[c]	3.7	3.7[c]
Metallic ions			
Al^{3+} (mg L^{-1})	ND	0.018 ± 0.002	316.4 ± 0.13
B^{3+} (mg L^{-1})	0.016 ± 0.007	4.49 ± 0.10	0.511 ± 0.007
Ca^{2+} (mg L^{-1})	ND	387.4 ± 1.17	1055 ± 2.01
Cd^{2+} (mg L^{-1})	ND	ND	ND
Co^{2+} (mg L^{-1})	ND	ND	ND
Cr^{6+} (mg L^{-1})	ND	ND	ND
Cu^{2+} (mg L^{-1})	ND	ND	ND
Fe (total dissolved) (mg L^{-1})	ND	ND	24.7 ± 0.06
Mg^{2+} (mg L^{-1})	ND	1118.3 ± 1.95	97.01 ± 1.24
Mn^{2+} (mg L^{-1})	ND	ND	1.89 ± 0.05
Pb^{2+} (mg L^{-1})	ND	0.03 ± 0.01	0.05 ± 0.01
S (mg L^{-1})	ND	947.95 ± 0.88	122.07 ± 0.91
Zn^{2+} (mg L^{-1})	ND	ND	1.29 ± 0.08

[a] After adjusted of pH with NaOH.
[b] ND: not detected.
[c] After adjusted of salinity.