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Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources

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HIGHLIGHTS

- We applied wastewater from a municipal WWTP for microalgae cultivation.
- The isolates originated from an anaerobic digester grew without a lag period.
- The high ammonia concentration in anaerobic digester effluent should be diluted.
- The highest biomass productivity was obtained from a combined wastewater.
- The effluent of a 1st settling tank enhances separation between solid and liquid.

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ABSTRACT

In order to reduce input cost for microalgal cultivation, we investigated the feasibility of wastewater taken from a municipal WWTP in Busan, Korea as wastewater nutrients. The wastewaters used in this study were the effluent from a primary settling tank (PS), the effluent from an anaerobic digestion tank (AD), the conflux of wastewaters rejected from sludge-concentrate tanks and dewatering facilities (CR), and two combined wastewaters of AD:PS (10:90, v/v) and AD:CR (10:90, v/v). *Chlorella* sp. ADE5, which was isolated from the AD, was selected for the feasibility test. The highest biomass production (3.01 g-dry cell weight per liter) of the isolate was obtained with the combined wastewater ADCR, and it was 1.72 times higher than that with BG 11 medium. Interestingly, the cells cultivated with wastewater containing PS wastewater were easily separated from the culture and improved lipid content, especially oleic acid content, in their cells.

1. Introduction

For sustainable support of modern society, it is urgent to develop new energy sources other than petroleum-based energy sources. Biodiesel production from microalgae has been expected to replace the petroleum-based energy sources owing to its high areal productivity and lipid contents due to the higher energy yield: 7–31 times higher than palm (Kent and Andrews, 2007) and several tens–several hundred times than other oily plants such as corn, soybean, canola, jatropha, coconut and oil palm (Chisti, 2007).

However, large-scaled algae cultivation for bioenergy production should be significantly progressed to compete with the cost of energy production from other resources, especially petroleum based fuel. The difference between the cost of bioenergy production from microalgae and that from present fossil-based energy is still the main hurdle that blocks for industrialization of the microalgal bioenergy (Kovacevic and Wesseler, 2010). The main expenditure would have to cover the costs of large amount of water with nutrients supplement, biomass harvest, lipid extraction from biomass and additionally illumination for high biomass production required to promote their photosynthesis.

In order to reduce the cost for fertilizer and freshwater cost as well as reducing green house gases, it has been suggested to integrate algal cultivation and wastewater treatment with CO_2 supplementation (Clarens et al., 2010; Pittman et al., 2011).

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Nutrients concentration and composition affect biomass and lipid productivity in microalgae culture; lipid accumulation with nitrogen (below 2.5 mg L^{-1}) and phosphorus (below 0.1 mg L^{-1}) limitation (Xin et al., 2010), the increase of lipid content and productivity on urea feeding time (Hsieh and Wu, 2009), the differences of dry biomass and lipid productivity of microalgae cultivated at different salinities (Araujo et al., 2011) and nitrogen sources (Li et al., 2008; Cho et al., 2011b), effect of organic matters on biomass and lipid production (Heredia-Arroyo et al., 2010) and the differences under different trace metals (Abou-Shanab et al., 2012).

Several studies have already focused on utilizing wastewaters in wastewater treatment plant for microalgae cultivation (WWTP) (Park et al., 2010; Wang et al., 2010). However, many things should be understood and controlled more practically for stable application of wastewaters as a nutrient source for mass-culture of microalgae. For an example, many studies have underestimated bacterial activities since the wastewaters are autoclaved or filtered by using a smaller pore size of filter to remove bacterial activities (Gonzalez et al., 1997; Wu et al., 2012; Zhou et al., 2012).

We investigated the effects of wastewaters collected from the effluent from a primary settling tank (PS), the effluent from an anaerobic digestion tank (AD), the conflux of wastewaters rejected from unit-processes (CR), and artificially two combined wastewaters of AD:PS (10:90, v/v), AD:CR (10:90, v/v) as nutrient additives for microalgae cultivation and biomass productivity after harvest from a municipal WWTP. All wastewaters were used after filtration with 1.2 μm-pore size GF/C filter (Whatman co.).

2. Methods

2.1. Wastewaters

The wastewaters used in this study were collected from the effluent line of a primary settling tank (PS), the effluent line of an anaerobic digestion tanks (AD), and the conflux line of the wastewaters (CR) rejected from the 1st and 2nd sludge-concentrate tanks (77.6%, v/v) and the dewatering water of anaerobic digester sludge (22.4%, v/v) at the Su-young WWTP in Busan, Korea. The effluent of AD was taken before adding flocculants to enhance dewatering efficiency of the sludge from anaerobic digester.

Several dilution ratios (2, 3.5, 5, 10, 20 times) of the AD wastewater using tap water were tested because microalgal growth in the raw AD wastewater was inhibited at our previous experiment (Supplementary 1). High ammonia toxicity may result in the growth inhibition in the raw AD wastewater (Kallqvist and Svenson, 2003; Li et al., 2008), and ten folds dilution was determined as the suitable dilution ratio of the AD through the repeated experiments. Then, the tap water was replaced by the wastewater from PS or CR for practical application of the wastewater for microalgae cultivation. Hereafter, the mixed wastewaters of with 10% (v/v) AD wastewater in PS (90%, v/v) and CR (90%, v/v) are referred as ADPS and ADCR, respectively.

All wastewaters were filtered using 1.2 μm-pore size GF/C filter (Whatman co.), through which most eukaryotes would be removed but a part of bacteria and cysts of protozoa remain (Madigan et al., 2012), before they were applied for microalgae cultivation.

2.2. Determination of microalgae growth

Biomass production in biological processes has been often expressed as a dry cell weight (DCW) by measuring the total suspended solid (TSS) concentration in cultures by following standard methods (APHA et al., 2005). However, low concentration of biomass (<1 g L^{-1}) measured by using a small volume is somewhat error-prone. Instead of TSS in the cases, the optical density (OD) at 730 nm was measured by the Spectrophotometer (Spectronic Instruments 20D+, USA), and the light absorbance of culture suspension was transferred to microalgal cell density by using a suitable standard curve expressed as the following equation. The relationship equation between dry cell weights and ODs was determined by pre-experiments as:

$$\text{Dry cell weight (mg-DCW L}^{-1}) = 869.4X - 18.1 \quad (R^2 = 0.9718)$$

where X refers to the OD at 730 nm and R^2 refers to the regression coefficient.

2.3. Isolation and identification of microalgae species from the AD

Chlorella sp. 227, obtained from the Culture Collection of National Institute for Environmental studies (NEIS) in Japan, was used for a control strain and maintained in a modified BG 11 medium reported by Stanier et al. (1971). The medium consists of the following components; $NaNO_3$ (1.5 g L^{-1}), K_2HPO_4 0.04 (g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.075 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.036 g L^{-1}), citric acid (0.01 g L^{-1}), ferric ammonium citrate (0.006 g L^{-1}), $Na_2{\cdot}EDTA$ (0.001 g L^{-1}), Na_2CO_3 (0.02 mg L^{-1}) and 1 mL of trace metal solution per liter. The trace metal solution contains H_3BO_3 (61.0 mg L^{-1}), $MnSO_4{\cdot}H_2O$ (169.0 mg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (287 mg L^{-1}), $CuSO_4{\cdot}5H_2O$ (2.5 mg L^{-1}), and $(NH_4)_6MoO_4{\cdot}4H_2O$ (12.5 mg L^{-1}).

To get domestic microalgae strains to grow fast in the AD wastewater, several microalgae were isolated from the AD wastewater by a plate method. The AD was diluted serially and spread on BG 11 agar plates. Then, the plates were incubated at a light incubator of 25 ± 1 °C illuminating of 200 μmol m^{-2} s^{-1} with 16:8 h light dark cycle (Jeiotech, GC-300TLH). The individual colonies were microscopically observed, and the partial 18S rRNA sequence of the selected isolate, ADE5, for further experiments was analyzed for its classification. The primers to amplify the partial 18S rRNA gene of the selected isolate were the forward primer of P45 (5′-ACC TGG TTG ATC CTG CCA GT-3′) and the reverse primer of P47 (5′-TCT CAG GCT CCC TCT CCG GA) (Dorigo et al., 2002). The sequence was used for identification through the BLAST search at NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/).

2.4. Microalgal cultivation in different wastewaters

Flask culture was done in 1 L Erlenmeyer flasks containing the AD wastewater of 300 mL as the working volume at a shaking incubator (HB 201 SF-L Hanbaek-Scientific Co) of 25 ± 1 °C, rotation speed of 160 rpm and continuous illumination with 60 μmol m^{-2} - s^{-1} cool-white fluorescent light. The growth of five isolates was compared with that of *Chlorella* sp. 227 through the flask culture at which 0.05 M sodium hydrogen carbonate was added in the wastewaters as the inorganic carbon source.

The isolate, *Chlorella* sp. ADE5 which showed the fastest biomass production among the isolates, was selected and cultivated with the different wastewaters in a cylindrical photo-bioreactor of 1 L (inner diameter 5 cm, height 75 cm, working volume 700 mL). At all cultivation experiments, the initial concentration of the microalgae was controlled by 0.12–0.13 optical density (OD) at 730 nm, which corresponds to 1.2–1.3 × 10^6 cells per mL. The cool-white fluorescent light of 200 μmol m^{-2} s^{-1} was illuminated to the reactor continuously and the gas of 1% CO_2 (0.4 L min^{-1}) supplemented by air was supplied from bottom of the reactor. The initial pH and temperature were controlled to pH 7 ± 0.1 and 30 ± 2 °C. The biomass of the culture was periodically sampled and used to analyze the production of biomass and