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Photoautotrophic cultivation of mixed microalgae consortia using various organic waste streams towards remediation and resource recovery

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ABSTRACT

In this study, mixed microalgae consortia was cultivated using digestate (D), animal manure (AM) and textile wastewater (TW) as growth medium providing mainly N (nitrogen) and P (phosphorous) sources without any extra nutrient addition. The corresponding total nitrogen (TN) and total phosphorous (TP, PO3-P) concentrations were noted as 323 and 21 for AM, 481 and 31 for TW and 747 and 55 mg/L for D, respectively. After a cultivation period of 13 days, P were completely removed (100%), however, N was still remain and attained the removal rate of 70.1, 72.3 and 16.7% for TW, AM and D, respectively. The peak growth rate and biomass production of 0.419 d^{-1} and 0.4 g/L (in terms of volatile solids, VS) was achieved using TW as growth medium.

1. Introduction

Global energy-related CO_2 emission increase significantly until 2013. Due to this growth of emissions, renewable energy plans were came into action a result of that the preliminary data in 2014 shows the slowdown of the emissions as the green/renewable energies are growing and resulting in less CO_2 level in atmosphere. The global development of renewable energy is a great success story as 44 of 58 countries ranked have the double-digit growth rates (BP, 2016; Burck et al., 2015).

Dwindling petroleum resources and global warming has raised the awareness about clean energies especially, biofuel production technologies using agricultural, forestry, and cellulosic based biomass. Although, it is not sustainable as it has been pointed to be the challenge of 'food vs fuel' (Behera et al., 2015). In this spotlight, microalgae have gained more attention to researchers considering as one of the popular feedstocks for biofuel production mainly because of its potential towards high rapid productivity of biomass and could be grown on most of the water sources (fresh, brackish, saline and wastewater) (Jagger, 2011; Stephens et al., 2010). As well as, most of the algae rich in lipid content in their cell and capable of producing energy-rich oil, and microalgae dry biomass naturally contain high oil level (Abdel-Raouf et al., 2012; Hannon et al., 2010). Furthermore, algae can be grown in either be autotrophic or heterotrophic mode using various organic and inorganic carbon sources. The autotrophic algae require only inorganic compounds such as CO_2, salts, and a light energy source and utilize those substance to conduct biomass growth, while the heterotrophs are non-photosynthetic, which external source such as organic compounds are required as well as nutrients as energy sources (Brennan and Owende, 2010; Kumar et al., 2017).

Moreover, conversion technologies from algae biomass have been studied for the eco-friendly processes producing different many kinds of biofuels such as biodiesel, bioethanol, biogas and other valuable co-products (Ganesh et al., 2017; Vijayan et al., 2016; Zhen et al., 2016). Several advantages in algal biofuels have been regarded to replace liquid petroleum fuel as a low land requirement for high rate biomass productivity as well as the contribution to sustainability (Behera et al., 2015; Kruse and Hankamer, 2010). Recently, some researchers have conducted studies on cultivation technique throughout different kind of growth medium which enhance more the biomass productivity for novel selection feedstock for microalgae (Chia et al., 2013). Most of the studies have pointed out Basal Bold's medium giving better growth, however in terms of scale up and mass production in industrial level that will be not feasible. Thus, finding more cheaper nutrient sources are highly recommended and also eco-friendly (Cea-Barcia et al., 2014)

Overlook through microalgae and wastewater, microalgae has played an important role for treating organic and inorganic wastewater, especially, since microalgae cultivation has been applied to many kinds of wastewater treatment facilities (Wang et al., 2010b). Moreover, It has the ability to eliminate some toxic organic compounds and the

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Table 1
Physical/chemical characterization of various organic waste streams (growth medium) used in this study.

Growth medium used	pH	TS g/l	VS g/l	tCOD g/l	sCOD g/l	TN mg/l	TP mg/l
Textile wastewater	8.89	5.11 ± 0.03	3.24 ± 0.05	1.9 ± 0.1	0.75 ± 0.05	480.5	31
Animal Manure	8.83	27.92 ± 0.47	16.18 ± 0.24	28.1 ± 0.1	18.45 ± 0.05	323	21
Digestate	8.84	14.68 ± 0.64	13.04 ± 0.16	26	16.85 ± 0.15	746.5	55

potential of breaking down and utilize nutrients in wastewater to give high-value production of biomass itself (Abdel-Raouf et al., 2012; El-kassas, 2014; Monfet and Unc, 2016). Since microalgae cultivation strategies were promising to produce sustainable energy for future aspects, reasonable and reached nutrient growth mediums were still a big challenge.

To counter these above mentioned issues, this study aimed to investigate the possibility of textile wastewater, animal manure, and digestate as a nutrient source for microalgae cultivation, further evaluation on their treatment efficiencies and algae biomass production could be further proficient regarding consideration of eco-friendly and environmentally friendly cultivation strategies.

2. Materials and methods

2.1. Collection and cultivation of mixed microalgae consortia

Mixed microalgae sample was collected from an opened wastewater pond which is located nearby cafeteria in Daegu University, Daegu, South Korea. The culture was collected with 5 L sampling bag, then stored inside 4°C cold temperature room. Before used, in real experiments collected sample was cultivated in Basal Bold's Medium which is commonly used for freshwater microalgae. The first culture cultivation was designed in a way that consisted of 200 mL working volume in a 250 mL conical flask with 30°C in the growth chamber to get more biomass. pH was maintained in between 8.0 and 9.0 in order to enhance growth and avoid bacteria contamination. Cossio et al., reported that microalgae was genially cultivated within the pH range 7–9 but the optimum pH condition was between 8.2 and 8.7 (Cossio et al., 1996). Light intensity inside the growth chamber was 212.77 mol m^{-2} s^{-1} with the 12 h light: 12 h dark cycle. Further it has been transferred 1 L plastic bottles and subsequently to 3 L bags (more details are given in the next section) to Moreover, Microscopy observation was performed to evaluate the microalgae species. 4 Subcultures were performed continuously resulted in almost only monoculture of *Chlorella species* was found before the study. In this study, mixed microalgae were designed consisted of 3 L working volume with 3 different organic wastewaters (textile wastewater, animal manure, and digested sludge) using 4 L sampling bag with an aeration rate of 2 L/min. Culturing process was shown in Supplementary Fig. 1 (Fig. S1). After the final sub culturing, microscopical analysis revealed that the consortia are highly dominated by *Chlorella* Species and small portions followed by *Scenedesmus* sp.

2.2. Experimental setup

In this study, mixed microalgae were cultivated inside the 4 L (working volume, 3 L) transparent plastic sampling bag with a dimension of 25 cm length, 11.8 cm width and 24 cm height which is allowed light to penetrate inside photo-bioreactor easily. 300 mL of fresh microalgae consortia was mixed with 2.7 L of various organic substrates (textile wastewater, animal manure, and digested sludge) to make up the final volume of 3 L. The experimental configuration was shown in Supplementary Fig. 2 (Fig. S2). Photobioreactors frame was designed with the steel frame of 15 L length × 4 L width × 4 L height with disposable cardboard covered the whole structure as walls in order to prevent light escaping. The light source was provided with 3 LED lights (SS light 50 W, model KFL 50 white color) fixed with a timer to control the light cycle. Light cycle was performed with 12 h dark and 12 h light. As well as, the Light intensity was measured at the photo-bioreactor outside the surface area by digital portable light lux meter (UYIGAO, model UA1010B, China) resulted in 212.77 ± 22.22 mol m^{-2} s^{-1}. Aeration pump (AMAZONPET, model SH-A2, China) was installed with stone sparger in order to provide CO_2 needed for microalgae photosynthesis. As well as, pH was noted in the range between 8.0 and 9.5.

2.3. Composition and characterization of TW, AM and D

In order to enhance cost-effective strategy for growth medium challenge, mixed microalgae were cultivated with three kinds of nutrient source to evaluate the growth rate. Textile wastewater, animal manure, and digested sludge were selected as growth medium as they were enriched of nutrients composition. Nutrients composition of each substrate is shown in Table 1. Due to the high concentration of organic content animal manure and digestate was decided to be diluted in order to reduce the amount of initial composition such as TSS and TCOD. However, as the initial concentration of nutrient was low, animal manure and digestate could only be diluted twice.

2.4. Regression and statistical calculations

This study was carried out in triplicate. The statistical analyses were conducted using Microsoft Office (Excel 2013). Results were shown as mean value ± standard deviation.

2.4.1. SD and mean values

Standard deviation Equation $\sigma = \sqrt{\frac{1}{N}\sum_{t=1}^{N}(x_i-\mu)^2}$

whereas x_i = individual data
μ = mean values
N = Number of sum

2.4.2. Growth rate

The growth rate of the exponential phase was analyzed by following equation:

$$\mu_0 = \frac{Ln(ABS_{exp}) - Ln(ABS_0)}{t_{exp} - t_0}$$

where, ABS_0 = absorbance at 680 nm at the beginning of the experiment
ABS_{exp} = absorbance at 680 nm at the exponential phase
t_{ext} = Exponential time (days)
t_0 = Beginning time (days)

2.4.3. Organic removal efficiency equation

$$Efficiency = \frac{C_i - C_f}{C_f} \times 100$$