



# Parametric study of brewery wastewater effluent treatment using *Chlorella vulgaris* microalgae

Hee-Jeong Choi<sup>†</sup>

Department of Energy and Environment Convergence, Catholic Kwandong University, Beomil-ro 579, Gangneung 25601, Republic of Korea

## ABSTRACT

The aim of this study was to evaluate the biomass and lipid production of *Chlorella vulgaris* and its nutrient removal capability for treatment of brewery wastewater effluent. The results indicate that the maximum biochemical oxygen demand (BOD) (91.43%) and chemical oxygen demand (COD) (83.11%) were removed by *C. vulgaris* with aeration in the absence of light. A maximum of 0.917 g/L of dry biomass was obtained with aeration in the dark conditions, which also demonstrated the highest amount of unsaturated fatty acids at 83.22%. However, the removal of total nitrogen (TN) and total phosphorus (TP) with these aeration and light conditions was 9.7% and 11.86% greater than that of other conditions. The removal of BOD and COD and the production of biomass and lipids with aeration in the dark and the TN and TP removal with aeration and light were more effective than other conditions in the brewery wastewater effluent in the presence of *C. vulgaris*.

**Keywords:** A Biomass, Brewery wastewater, Lipid content, Microalgae, Nutrient removal

## 1. Introduction

The brewery industry uses large quantities of water and generates enormous amounts of wastewater. The amount of brewery wastewater depends on the production type and the specific water usage [1, 2]. Recently, brewery wastewater production in Korea has increased to account for up to 12% of the total wastewater. The primary characteristics of brewery wastewater are high organic matter content, no toxicity, low content of heavy metals, and easily biodegraded [3]. However, wastewater from these industries can pose a serious risk to human beings, the environment, and aquatic life if it is not properly treated prior to consumption or disposal. Therefore, the treatment and safe disposal of brewery wastewater have become important aspects in brewery operations [4].

Typically, brewery wastewater treatment has included aerobic treatment and, more recently, anaerobic systems combined with aerobic treatment as attractive options for energy recovery and highly efficient nutrient removal. Compared to other methods, biological methods have the advantage of high efficiency of chemical oxygen demand (COD) and the biochemical oxygen demand (BOD) removal, low investment cost, and an eco-friendly system. In addition, many different methods have been applied for the treatment of brewery wastewater, such as quenched plasma [5],

upflow anaerobic sludge blanket (UASB) [6], membrane bioreactor (MBR) [7], the electrochemical method [8], microbial fuel cell [9], anaerobic sequencing batch reactor (ASBR) [10], and reverse osmosis [11]. These various methods have been reported to remove 73-98% of the nutrients in brewery wastewater. After treatment, BOD, COD, and suspended solids (SS) of treated brewery wastewater have been shown to be markedly reduced by the methods noted above. However, brewery effluent still contains nitrogen, phosphorus, and other nutrients. Many studies have reported that a 246-931 mg/L COD, 80-180 mg/L BOD, 10-34 mg/L total nitrogen (TN), and 5-27 mg/L total phosphorus (TP) remain from initial concentrations of 2,300-6,700 mg/L, 1,500-3,900 mg/L, 38-89 mg/L, and 34-57 mg/L of COD, BOD, TN, and TP, respectively [12, 13]. Regarding the potential of using microalgae for brewery wastewater treatment few studies still exist. The possibility of reducing the nutrient using *Chlorella vulgaris* by Raposo et al. [14] studied, concluding that it is an effective treatment more than 90% reduction of BOD and COD was obtained. However, Matat et al. [12] investigated the potential of using microalga *Scenedesmus obliquus* for a brewery wastewater treatment and biomass production. It reported that the maximum of 0.9 g of dry biomass per liter of culture after 9 d, and 57.5% of COD and 20.8% of TN removal after 14 d.



This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2016 Korean Society of Environmental Engineers

Received February 10, 2016 Accepted July 25, 2016

<sup>†</sup> Corresponding author  
Email: [hjchoi@cku.ac.kr](mailto:hjchoi@cku.ac.kr)

Tel: +82-33-649-7297 Fax: +82-33-647-7635

Due to the ability of microalgae to use wastewater as fuel for growth, microalgae are particularly useful for the reduction of concentrations of inorganic nitrogen and phosphorus in wastewater [15, 16]. Microalgae cultures in wastewater can significantly contribute to the management of water ecosystems by providing an inexpensive, environmentally friendly system for wastewater treatment. Using brewery effluent for microalgal cultures is beneficial for minimizing the use of freshwater, reducing the cost of nutrient addition, removing the remaining nitrogen and phosphorus, and producing microalgal biomass as bioresources for biofuel or high-value by-products [17]. However, there are many disadvantages of this method, e.g., microalgae cultivation in brewery effluent requires suitable temperatures, air, pH, and light, though the neutral pH (6.1-8.5) and temperature (28-32°C) in brewery effluent are suitable for microalgae cultivation. Few studies have investigated the potential use of microalgae for brewery wastewater treatment. Therefore, the objective of this study was to provide suitable light and air for the efficient treatment of nutrients and the production of biomass in brewery effluent. An additional objective of the present study was to provide a robust method for the economically efficient treatment of brewery effluent on a large scale with high yield using the smallest possible amount of chemicals.

## 2. Materials and Methods

### 2.1. Characteristics of Brewery Wastewater Effluent

Brewery wastewater effluent was collected from Breweries Ltd., located in Gangneung city in Korea. The company produces large amounts (approximately 1,500 m<sup>3</sup>/d) of wastewater. The water quality characteristics of the brewery wastewater are summarized in Table 1. The ratio of COD and BOD ranged from 1.78 to 2.30 in both the influent and effluent, respectively. The average pH was relatively low and measured at 3.3-5.1 for the influent tank and 6.1-8.5 for the effluent.

### 2.2. Experimental Design

The nutrient removal is basically an effect of assimilation of nutrient as the microalgae grow. The growth rate of microalgae is influenced by physical, chemical and biological factors [1]. Examples of physical factors are light and temperature. Chemical factors can be availability of nutrients and carbon dioxide, and biological factors

are e.g. competition between species, predation by zooplankton and pathogens (bacteria, fungi, viruses) infections [12, 15]. In order to evaluate the brewery wastewater effluent treatment by microalgae as well as the microalgae growth rate and to monitor the reduction in wastewater contaminants, the brewery wastewater was used as a culture medium for microalgae, *C. vulgaris* (KMMCC-143). *C. vulgaris* is a good source of ethanol due to its high starch content (~37% dry wt.) and up to 65% ethanol conversion efficiency [18]. For the seed culture, *C. vulgaris* (FC-16) cells were cultivated in Jaworski's medium (JM) [19], which was prepared using deionized water under LED lamps at ambient temperature. In JM medium, 100 mL of cultured *C. vulgaris* was added to 1 L of brewery wastewater effluent. The initial concentration of the inoculated *C. vulgaris* was  $0.367 \pm 0.06$  g/L. The experiments were conducted at a neutral pH ( $7.3 \pm 0.4$ ) under 8 h dark and 16 h light cycles, with the samples shaken at a rate of 80 rpm on an orbital shaker. The temperature was maintained at 28-32°C for 15 d. Continuous illumination was maintained at a light intensity of approximately 200-220  $\mu\text{mol photons/m}^2/\text{s}$  on the flask surface. For the samples grown in the absence of light, the flasks were wrapped in aluminum foil. The samples were aerated continuously at a rate of 0.5 L/min. CO<sub>2</sub> at an equivalent aeration rate of 0.02 vvm was used for cultivation. CO<sub>2</sub> with a flow rate of 0.74 L/min was introduced into the reactor. The parameters investigated in this study were as follows:

- light (without and with light of 200-220  $\mu\text{mol photons/m}^2/\text{s}$  in 8 h dark and 16 h light cycles) and
- aeration (without and with an average aeration rate of 0.5 L/min).

### 2.3. Analytical Methods

To determine the biomass concentration, a sample of microalgae in growth medium was centrifuged for 10 min at 628 g, washed with distilled water, and then dried in an oven at 105°C for 24 h to a constant weight. The algal biomass for lipid extraction was prepared through centrifugation and drying. After oven drying, the algae were pulverized and subjected to Soxhlet extraction. All of the Soxhlet extractions were performed for 72 h using 500 mL solvent for 1 g of pulverized dry algae with a cycle time of 10-15 min. Soxhlet extraction with hexane was selected because the method is suitable for extraction of all lipids, including triglycerides, phospholipids, and other pigments [19]. The excess hexane was evaporated using rotary evaporation until the total volume

**Table 1.** Water Quality Characteristics of the Brewery Wastewater

Parameters	Influent [mg/L]	Effluent [mg/L]	Average of the effluent
pH	3.3-5.1	6.1-8.5	7.3
TSS	1,997-2,865	246-586	302.5 $\pm$ 19.4
COD	2,987-5,864	856-1,698	1,250 $\pm$ 104.2
BOD	1,678-3,267	364-698	543 $\pm$ 32.5
TN	48.6-76.3	18.9-34.7	20.3 $\pm$ 3.1
TP	33.7-53.9	4.8-12.8	6.4 $\pm$ 4.2
Heavy metal	very low	very low	
Water to beer ratio	4-10 hL water/hL beer	-	
Wastewater to beer ratio	1.3-1.8 hL/hL less than water to beer ratio	-	

reached 30-40 mL. The solutions were diluted to 50 mL and used to determine triacylglycerol (TAG) content. The amount of TAG was determined using a Fourier transform infrared (FTIR) spectrometer (Spectrum RX 1, Perkin Elmer) according to the carbonyl stretching absorption at 1,740/cm. The amount of TAG in the extract solutions was determined using a standard graph and was calculated in dry algae (% w/w).

The fatty acid composition of the algae oil was determined using the standards EN ISO 5508 and EN ISO 5509. The analysis was performed with a Clarus 500 (Perkin Elmer) gas chromatograph. The conditions for analysis were as follows: Capillary column Alltech AT-FAME (30 m – 0.25 mm – 0.25  $\mu$ m), initial oven temperature of 210°C held for 5 min then increased at 20°C/min from 210°C to 230°C and held at 230°C for 12 min. Nitrogen was used as the carrier gas. The injector temperature was 250°C. The fatty acids were identified by comparing their retention times with those of the standards. The experiments were performed five times, and then the mean values and standard deviations were calculated.

The Chlorophyll-a (Chl-a) concentration in the extract was calculated by reading the absorbance (A) of the pigment extract in a spectrophotometer at a given wavelength against a solvent blank using Eq. (3) as follows:

$$C_{chl-a} = 0.0127A_{663nm} - 0.00269A_{645nm} \quad (1)$$

$$C_{chl-b} = 0.0229A_{645nm} - 0.00468A_{663nm} \quad (2)$$

Total Chlorophyll

$$(C_{chl-a} + C_{chl-b}) = 0.00802A_{663nm} + 0.2020A_{645nm} \quad (3)$$

The BOD, COD, TN, and TP were measured as indicated in the American Public Health Association's (APHA) standard procedures [20]. The experiments were performed five times, and the mean values and standard deviation were calculated.

## 2.4. Statistical Analysis

The data presented in the tables and figures are the mean values  $\pm 3\sigma$  of five replications. Where error bars are not visible, the errors were smaller than or equal to the symbols. Differences between mean values were calculated using Tukey's test at the 0.05 level with Origin software (v.7.5, OriginLab, Northampton, MA, USA).

## 3. Results and Discussion

### 3.1. Production of Biomass and Chlorophyll

The efficiency of brewery wastewater treatment by microalgae was directly or indirectly controlled by growth rate, depending on metabolism and availability of nutrients, the operating conditions of the system under operation, and the harvesting of the produced biomass for effective nutrient removal [21, 22]. In this study, brewery effluent treatment using microalgae was investigated with/without light and aeration. Considering the operating conditions, the presence of light and aeration influence the growth

of microalgae. Fig. 1 shows the biomass growth curves in the brewery effluent with/without light and aeration. In five days, biomass increased with light faster than that without light. However, the biomass with light conditions was unchanged after five days in comparison to that in the dark conditions. The maximum biomass (dry weight) in the brewery effluent reached 0.806 g/L with light and 0.882 g/L without light. The biomass without light was 8.62% greater than with light. These results are in agreement with previous reports that heterotrophic growth significantly increases the microalgal (*Chlorella* sp.) cell concentration and volumetric productivity in batch systems [22-24]. A possible explanation could be sought in the fact that the anabolism in heterotrophic cultures is accelerated due to adenosine triphosphate formed in heterotrophic reactions [25]. After 10 d, the biomass in both conditions decreased significantly. These results suggested that microalgae were rapidly consumed in 10 d nutrient and thereby the nutrients in brewery effluent for growth due to lack after 10 d.

In the aerated microalgae cultures in the brewery effluent, more algal biomass was obtained than for the non-aerated cultures. The maximum biomass productivity was determined to be 0.82 g/L with light and 0.917 g/L without light in the aerated cultures. This result indicated that the aerated cultures obtained 1.71% more biomass productivity with light and 3.82% without light than those without aeration. In addition, the growth curve of the microalgae with aeration did not decrease after 10 d, while that of the non-aerated cultures significantly decreased after 10 d. This result indicates that the CO<sub>2</sub> (0.02 vvm) in the aeration was a continued carbon source for the microalgae cultures.

Chlorophyll is an extremely important biomolecule that is critical in photosynthesis and that allows plants to absorb energy from light. Chlorophyll is a chlorin pigment, which is structurally similar to porphyrin pigments and is produced by the same metabolic pathway used for other porphyrin pigments such as hemes. Bound at the center of the chlorin ring is a magnesium ion. The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystem [26, 27]. The ratio of chlorophyll-a (Chl-a) to chlorophyll-b (Chl-b) in plants is normally 3:1 [28, 29]. In this study, the contents of Chl-a and Chl-b

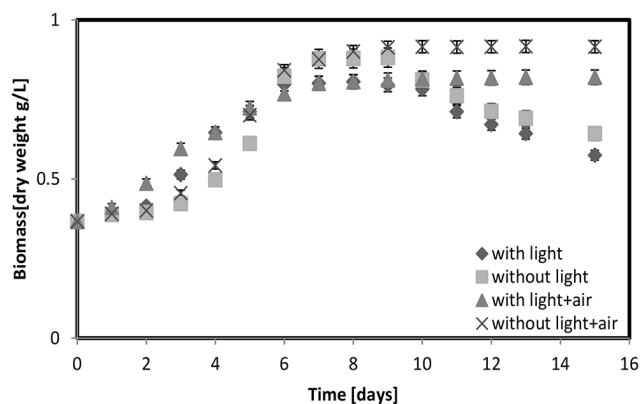


Fig. 1. Growth curve of *C. vulgaris* in brewery effluent with different conditions.

**Table 2.** Photosynthetic Pigments of *C. vulgaris* under Different Conditions

Growth conditions	Pigment content ( $\mu\text{g}/10^7\text{cells}$ )		
	Chl- <i>a</i>	Chl- <i>b</i>	Chl- <i>a</i> /Chl- <i>b</i> ratio
With light	$0.78 \pm 0.02$	$0.24 \pm 0.02$	$3.25 \pm 0.08$
Without light	$0.59 \pm 0.02$	$0.21 \pm 0.01$	$2.81 \pm 0.04$
With light +air	$0.81 \pm 0.02$	$0.26 \pm 0.02$	$3.12 \pm 0.06$
Without light +air	$0.65 \pm 0.02$	$0.23 \pm 0.01$	$2.83 \pm 0.03$

\* Data from 15-d cell growth was used for the determination.

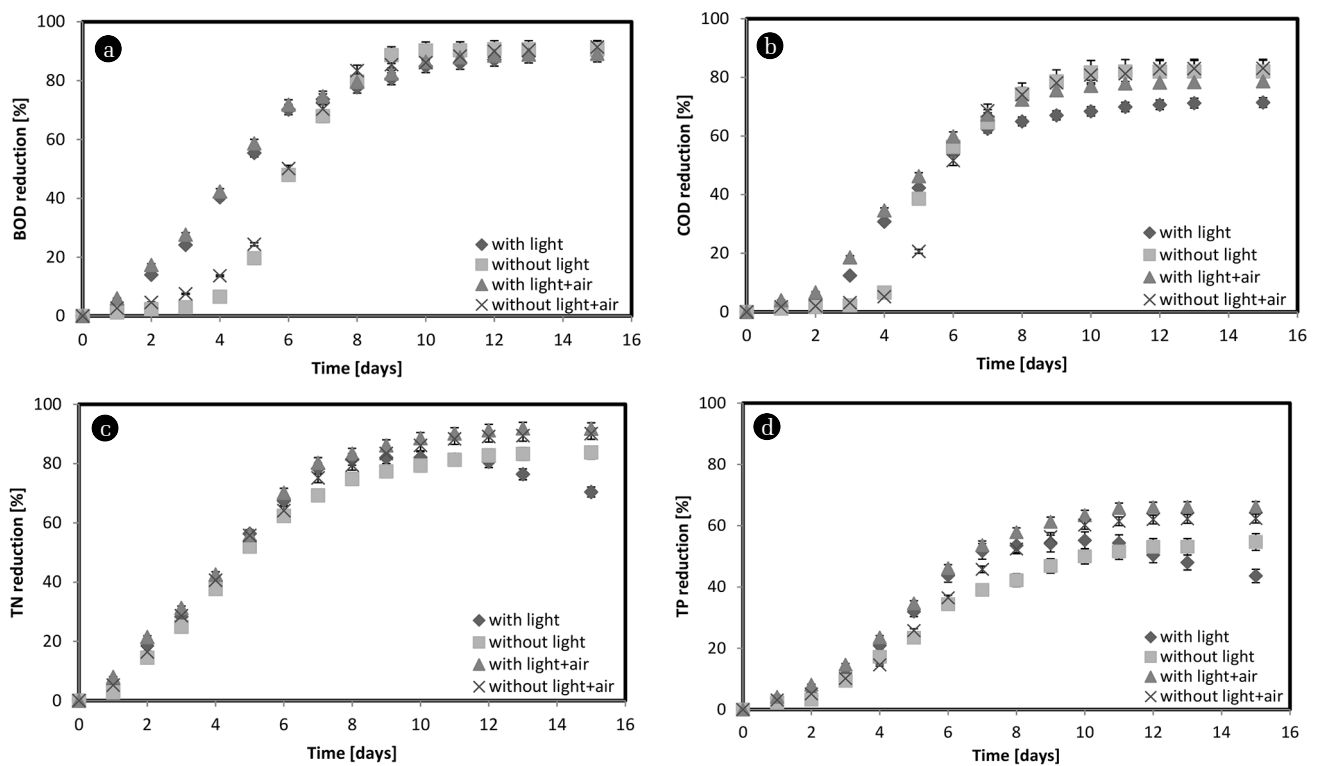
Notes: Values are mean  $\pm$  SD, N = 5; same letters denote statistically significant differences ( $P < 0.05$ )

were lower without light than with light. The ratio of Chl-*a* to Chl-*b* was approximately 3.25:1 with light and 2.81:1 without light (Table 2). The Chl-*a* to Chl-*b* ratio obtained with light was 13.54% higher than that without light. Chl-*b* absorbs light at different wavelengths than Chl-*a* and extends the range of light available for photosynthesis. The pigment content of the microalgae in the aerated cultures was slightly higher than that in the cultures that were not aerated. The Chl-*a*/Chl-*b* ratio was 3.25:1 to 3.12:1 for non-aeration and aeration with light, respectively, and 2.81:1 to 2.83:1 for non-aeration and aeration without light, respectively. The Chl-*a*/Chl-*b* ratio of the microalgae was higher without light and decreased with light in the presence of aeration. However, the results indicate that the Chl-*a*/Chl-*b* ratio is not significantly changed according to the presence of air.

### 3.2. Nutrient Removal

The calculated BOD and COD reductions were 88.58% and 71.44%

with light (Fig. 2a) and 90.72% and 82.04% without light (Fig. 2b) in 15 d. The BOD and COD concentrations remained at 50.39 and 224.5 mg/L without light and at 62 and 357 mg/L with light in the brewery effluent. By analyzing the reductions in BOD and COD in the brewery effluent in different conditions, it was determined that the *C. vulgaris* cultured without light was a more efficient treatment. The growth of *C. vulgaris* without light showed a 2.14% BOD and 10.60% COD reduction in comparison to the *C. vulgaris* cultivated with light. In addition, the culture without light obtained 8.62% higher values of *C. vulgaris* biomass (Fig. 1) than that in the light conditions. Thus, culture in the dark helped to reduce the BOD and COD and increased the effectiveness of the reduction. In addition, the BOD and COD reduction without light in the brewery effluent was found to decrease significantly in three days (less than 3%). These results were related to the *C. vulgaris* growth rate. The increased activity and faster growth of *C. vulgaris* was more effective for BOD and COD reduction



**Fig. 2.** Reductions in BOD (a), COD (b), TN (c), and TP (d) from brewery effluent using *C. vulgaris* in different operation conditions.

in the brewery effluent. The calculated BOD and COD reduction values were 89.13% and 78.65% with light (Fig. 2a), respectively, and 91.43% and 83.11% without light (Fig. 2b) after 15 d with aeration. The removal of nutrients in the brewery effluent with aeration was 0.55% and 7.21% more effective with light and 0.71% and 1.07% more effective without light, respectively, than that without aeration.

The maximum TN and TP reductions in the brewery effluent were 83.74% and 54.69% after 15 d without light and 82.05% and 54.32% after 10 d with light (Fig. 2c, Fig. 2d). The TN and TP reductions in the brewery effluent treated with *C. vulgaris* were not significantly different with and without light. However, TP consumption in particular was approximately 30% lower than that of the TN, in both conditions. In a previous study, *C. vulgaris* consumed relatively small amounts of TP, and the TP influence on lipid content and mass cultivation was smaller than that of TN [29]. The maximum TN and TP reductions in the brewery effluent with aeration were 90.11% and 62.34% in 15 d without light, respectively, and 91.75% and 66.18% in 10 d with light (Fig. 2c, Fig. 2d). The *C. vulgaris* cultivated with air removed 6.37% (without light) and 9.7% (with light) more TN in comparison to that without aeration. The TP consumption with aeration was approximately 7.65% (without light) and 11.86% (with light) higher than that without aeration. By analyzing the reductions in BOD, COD, TN, and TP in the brewery effluent in different conditions, it was determined that *C. vulgaris* cultured with aeration and without light was more efficient for removal of nutrients.

### 3.3. Relationship between Biomass and Nutrient Reduction

By analyzing the graphs shown in Fig. 3, it was determined that there was a general increase in BOD and COD reductions with an increase in biomass. The results indicate that the microalgae consumed more BOD than COD in the brewery effluent for biomass production. Fig. 3 shows the linear relationship between biomass productivity and BOD, COD, TN, and TP in the brewery effluent. The coefficient ( $R^2$ ) of determination is an important tool for determining the degree of linear correlation of variables in regression analysis [2]. In this experiment, relatively low correlation coefficients ( $R^2$ ) of 0.6834, 0.6771, 0.8003, and 0.7987 with light and 0.7682, 0.7022, 0.7585, and 0.6957 without light were obtained for BOD, COD, TN, and TP, respectively. The BOD, COD, TN, and TP concentrations in the brewery effluent decreased with an increase in biomass productivity. The relationships between biomass productivity and BOD and COD reductions without light were relatively higher than those with light. In contrast, the relationships between biomass productivity and the TN and TP reductions without light were relatively lower than those with light. The biomass productivity influenced the BOD, COD, TN, and TP reduction. In particular, *C. vulgaris* cultured with light was more effective for TN and TP reduction than that cultured without light.

The linear relationships of biomass productivity and BOD, COD, TN, and TP in the brewery effluent with aeration are shown in Fig. 3. In this experiment, relatively high correlation coefficients ( $R^2$ ) of 0.9719, 0.951, 0.9672, and 0.9238 with light + air and 0.932, 0.9373, 0.9746, and 0.9507 without light +

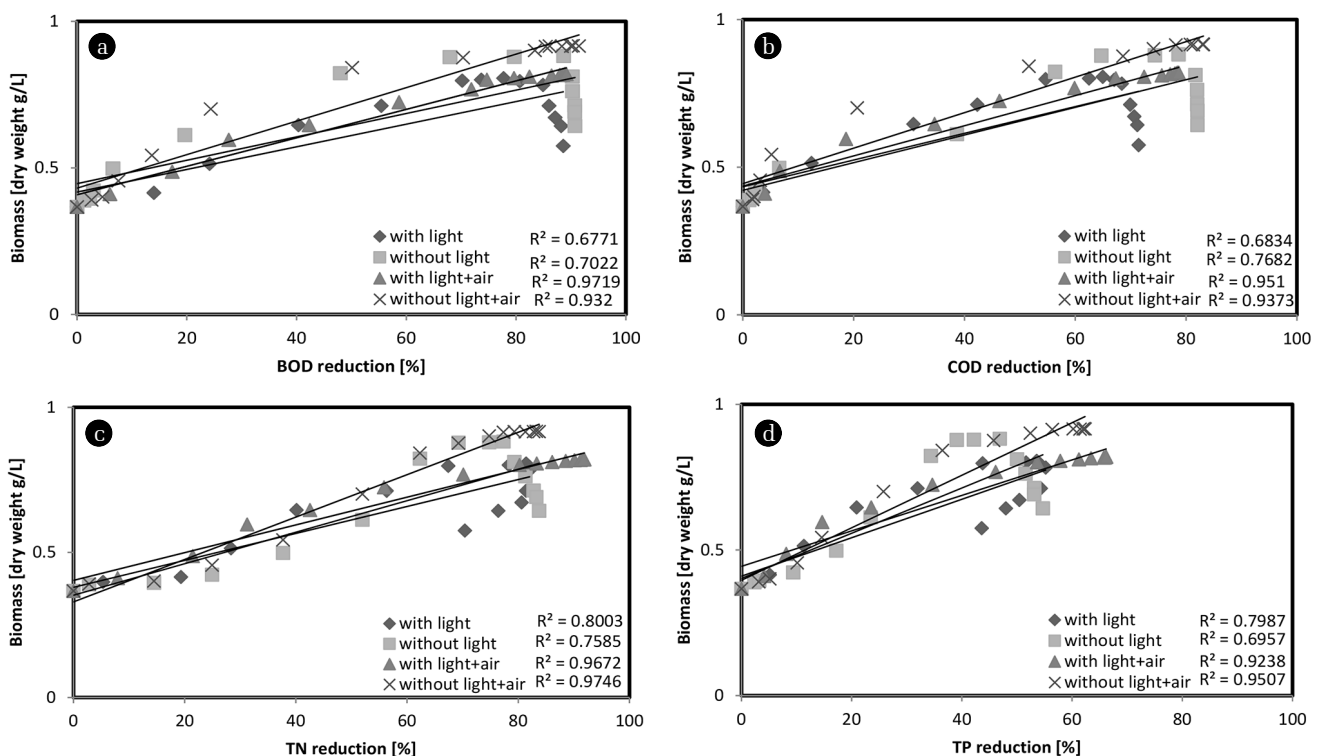


Fig. 3. The relationship between TN biomass and BOD, COD, TN and TP reduction in brewery effluent: (a) BOD, (b) COD, (c) TN and (d) TP.

air were obtained for BOD, COD, TN, and TP, respectively. The relationships between biomass productivity and the BOD and COD reductions without light + air decreased slightly in comparison to those with light + air. In contrast, the relationships between biomass productivity and the TN and TP reductions without light + air increased in comparison to those with light + air. Biomass productivity had an influence on BOD, COD, TN, and TP reduction.

### 3.4. Total Fatty Acid Composition

This study demonstrated the feasibility of microalgae for treatment of and cultivation in brewery effluent. The lipid content and effectiveness of microalgae growth for biodiesel production are important. Improved oil accumulation with slower growth can result in lower oil yields compared to that with faster growth and less oil accumulation. The fatty acid profiles of the algae oil are shown in Table 3. The saturated fatty acid contents of the *C. vulgaris* were 22.65% (with light), 21.39% (with light + air), 17.32% (without light), and 16.78% (without light + air), and the amounts of unsaturated fatty acids were 77.35% (with light), 78.61% (with light + air), 82.68% (without light), and 83.22% (without light + air) in the brewery wastewater. The culture conditions without light and without light + air resulted in lower saturated fatty acid and higher unsaturated fatty acid contents in comparison to those cultured in light conditions. The qualities of parameters of biodiesel are influenced by the fatty acid composition of the oil [30]. Fatty acid content was found to be changed in the different culture conditions. This results which is in good agreement with Ryu et al. [3] and Meesters et al. [31] who used *Cryptococcus curvatus* and *Rhodotorula gracilis*, led to a plausible

conclusion that a change in lipid composition according to culture conditions is a general trait of oleaginous microalgae. The results of the present study showed that a small amount of saturated fatty acids (16.78%) and a large amount of unsaturated fatty acids (83.22%) were characteristic of aeration and no light. The content of linolenic acid in the algae species corresponded to the requirements of the standard EN 14214, which states that the content of linolenic acid methyl ester in biodiesel fuel should not exceed 12%. The content of linolenic acid methyl ester for the microalgae investigated reached 3.65 to 6.04% for all of the conditions in the brewery wastewater effluent treatment. Therefore, microalgae cultivation can be integrated into and contribute to in-brewery wastewater treatment systems, and it could offer a cost saving potential with energy recovery.

The reduction in the nutrients in the brewery effluent using microalgae was the result of both treatment of the brewery wastewater and biomass production [22]. In the published literature, most studies have used municipal wastewater for microalgal cultivation [29, 30, 32]. One of the primary reasons for this is that municipal wastewater contains a small amount of heavy metal or chemicals. Therefore, the use of microalgal cultures in domestic wastewater is both safer and easier than their use in industrial wastewater [13]. However, the properties of brewery wastewater showed great potential for microalgal biomass and lipid production, and breweries generate large volumes of highly polluted wastewater. Currently, there are several methods and technologies for the treatment of brewery wastewater, and the most important criterion for the selection of which treatment system to install is flexibility to overcome the constant fluctuations in organic load and to keep the process economically viable [13, 24]. The microalgae

**Table 3.** Total Fatty Acid Composition of *C. vulgaris* in Different Culture Conditions in the Brewery Effluent

Fatty acid (% total fatty acids*)	Culture conditions			
	With light	With light +air	Without light	Without light +air
Saturated	22.65	21.39	17.32	16.78
14:0	4.15	3.28	2.57	2.31
16:0	14.32	13.52	11.07	11.02
17:0	0.67	0.35	0.31	0.27
18:0	1.34	1.67	1.38	1.35
20:0	0.75	0.53	0.49	0.45
22:0	0.63	0.45	0.36	0.34
24:0	0.24	0.16	0.15	0.12
Others	0.55	1.41	0.99	0.92
Unsaturated	77.35	78.61	82.68	83.22
16:1	1.61	1.85	1.92	2.13
18:1	15.65	17.35	17.46	19.99
18:2	54.46	53.15	53.68	55.01
18:3	3.65	4.12	4.32	6.04
20:1	0.25	0.32	0.35	0.51
22:1	0.14	0.15	0.19	0.24
24:1	0.01	0.02	0.01	0.02
Others	1.58	1.65	1.75	1.28

\* Based on data from 15 d of growth.

Notes: Values are mean  $\pm$  SD, with N = 5; same letters denote statistically significant differences (P < 0.05).

*Chlorella* has been reported to obtain high levels of nutrient pollution removal and efficiency when the algal cells were cultured in brewery wastewater [12]. In the present study, the maximum nutrient removal efficiency rates were 91.43% of BOD and 83.11% of COD in the without light + air, and 91.75% of TN and 66.18% of TP in the with light + air conditions.

Many researchers investigated using various carbon sources to increase the growth rate and lipid content of microalgae [33-37]. However, these methods are cost-intensive. The price of glucose, glycerol, and acetate are from 0.5 to 1.8 US dollars per kilogram according to the quality [38]. In contrast, using brewery effluent for microalgal cultures is beneficial for minimizing the use of freshwater, reducing the cost of carbon sources addition, removing the remaining nutrient, and producing microalgal biomass. Therefore, using brewery effluent for microalgal cultures could be a useful and practical strategy as an advanced, environmentally friendly treatment process.

#### 4. Conclusions

The aim of this study was to provide suitable light and air for the efficient treatment of nutrients and the production of biomass in brewery effluent. The result indicated that the maximum biomass (dry weight) reached 0.917 g/L without light in the aerated cultures in the brewery effluent. The Chl-*a*/Chl-*b* ratio was changed to 3.25 to 3.12 for non-aeration and aeration with light, respectively, and to 2.81 to 2.83 for non-aeration and aeration without light. By analyzing the reductions in BOD and COD in the brewery effluent in different conditions, it was determined that *C. vulgaris* cultured with aeration and without light was more efficient for treatment of nutrients. However, TN and TP were more effectively removed with aeration and light conditions than in any of the other conditions. The calculated BOD and COD reductions were 91.43% and 83.11% without light, respectively, in approximately 15 d with aeration. However, TN and TP were more effectively removed with aeration and light conditions than in any of the other conditions. In these conditions, 9.7% more TN and 11.86% more TP were removed than in the other conditions. The BOD, COD, TN, and TP concentrations in the brewery effluent were decreased with an increase in biomass productivity. Moreover, the highest amount of unsaturated fatty acids was achieved at 83.22% without light and in aerated culture conditions. Microalgae cultured without light and aeration produced more biomass and unsaturated fatty acids, and removed more nutrients from the brewery wastewater effluent. Brewery effluent treatment using microalgae can be removing the remaining nutrient and saving the cost of carbon sources addition.

#### Acknowledgements

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2013006899/2016005271).

#### References

1. Drissen W, Vereijken. Recent developments in biological treatment of brewery effluent. The institute and Guild of Brewing Convention; 2-7 March 2003; Livingstone, Zambia.
2. Choi HJ. Effect of Mg-Sericite flocculant for treatment of brewery wastewater. *Appl. Clay Sci.* 2015;115:145-149.
3. Ryu BG, Kim J, Kim K, Choi YE, Han JI, Yang JW. High-cell-density cultivation oleaginous yeast *Cryptococcus curvatus* for biodiesel production using organic waste from the brewery industry. *Bioresour. Technol.* 2013;135:357-364.
4. Farooq WF, Lee YC, Ryu BG, et al. Two-stage cultivation of two *Chlorella* sp. strains by simultaneous treatment of brewery wastewater and maximizing lipid productivity. *Bioresour. Technol.* 2013;132:230-238.
5. Doubla A, Laminsi A, Nzali A, Njoyim E, Kamsu-Kom J, Brisset JL. Organic pollutants abatement and biodecontamination of brewery effluents by a non-terminal quenched plasma at atmospheric pressure. *Chemosphere* 2007;69:332-337.
6. Parawira W, Kudita I, Nyandoroh MG, Zvauya R. A study of industrial anaerobic treatment of opaque beer brewery wastewater in a tropical climate using a full-scale UASB reactor seeded with activated sludge. *Process Biochem.* 2005;40: 593-599.
7. Dai H, Yang X, Dong T, Ke Y, Wang T. Engineering application of MBR process in the treatment of beer brewing wastewater. *Modern Appl. Sci.* 2010;4:103-109.
8. Wang X, Feng YJ, Lee H. Electricity production from beer brewery wastewater using single chamber microbial cell. *Water Sci. Technol.* 2008;57:1117-1121.
9. Mathuriya AS, Sharma VN. Treatment of brewery wastewater and production of electricity through microbial fuel cell technology. *Int. J. Biotechnol. Biochem.* 2010;6:71-80.
10. Shao X, Peng D, Teng Z, Ju X. Treatment of brewery wastewater using anaerobic sequencing batch reactor (ASBR). *Bioresour. Technol.* 2008;99:3182-3186.
11. Madaeni SS, Mansourpanah Y. Screening membranes for COD removal from dilute wastewater. *Desalination* 2006;197:23-32.
12. Mata TM, Melo AC, Simões M, Caetano NS. Parametric study of a brewery effluent treatment by microalgae *Scenedesmus obliquus*. *Bioresour. Technol.* 2012;107:151-158.
13. Simate GS, Cluetta J, Iyukea SE, et al. The treatment of brewery wastewater for reuse: State of the art. *Desalination* 2011;273: 235-247.
14. Raposo MFJ, Oliveira SE, Castro PM, Bandarra NM, Morais RM. On the utilization of microalgae for brewery effluent treatment and possible application of the produced biomass. *J. Int. Brew.* 2010;116:285-292.
15. Chiu SY, Kao CY, Chen TY, Chang YB, Kuo CM, Lin CS. Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource. *Bioresour. Technol.* 2015;184:179-189.
16. Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour. Technol.* 2011;102:17-25.
17. Schneider T, Graff-Hönninger S, French WT, et al. Lipid and carotenoid production by oleaginous red yeast *Rhodotorula*

- glutinis* cultivated on brewery effluents. *Energy* 2013;61:34-43.
18. Abreu AP, Fernandes B, Vicente AA, Teixeira J, Dragone G. Mixotrophic cultivation of *Chlorella vulgaris* using industrial dairy waste as organic carbon source. *Bioresour. Technol.* 2012;118:61-66.
  19. Choi HJ, Yu SW. Influence of crude glycerol on the biomass and lipid content of microalgae. *Biotechnol. Biotechnol. Equip.* 2015;29:506-513.
  20. APHA. Standard methods for the examination of water and wastewater. 22nd ed. Washington D.C. American Public Health Association ; 2012.
  21. Mata TM, Mendes AM, Caetano NS, Martins AA. Sustainability and economic evaluation of microalgae grown in brewery wastewater. *Bioresour. Technol.* 2014;168:151-158.
  22. Simate GS. Water treatment and reuse in breweries. *Brewing Microbiol.* 2015;425-456.
  23. Mitra D, Van Leeuwen J, Lamsal B. Heterotrophic/mixotrophic cultivation of oleaginous *Chlorella vulgaris* on industrial co-products. *Algal Res.* 2012;1:40-48.
  24. Gupta PL, Lee SM, Choi HJ. A mini review: Photobioreactor for large scale algal cultivation. *World J. Microbiol. Biotechnol.* 2015;31:1409-1417.
  25. Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y. Heterotrophic cultures of microalgae: Metabolism and potential products. *Water Res.* 2011;45:11-36.
  26. Choi HJ, Lee SM. Effect of optical panel thickness for nutrient removal and cultivation of microalgae in the photobioreactor. *Biopro. Biosyst. Eng.* 2014;37:697-705.
  27. Choi HJ. Effect of optical panel distance in a photobioreactor for nutrient removal and cultivation of microalgae. *World J. Microbiol. Biotechnol.* 2014;30:2015-2023.
  28. Devi MP, Subhash GV, Mohan SV. Heterotrophic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases: effect of nutrient supplementation. *Renew. Energy* 2012;43: 276-283.
  29. Choi HJ, Lee SM. Effect of the N/P ratio on biomass productivity and nutrient removal from municipal wastewater. *Biopro. Biosys. Eng.* 2015;38:761-766.
  30. Ebrahimian A, Kariminia HR, Vosoughi M. Lipid production in mixotrophic cultivation of *Chlorella vulgaris* in a mixture of primary and secondary municipal wastewater. *Renew. Energy* 2014;71:502-508.
  31. Meesters PAEP, Huijberts GNM, Eggink G. High cell density cultivation of lipid accumulating yeast *Cryptococcus curvatus* using glycerol as a carbon source. *Appl. Microbiol. Biot.* 1996;45: 575-579.
  32. Liang Y, Sarkany N, Cui Y. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol. Lett.* 2009;31:1043-1049.
  33. Qiao H, Wang G, Zhang X. Isolation and characterization of *Chlorella sorokiniana* GXNN01(Chlorophyta) with the properties of heterotrophic and microaerobic growth. *J. Phycol.* 2009;45:1153-1162.
  34. Gao C, Zhai Y, Ding Y, Wu Q. Application of sweet sorghum for biodiesel production by heterotrophic microalga *Chlorella protothecoides*. *Appl. Energy* 2009;87:756-761.
  35. Yeh KL, Chang JS. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31. *Bioresour. Technol.* 2012;105:120-127.
  36. Heredia-Arroyo T, Wei W, Ruan R, Hu B. Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials. *Biomass Bioenergy* 2011;5:2-10.
  37. Choi HJ, Lee JM. Application of saccharified acorn-starch for biomass and lipid accumulation of microalgae. *J. Korean Soc. Water Environ.* 2016;32:197-204.
  38. Lin TS, Wu JY. Effect of carbon source on growth and lipid accumulation of newly isolated microalga cultured under mixotrophic condition. *Bioresour. Technol.* 2015;184:100-107.