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Wastewater Treatment in the Brewing Industry Using *Chlorella vulgaris*

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Abstract

We studied the wastewater treatment efficiency of brewing industry effluents using the growth and biomass of the microalgae *Chlorella vulgaris*. Wastewater treated at% concentrations of 0, 20, 40, 60, 80 and 100 was examined and evaluated using the parameters of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solids (SS), Phosphate (PO_4^{3-}) as ascorbic acid), TKN Nitrogen (Total Kjeldahl Nitrogen) and Total Dissolved Solids (TDS). Treatment reductions noted were 52.48% BOD, 52.42% COD, 79.12% SS, 40.82% Phosphate, 98.72% TKN and 43.96% TDS. During treatment, fats and oils measured as Oil and Grease (OG) increased to a maximum of 70.55% and *Chlorella vulgaris* at day 5 had the highest growth at $7.8x10^5$ cells per mL cultured in wastewater in a concentration of 100%. A maximum of 0.1541 g/L of dry biomass was obtained in 80% wastewater concentration. The autoclave method was the best method to extract oil by *Chlorella vulgaris* with an extraction value at 6.3%.

Introduction

Today, the problem of water pollution is becoming more and more serious due to population growth and industrial production required to meet the demand of the expanding population. Wastewater effluents from increased industrial activity pose risks to human health and the environment. Energy demands to operate treatment facilities (e.g., power for pumps and oxygenation equipment) are increasing every year (Chia et al., 2018) while fossil fuels are rapidly being depleted and are also losing favor because of their contribution to climate change. Renewable energy options available in Thailand have the potential to be developed as substitutes for petroleum-based fuels. Solar energy, biomass, biogas and energy from wind turbines are good examples. Biodiesel is one excellent source of sustainable energy as long as it does not use biomass from agriculture. The production of biodiesel usually requires the extraction of oil from food plants which is an ineffective use of resources since it reduces the food supply in Thailand and also increases the costs of biodiesel. Microalgae offer a good alternative to produce biodiesel since they contain nutrients such as nitrogen and phosphorus and can be produced with fatty acids stored in the cells in large quantities (de Alva et al., 2013). Small algae can

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be easily cultured due to their relatively rapid growth rate and low space requirements. Microalgae are able to photosynthesize and convert solar energy into biomass, fat and protein for use as algae biomass as food and for extracting oil to produce biodiesel. The beer fermentation industry uses large amounts of water, which also produces a large amount of wastewater. Wastewater effluent has major impacts on the environment and typically contains many nutrients that are necessary for the growth of microalgae. 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 (Schneider et al., 2013). We studied and report the wastewater treatment efficiency of brewing industry effluents using the growth and biomass of the microalgae *Chlorella vulgaris*.

Materials and methods

1. Wastewater sampling and analysis

Samples of wastewater were collected from a brewery (TAP Brewery, which brews Heineken, among others) in the SaiNoi, Nonthaburi Province. Samples were taken from a holding tank with treated wastewater prior to discharge from the brewery. The wastewater samples were autoclaved and analyzed to determine the quality of the wastewater prior to their use in experiments. Table 1 lists the parameters examined.

Table 1 Parameter analysis method

Parameters	Method
pH	pH meter (HANNA, HI98128)
TDS	TDS meter (HANNA, HI98312)
SS	Gravimetric analysis (Skoog et al., 1996)
BOD	Azide modification method (APHA, 2012)
COD	Closed reflux method (APHA, 2012)
Oil and Grease	Gravimetric analysis (Skoog et al.,1996)
TKN	Kjeldahl method (APHA, 2012)
Phosphate	Ascobic acid method (APHA, 2012)

2. Algae cultivation

Samples of microalgae (*Chlorella vulgaris*) were taken from the Aquatic Animal Feed Research and Development Division, 5th Floor, Department of Fisheries, Chatuchak, Bangkok, to cultivate with community wastewater. In this study, the starting cells were $5x10^5$ cells/mL with a total amount of microalgae suspension of 350 mL. The cells were placed in 18 flasks of 500 mL each and then shaken every day. Afterward, the experiment was divided into 6 treatments (3 replications). The ratio of wastewater: deionized water was 100:0, 80:20, 60:40, 40:60, 20:80, 0:100. Microalgae was cultured indoors at room temperature in covered flasks, light intensity 4,000 lux, 12 h day: 12 h night for 7 days (Verma et al., 2020).

3. Algae biomass analysis

Microalgae was harvested in the stationary growth 350 mL of cultured wastewater samples were separated and filtered through CF/C filter paper, and then treated. The algae filter was dried at 105°C for 2 h. The algae were weighed and calculated according to the following equation.

$$\text{Dry weight of algae (g/L)} = \frac{(A - B) \times 1{,}000 \text{ mL}}{\text{Total algae}}$$

Where: A = after-filter fiber weight
B = pre-filter fiber weight

4. Algae oil extraction

The algal oil was extracted by the following three methods: manual, sonication, and autoclave.

4.1 Manual method

Algae was separated by using a centrifuge at 8,000 rpm, 2 min at 4°C, dried at 80°C for 17 h, dried algae were minified by crushing with medicinal mortar, and placed in 500 mL separator hoppers using 10 g of dried algae in 10 mL of hexane solution, shaken for 2 min. After that, the solvent was filtered and separated from the algae oil by evaporating on a hot water bath at 70°C. The evaporation bowl and samples were placed in the desiccant jar for 1 h.

4.2 Sonication method

Algae was separated by using a centrifuge at 8,000 rpm, 2 min at 4°C and mixed with 10 mL of DI water, then put into a 250 mL beaker. Sonication with a sonicator at 2 KHz for 20 min, then centrifuge again. The algae were then placed in a 500 mL separator funnel using 1 g of algae in 1 mL of hexane solution and shaken for 2 min. After that, the solvent was filtered and separated from the algae oil, and then the evaporation was carried out in a 70°C water bath.

4.3 Autoclave method

300 mL of algae was autoclaved at 125°C at 1.5 pounds/square inch for 5 min, and then the algae were placed in a 500 mL separator funnel using a 1 g of algae in 1 mL of hexane solution and shaken 2 min. After that,

the solvent was filtered and separated from the algae oil by evaporating in a hot bath at 70°C, and then the evaporation bowl and samples were weighed.

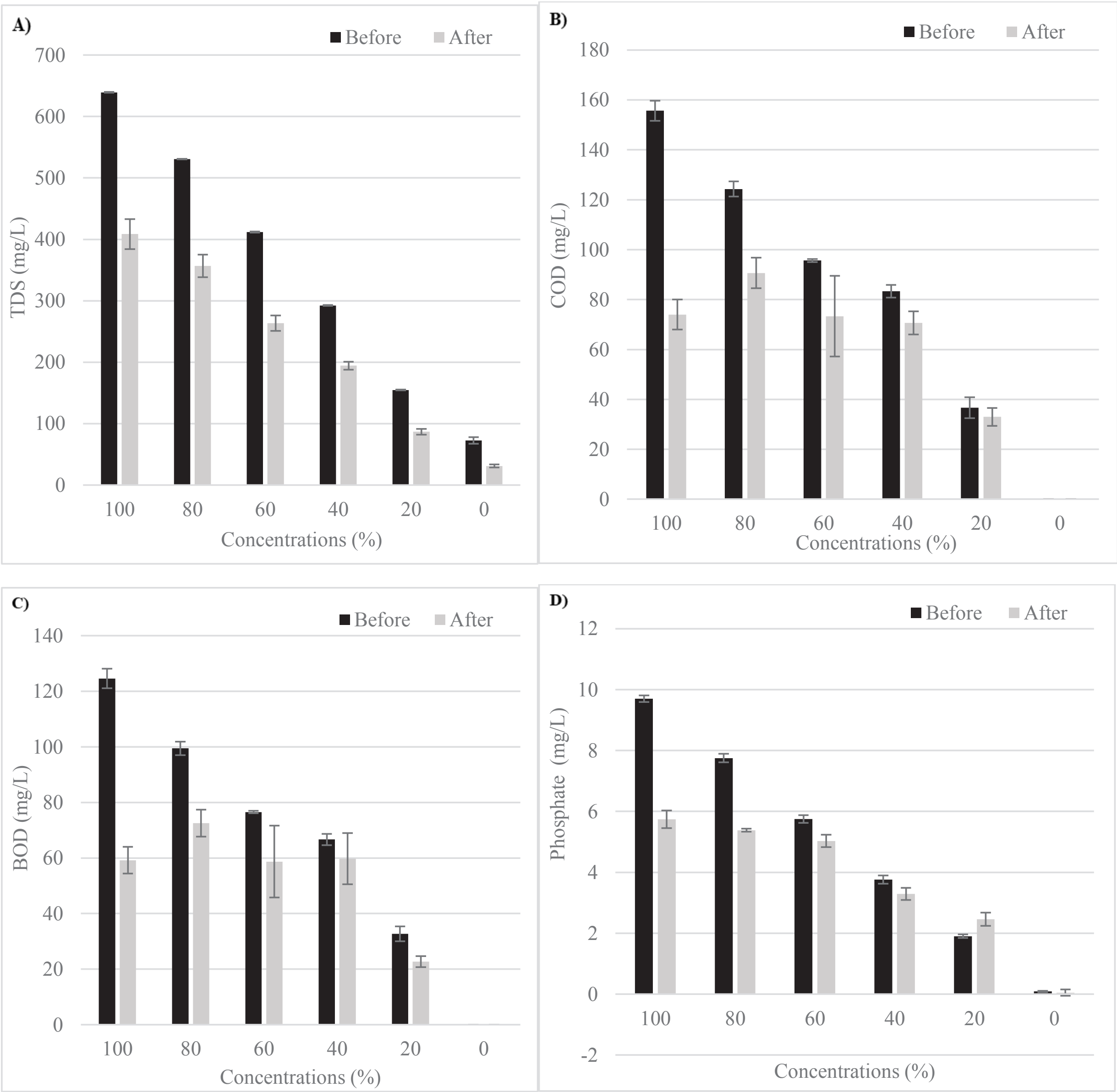
5. Statistical analysis

Data were analyzed by using One-Way ANOVA and compared with Duncan's New Multiple Range Test (DMRT) method at 95% using SPSS version 20.

Results and discussion

1. Wastewater quality analysis

Wastewater quality before and after microalgae culturing for 7 days in the concentrations 100%, 80%, 60%, 40%, 20%, 0% shown in Fig 1.



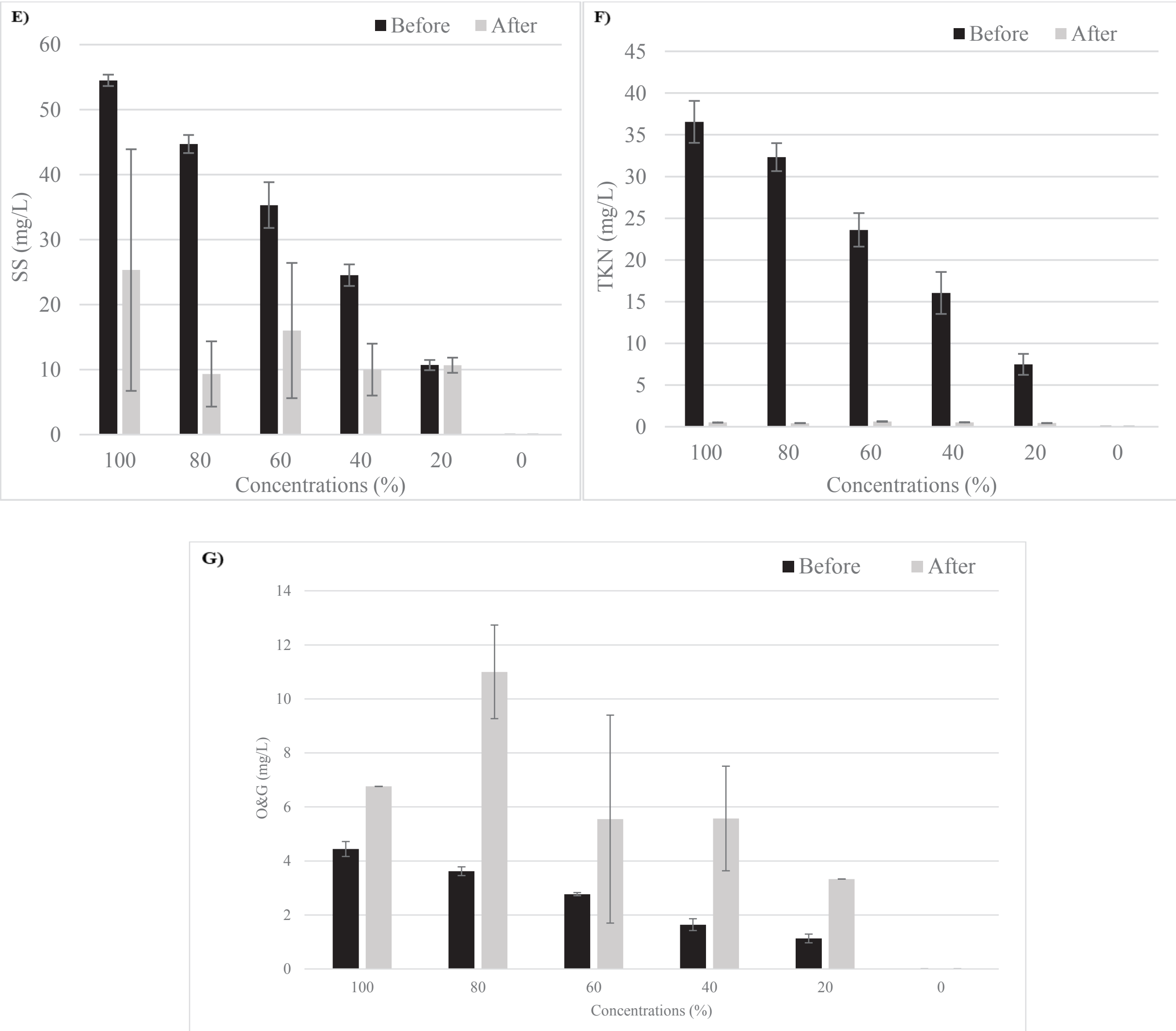


Fig. 1 Wastewater quality before and after microalgae culturing for 7 days A) TDS B) COD C) BOD D) Phosphate E) SS F) TKN G) O&G

2. Analysis of removal of physico-chemical parameters

The physico-chemical parameters of wastewater before and after microalgae culturing were shown in Fig 1. The selected parameters, such as TDS, COD, BOD, SS, PO_4^{3-}, TKN of wastewater decreased except for O&G which increased after *Chlorella vulgaris* culture. The study of all parameters after algae culture for 7 days showed that TDS was significantly reduced because part of the organic matter that plants use for their growth come from the dissolved form of organic matter. This was consistent with the research of Murugesan et al. (2010) culturing microalgae with wastewater of poultry farms, with the highest reduction in total dissolved solids in different trials at 38.92%. COD was significantly reduced clearly because algae can absorb the COD that is a source of carbon to algae for photosynthesis. This was consistent with research of Lin et al. (2007) that algae can absorb organic carbon as an energy source as well. BOD was significantly reduced because BOD contains a large number of dissolved organic compounds. Algae can make the biodegradation to be used as a source of energy that is consistent with the research of Praditwatthanakit (2012) with the average BOD in the range of 15.02-18.53 mg/L. SS was significantly reduced because suspended solids are the major source of nutrients present in brewery wastewater and were taken up during

Chlorella growth. Suspended solids consist of organic matter and many types of inorganic substances which are used during algae culture and its related anaerobic metabolism. Kotteswari et al. (2012) reported that total suspended solids decreased to 74.37% when dairy farm effluent was treated with *Spirulina platensis*. Phosphate (PO_4^{3-}) decreased the most with 40.82 percent because phosphorus is an essential element for algae growth which plays a role in various processes in cells, especially energy transfer and the process of creating nucleic acids including acting as a buffer to help stabilize the pH in cells, which was consistent with the study of Phromya et al. (2001) who cultured spirulina in various mediums. It was found that the syringe formula had a better growth risk because it contained a carbon source, nitrogen source, phosphorus and potassium. Phosphate (PO_4^{3-}) values are reduced because phosphorus is an essential nutrient for algae growth which plays a role in various processes in cells, especially energy transfer and the process of creating nucleic acids. It also acts as a buffer to stabilize the pH in cells, which was consistent with a study by Henkanatte-Gedera et al. (2015) who examined the BOD: N: P ratio in community wastewater by microalgae. The BOD: N: P value of 16.5: 13.4: 1 was able to reduce phosphate values in community wastewater by 98% over 3 days. *Galdieria sulphuraria* can be treated with 99% phosphate in 7 days. TKN was reduced by 90% because TKN is a source of nitrogen for algae and is also the main nutrient for algae. Algae cannot fix nitrogen in the atmosphere for use, therefore, nitrogen in organic and inorganic form in the water supporting growth is consistent with research by Feng & Zhang (2011) microalgae can reduce ammonia (nitrogen) in wastewater by 97%. Lipid and oil analysis (O&G) showed an increase in fat and oil contents due to stress because of the reduction of nutrients in wastewater. Likewise Mujtaba et al. (2012) found *Chlorella vulgaris* increased oil content from 20% to 40% of dry weight when the algae were under unsuitable growth conditions. The pH value selected is important in the cultivation of microalgae as unfavorable pH values will stress the microalgae, which ultimately affects the efficacy of nutrient absorption and metabolites produced (Morais et al., 2015). The pH values in the holding tank of brewery wastewater in 100% concentration was 8.8. In experiments, the wastewater samples were autoclaved to eliminate algae and other microorganisms that were not part of the experiment, thus the pH value in the experiment was 9.92 increasing because of oxygen decay, with the hydrogen value increasing slightly. The alkaline pH after culturing microalgae was 10.68 due to the positively charged micronutrients used by algae and the nutrients in the water contain phosphates, which are negatively charged micronutrients. The phosphates increasing pH after culturing algae is consistent with that reported in Saekoo & Panich-pat (2020) using microalgae cultured in piggery farm wastewater which increased pH from 8.11 to 8.84 at a wastewater concentration 100%. Gong et al. (2014) demonstrated the potential use of *Chlorella vulgaris* to mitigate the toxic effects of cadmium in the water and found under normal cultivation, the optimum pH for the growth of *Chlorella vulgaris* falls between 10.0 and 10.5 and that constant pH adjustment to optimum pH enhances the growth of microalgae and prevents contamination.

3. Growth of *Chlorella vulgaris* algae

The growth of microalgae cultured in wastewater from the brewing industry was studied in different concentrations of 100%, 80%, 60%, 40%, 20%, and 0% that measured the growth for 7 days. It was found that the concentration of 100 percent wastewater supported the highest growth of algae. At day 5, wastewater contained more nutrients than seen in other experiments, and the nutrients were sufficient to both meet the required needs of the algae (Lam et al., 2017) and to support the number of cells equal to 7.8 x 10^5 cells/mL which is the best growth concentration (Fig 2). Thus, the 100% concentration supported the highest live algae biomass when compared to lower concentrations of effluent.

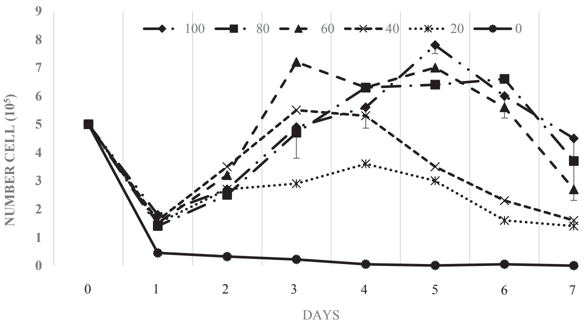


Fig. 2 Growth of *Chlorella vulgaris* in different concentrations for 7 days

4. Amount of biomass obtained from algae culture

The results showed that biomass yields cultured in different concentrations ranged from 0.0165-0.1541 g/L, with the highest concentration of biomass being 80% as followed 100%, 60%, 40%, 20%, 0%, equal to 0.1541, 0.1157, 0.0984, 0.0546, 0.0165 and 0 g/L, respectively, which was consistent with the work of Feng & Zhang (2011) that microalgae can be cultured in treated wastewater. The value of dried algae was 0.5-0.28 g/L. The concentration of biomass at 80% was not statistically significantly different at $p>0.05$ but 100% and 60% were statistically significantly different ($p<0.05$) as same as 40%, 20%, 0%. Dried algae at a concentration of 80% should be used to extract oil further.

5. Amount of oil extraction obtained from algae culture

In the study, it was found that the extraction of oil from algae at the concentration of 80% by the autoclave method had the highest percentage yield, followed by the sonicate and manual methods with extraction rates of 6.3%, 5.05% and 4.29%, respectively. These three methods of oil extraction are the most widely used ones. The autoclave method was the best oil extraction technique because the pressure during the autoclave treatment destroyed cell walls of the algae which facilitated oil release. One study using HPLC testing for saturated and unsaturated fats to determine the quality of biodiesel production in cultured microalgae reported that the resulting fat was in a saturated form that increased the quality of biodiesel (Rinna et al., 2017). For this study, the extracted algae oil was insufficient for biodiesel extraction because the extraction of biodiesel depends on the amount of algae that need to be cultured in larger quantities by using larger scale cultures than the laboratory scale. Lipid production in *Chlorella vulgaris* increases under nitrogen starvation conditions but this also reduces the total biomass of microalgae which reduces the overall lipid yield (Prîbyl et al., 2012). Hence, in order to solve this problem without affecting the overall lipid yield, it is suggested that microalgae are cultured in nitrogen-rich conditions initially to enhance growth and increase biomass before transferring them to a nitrogen deficient environment to stimulate lipid production (Mujtaba et al., 2012).

Conclusion

Chlorella vulgaris was very effective in reduction of chemical concentrations (BOD, COD, SS, Phosphate, TKN and TDS). TKN removal was achieved up to 98.72%. The maximum growth of algae at day 5 was 7.8×10^5 cells/mL at 100% concentration of wastewater. The highest algal biomass was 0.1541 g/L at 80% concentration of wastewater. The results of microalgae oil extraction showed that the autoclave method was the best algae oil extraction method with 6.3%. Although the autoclave method was the best oil extraction, the sonicate method is probably the most cost-effective way of oil extraction in terms of energy consumption.

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