

Nutrient Removal from the Effluent of Swine Slaughterhouse Wastewater by *Chlorella vulgaris* TISTR 8580

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Abstract— *Chlorella vulgaris* TISTR8580 was investigated for its ability to remove nutrient from the effluent of swine slaughterhouse wastewater which was diluted in three different proportions (namely, 75%, 50% and 25%). On day 4, which was the end of the batch experiment with 3,000 lux daylight fluorescent lamp, light/dark photoperiod of 12 hr:12 hr, at $28\pm 2^\circ\text{C}$, it was found that the alga cultured in the 25% effluent wastewater exhibited the highest growth production with the cell density of $8.51\pm 2.77\times 10^6$ cells/ml. However, considering generally not only the nutrient removal efficiency but also the net lipid production, the algae cultured in the 50% effluent wastewater indicated the best results, i.e. 28.69% nitrate removal, 28.75% total phosphorus removal, 15.2 mg/L lipid weight production, and 20.9% lipid content. Therefore, *Chlorella vulgaris* can potentially be applied as an alternative for nutrient removal from swine slaughterhouse effluent wastewater as well as biofuel production.

Keywords—*Chlorella vulgaris*, Biofuel, Nutrient removal, Swine slaughterhouse effluent wastewater

I. INTRODUCTION

About 18.15 kiloton of pork products was produced in Thailand in the year 2015 [1]. Swine slaughterhouse generates a large volume of wastewater composting of high organic load and nutrient [2], [3]. For example, 1.6–8.3 m³ of water per tonne of carcase is generated in swine slaughterhouse [4]. However, some anaerobic reactors effluents do not meet the standards of the legislation and a post-treatment process to improve the removal of organic matter, nitrogen and phosphorus is required as reported in [5]. Nitrogen and phosphorus without treatment may cause eutrophication in river, lake, sea, upset the balance of the ecosystem and other negative effects such as algal bloom, low dissolved oxygen concentration, fish kill, undesirable pH shifts and cyanotoxin production. Nutrient removal by chemical and physical treatment can be costly for energy and chemical consumption [6]-[7]. While cultivation of alga in wastewater is considered a potential option for sustainable production of algal biomass by

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not only reduction in the production cost but also wastewater cleaning by environmental friendly method [8]. Microalgae could grow in different types of wastewaters, such as septage effluent, cattle slaughterhouse, poultry manure and primary piggery wastewater [9]-[14].

The objectives of this study were to assess growth, nutrient uptake and lipid production of *Chlorella vulgaris* TISTR 8580 from the effluent of swine slaughterhouse wastewater which was diluted in three proportions at 25, 50 and 75% under experimental condition which has not been published in previous studies. These experiments were performed in order to investigate the potentiality of *Chlorella vulgaris* TISTR 8580 as an option for nutrient removal and biofuel production from the effluent of swine slaughterhouse wastewater.

II. MATERIALS AND METHODS

A. Microalgal Strain and Culture Medium

The microalgal strain of *Chlorella vulgaris* TISTR 8580 used in this study was purchased from Thailand Institute of Scientific and Technological Research. Then, it was purified by isolation techniques on Tris-Acetate-Phosphate (TAP) agar [15]. The algal stock was kept in TAP agar slant under 3,000 lux daylight fluorescent lamp, light/dark photoperiod of 12 hr:12 hr, at $28\pm 2^\circ\text{C}$ and it was subcultured every two weeks.

The TAP medium was used immediately after preparation for next experiments.

B. The Effluent of Swine Slaughterhouse Wastewater

The effluent of swine slaughterhouse wastewater was collected after anaerobic digestion from Samphran slaughterhouse Limited Company in Nakhon Pathom, Thailand. In order to make it more practical and related to the real application in the field as well as according to the more efficiency from effluent wastewater without filtration which was recorded in previous study [16], the effluent was used as it was without filtration and its nutrient concentration was shown as in Table I. For further experiments, it was diluted with distilled water (DW) to obtain three different proportions, viz. 25, 50 and 75%.

TABLE I: NUTRIENT CONCENTRATIONS OF THE EFFLUENT OF SWINE SLAUGHTERHOUSE WASTEWATER USED IN EXPERIMENTS

Nutrient	Concentration (mg/L)
NO ₃ ⁻	52.9
Total phosphorus	11.4

C. Growth of *C. vulgaris* TISTR8580 in the Effluent of Swine Slaughterhouse Wastewater

Starter cultures of *C. vulgaris* TISTR8580 were prepared by transferring algal colonies on TAP agar slant into 800 ml of TAP medium in 1,000 ml sterilized glass bottles. They were incubated under 3,000 lux daylight fluorescent lamp, light/dark photoperiod of 12 hr:12 hr, at $28\pm 2^\circ\text{C}$ and continuously aerated with filtered air at the rate of 0.4 vvm (volume of air per volume of culture per minute). Cell density was counted daily by haemocytometer until log phase of the growth curve was obtained with the cell density of $6,350\times 10^4$ cell/ml. Then, the algae was separated from 80 ml of the starter culture by centrifugation at 3,000 rpm for 15 minutes and it was inoculated into each test bottle containing 800 ml of the selected dilution of the effluent of swine slaughterhouse wastewater for treatment experiments or TAP medium. There were 3 sets of treatment experiments, viz. wc25, wc50 and wc75 which were treatment experiments with test algae in the effluent of swine slaughterhouse wastewater which were diluted in three proportions of 25, 50 and 75%, respectively. Batch experiments were performed under the same condition as starter cultures with two replications. All experiments were stopped when algal growth of at least an experiment was in stationary phase because it was reported that the highest lipid content was obtained at the stationary phase of algal growth [17]. Algal growth was monitored by cell counts daily with haemocytometer, then cell density was calculated. Cell size of test algae was also measured by using compound microscope at magnification of 40x with Image Analyzer software. Cell density and cell size of the algae in all test experiments (wc25, wc50, wc75 and TAP medium) were compared.

D. Nutrient Removal Efficiency from the Effluent of Swine Slaughterhouse Wastewater by *C. vulgaris* TISTR8580

Batch experiments were conducted as explained in section C. The initial and final concentration of NO_3^- and total phosphorus in test swine slaughterhouse wastewater for every dilution of treatment experiments were analyzed by electrode method and persulfate method, respectively, with duplications.

E. Lipid content

At the end of each experiment, the algal cells were harvested by centrifugation at 8,500 rpm for 5 min. Then, the pellets were freeze-dried and stored at -20°C before analysis. Total lipids were determined by the method of Bligh and Dyer [18]

The lipid content of the microalgae was calculated from the equation:

$$\text{Lipid content (\%)} = \frac{\text{Weight of extracted lipid}}{\text{Weight of dry biomass}} \times 100 \quad (1)$$

III. RESULTS AND DISCUSSION

A. The Growth of *C. vulgaris* TISTR8580

Algal growth can be divided into four different stages, namely (1) lag phase, (2) log phase, (3) stationary phase, and

(4) death phase [19]. The results of this study follow this growth curve and were expressed in Fig.1- Fig.2

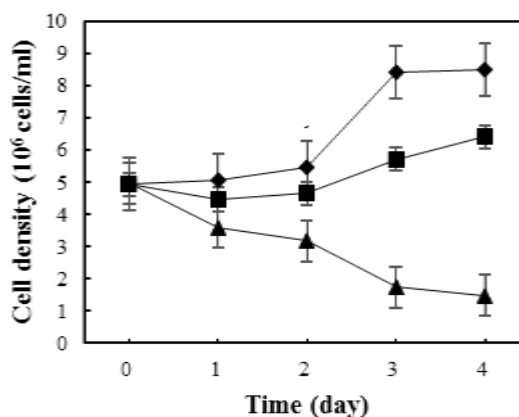


Fig. 1. Cells density of *C. vulgaris* TISTR 8580 cultivated in the effluent of swine slaughterhouse wastewater at the dilution of \blacklozenge 25% (wc25), \blacksquare 50% (wc50) and \blacktriangle 75% (wc75)

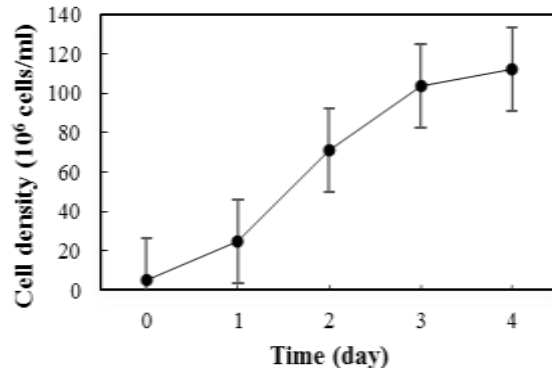


Fig. 2. Cells density of *C. vulgaris* TISTR 8580 cultivated in \bullet TAP medium

From Fig. 1 and Fig. 2, within 4 days the alga cultured in TAP medium and in 25% of the effluent wastewater (wc25) grew up to stationary phase while those in 50% of the effluent wastewater (wc50) were still in log phase. In the case of alga cultured in 75% of the effluent wastewater (wc75) did not resemble the normal growth of algae but they were in declining stage which were indicated by the decreasing line in Fig. 1. In addition, the change of their cell size was relatively the least and was different from those in others [Fig.3]. This was in accordance with the observation of changing of algal color from green to slightly yellow color in wc75 treatment experiment. This result agreed with the study of Wrigley and Toerien [20] which reported that wastewater often had high concentration of nutrients and much of the nitrogen in the form of $\text{NH}_4\text{-N}$ could inhibit algal growth at high concentration. The lag phase of the alga cultured in TAP medium occurred probably > 1 day, log phase from 1 day (or probably > 1 day) to 3 days, stationary phase from 3 to 4 days (the final day of the experiment). For treatment experiments, wc25 experiment exhibited the highest growth production with the cell density of $8.51\pm 2.77\times 10^6$ cells/ml at the end of

the experiment, however, it is approximately 13 time less than that in TAP medium (the rich medium for the algal growth). The results in this study were in consistent with the result of Rowley [21] who suggested that the N:P ratio should be at least 3:1 in order to maximize *C. vulgaris* growth. Because the N:P ratios for wc25 and wc50 in this study are 2.69 and 2.20, respectively. Therefore, the ratio for wc25 is close to the suggested ratio more than that for wc50 resulted in higher growth as expressed in the term of cell density [Fig. 1]. For wc25 and wc50 treatment experiments, the lag phase occurred the same period of time (between 0 and 2 days). But the further periods of their growth curve were different. For wc25 experiment, log phase occurred from 2 to 3 days and stationary phase from 3 to 4 days but for wc50 experiment the log growth phase was continued until the end of the experiment (4 days) and has not reach the stationary and declining phases yet.

Cells size of microalgae in TAP medium had smaller size than wc25, wc50 and wc75. This should be because Tap medium was more suitable medium for algal growth than other treatment experiment, therefore, TAP medium can support algal cell division more than others [Fig. 3].

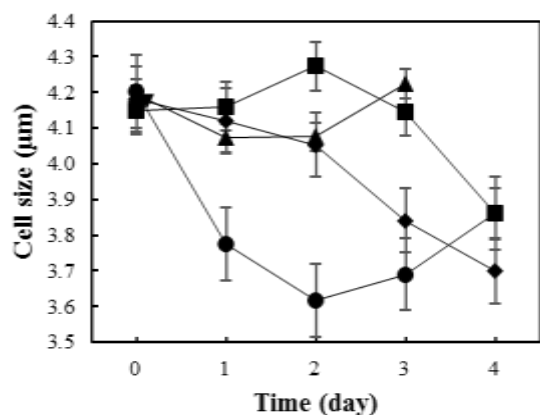


Fig. 3. Cells size of *C. vulgaris* TISTR 8580 cultivated in ●TAP medium and in the effluent of swine slaughterhouse wastewater at the dilution of ◆ 25% (wc25), ■ 50% (wc50) and ▲ 75% (wc75)

B. Removal of nitrate and total phosphorus from the effluent of swine slaughterhouse wastewater

Nitrate and total phosphorus can be removed from wastewater by their uptake to microalgal cells. The living cells drew nitrate and phosphorus in the surrounding environment for syntheses of amino acids, pigment, nucleic acids, ATP, biochemical precursors, etc. Limitation of these nutrients would lead to retardation in growth, metabolic efficiency and ultimately survival in the environment [22]. In addition, the majority of dissolved inorganic nitrogen in effluent wastewater was in the form of NO_x-N and it was reported that the majority of nitrogen in the form of NO_x-N was removed in effluent wastewater [23]. Moreover, nitrate is a commonly studied N source for understanding nutrient deprivation to induce lipid accumulation [24], therefore nitrate and total phosphorus were chosen as the representative

for N and P sources in effluent wastewater in the present study.

The results for nitrate and total phosphorus removal from the effluent of swine slaughterhouse wastewater by *C. vulgaris* were expressed in Table II. The best results for nitrate and total phosphorus removal were achieved in wc25 and wc75 treatment experiments with the values of 53.06% and 32.35%, respectively. While considering for both nitrate and total phosphorus in general, wc50 treatment experiment may provide better result. However, these were lower than the results for NO_x-N and total phosphorus removal efficiency from municipal wastewater by *C. vulgaris* which were reported at more than 88% and 90%, respectively [23]. This might be due to different in many parameters such as effluent preparation, culturing condition, and incubation time.

TABLE II: THE REMOVAL OF NITRATE AND TOTAL PHOSPHORUS FROM THE EFFLUENT OF SWINE SLAUGHTERHOUSE WASTEWATER

Nutrient	Treatment	Initial concentration (mg/L)	Final concentration (mg/L)	Removal (%)
NO ₃ ⁻ (mg/L)	wc25	12.25±0.78	5.75±0.07	53.06
	wc50	17.60±0.28	12.55±0.07	28.69
	wc75	40.45±0.35	39.95±0.07	1.24
TP (mg/L)	wc25	4.55±0.07	3.75±0.07	17.58
	wc50	8.00±0.00	5.70±0.00	28.75
	wc75	10.20±0.28	6.80±0.00	32.35

C. Lipid content

The lipid content of test microalgae was calculated by equation 1 and the results were expressed in Table III. All lipid content from treatment experiments (wc25, wc50 and wc75) were higher than that from TAP medium, this might be as a result of changing from rich medium to effluent wastewater in treatment experiments may cause stress to the algae. Because previous research [25] concluded that lipid content of *C. vulgaris* increased from 20% to 43% of dry weight under nitrogen starvation conditions. On the contrary, dry cell weight, and lipid weight of the alga from TAP medium were higher than those from the treatment experiments. Among these treatment experiments, wc50 exhibited the best for net lipid production [Table III].

TABLE III: DRY CELL WEIGHT, LIPID WEIGHT AND LIPID CONTENT OF *CHLORELLA VULGARIS* TISTR 8580

Experiment	Dry cell weight (mg)	Lipid weight (mg)	Lipid content (%)
TAP	393.50±75.57	65.24±1.67	16.58±0.00
wc25	52.66±1.15	9.34±0.13	17.73±0.00
wc50	58.22±4.72	12.16±2.37	20.90±0.00
wc75	46.97±6.05	10.76±0.07	22.91±0.00

IV. CONCLUSION

In this study, *Chlorella vulgaris* TISTR8580 was used to remove nutrient in the effluent of swine slaughterhouse

wastewater. The results indicated that *C. vulgaris* TISTR8580 grew only in wc25 and wc50 experiments. However, the alga in wc25 provided the best growth. The best removal efficiencies for nitrate and total phosphorus were obtained from wc25 and wc75 experiment, respectively. Nevertheless, wc50 experiment showed good results considering for both nitrate and total phosphorus removal. The alga in wc75 experiment resulted in the highest lipid content but the alga in wc50 experiment could provide the best net lipid production. Then, *C. vulgaris* culture in the effluent of swine slaughterhouse wastewater can be an alternative for nutrient removal and biofuel production.

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studying nano biointeraction: The effect of TiO₂ nanoparticles on *E. coli*." *The Proceeding of the 37th Congress on Science and Technology of Thailand*, ed. S. Chongthammakun, pp. 209-210, vol. 37, Thailand: The Science Society of Thailand under the Patronage of His Majesty the King, 2011. His research interests are algal cultivation and energy.



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