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Production of microalgal biomass using raw wastewater from instant noodle factory

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KEYWORDS

Wastewater, *Scenedesmus sp.*, Phosphate addition, External carbon dioxide, Microalgae cultivation, Instant noodle factory, COD reduction, Mixed liquor suspended solids (MLSS)

A B S T R A C T

Wastewater treatment using microalgae can be applied for nutrients (N and P) removal together with carbon dioxide fixation through photosynthesis. Moreover, algal biomass produced after wastewater treatment can be further utilized as fertilizer or biodiesel extraction. The objective of this research is to enhance microalgae biomass production for recovering nutrients in wastewater from instant noodle factory. The experiment was performed using 2000 ml Duran bottle as culture vessel under laboratory condition. Raw wastewater as mixed liquor suspended solids (MLSS) and effluent after sedimentation were chosen as the nutrient source for the microalga *Scenedesmus* sp. cultivation. It was found that MLSS wastewater and effluent can be used as a sole nutrients source for *Scenedesmus* sp. After phosphate addition, treatment with phosphate addition exhibited higher growth and biomass (482.02 ± 144.46 mg/L and 300.73 ± 182.35 mg/L for MLSS and effluent, respectively) than treatment without phosphate addition (200.19 ± 20.13 mg/L and 174.15 ± 45.08 mg/L for MLSS and effluent, respectively). After treatment, total COD reduction was highest by 89.37% and 31.48% for MLSS and effluent, respectively. However, external carbon dioxide supplement was not significantly enhanced microalgal growth in MLSS wastewater from instant noodle factory.

Introduction

Discharge of wastewater from human activities is the major cause of nutrients loading into aquatic environments, which leads to eutrophication and algal blooms (Abdel-Raouf *et al.*, 2012; Cai *et al.*, 2013). Wastewater treatment requires significant investment and operation which is an additional cost for industries. Uses of aquatic plants and algae for wastewater treatment have been studied (Olguın, 2003; Rawat *et al.*, 2011). There were several reports commencing the use of microalgae for secondary wastewater treatment. The benefits of microalgae include nutrients assimilation, carbon dioxide removal, heavy metal removal, and low operating cost. Wastewater treatment using microalgae can be applied for nutrients (N and P) removal together with carbon dioxide fixation through photosynthesis. Moreover, algal biomass produced after wastewater treatment can be further utilized as fertilizer or biodiesel extraction.

Wastewater treatment using microalgae can be defined as the high rate algal pond (HRAP). With HRAP, algal growth is provided by well mixing of mechanical agitation and light availability is generally enhanced by shallow raceway pond. The main disadvantage of HRAP is the requirement of large pond area and water treatment efficiency is still low. To develop the high efficiency phycoremediation process, photobioreactor can be applied instead of large shallow pond and, moreover, carbon dioxide from industrial process can be utilized as external carbon source (Graham *et al.*, 2009; Langley *et al.*, 2012). Algal biomass produced from wastewater treatment might be one of the raw materials for biofuel production in the future. The objective of this research is to enhance microalgae biomass production using wastewater from instant noodle factory by phosphate addition and external carbon dioxide supplement.

Materials and Methods

Source of microalgae

Scenedesmus sp. was obtained from culture collection of the Center of Excellence for Marine Biotechnology, Chulalongkorn University. *Scenedesmus* sp. was incubated under continuous light (5000 lux) at 25± 1 ^{o} C in BG-11 medium containing (g/L): $K_2HPO_4 \cdot 3H_2O$, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; EDTA, 0.001; $NaNO_3$, 1.5; Na_2CO_3, 0.02; trace metal mix A5, 1.0 ml which consisted of (g/L) H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 7H_2O$, 0.222; $NaMoO_4 \cdot 2H_2O$, 0.39; $CuSO_4 \cdot 5H_2O$, 0.079; and $CoCl_2 \cdot 6H_2O$, 0.05 (Zhou *et al.*, 2012).

Wastewater collection and analysis

Wastewater was taken from the different stages of the activated sludge wastewater treatment plant of Wan Thai Foods Industry Co., Ltd, which is instant noodle factory, in Bangkok. Mixed liquor suspended solids (MLSS) collected form aeration tank and effluent collected from final sedimentation tank were chosen for this study. Wastewater was transported to the laboratory and kept frozen at -20^{o} C prior to cultivation (Zhou *et al.*, 2012).

Dissolved oxygen (DO), Temperature, and pH were measured immediately during water sampling. Wastewater parameters including total nitrogen (TN), total ammonia nitrogen (TAN), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), phosphate (PO_4^{3-}), total suspended solids (TSS) and chemical oxygen demand (COD) were determined according to standard methods for

wastewater analysis (Strickland and Parsons, 1972; APHA, 2005).

Effect of phosphate addition to enhance microalgae growth in wastewater from instant noodle factory

In general, phosphate concentration in the water is the limiting factor for microalgal growth (Graham *et al.*, 2009). MLSS and effluent without dilution were used as the culture medium. A 10 days-batch experiments were conducted using 2000 ml Duran bottle as the culture vessel (Fig 1). Culture condition was 33.2 ± 0.7 ^{o}C under continuous light at approximately 5000 lux and continuous aeration. All batch cultivation was carried out in triplicate. Duran bottles were filled with 1800 ml of wastewater and 200 ml of *Scenedesmus* sp. stock culture. Continuous aeration was supplied to all reactors by air pump and continuous artificial light at approximately 5000 lux was provided by cool white fluorescence lamps. The experiment consisted of T1 (MLSS), T2 (MLSS with phosphate addition), T3 (effluent), and T4 (effluent with phosphate addition). K_2HPO_4 was added in the 4th day to compensate phosphate depletion during cultivation. Ten milliliters of wastewater was collected daily during ten days cultivation in order to observe microalgal growth (cell count with hemocytometer) and nutrient removal.

Effect of lighting period and external carbon dioxide supplement on growth of microalgae cultivated in wastewater

According to previous results, MLSS was selected for *Scenedesmus* sp. cultivation. Two percent carbon dioxide mixed with air (volume/volume) derived from a liquid carbon dioxide storage tank (Figure 2) was supplied to culture vessels while 100% air supplement were assigned as a control. The experiment consisted of T1 (control; continuous light and continuous aeration), T2 (carbon dioxide supplement under continuous light), T3 (12:12h light:dark cycle with continuous aeration), and T4 (12:12h light:dark cycle with carbon dioxide supplement). Light source used in this study was cool white fluorescence lamps providing approximately 5000 lux light intensity. Ten milliliters of wastewater was collected daily during ten days cultivation in order to observe microalgal growth (cell count with hemocytometer) and nutrient removal.

Wastewater analysis

Ten milliliters of water samples for nutrient analysis were collected with daily basis. Water sample was filtered with GF/C filter (Whatman) and kept ay 4 ^{o} C prior analysis. Analysis method of wastewater parameter was determined according to standard method (Strickland and Parsons, 1972; APHA, 2005).

Measurement of microalgal growth

The microalgal cell density was counted daily under microscope observation using a hemocytometer. Biomass (dry weight) was calculated using linier relationship between dry weight biomass and cell density counted with hemocytometer (Appendix B) according to the equation:

Dry weight (mg/L) = (Cell density (10^4cell/ mL) + 7.2328)/ 0.2945 (1)

A growth curve was plotted between biomass or cell concentration and time. Specific growth rate (μ) of the cells during exponential phase was calculated with the following equation:

$$\mu = \frac{lnN2 - lnN1}{T2 - T1} \quad (2)$$

Where N1 and N2 are cell densities at times T1 and T2 respectively.

Result and Discussion

Characteristics of wastewater from instant noodle factory

Wastewater from instant noodle factory (Wan Thai Foods Industry Co., Ltd., Bangkok, Thailand) was collected from two stages of activated sludge treatment process; mixed liquor suspended solids (MLSS) and effluent. Their characteristics were shown in Table 1.

Table 1 show that MLSS from instant noodle factory contained high suspended solids and high COD. Due to the high TSS (5500.00 ± 244.34 mg/L) in MLSS, nitrogen and phosphorus were mostly deposited in insoluble organic particle. Ammonia, nitrite, and nitrate in MLSS were found in low concentration (1.32 ± 0.15 mg N/L, 0.04 ± 0.00 mg N/L and 44.81 ± 3.63 mg N/L, respectively). In general, decomposition of organic compounds could release high amount of ammonia into the water due to ammonification process. This ammonia is the nitrogen source that enhanced growth of the microalgae. Effluent, on the other hand, had lower TSS (126.67 ± 5.77 mg/L) so it could be state that almost nutrient in effluent was soluble form.

Effect of phosphate addition to enhance microalgae growth in wastewater from instant noodle factory

Growth of *Scenedesmus* sp. in wastewater from instant noodle factory was shown in fig 3 and Table 2. Changes of inorganic nutrients concentration including total ammonia nitrogen, nitrite, nitrate, and phosphate were shown in Fig 4. It was found that total ammonia in MLSS treatments (T1 and T2) was significantly higher than effluent. This was due to solid decomposition with ammonia as the end product. High ammonia concentration was found in MLSS and high nitrate was in effluent during cultivation. This finding leads to the assumption that ammonia was the nitrogen source for algae growth in MLSS while growth of algae in effluent was due to nitrate utilization. The uptake of ammonium and nitrate both removed nitrogen from wastewater but most of the microalgae prefer ammonia instead of nitrate as the nitrogen source for cell growth.

Phosphate in all four treatments, on the other hand, depleted from the culture medium within four days of algal culture. Therefore, K_2HPO_4 was added as phosphate supplement for T2 and T4 to investigate effect of phosphate addition for microalgae growth. After phosphate addition, T2 and T4 exhibited higher growth and biomass than T1 and T3. This result suggested that phosphate addition can enhance growth of microalgae cultivated in wastewater from instant noodle factory. Noted that, biomass of microalgal grew in MLSS wastewater was higher than effluent. MLSS wastewater was therefore chosen for further investigation.

Phosphorus is the essential nutrient for plant as the cellular component and biosynthesis of nucleic acids. Reactive form of phosphorus for microalgal utilization is orthophosphate form (PO_4^{3-}). In general, phosphorus concentration in the water is the limiting factor for microalgal growth. With this experiment, the results showed that low phosphate concentration in wastewater is the limiting factor for *Scenedesmus* sp. growth. The results after phosphate addition, as shown in Fig 4, indicated the significant of phosphate supplement for microalgal wastewater treatment process. On the other

hand, nitrogen availability was not a problem with wastewater containing high suspended solid (MLSS) since ammonia and nitrate were found at high concentration (Fig 4 a).

Moreover, cultivation of *Scenedesmus* sp. had capable to reduce total chemical oxygen demand (COD) by 89.37 % and 31.38 % for MLSS and effluent respectively. On the other hand, reduction of soluble COD was 89.13 and 30.95 % for MLSS and effluent respectively (Fig 5). COD reduction is generally the results from bacterial decomposition in wastewater treatment. With this study, wastewater from instant noodle factory without sterilization contained natural bacteria so COD removal was related to both algal and bacterial activities. With the effluent treatments, COD after treatment was under the Thailand authority standard of effluent from industries (Pollution Control Department, Thailand, 1996).

Effect of lighting period and external carbon dioxide supplement on growth of microalgae cultivated in wastewater

According to previous experiment, MLSS was selected as the wastewater in this study. Fig 6 shows growth of *Scenedesmus* sp. with 2% carbon dioxide supplement. The growth analysis results indicated that, under laboratory condition, external carbon dioxide supplement did not clearly enhance growth of microalga *Scenedesmus* sp. in all treatments using MLSS wastewater from instant noodle factory. In addition, light period either continuous light or 12:12 light: dark cycle also provided similar growth of *Scenedesmus* sp.

In general, fifty percent of algal biomass is carbon which derived from carbon dioxide fixation through photosynthesis. Carbon supplement plays a vital role for the success of algal cultivation. Many studies showed that carbon dioxide can enhance growth of microalgae (Xu *et al.*, 2009; Kong *et al.*, 2010; Pegallapati and Nirmalakhandan, 2013). However, the results illustrated that external carbon dioxide supplement in this study did not clearly affect algal growth. Apart of photoautotrophic growth, sugar or acetate can be supplied as organic carbon for some heterotrophic or mixotrophic algae. For instance, *Chlorella* sp. can grow in heterotrophic condition using appropriate organic carbon source such as acetate (Perez-Garcia *et al.*, 2011). Since there were several reports on mixotrophic or even heterotrophic cultivation of *Scenedesmus* spp. (Shamala *et al.*, 1982; Sheekh Mostafa *et al.*, 2012; Zhang *et al.*, 2013) use of organic carbon under mixotrophic condition could be occurred along with inorganic carbon uptake by photosynthesis. It was likely that mixotrophic growth of *Scenedesmus* sp. occurred in this experiment so the alga could consume both carbon dioxide from air bubbling and organic carbon from wastewater.

Conclusion

K_2HPO_4 was added as phosphate supplement for MLSS and effluent to investigate effect of phosphate addition for microalgae growth. After phosphate addition, treatment with phosphate adding exhibited higher growth and biomass (482.02 ± 144.46 mg/L and 300.73 ± 182.35 mg/L for MLSS and effluent, respectively) than treatment without phosphate addition (200.19 ± 20.13 mg/L and 174.15 ± 45.08 mg/L for MLSS and effluent, respectively). This result suggested that phosphate addition can enhance growth of microalgae cultivated in wastewater from instant noodle factory. MLSS wastewater was therefore chosen for next section. In addition, growth

analysis results indicated that, under laboratory condition, external carbon dioxide did not clearly enhance growth of microalga *Scenedesmus* sp. in all treatments using MLSS wastewater from instant noodle factory. In addition, light period either continuous light or 12:12 light: dark cycle also provided similar growth of *Scenedesmus* sp.

Table.1 Physical and chemical characteristics of wastewater from instant noodle factory

Parameter (Unit)	**Wastewater**	
	MLSS	**Effluent**
	Average ± SD (min - max)	**Average ± SD**
pH	7.17 ± 0.06 (6.98 – 8.23)	7.43 ± 0.27
Total nitrogen (mg N/L)	189.87 ± 14.80 (68.44 – 211.15)	68.74 ± 11.90
Soluble total nitrogen (mg N/L)	32.09 ± 7.43 (28.56 – 63.22)	45.07 ± 2.66
Total ammonia nitrogen (mg N/L)	1.32 ± 0.15 (nd. – 24.23)	1.24 ± 0.10
Nitrite (mg N/L)	0.04 ± 0.00 (nd. – 2.65)	nd.
Nitrate (mg N/L)	44.81 ± 3.63 (6.33 – 63.29)	46.28 ± 0.32
Total phosphorus (mg P/L)	5.93 ± 0.37 (4.21 – 30.28)	3.13 ± 0.13
Soluble total phosphorus (mg P/L)	0.40 ± 0.07 (0.35 – 20.16)	1.33 ± 0.07
Phosphate (mg P/L)	1.82 ± 0.38 (0.23 – 18.22)	1.10 ± 0.04
Total suspended solids (mg/L)	5500.00 ± 244.34 (3408.25 – 9023.55)	126.67 ± 5.77
Chemical oxygen demand (mg/L)	3366.00 ± 200.92 (2908.11 – 11005.00)	198.00 ± 6.51
Soluble chemical oxygen demand (mg/L)	367.16 ± 8.08 (290.43 – 657.17)	148.88 ± 10.81

Remark *MLSS was collected seven times during 2013 and 2014 While effluent was collected only once in 2013

Table.2 Growth performance of *Scenedesmus* sp. cultivation in MLSS or effluent

Treatment	Initial biomass (mg/L)	Maximum biomass (mg/L)	Duration to reach maximum biomass (day)	Specific growth rate (day^{-1})
T1 (MLSS)	47.57 ± 2.13[a]	200.19 ± 20.13[a]	4	0.94 ± 0.50[a]
T2 (MLSS with phosphate addtion)	48.33 ± 2.04[a]	482.02 ± 144.46[b]	5	1.12 ± 0.15[a]
T3 (Effluent)	48.71 ± 1.18[a]	174.15 ± 45.08[a]	4	0.79 ± 0.20[a]
T4 (Effluent with phosphate addtion)	46.13 ± 0.71[a]	300.73 ± 182.35[ab]	5	0.71 ± 0.37[a]

Fig.1 A 2000 ml Duran bottle used as the culture vessel for mi-croalgae growth under batch experiment

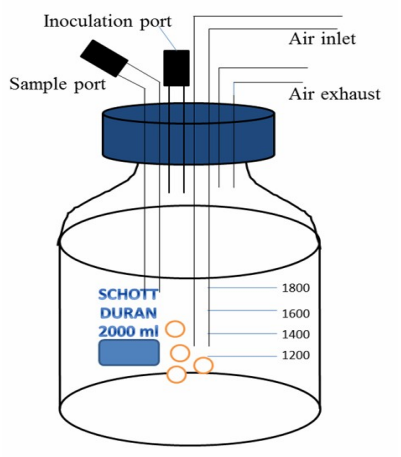


Fig.2 Diagram of external CO_2 mixing device in this experiment

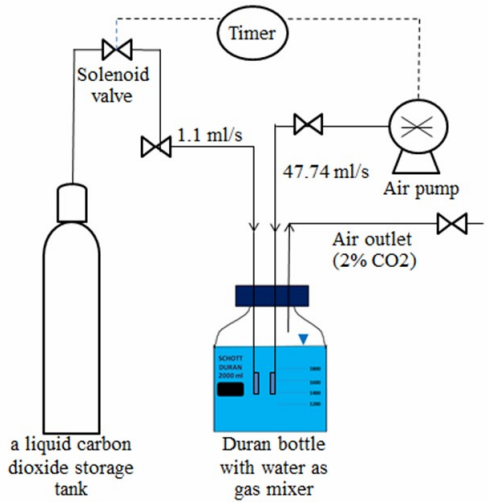


Fig.3 Growth curve of *Scenedesmus* sp. cultivation in MLSS or effluent. Dash line indicates phosphate addition in T2 and T4

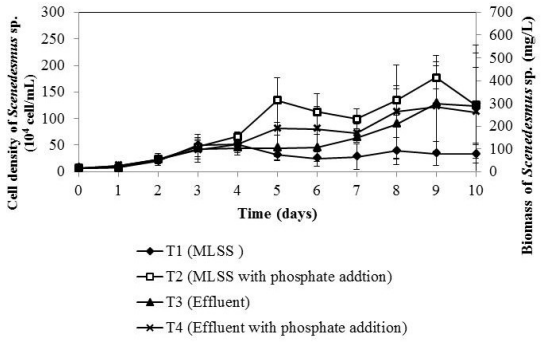


Fig.4 Inorganic nitrogen and inorganic phosphorus profile during *Scenedesmus* sp. cultivation in MLSS or effluent. (a) total ammonia nitrogen (b) nitrite (c) nitrate and (d) phosphate. Dash line indicates phosphate addition in T2 and T4

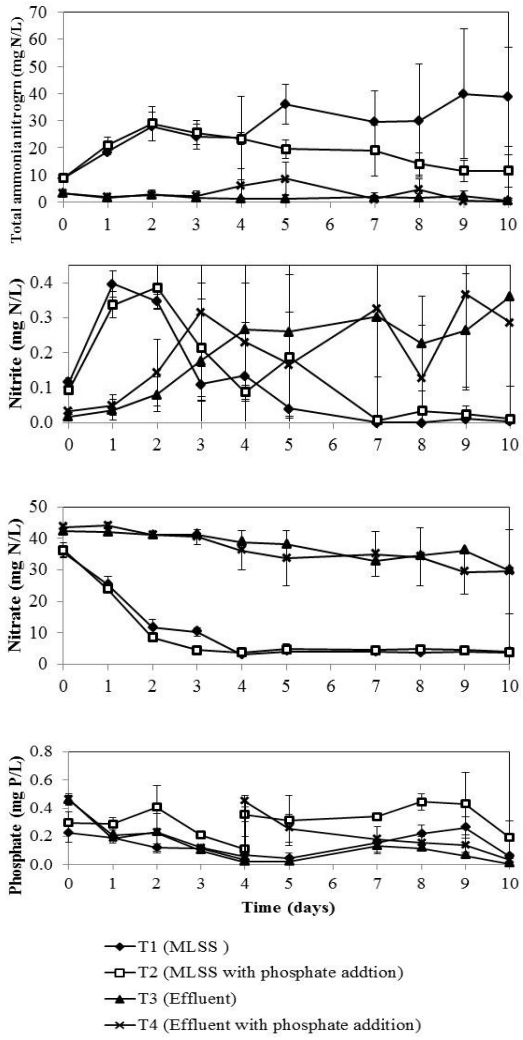


Fig.5 Chemical oxygen demand (COD) during initial and final day of microalgae cultivation in MLSS or effluent. (a) total COD (b) soluble COD

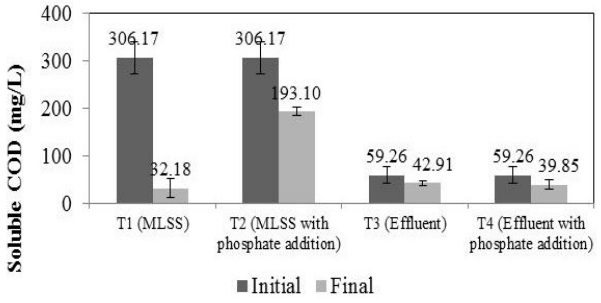
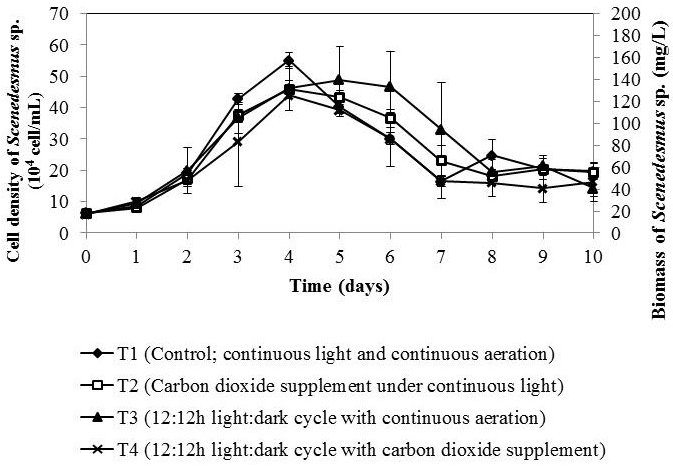


Fig.6 Growth curve of *Scenedesmus* sp. using MLSS wastewater from instant noodle factory as a medium with aeration or external carbon dioxide supplement under continuous illumination or 12:12 h light: dark cycle



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