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มวลชีวภาพและปริมาณลิพิดจาก *Scenedesmus acutus* UD01 ที่เพาะเลี้ยงในน้ำทิ้ง

Biomass and lipid content of *Scenedesmus acutus* UD01 cultured in wastewater effluent

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Abstract

The cultivation of microalgae as an alternative energy source has increasingly become popular in various parts of the world. However, the high cost of the media used in cultivation has directly affected the manufacturing cost. In this study, we have aimed to culture *Scenedesmus acutus* UD01 in the effluent acquired from the biogas system of a piggery farm (BGF), the wastewater of a sugar factory (SF), and modified JM(MJM). After 7 days of cultivation under white fluorescence at room temperature, the highest growth rate was found in BGF and the second highest was recorded in MJM. In the study on the lipid contents, the results revealed that 20.09±0.18% of lipid was obtained from *S. acutus* UD01 cultured in SF, while 17.72±1.05% of lipid was recorded in BGF. In considering the lipid composition and the process of culturing conducted in the low-cost media, there is a desire to find an achievable economic procedure for algal biofuel production.

Keywords: *Scenedesmus*, lipid, biomass, wastewater, piggery, sugar factory

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1. Introduction

Presently, petroleum is recognized as a finite resource that is running out, while global energy consumption is increasing. Therefore, alternative sources of energy need to be identified and developed as substitutes for fossil-based energy sources [1]. Bio-energy is being considered worldwide as a future energy source of great significance. Microalgae have emerged as a highly productive organism and are recognized as a novel energy resource. The high efficiency of the photosynthetic conversion and the high lipid contents of microalgae have been widely acknowledged in studies [2-3]. Moreover, biofuels, especially biodiesel generated from microalgae have been noted as having an ability to compete with petro fuels [4]. Microalgae have displayed the ability to grow under various weather conditions and in a variety of problem soil areas that are unable to sustain any agricultural activity. In addition, microalgae have emerged as having a lower cost and high potential to reduce greenhouse gas emissions [5]. Some microalgae (such as *Scenedesmus* and *Chlorella*) have displayed a very short duplicating time while producing a high yield of lipid per hectare. Therefore, the environmental and economical sustainability of microalgae as a potential new biofuel source has been suggested [6].

Microalgae are considered a novel substitute as an energy resource. However, microalgae require a huge amount of water for cultivation, as in 11-13 million liters per hectare. In addition, the cost of macro-nutrients are significant factors in determining the overall cost of algal production (N and P) [7-8]. The cost of N and P fertilizer has increased continuously and this has directly affected the cost of the biomass and bio-lipid production. Therefore, It is essential to explore any potential alternate nutrient sources for large-scale algal cultivation. Wastewater has been suggested as a nutrient source due to the richness of the organic and inorganic nutrients that it contains. As a result, it has been suggested that wastewater could used in the place of freshwater that has been supplemented with fertilizer [8-9].

Many species of microalgae are known to have the ability to rapidly grow in wastewater, as they are able to consume the abundant organic and inorganic nutrients present in the effluent. Consequently, the idea of growing algae at wastewater treatment plants has long been promoted [10]. Several studies have investigated the use of wastewater as a source of algal nutrients. Caprio *et al*. [11] published a study on the cultivation of *Scenedesmus* sp. in olive mill wastewater. This study was conducted by mixing wastewater with BG11 and Basal Medium. Bhatnagar *et al*. [12] found that poultry litter could effectively support *Scenedesmus bijuga* in growth when compared to BG11 and wastewater. The wastewater from a piggery farm to produce lipids has revealed that this wastewater effectively

provided the necessary nutrients for potentially profitable products such as biomass and lipids [13].

Scenedesmus are usually found as the dominat geneus in the oxidation ponds of treatment systems [14].The lipid content up to 58% (dry cell weight) was reported from the Genus *Scenedesmus* [14-20]. The present study was determined the optimum concentration of wastewater from a piggery farm and sugar factory. *Scenedeusmus acutus* UD01 was selected and isolated from a local water resource.

2. Materials and Experiment

Algal preparation

Scenedeusmus acutus UD01 was isolated from the artificial water resource in UdonThani Province. The alga was grown in Jaworski's medium (JM) [21] using a white fluorescent lamp at room temperature for 7 days as the stock culture.

Growth condition

In terms of sorting out the best conditions for cultivation, 10 media were designed. JM was modified into 3 conditions using 16-16-16 fertilizer, modified-JM1 (MJM1); JM supplemented with a gram of fertilizer/500 ml, modified-JM2 (MJM2); JM supplemented with 2.5 g of fertilizer/500 ml and modified-JM3 (MJM3); JM supplemented with 5 g of fertilizer/500 ml. The media from the effluent of the biogas system of a piggery farm (BGF) and the effluent of a sugar factory (SF) were prepared in 5%,10% and 15% solutions respectively. All conditions were carried out under white fluorescent lamp at room temperature for 7 days. The algal growth was evaluated in every day by measuring the absorbance at 665 nm using Hach: DR2700 and cell-counting using a haemacytometer (colony/ml). The cell dry-weight was estimated at the 1st and on 7th day of cultivation. The dried weight was used to determine the best growth promoting conditions of MJM, BGF and SF. The selected conditions were re-cultured in order to study the lipid contents.

Cell harvesting and dewatering

The biomass of the culture was harvested by centrifugation at 3500 rpm for 15 minutes. The medium was discarded and the remaining cells were collected. The de-ionized water was used to rinse the substance and for re-centrifugation. The algae cells was dried at 60°C for 16 h.

Lipid content examination

Dried algal powder was extracted the total lipids using modified procedures from Bligh and Dyer's method [22]. A gram of algal powder was mixed with 30 ml of the mixture solution [chloroform: methanol (2:1, v/v)]. Then, cells were breaked using a probe sonicator (Vibra-cell; Sonics VCX-750). The mixtures were left at room temperature for 24 h, centrifuged at 6000 rpm for 15 minutes and the supernatant was transferred into a separatory funnel. After that, 5 ml of normal saline was added and the solution was shaken for 5 minutes. The lipid fraction was separated and this fraction was collected into a pre-weighed flask. Heating to achieve dryness was done in the oven (60°C) before measuring the lipid content.

Statistical analysis

The growth rate and the lipid content were compared using the one-way ANOVA with Tukey-b test. The level of significant difference was at $P < 0.05$.

3. Result and Discussion

According to the absorbance at 665 nm, the results of MJM were not differentiated when compared to JM, which is considered the conventional media (Fig. 1). This was in contrast to the results from BGF, which revealed that 10% of BGF showed the highest absorbance (OD_{665}=1.137) at day 5th and this value was higher than that of JM (Fig. 2). Whereas, the absorbance of the alga cultured in SF, it was revealed that all conditions of this media were clearly lower than JM (Fig. 3). However, the study of the growth of SF using the cell-counting method revealed a higher anumber of colonies per milliliter (Fig. 6). The MJM media also revealed the same growth pattern as the JM (Fig. 4). However, all conditions of BGF still showed higher values than the other conditions. The highest value of cell number was found in 10% BGF at 7 days (1040 colony/ml) (Fig. 5).

After seven days, the dried weight cell of *Scenedesmus acutus* UD01 in 10% of BGF showed the highest value (Table 1). The second highest value was found with MJM1, which was 1.79±0.20 g/100ml. In comparison with the conventional media(JM), MJM1, MJM2, BGF5%, BGF10%, and SF10% revealed significant supporting the growth rate of the microalgae.

Under MJM conditions, the addition of fertilizer could promote the growth rates as presented in MJM1 and MJM2, but the addition of fertilizer by up to 5 grams could negatively affect the growth rate (Table 1 and Fig. 7), it might be affected by the osmotic pressure. Similarly, result of BGF medium, the concentration of bio-gas

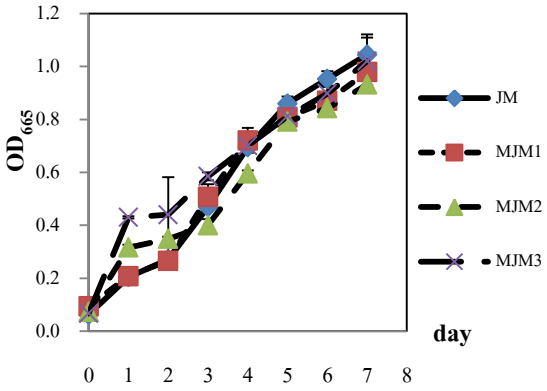

Fig. 1 Absorbance of *S. acutus* UD01 in MJM media

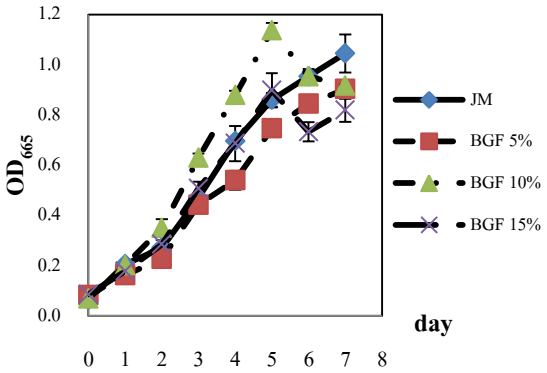

Fig. 2 Absorbance of *S. acutus* UD01 in BGF media

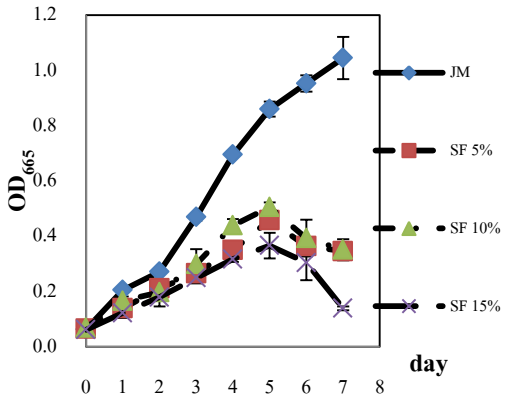

Fig. 3 Absorbance of *S. acutus* UD01 in SF media

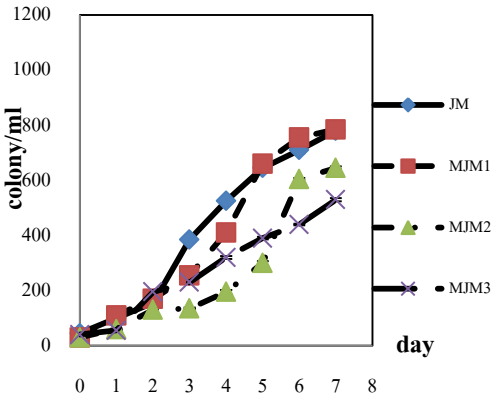

Fig. 4 The colony numbers of *S. acutus* UD01 in MJM media

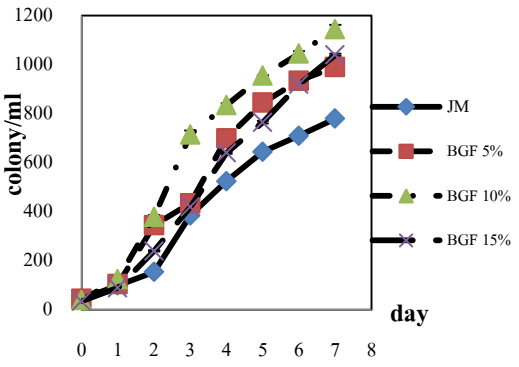

Fig. 5 The colony numbers of *S. acutus* UD01 in BGF media

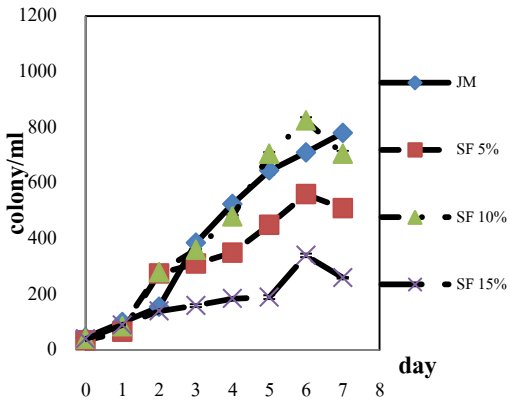

Fig. 6 The colony numbers of *S. acutus* UD01 in SF media

Table 1: Dried weight of *S. acutus* UD01 in wastewater and modified media

Media	Dried weight cell of *S. acutus* UD01 (g/100ml)
JM	1.71 ± 0.17^{bc}
MJM1	2.11 ± 0.09^{b}
MJM2	1.89 ± 0.16^{bc}
MJM3	1.48 ± 0.30^{c}
BGF 5%	1.78 ± 0.53^{bc}
BGF 10%	2.71 ± 0.53^{a}
BGF 15%	0.81 ± 0.07^{d}
SF 5%	1.58 ± 0.08^{bc}
SF 10%	1.79 ± 0.20^{bc}
SF 15%	1.36 ± 0.24^{c}

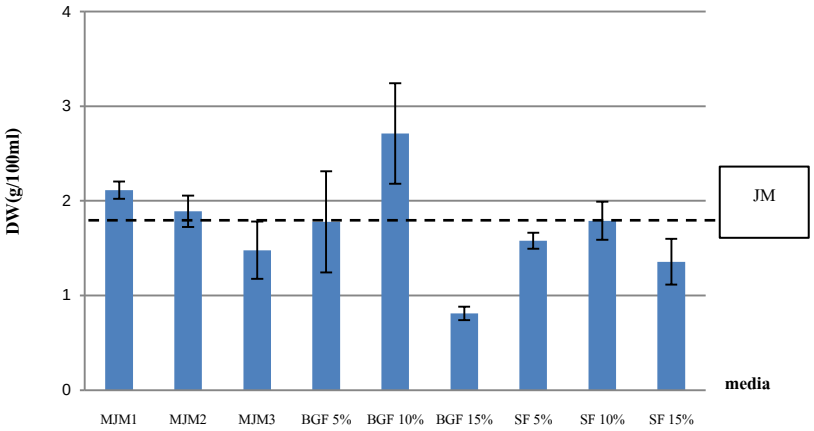

Fig. 7 Dried weight of *S. acutus* UD01 in wastewater and modified media

effluent was recorded at 5% while a 10% value produced a higher growth rate than 15%. In contrast with the SF, 5% and 15% of the sugar factory wastewater revealed lower values. The best concentration of SF was found in 10% (Table 1 and Fig. 7).

The best conditions for biomass production were found in 10% of BGF and 10% of SF (Table 1 and Fig. 7). These findings seem to involve higher concentrations of wastewater that were used in algal cultivation for lipid and biomass production when compared to previous studies which had used 0.5-2% of wastewater [18-20]. Additionally, Ji et al. [18] and Caprio et al. [11] reported only a few higher values of biomass production in this report, but Bold's Basal Medium (BBM) and BG11 were used as the culture media. In contrast to this study, only wastewater diluted with distilled water was used. Although the biomass production did not necessarily seem to yield high values, the required minerals were not added. This essentially decreased the cost of manufacturing.

The best conditions in terms of the growth rates of each medium were used for the study of the lipid content, which was considering by dried weight. MJM1, SF10% and BGF10% were selected for this experiment. The lipid contents produced by MJM1 and JM were not significantly different from JM. BGF media produced the second highest lipid content in the algal cells, significantly. The highest lipid content was found in the cells from 10% of SF, which produced up to 20%(w/w) (Table 2). However, regarding the percentage of lipid content and dried weight, 10% of BGF showed the best values ($p<0.05$), followed by 10% of SF, while MJM1 and JM produced similar amounts (Table 2). Further, the highest lipid content in the present study was greater than that of *Chlorella pyrenoidasa* and *Scenedesmus sp.* grown in piggery wastewater and fermented with swine

urine [9; 23]. Additionally, *S. bijuga* grown in anaerobically digested poultry litter exhibited the equal lipid content with this study [12].

The lipid productivity levels of the present study were not found to be superior to the previous studies. Ji *et al*. [18] reported that the lipid content obtained from *S. obiquus* grown in food wastewater diluted with BBM was 13.3 mg/l. In Ashokkumar's study, 63 mg/l·d of lipid was obtained from *S. bijugatus* grown in the custom-made vertical tubular photo-reactor [24]. Ji *et al*. [19] reported 10-11 mg/l·d of lipids produced from *S. obiquus* that was grown in food wastewater with flue gas CO_2, which could have enhanced both the levels of growth and lipid productivity. It should be noted that all of the above detailed conditions involved variable levels of nutrient contents and a range of conditions in order to sort out the best possible procedure for lipid production from the wastewater which had been mixed with the complete media, such as BM and BG11. These factors could clearly increase the cost of lipid production. In addition, many reports have described certain procedures in providing the algal stresses to increase the lipid contents such as growing under nutrient depletion conditions (nitrogen and phosphorus) [25-26], culturing in mixotrophic under light limitation conditions [27-29]. All of these conditions can be used to provide a high yield of lipid production, but the complexities of the procedure were also increased.

Table 2: Lipid contents of *Scenedesmus acutus* UD01 cultured in wastewater and modified media

Treatments	Lipid	
	Lipid content(%)	(mg/L)
JM	13.40±1.24^{c}	29.48^{c}
MJM1	13.28±0.67^{c}	29.22^{c}
SF 10%	20.09±0.18^{a}	44.20^{b}
BGF 10%	17.72±1.05^{b}	56.70^{a}

Table 3: Nutrient compositions of BGF and SF

Nutrients	BGF	SF
Ammonium nitrogen (mg/l)	157	9.1
Soluble reactive phosphorus (mg/l)	162	20.8
Nitrate nitrogen (mg/l)	1.8	8

Nutrients of cultured media directly affect the biomass production and the lipid composition. Phosphorus and Nitrogen available in BGF were higher than JM (Table 3). This related to the greatest values of growth rate and biomass production of BGF. On the other hand, nitrogen insufficient of SF affected to the highest level of lipid content (Table 2). Nitrogen-deficiency has been widely shown to affect markedly the lipid composition [1].

In the present study, we revealed the values of the biomass and lipid contents, which were produced by the simplified method, cultured under autotrophic conditions at room temperature and used only wastewater that had been diluted with distilled water. Although the biomass and

lipid contents did not seem to be of particularly high values, the reduced level of complexities involved with establishing the open algal pond as a small business enterprise would be beneficial for successful and affordable biomass and lipid production.

4. Conclusions

This study investigated the growth rates and lipid contents of *Scenedesmus actus* UD01 which cultivated in the effluent acquired from the biogas system of a piggery farm (BGF), the wastewater of a sugar factory (SF) and modified JM. The best conditions in terms of the growth rates considering by dried weight was 10% of BGF. The highest lipid content was found in the cells from 10% of SF, which produced up to 20%(w/w). However, regarding the percentage of lipid content and dried weight, 10% of BGF gave the best values ($p<0.05$). These results indicated that growing *S. acutus* in UD01 BGF and SF provided the profitable products from wastewater. In addition, these could reveal the economic information in producing biomass and lipid from microalgae. However, the scale up of cultivation to industrial production is needed.

5. Acknowledgement

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