



# Effective biogas upgrading and production of biodiesel feedstocks by strategic cultivation of oleaginous microalgae

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## ABSTRACT

This study has shown the strategies to upgrade biogas and produce lipids as biodiesel feedstocks by cultivation of oleaginous microalgae. Three important growth factors for microalgae including light intensity, nitrogen source and CO<sub>2</sub> supply, were strategically stepwise-increased during cultivation. The stepwise-increasing of CO<sub>2</sub> supply was suitable for cell growth and lipid production while the stepwise-increasing of light intensity was more suitable for CO<sub>2</sub> removal efficiency. Among the strategies attempted, the simultaneous stepwise-increasing of all three growth factors most effectively enhanced the performance of microalgae. Through this strategy, >96% of CO<sub>2</sub> was continuously removed from biogas and the CH<sub>4</sub> content in the purified biogas was >98%. This process also generated microalgal biomass at  $4.40 \pm 0.04 \text{ g L}^{-1}$  with a lipid content of  $34.10 \pm 2.26\%$ . The CO<sub>2</sub> removal rate by this process was as high as  $6.50 \pm 0.21 \text{ g-CO}_2 \text{ day}^{-1}$  per 1 L microalgal culture. The microalgal lipids contained long chain fatty acids (C16–C18) >94% and their prospect fuel properties indicated their suitable use as biodiesel feedstocks. The integrated processes and strategies in this study would contribute greatly to the production of biogas and biodiesel feedstocks.

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## 1. Introduction

Biogas has been receiving increasingly interest as a renewable and sustainable source of energy. Biogas is produced from anaerobic digestion of organic wastes. Generally, crude biogas is composed of 40–75% CH<sub>4</sub>, 25–60% CO<sub>2</sub> and trace amounts of other components [1]. But only biogas with CH<sub>4</sub> content >90% (v/v) meets requirement for being used as biofuel with high-efficiency combustion and high heating value [2,3]. The removal of CO<sub>2</sub> is an important key to increase CH<sub>4</sub> content in biogas. Despite physical/chemical absorption, water scrubbing, membrane separation, and pressure-swing adsorption supporting a removal of CO<sub>2</sub> from biogas, these technologies require considerable large amount of energy, auxiliary materials, and chemicals. Moreover, they also generate wastes and wastewater that can pollute the environment [4]. Recently, the biological removal of CO<sub>2</sub> using cultivation of microalgae has received considerable attention for improving CH<sub>4</sub> content in biogas. This is because the CO<sub>2</sub> fixation capability of the microalgae is high and this process also gives high productivity of

microalgal biomass [5]. The CO<sub>2</sub> removal from biogas has been studied by using several species of microalgae i.e. *Scenedesmus obliquus*, *Nannochloropsis* sp., *Chlorella vulgaris* and *Neochloris oleoabundans*. The results showed that more than 90% of CO<sub>2</sub> could be removed from biogas while only 0.2–1% O<sub>2</sub> was detected in the gas phase. It should be noted that the use of specific microalgae that could produce high value products, would be more attractive in order to offset the costs of this process. However, a little report has evaluated the use of oleaginous microalgae for removing CO<sub>2</sub> from biogas [6,7]. Due to the high potential of this technique for environmental friendly and renewable energy production, the promising strategy to improve this technique should be developed to make it more viable at industrial scale.

The light intensity, nitrogen source and carbon dioxide concentration are main important growth factors that determine the CO<sub>2</sub> fixation capacity and lipid production of the microalgae. It has been reported that an insufficient light intensity could limit the microalgae growth and CO<sub>2</sub> fixation rate and the microalgae might even consume its storage lipid during photolimitation phase [8]. Nitrogen source is one of important growth factors for microalgae reproduction as it involves in the synthesis of nucleic acids, phospholipids and proteins. It has been reported that increasing

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nitrogen concentration could lead to the increase in biomass [10,11]. Although increasing the growth factors at initial could increase the ability of the microalgae, when their levels are above the optimal level the inhibition occurs. For example, an excessive light intensity could bleach the pigments and produce an overloaded photosystem which finally inhibited the microalgae growth and CO<sub>2</sub> fixation [7,9]. Similar trends have also been observed for the effects of nitrogen source and carbon dioxide concentration [10,11]. Although increasing of CO<sub>2</sub> supply could increase the microalgae growth, the CO<sub>2</sub> supply with high gas flow rate would rapidly lower the pH of the medium and decrease the performance of the microalgae. In addition, Kao et al. [12] also reported that the CO<sub>2</sub> utilization by microalgae decreased at high gas flow rate due to the decrease in retention time of large bubbles in the medium. Therefore, it is necessary to determine the optimal initial levels of light intensity, nitrogen source and CO<sub>2</sub> supply in order to avoid their inhibition effects. However, the more microalgae grow during cultivation the higher levels of these growth factors are needed. Hence, the strategies to efficiently provide these growth factors during cultivation should be determined.

In this study, oleaginous green microalga *Scenedesmus* sp. was used as potential microalgae to biologically remove CO<sub>2</sub> from biogas and simultaneously produce lipids for being used as biodiesel feedstocks. Firstly, the important growth factors including light intensity, nitrogen source (KNO<sub>3</sub>) and CO<sub>2</sub> supply (biogas flow rate) were individually stepwise-increased to support the increased microalgae cells during cultivation. Secondly, the simultaneous stepwise-increasing of either two or three growth factors were attempted. Finally, the fatty acid compositions and prospect biodiesel properties of microalgal lipid were evaluated.

## 2. Materials and methods

### 2.1. Microalgae strain and culture medium

The microalgae strain used in this study was oleaginous *Scenedesmus* sp. from Bioprocess Engineering Laboratory at Prince of Songkla University, Thailand. The modified Chu13 medium used as basic medium consisted of 0.8 g KNO<sub>3</sub> as a nitrogen source, 0.04 g K<sub>2</sub>HPO<sub>4</sub> as a phosphorus source, 0.1 g citric acid, 0.01 g Fe citrate, 0.1 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.036 g NaHCO<sub>3</sub>, and 1 mL of trace metal solution per 1 L. The trace metal solution consisted of 2.85 g H<sub>3</sub>BO<sub>3</sub>, 1.8 g MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.02 g ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.08 g CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.08 g CoCl<sub>2</sub>·6H<sub>2</sub>O, and 0.05 g Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O per 1 L, pH was 6.8 [6].

### 2.2. Stepwise-increasing of growth factors for cultivation of microalgae

The oleaginous *Scenedesmus* sp. was cultured in 500 mL Duran bottles containing 400 mL of modified Chu13 and agitation was performed by a magnetic stirrer at 150 rpm. The culture was bubbled with simulated biogas (CO<sub>2</sub>:CH<sub>4</sub> 40:60) at a gas flow rate of 0.3 L h<sup>-1</sup> per 1 L microalgal culture. The light illumination was provided by cool-white fluorescent lamps with photoperiod 24 h light: 0 h dark. The initial light intensity was set at 5.5 klux and the initial cell concentration was 10<sup>7</sup> cells mL<sup>-1</sup>. The cultures were incubated at 30 °C for 8 days. Three growth factors including light intensity, nitrogen source and CO<sub>2</sub> supply each and their combination were stepwise-increased to support the increased biomass of the microalgae during cultivation. The stepwise-increasing of light intensity was performed by increasing the light intensity at a step of 0.5 klux every 2 days (Stepwise LI). The stepwise-addition of nitrogen source was performed by intermittently adding 0.2 g L<sup>-1</sup> KNO<sub>3</sub> every 2 days (Stepwise N). The stepwise-increasing of CO<sub>2</sub> supply (Stepwise GFR) was performed by increasing biogas flow

rate at a step of 0.03 L h<sup>-1</sup> per 1 L microalgal culture every 2 days. The simultaneous stepwise-increasing of light intensity and nitrogen source (Simultaneous Stepwise LI + N) and those of three factors (light intensity, nitrogen source and CO<sub>2</sub> supply; Simultaneous Stepwise LI + N + GFR) were also performed.

### 2.3. Analytical methods

Cell concentration was determined by a direct microscopic count method using a hemocytometer and turbidimetrically at 660 nm using a spectrophotometer (Technical Cooperation, USA). The dry microalgal biomass was determined as follows: 10 mL of microalgal suspension was centrifuged at 3500 × g for 15 min and the supernatant was used to estimate the nitrate-nitrogen (N-NO<sub>3</sub><sup>-</sup>) concentration by brucine method [13]. Then, the cell pellet was dried at 60 °C until constant weight and weighted. The specific growth rate (μ) was calculated using data in the exponential phase using the following equation:

$$\mu \text{ (day}^{-1}\text{)} = (\ln X_2 - \ln X_1) / (t_2 - t_1) \quad (1)$$

where X<sub>1</sub> and X<sub>2</sub> are the concentrations of microalgal cells (g L<sup>-1</sup>) at time t<sub>1</sub> and t<sub>2</sub>, respectively.

The lipid content of the dried microalgal biomass was determined using liquid extraction by a mixed solvent solution of methanol and chloroform (2:1 v/v). Dry microalgal biomass was mashed and mixed with the solvent solution before sonication for 30 min. The suspension was centrifuged at 3500 × g for 15 min. The supernatant was collected and the cell pellets were extracted twice more. After extraction, the solvent solution was evaporated overnight and the extracted lipid was determined gravimetrically. The lipid productivity (P<sub>lipid</sub>) was calculated by the following equation:

$$P_{\text{lipid}} \text{ (mg L}^{-1}\text{ day}^{-1}\text{)} = (L_2 - L_1) / (t_2 - t_1) \quad (2)$$

where L<sub>1</sub> and L<sub>2</sub> were the lipid production (mg L<sup>-1</sup>) at time t<sub>1</sub> (start point of cultivation) and t<sub>2</sub> (the point that the maximum lipid was obtained), respectively. The lipid content (%w/w) was calculated with the following equation:

$$\text{Lipid content} = [\text{lipid production (g L}^{-1}\text{)} / \text{dried microalgal biomass weight (g L}^{-1}\text{)}] \times 100 \quad (3)$$

The biogas compositions analysis, the compositions of biogas were determined using a Gas Chromatograph (GC 6890) with a cross-linked capillary HP-PLOT Q column (length 30 m, 0.54 mm I.D, 0.04 μm film thickness) and a thermal conductivity detector. Helium was used as carrier gas [6]. The percent of CO<sub>2</sub> removal from biogas and CO<sub>2</sub> removal rate (g-CO<sub>2</sub> day<sup>-1</sup> per 1 L-microalgal culture) were determined as follows:

$$\% \text{CO}_2 \text{ removal} = [\text{initial CO}_2 \text{ (\%)} - \text{final CO}_2 \text{ (\%)} / \text{initial CO}_2 \text{ (\%)}] \times 100 \quad (4)$$

$$\text{CO}_2 \text{ removal rate} = \text{biogas flow rate (L day}^{-1}\text{ L}^{-1}\text{)} \times \text{initial CO}_2 \text{ (\%)} \times \% \text{CO}_2 \text{ removal} \times 1000 \text{ g-CO}_2 / 556.2 \text{ L} \quad (5)$$

The extracted lipids were hydrolyzed and esterified to fatty acid methyl esters (FAME) [14]. The fatty acid profiles of the FAME were analyzed using a HP6850 Gas Chromatography equipped with a cross-linked capillary FFAP column (length 30 m, 0.32 mm ID, 0.25 μm film thickness) and a flame ionization detector. Operating condition were as follows: inlet temperature 290 °C; oven temperature initial 210 °C held for 12 min, then ramped to 250 °C at 20 °C/min; held for 8 min and the detector temperature was 300 °C.

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