www.rericjournal.ait.ac.th

Development of a Microalgae based System for Biogas Upgrading and Oil Production from Waste Biomass

Mathin Jaikua*, Sahataya Thongsan*[1], and Sirilux Chaijamrus[+]

Abstract – *A study of a system for carbon dioxide reduction from biogas to increase the methane and accumulate oil in the form of lipid in the Chlorella vulgaris TISTR 8580 was proposed. The system coupled together with the CO_2 reduction and oil production systems. Microalgae were cultivated in a photobioreactor bubble column with cell turbidity at 540 nm, where the specific growth and doubling time of the algae were calculated. Biogas was produced from the cow manure, which was injected directly into the C. vulgaris culture system with the different flow rates. The optimum biogas flow rate to affect the highest carbon dioxide fixation efficiency and methane content was tested. By applying the flow rate of 100 mL/min and a concentration of 1.9 x 10^7 cell/mL of C. vulgaris culture, which is shown that the maximum efficiency of CO_2 reduction was 84.48%, while the methane in the biogas effluent increased to 89.40%. The highest biomass productivity, lipid production and content levels of 0.33 mg/L d^{-1}, 4.74 mg/L d^{-1} and 14.35 % were achieved, respectively. These results show that the utilization of microalgae C. vulgaris is the effective alternative process for reducing the carbon dioxide content, while the biogas methane volumes are increased. The lipids accumulation in the microalgae C. vulgaris culture were 75.22% of unsaturated fatty acid.*

Keywords –biogas upgrading, carbon dioxide reduction, *Chlorella* sp., *Chlorella vulgaris*, oil production.

1. INTRODUCTION

Fossil fuels are still commonly used for power generation and transport worldwide. While Thailand has an oil production industry, it still imports a significant proportion of its oil consumption requirements, which continues to increase with industrial development and population. Both government and private sector organizations now place emphasis on continuing to investigate new energy sources apart from fossil fuels. Natural gas is a fossil fuel that is increasing in use, particularly to replace coal-fired power generation capacity, as well as a motor vehicle fuel, and for domestic use in heating and cooking. Another significant benefit of using natural gas as a power source include its compress ability, where huge volumes can be transported in concentrated, liquid form, as compared to the transportation requirements of coal and oil. Natural gas is a hydrocarbon gas mixture consisting primarily of methane [1], but also contains carbon dioxide, nitrogen, and hydrogen sulfide. Production of natural gas in Thailand began in 1981 from the Erawan field, with additional major gas fields more recently discovered at Bong Kot, the Thailand-Malaysia Joint Development Area, and the Arthit and Pailin fields [2]. Notwithstanding these discoveries, high demand growth over the past two decades has led Thailand to become a net importer of natural gas, which is an expensive activity. Therefore, researchers are now investigating alternative means of generating natural gas in sufficient quantities, economically and in an environmentally friendly manner.

Biogas is a renewable energy source with potential as it can be put to multiple uses, including power generation, as a motor vehicle fuel and for domestic heating and cooking, in the same way as natural gas can be used. It is therefore a convenient and useful substitute for natural gas, if available in sufficient quantities at a reasonable cost. However, biogas contains undesirable substances, particularly carbon dioxide that must be removed before the biogas is ready for use.

One process for generating biogas is anaerobic digestion. Biogas is produced by the degradation of organic matter by chemical and biological reactions in anaerobic conditions. These conditions occur naturally, or can be created under controlled conditions using appropriate technology, such as an anaerobic reactor or digester. Biogas produced in this way is mostly methane (50-70%), carbon dioxide (30-50%) with smaller volumes of other gases such as hydrogen sulphide, oxygen and nitrogen. The combustion of methane releases energy in the form of heat, and when burned in compressed form is a good source of mechanical energy, but the level of performance and engine power depends substantially on the proportion of methane in the biogas.

The carbon dioxide (CO_2) content of the biogas is not combustible, but acts to lower the concentration of methane in the biogas. Therefore, removing the CO_2 is an important step in the process of producing biogas with a high methane concentration, approaching or equaling the same standards as fossil natural gas, which itself must undergo a cleaning process to become biomethane. The most prevalent methods of biogas upgrading currently in use are chemical absorption, the adsorption process, membrane separation and water scrubbing. However, these processes have high power consumption requirements, and in some, high water consumption with high levels of polluted waste water

*School of Renewable Energy Technology, Naresuan University, Phitsanulok, Thailand 65000.

[+]Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand 65000.

[1]Corresponding author;
Tel: + 665 596 3395, Fax: + 665 596 3182
E-mail: sahatayal@nu.ac.th.

resulting. They also require a high initial and on-going investment cost. All of these factors have meant that the production of biogas has been a high cost and high resources usage process.

One avenue of research now is in the beneficial use of the CO_2 component of biogas produced the anaerobic digestion process. CO_2 is consumed in the process of photosynthesis of plant matter. In the current research, algae is the subject of our investigations, as a way to use the CO_2 which is otherwise considered a nuisance waste gas requiring cost and effort to remove. The fact that CO_2 is a source of nutrition for algae offers an environmentally friendly, and cheap way, to use the CO_2 in the production of useable oils in the algae.

Chlorella vulgaris is one of the best algae species for use in the capture of carbon dioxide given its fast growth rate and easy cultivation. *C. vulgaris* converts inorganic carbon into organic carbon. CO_2 is an inorganic carbon source. *C. vulgaris* can double its volume in less than 20 hours [3], and can be grown in autotrophic, heterotrophic, or mixotrophic conditions. *Chlorella* sp. has been reported, at a biomass concentration of 1.2 g/L, to be able to fix CO_2 at a rate of 70% of CO_2 volume on cloudy days, and 80% on sunny days [4]. In [5], improving carbon dioxide fixation efficiency by optimizing Chlorella PY-ZU1 culture conditions in sequential bio-reactors was studied, and peak carbon dioxide fixation efficiency was assessed at 85.6%. Another study [6] investigated CO_2 bio-mitigation by using CO_2 as a fuel for the growth of *Chlorella vulgaris*, and showed that CO_2 bubbling could accelerate growth by 5.2 times when compared to that achieved with $NaHCO_3$. However, a significantly lower CO_2 removal efficiency was observed. Interestingly, when $NaHCO_3$ was supplied as the carbon source, *C. vulgaris* preferred to utilize free CO_2 molecules at acidic cultivation conditions (pH 4) instead of bicarbonate ions at alkaline conditions (pH 8.5), at a CO_2 removal efficiency of 82.5%–99%. In addition, the volume of lipids produced in the growth of *C. vulgaris* was significantly increased by up to 56.6% of the dry biomass weight [7.]

To cultivate algae to accumulate oil in high volume, close control of the various conditions that affect the growth and oil accumulation of the algae cells is essential. These growing conditions include the food source, carbon source, lighting range and temperature. Carbon dioxide is the primary food source for the cultivation of algae, and is used in the process of photosynthesis and growth of the microalgae. As such, carbon dioxide enhances the accumulation of high volumes of oil which can be used to produce biodiesel similar biodiesel from vegetable oil. Biogas is one type of renewable energy that has a high carbon dioxide content that needs to be removed before using. Clearly, if the carbon dioxide content in biogas could be used as a food for the microalgae, this would be of considerable benefit in the biogas and biodiesel production cycle.

Therefore, this research was a study of the efficiency of carbon dioxide reduction methods and methane enrichment in biogas and the subsequent use of the waste carbon dioxide as feedstock for growing microalgae as a source of oil production. *C. vulgaris* TISTR 8580, which grows in profusion in Thailand, was grown in a photobioreactor using bubble columns of the waste carbon dixoide to feed the algae, in a combined, integrated system.

2. MATERIALS AND METHOD

2.1. Materials

2.1.1 Microalgae

Chlorella vulgaris TISTR 8580 was purchased from the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. The *C. vulgaris* was grown in 100 mL of a desirable medium contained in 250 mL Erlenmeyer flasks. The culture was incubated at room temperature in a shaking incubator, at the agitation speed of 150 rpm, under fluorescent light in a 16-h light and 8-h dark regime. During the light period, the light intensity was 3,000 Lux using cool white fluorescent lamps.

The cultivation medium was chlorella broth, which had been purchased from commercial suppliers (HiMedia, India) in powder form and reconstituted with 17.6 g of powder distributed in 1,000 mL distilled water using a stirrer, then sterilized by autoclave at 121°C at 15 lbs pressure for 15 minutes. The medium consisted of the following components (per liter of distilled water): 8 µg of $CuSO_4.5H_2O$, 50 µg of Na_2MoO_4, 0.22 mg of $ZnSO_4.7H_2O$, 0.28 mg of H_3BO_3, 1.4 mg of $MnSO_4.H_2O$, 1.5 mg of $FeSO_4$ $7H_2O$, 32 mg of $C_6H_5K_3O_7$, 217 mg of K_2SO_4, 2.4 g of $MgSO_4$, 2.45 g of KH_2PO_4, 2.5 g of KNO_3 and 10 g of dextrose.

2.1.2 Biogas

Fresh cow manure was collected from the farm in Phitsanulok, Thailand. The cow manure was diluted with water at the ratio 1:1, and then glucose added into cow manure was 0.1% v/v, which was used as inoculums. Biogas was produced under anaerobic digestion of 200 liter reactor, with a working volume at 150 liter. Biogas was stored in floating drum system. The location of biogas production at the biogas plant is at the School of Renewable Energy Technology, Naresuan University, Thailand. The biogas consisted of 68 ± 0.5% CH_4, 28 ± 0.7% CO_2, 0.5 ± 0.2% O_2 and H2S concentration below 100 ppm.

2.1.3 Photobioreactor Bubble Column

The photobioreactor bubble column was designed with a height of 52.5 cm and a diameter of 3.5 cm [8]. Acrylic polymer was used to construct the photobioreactor bubble column. A Pasteur pipette with the diameter of 1 mm was used as an air sparger in the photobioreactor bubble column. The working volume was 0.45 L in 0.5 L reactor. The column was placed at the vertical center of the bubble column to ensure the carbon dioxide gas was uniformly exposed to the 3,000 lux light intensity (Figure 1).

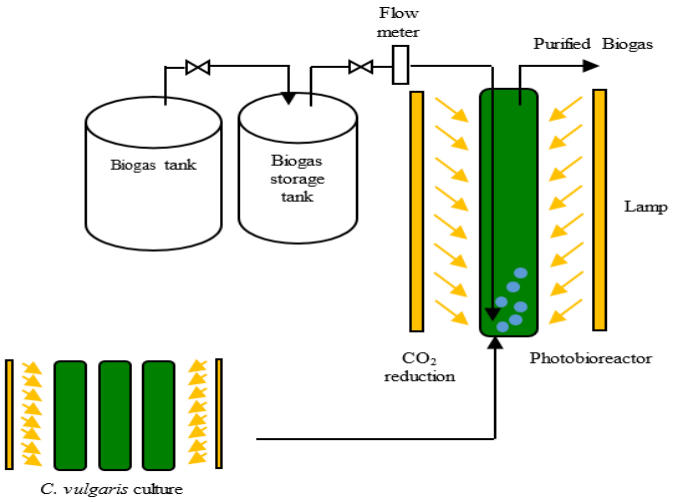

Fig. 1. Diagram of a process for carbon dioxide reduction using *C. vulgaris* cultures.

2.2 Methods

2.2.1 Growth Curve of C. vulgaris

The turbidity of the initial *C. vulgaris* cell growth medium in the erlenmeyer flasks was measured at 0.5178 of an optical density of 540 nm by a spectrophotometer (Analytik Jena specord 40, Germany). Fresh medium not containing algae cells had been set to a spectrophotometer level of zero or blank. Initial cell concentration for all experiments was always 0.08 g/L dry cell weight. The cultures were incubated at room temperature in a shaking incubator at 150 rpm under a light to dark regime of 16:8 h light, 8 h dark with the light intensity on the surface of vessels of 3,000 Lux and a 2 mL sample taken every day for 14 days to measure growing medium turbidity, and the cells counted using a haemacytometer. The OD and total cell were presented in the growth curve, with the specific growth rate (μ) calculated by using Equation 1 [16]:

$$\mu(\text{day}^{-1}) = \frac{\ln(N_2 / N_1)}{t_2 - t_1} \quad (1)$$

The biomass productivity calculated by using Equation 2:

$$P(\text{g/L/day}) = \frac{N_2 - N_1}{t_2 - t_1} \quad (2)$$

The population doubling time (PDT) was calculated by using Equation 3:

$$PDT = \frac{(t_2 - t_1)\log 2}{\log N_2 - \log N_1} \quad (3)$$

where N_1 and N_2 are defined as biomass (g/L) a time t_1 and t_2, respectively.

2.2.2 Experiment of a Continuous Process for CO_2 Reduction

To study carbon dioxide reduction in biogas when the biogas is injected directly into the *C. vulgaris* culture (Figure 1), biogas consisting of 68 ± 0.5% CH_4, 28 ± 0.7% CO_2, 0.5 ± 0.2% O_2 and H_2S concentration below 100 ppm was injected into a continuous 0.5 liter photobioreactor bubble column at a flow rate of 100, 150, 200 and 250 mL/min. The sample biogas input and output of the *C. vulgaris* cultivation system was taken for analysis. These were processed with a portable gas analyzer (GAS DATA GFM416), with the biogas influent and effluent of the system was analyzed every 30 minutes. The methane enrichment was determined using Equation 4 [12]:

$$CH_4\text{enrichment}(\%) = \frac{CH_{4\,inf} - CH_{4eff}}{CH_{4\,inf}} \times 100\% \quad (4)$$

The carbon dioxide reduction efficiency of biogas upgrading system was calculated by using Equation 5 [4], [5], [12]:

$$CO_2\text{reduction}(\%) = \frac{CO_{2\,inf} - CO_{2eff}}{CO_{2\,inf}} \times 100\% \quad (5)$$

The CO_2 fixation rate (P_{CO2}, $gCO_2/L/d$) of microalgae was calculated by using Equation 6 [35], [36]:

$$P_{CO2} = 1.88 \times P_{max} \quad (6)$$

where P_{max} defined as maximum biomass productivity (g/L/d) and 1.88 is the theoretical value of CO_2 fixated in grams per gram of biomass produced, assuming that derived from a mass balance with the typical molecular formula of microalgae biomass, that is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ [37], [38].

2.2.3 Experiment of Variation pH

To study the variation in the pH of the *C. vulgaris* culture in the CO_2 reduction process when biogas was injected directly into *C. vulgaris* culture, the initial pH of *C. vulgaris* culture was measured (pH = 7.0), and the variations in the pH level of the culture were measured using a pH meter every 30 minutes during the period in

which the biogas was being injected into the *C. vulgaris* cultures at a flow rate 100, 150, 200 and 250 mL/min. The results of investigation are presented in a pH profile curve in Figure 5 which appears below in Section 3.3.

2.2.4 Biomass Measurement

The growth of the *C. vulgaris* was measured by the culture turbidity, at the optical density of 540 nm, by spectrophotometer (Analytik Jena Specord 40, Germany). The culture broth was sampled every 90 min for 7.5 hours. The number of cells in the sample microalgae suspension was directly counted by heamacytometer under a light microscope.

The cell suspension was filtered with a Whatman No. 5 membrane filter, and washed with distilled water twice. The pellet cells were placed on a petri-dish plate and dried at 45℃ for 24 hours. The dried cells was weighed and crushed into a fine powder [9]. The biomass productivity P_B (mg/L d^{-1}) was calculated by Equation 8.

2.2.5 Lipid Extraction

The dried algae (0.35 g) was extracted with 200 mL hexane placed by soxhlet at 60°C for 3 hours [10], and the extracts then filtered to separate the disrupted cells and the oil. The sludge was dried in a hot air oven at 80°C for 24 hours. The solvent was evaporated from the mixture by a rotary evaporator at 40°C. The extracted oil was weighed.

The lipids content L (%) was calculated by Equation 7:

$$L(\%) = \frac{W_L}{W_B} \times 100 \quad (7)$$

where W_L is the weight of the extracted lipids and W_B is the weight of the dry biomass. The biomass production P_B (mg/L d^{-1}) was calculated by Equation 8.

$$P_B = \frac{W_{BF} - W_{B0}}{t} \quad (8)$$

where W_{B0} and W_{BF} are the weights of dry biomass at the begin and the end of a batch run and t is the overall culture time.

The lipid productivity P_L (mg/L) was calculated as the product of biomass production and lipid content according to Equation 9:

$$P_L = P_B \times L\ (\%) \quad (9)$$

2.2.6 Statistical Analysis

The design aspect of the analysis of variance was used for Completely Randomized Design (CRD). The comparison of all sample averages used Duncan's New Multiple Range Test for Statistical analysis by SPSS (statistical package for the social sciences).

3. RESULTS AND DISCUSSION

3.1 Growth Curve of *C. vulgaris*

The growth curve of the *C. vulgaris* cultivated in the photobioreactor with chlorella broth media was analyzed. The turbidity of the initial cell growth medium was 0.5178 at OD 540 nm and the viable cells count was 6.15×10^6 cell/mL. Initial cell concentrations for all experiments were always 0.08 g/L dry cell weight. It was found that the maximum specific growth rate (μ) was 0.629 day^{-1} (Figure 2) The maximum biomass productivity was 1.875 DCW g/L/day. The population doubling time (PDT) was 38 hours (1.6 days), which is a significantly fast rate at least partly due to there being no lag phase in our sequence, and the exponential phase being 1.6 days. At 1.6 days the growth rate enters the stationary phase followed by natural death. According to Ruthairat's research on the optimal conditions for growth of *Chlorella* sp., the exponential phase of growth curve of *Chlorella* sp. K3 is 2 days, followed by the stationary phase, giving a total growth cycle period of 4 days. This was the same in all their experimental conditions [11]. In the current study, biogas was injected into the *C. vulgaris* cultures at the start of the exponential phase, which hastened the cell growth, resulting in the fast growth rate observed.

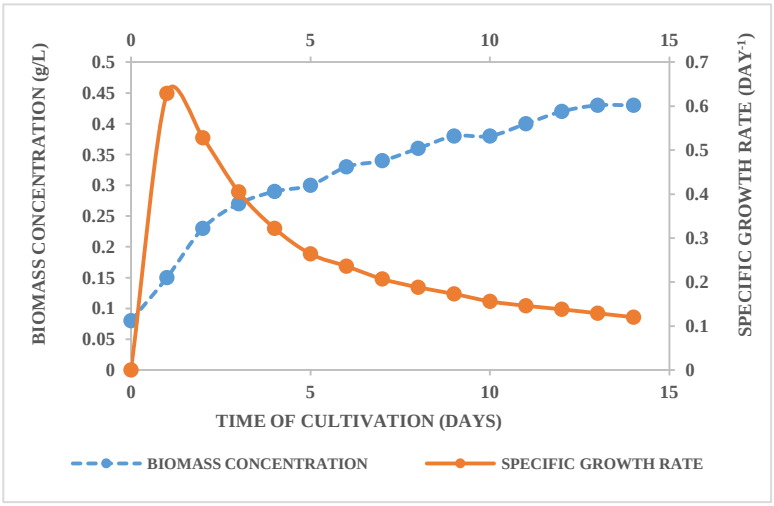

Fig. 2. Growth curve of *C. vulgaris* was measured by biomass concentration and specific growth rate of *C. vulgaris*.

3.2 Carbon Dioxide Reduction

We determined the efficiency of carbon dioxide reduction in the biogas by the action of the *C. vulgaris* by injecting the biogas directly into the *C. vulgaris* culture contained in 0.5 L. of a medium in the photobioreactor. The turbidity of the initial cell culture medium was 0.5 at OD 540 nm and the initial pH was 7.0. The biogas was injected at the different gas flow rates of 100, 150, 200 and 250 mL/min. Samples of the influent and effluent gas were taken every 30 minutes to calculate the amount of carbon dioxide reduction of biogas upgrading system, carbon dioxide fixation rate of *C. vulgaris* and methane enrichment. Figure 3 showed the efficiency of the carbon dioxide reduction over the time of *C. vulgaris* cultivation in the biogas upgrading system. (Table 1) The highest carbon dioxide fixation rate of *C. vulgaris* and efficiency of carbon dioxide reduction occurred from broth medium solubility and the photosynthesis of *C. vulgaris* achieved were 0.620 mg/L/d and 84.48%, respectively, at the gas flow rate of 100 mL/min and the methane in biogas effluent increased to 89.4%. Increased proportion of the maximum methane percentage was 30.32%. Experimental results at the 150, 200 and 250 mL/min flow rate the lower efficiency than 100 mL/min flow rate, the carbon dioxide fixation rate of *C. vulgaris* (0.508 mg/L/d, 0.376 mg/L/d, 0.301 mg/L/d, respectively), the carbon dioxide reduction of biogas upgrading system (77.78%, 65.85% and 58.22%, respectively), the methane effluent (87.3%, 85.2% and 82.6%, respectively) and the methane enrichment (28.19%, 26.22% and 21.65%, respectively), which was statistically significantly the different gas flow rates tested ($p<0.05$). Figure 4 showed the methane enrichment efficiency with each of the tested flow rates, with 100 mL/min gas flow rate the most efficient. We were, therefore, able to demonstrate that the maximum growth rate of the *C. vulgaris* in the photosynthesis process was closely associated with the volume of carbon dioxide reduction, and at the same time, the concentration of methane in biogas was significantly increased. The efficiency of carbon dioxide reduction of biogas upgrading by *C. vulgaris* was between 33.21% - 84.48% by volume, and the methane content of the biogas effluent increased to 89.4%.

Table 1. CO_2 reduction, CO_2 fixation rate, CH_4 enrichment and CH_4 effluent at different flow rate.

Biogas flow rate (mL/min)	CO_2 reduction (%)	CO_2 fixation rate (mg/L/d)	CH_4 enrichment (%)	CH_4 effluent (%)
100	84.48	0.620	30.32	89.4
150	77.78	0.508	28.19	87.3
200	65.85	0.376	26.22	85.2
250	58.22	0.301	21.65	82.6

Table 2. Cultivation compositions and CO_2 fixation of microalgae strain [27].

Strain	CO_2 (%)	pH	CO_2 fixation rate (g/L d)	Ref.
Botryococcus braunii	15	8.3	1.1	[28]
Chlorococcum littorale	40	5.5	-	[29]
Chlorella kessleri	18	6.4	0.087	[30]
Chlorella sp.	40	9.4	1.0	[31]
Chlorella sp. WT	25	-	0.376	[32]
Dunaliella tertiolecta	3	-	0.313	[33]
Haematococcus pluvialis	16-34	-	0.143	[34]
Spirulina sp.	12	7	0.413	[30]

Biogas was injected directly into the *C. vulgaris* culture in the photobioreactor with a bubble column system to improve the efficiency of carbon dioxide reduction. The efficiency of CO_2 reduction of biogas upgrading by *C. vulgaris* decreased at a higher flow rate [4] was due to the coalescence of gas bubbles that decreased the retention time of bubbles in the culture [12] and the decrease of surface area per unit gas volume of the bubbles can also reduce the CO_2 capture efficiency [12], [25], [26]. Therefore, the retention time of bubbles at flow rate of 100 mL/min higher than the others and affected to the CO_2 reduction efficiency increase. In addition, the efficiency of CO_2 reduction depended on (1) the microalgae species, (2) CO_2 concentration, (3) photobioreactor design and (4) operating conditions [24] (Table 2).

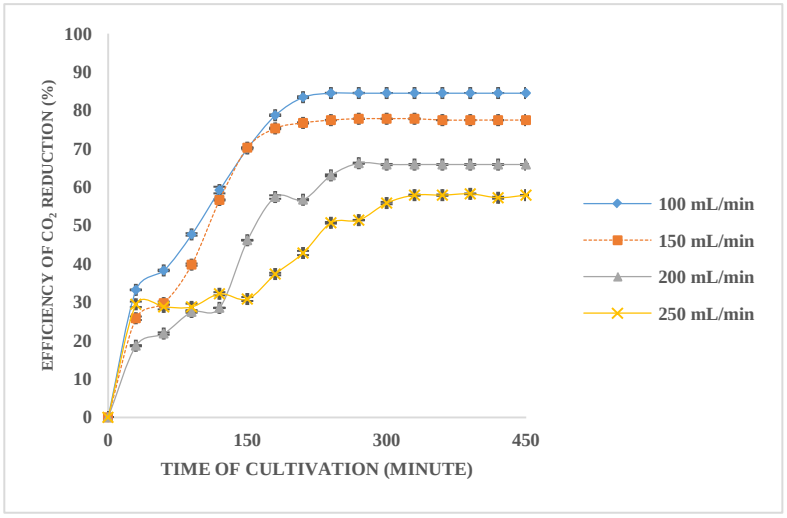

Fig. 3. Efficiency of carbon dioxide reduction in biogas upgrading by *C. vulgaris.*

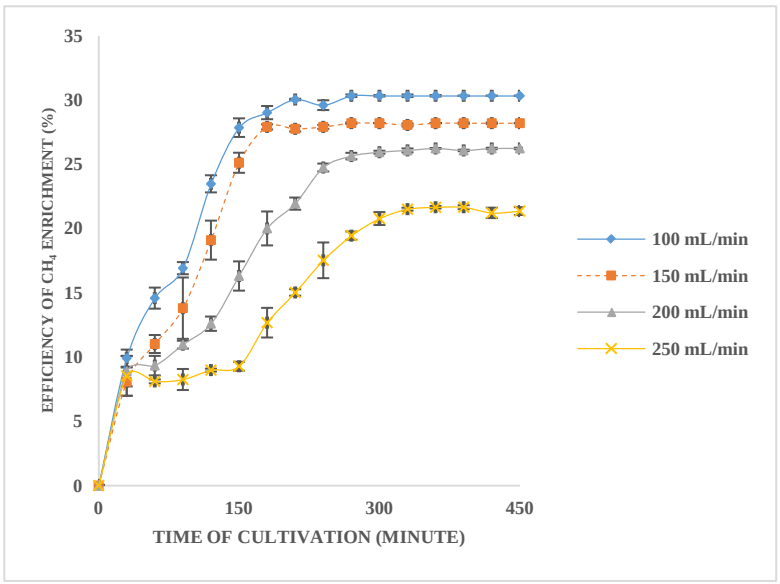

Fig. 4. Efficiency of methane enrichment in biogas upgrading by *C. vulgaris*.

3.3 Variation of pH

The pH of the *C. vulgaris* cultures was determined during while the biogas was being injected into the cultures, with pH readings taken every 30 min for a total time duration of 450 min. The pH levels for each biogas flow rate were: flow rate 100 mL/min, pH = 6.6-7.0, 150 mL/min, pH= 6.4-7.0, 200 mL/min, pH= 6.1-7.0, and 250 mL/min, pH= 5.9-7.0 (Figure 5). The change in pH levels at all flow rates is varied significant ($p<0.05$).

Our explanation for these readings is that the pH level of the cultures was reduced early was due to the availability of CO_2 in large amounts in the culture. The carbon dioxide reacted with water (H_2O) to form H_2CO_3 (carbonate acid) which was then dissociated into and H+. The accumulation of H+ ions caused the rise in the pH of the cultures. At the same time, the *C. vulgaris* was producing carbonic anhydrase to transform into CO_2 and OH-. This explained by the fact that carbon dioxide as an inorganic carbon source which was able to be used in the photosynthetic process, causing the pH level in the culture to increase [13]. According to research reports of the effects of biogas on microalgae after biogas injection into systems, pH levels decrease for each microalgae as follows: Chlorella sp. MM-2 (8.50 to 6.50) [12], *Chlorella* sp. MTF-7 (7.90 to 6.50) [17] and *Spirulina platensis* (9.60 to 7.00) [18].

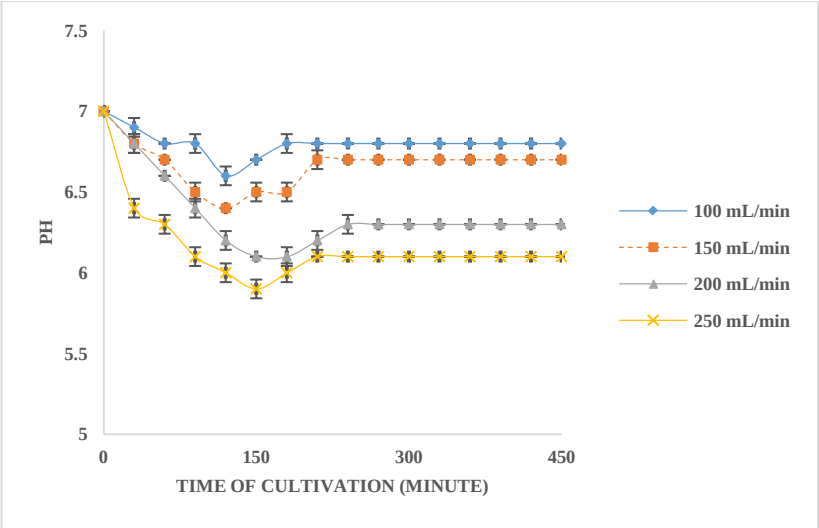

Fig. 5. Effect of pH in biogas upgrading by *C. vulgaris.*

3.4 Effect of Biogas on Growth Monitoring and Biomass Productivity

The growth monitoring of *C. vulgaris* was cultivated in photobioreactor using the biogas as a source of carbon. The biogas was injected at the different gas flow rates of 100, 150, 200 and 250 mL/min. The turbidity of the initial cell was used 0.5 at OD 540 nm and initial pH was 7.0, with taken a sample of influent and effluent microalgae every 90 min for 450 min to calculate biomass productivity. Figure 6 and Figure 7 show the growth monitoring of *C. vulgaris* when biogas injects into culture system at the difference flow rates. The result shows that the log phase was between 100 min to 300 min and the cell concentration and biomass productivity for each biogas flow rate were: flow rate 100 mL/min (1.9×10^7 cell/mL, 0.33 mg/L d^{-1}), 150 mL/min (1.8×10^7 cell/mL, 0.27 mg/L d^{-1}), 200 mL/min (1.79×10^7 cell/mL, 0.20 mg/L d^{-1}) and 250 mL/min (1.74×10^7 cell/mL, 0.16 mg/L d^{-1}) (Table 3). Therefore, cell concentration and biomass productivity at all flow rates varied significantly ($p<0.05$).

The carbon dioxide in the biogas affected the growth of *Chlorella vulgaris*, because carbon dioxide was the carbon source and main nutrient of the microalgae. The microalgae required carbon content in a range of 36-58% [19], where microalgae use carbon dioxide for photosynthesis. However, carbon dioxide has negative effects on microalgae when added too much to the culture and biomass productivity is decreased, as demonstrated in previous discussion [20]. According to Singh and Singh's research [21] on the effect of CO_2 concentration on algal growth, Chlorella species at 10% CO_2 concentration provided a maximum growth rate of up to 50% CO_2, while a 70% CO_2 concentration resulted at the end of the CO_2 fixation [21], [22]. Research on CO_2 utilization of *Nannochloropsis oculata* showed a maximum specific growth velocity at 2% CO_2 concentration, while biomass growth was inhibited at 5% CO_2 or higher, according to Chiu *et al.* [23].

The *C. vulgaris* of Figure 6 could grow faster than Figure 2, because the microalgae in Figure 6 were supplied with the higher CO_2 concentration, CO_2 came from medium and biogas. When considered about adaptation of the microalgae when they were fed with CO_2 from biogas, according to [40], [41], [43], kinetic study for the growth of *Chlorella vulgaris* and *Chlorella sp*. showed no lag phase was observed.

However, the growth rate of *C. vulgaris* in Figure 6 showed that microalgae were still growing even after 450 minutes, while the CO_2 reduction efficiency in Figure 3 was stagnated during 200-300 minutes. According to [6] could be used for discussion that Figure 6, the solubility of CO_2 was saturated, most of the CO_2 was released back to atmosphere (space on the top of a photobioreactor). The CO_2 solubility was an inorganic carbon source, which was able to be used in the photosynthetic process of microalgae, and another inorganic carbon source came from the nutrients from medium, causing the microalgae were still growing after 450 minutes. On the other hand, the efficiency of CO_2 reduction in Figure 3 was stagnated after 200-300 minutes, due to the limitation of the CO_2 solubility. It meant that after 200- 300 minutes CO_2 source from biogas was constant, therefore, only the nutrients from medium were affected to the growing of microalgae and this study focused on the CO_2 reduction efficiency in biogas.

Table 3. Specific growth rate and biomass productivity of *C. vulgaris*.

Flow rate (mL/min)	Dry cell weight (mg)	Specific growth rate (day^{-1})	Biomass productivity (mg/L d^{-1})
100	150	2.012	0.33
150	170	2.412	0.27
200	203	2.980	0.20
250	230	3.379	0.16

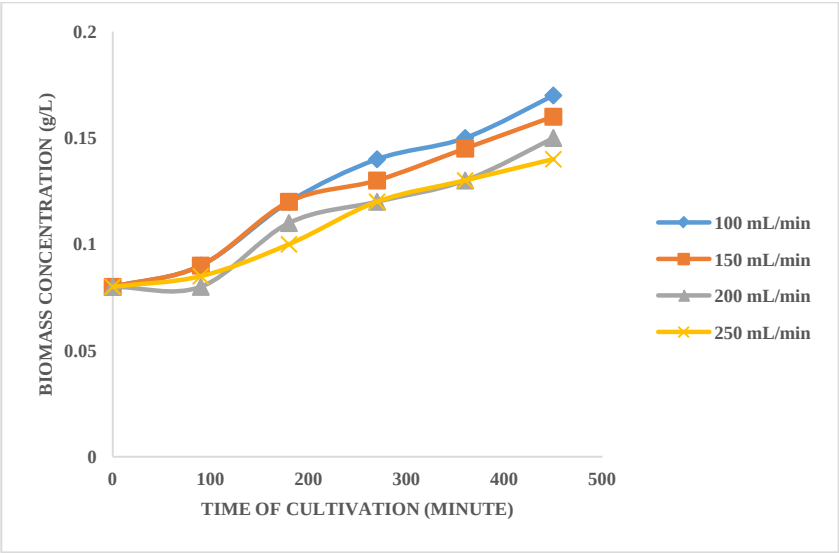

Fig. 6. Growth rate of *C. vulgaris* between biomass concentration and time of cultivation.

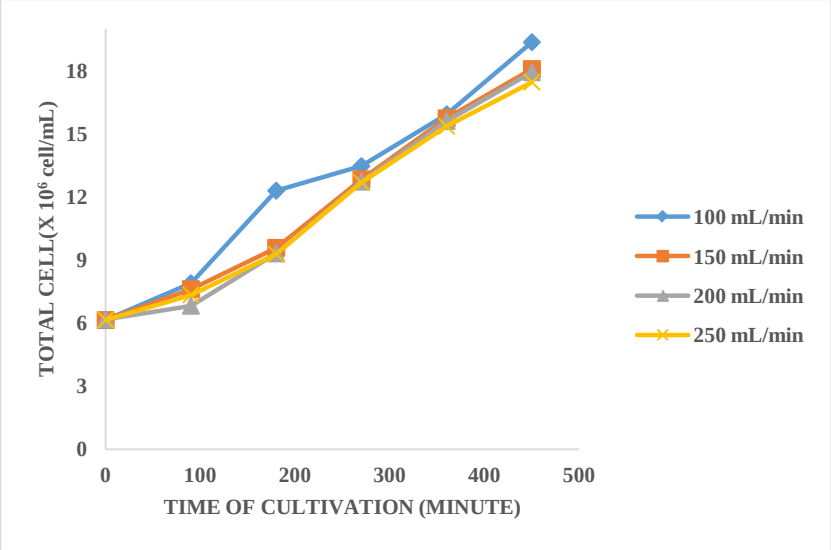

Fig. 7. Growth rate of *C. vulgaris* between total cell and time of cultivation.

3.5 Lipid Content and Productivity

In this study, the effects of lipid content in *C. vulgaris* culture when biogas was injected directly into their culture were operated at 0.5 L. medium in a photobioreactor. The turbidity of an initial cell was used 0.5 at OD 540 nm and initial pH was 7.0. Biogas was injected differently at the gas flow rate 100, 150, 200 and 250 mL/min. The experiment was took a sample of influent and effluent biomass every 90 min for 450 min to calculate lipid content and lipid productivity. Table 4 shows the lipid content and lipid productivity when biogas was injected directly into their culture at the flow rate 100, 150, 200 and 250 mL/min. The study found that the lipid content and lipid productivity for each biogas flow rate were: flow rate 100 mL/min (14.35%, 4.74 mg/L d^{-1}), 150 mL/min (14.29%, 3.86 mg/L d^{-1}), 200 mL/min (13.53%, 2.71 mg/L d^{-1}) and 250 mL/min (13.33%, 2.13 mg/L d^{-1}) significantly ($p<0.05$). Griffiths and Harrison, in 2009, suggested that lipid productivity is one of the key parameters in selecting microalgae species for biodiesel production [14] [15].

Table 4. The lipid content and lipid productivity at difference biogas flow rate.

Biogas flow rate (mL/min)	Lipid content (%)	Lipid productivity (mg/L d^{-1})
100	14.35	4.74
150	14.29	3.86
200	13.53	2.71
250	13.33	2.13

In the present study, microalgae lipid extraction was conducted hexane by soxhlet, which following by [39]. The composition of fatty acid extracted from *C. vulgaris* contains C10:0, C14:0, C16:0, C16:1, C16: 2, C16:4, C18:0, C18:1, C18:2 and C20:0 was analyzed by Gas Chromatograph-Mass Spectroscopy (Agilent-GC G1530N, MS G2573A). This result shows that the unsaturated fatty acid (75.22%) is higher than the saturated fatty acid (24.78%). The composition of fatty acid is shown in Table 5. Ahmad *et al.* in 2013 reported the percentage of unsaturated fatty acids (77.85%) [39]. Aguoru and Okibe, in 2015, reported the percentage unsaturated fatty acid (79.22%) [10].

Table 5. The composition of fatty acid extracted from *C. vulgaris.*

Fatty acid	Fatty acid names	Amount (%)
C10:0	Decanoic acid	1.96
C14:0	Tetradecanoic acid	2.33
C16:0	Hexadecanoic acid	18.61
C16:1	Hexadecenoic acid	11.54
C16:2	Hexadecadienoic acid	1.02
C16:4	Hexadecatetraenoic acid	6.21
C18:0	Octadecanoic acid	0.92
C18:1	Octadecenoic acid	25.32
C18:2	Octadecadienoic acid	7.35
C20:0	Eicosanoic	6.09

4. CONCLUSION

The efficiency of carbon dioxide reduction in biogas by *C. vulgaris* TISTR 8580 with different flow rates showed that a flow rate of 100 mL/min of biogas provided more efficient reduction of CO_2 than flow rates of 150, 200 and 250 mL/min. The maximum efficiency of carbon dioxide reduction was 84.48%, while the methane in biogas was at high concentrations from 68.6% to 89.4%. The variation of pH in the cultures was from 5.9-7.0 where the *C. vulgaris* can grow and use carbon dioxide for photosynthesis. The maximum cell concentration was 1.9 x 10^7 cell/mL and the biomass productivity was 0.33 at a flow rate of 100 mL/min. The highest lipid content was 14.35% when lipid productivity was 4.74 mg/L d^{-1} at a biogas flow rate of 100 mL/min. The lipids accumulation in the microalgae *C. vulgaris* culture were 75.22% of unsaturated fatty acid. The consideration of biogas feeding into *C. vulgaris* culture with different flow rates showed that the efficiency of carbon dioxide reduction, methane enrichment, variation of pH, biomass productivity, lipid content and lipid productivity were different statistically at a confidence level of 95 percent. This result indicated the efficiency of microalgae *C. vulgaris* TISTR 8580 to reduce carbon dioxide into organic compounds and produce oil via photosynthesis using the carbon dioxide as an energy source for growth. Therefore, utilization of the microalgae *C. vulgaris* TISTR 8580 as an alternative is of interest for carbon dioxide reduction and oil production from the biogas and can form a data base for research development into biogas upgrading.

ACKNOWLEDGEMENT

This research was financially supported by the national research council of Thailand for doctoral thesis program (FY2016). The authors would like to thank School of Renewable Energy of Technology and Department of Biology, Faculty of Science, Naresuan University for location of research and knowledge about biogas and microalgae. Many thanks to Mr. Thomas Elliott and Mr. Roy Morien of the Naresuan University Language Centre for their editing assistance and advice on English expression in this document.

REFERENCES

[1] Gianfrancesco A.D., 2013. *Materials for ultra-supercritical and advanced ultrasupercritical power plants*. Woodhead publishing series in energy.

[2] Energy Policy and Planning Office. 2014. *Energy Statistics of Thailand 2013*. Bangkok: Ministry of energy.

[3] Griffiths M.J. and S.T.L. Harrison. 2008. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Philology* 1-15.

[4] Kao C.-Y., Chiu S.-Y., Huang T.-T., Dai D., Wang G.-H., Tseng C.-C., Chen C.-H. and Lin C.-S., 2012. A mutant strain of microalga *Chlorella* sp. for the carbon dioxide capture from biogas. *Biomass and Bioenergy* 36: 132-140.

[5] Cheng J., Huang Y., Feng J., Sun J., Zhou J. and Cen K., 2013. Improving CO_2 fixation efficiency by optimizing *Chlorella* PY-ZU1 culture conditions in sequential bioreactors. *Bioresource Technology* 144: 321-327.

[6] Lam M.K. and K.T. Lee. 2013. Effect of carbon source towards the growth of *Chlorella vulgaris* for CO_2 bio-mitigation and biodiesel production. *International Journal of Greenhouse Gas Control* 14: 169-176.

[7] Liu Z.-Y.; Wang G.-C. and Zhou B.-C., 2008. Effect of iron on growth and lipid accumulation in

Chlorella vulgaris. *Bioresour Technol* 99: 4717–4722.

[8] Ni X. and S. Gao. 1996. Scale-up correlation for mass transfer coefficients in pulsed baffled reactors. *Chemical Engineering Journal* 63: 157–166.

[9] Hamedi S., Mahdavi M.A. and Gheshlaghi R., 2016. Improve lipid and biomass productivity in *Chlorella vulgaris* by differing the inoculation medium from the production medium. *Biofuel Research Journal*: 410-416.

[10] Aguoru C.U. and P.O. Okibe. 2015. Content and composition of lipid produced by *Chlorella vulgaris* for biodiesel production. *Advances in Life Science and Technology*: 96-100.

[11] Wisansuwannakorn R., 2005. Optimal conditions for growth of *Chlorella* sp. by using carbon dioxide as a carbon source in photobioreactor. MS. Thesis, Chulalongkorn University, Thailand.

[12] Kao C.Y., Chiu S.Y., Huang T.T., Dai L., Hsu L.K. and Lin C.H., 2012. Ability of a mutant strain of the microalga *Chlorella* sp. to capture carbon dioxide for biogas upgrading. *Applied Energy* 93: 176-183.

[13] Sumardiono S., Budiyono, Syaichurrozi I. and Sasongko S.B., 2014. Utilization of Biogas as Carbon Dioxide Provider for *Spirulina platensis* Culture. *Research Journal of Biological Sciences* 6(1): 53-59.

[14] Griffiths M.J. and S.T.L. Harrison. 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol.* 21: 493-507.

[15] Zhou X.P., and L.X., 2013. Feasibility of biodiesel production by microalgae *Chlorella* sp. (FACHB-1748) under outdoor conditions. *Bioresource Technology*: 131-135.

[16] Vonshak A., 1986. *Microalgae: Laboratory Growth Techniques and Outdoor Biomass Production*, In Coombs, J., Hall, D.O., Long, S.P. and Scurlock, J.M.O. (eds.). Techniques in Bioproductivity and Photosynthesis. *2nd Pergamon Press*.

[17] Chiu S-Y., Kao C-Y., Huang T-T., Lin C-J., Ong S-C., Chen C-D., Chang J-S. and Lin C-S., 2011. Microalgal biomass production and on site bioremediation of carbondioxide, nitrogenoxide and sulfur dioxide from flue gas using *Chlorella* sp. cultures. *Bioresource Technology* 102: 9135-9142.

[18] Sumardiono S., Yono B., Syaichurrozi I. and Sasongko S.B., 2014. Utilization of biogas as carbon dioxide provider for Spirulina platensis culture. *Biological Sciences* 6(1): 53-59.

[19] Sydney E.B., Sturm W., De Carvalho J.C., Thomaz Soccol V., Larroche C., Pandey A. and Soccol C.R., 2010. Potential carbon dioxide fixation by industrially important microalgae. *Bioresource Technology* 101: 5892-5896.

[20] Watanabe Y., Ohmura N. and Saiki H., 1992. Isolation and determination of cultural characteristics of microalgae which functions under CO_2 enriched atmosphere. *Energy Conversion Management* 33: 545-552.

[21] Singh S.P. and P. Singh. 2014. Effect of CO_2 concentration on algal growth: A review. *Renewable and Sustainable Energy Reviews* 38: 172–179.

[22] Don S.K., Lee J.S., Shin C.S. and Park S.C., 1999. Isolation of a new highly CO_2 tolerant fresh water Microalga *Chlorella* sp. KR-1. *Renewable Energy* 16(1–4): 1019–22.

[23] Chiu S.Y., Kao C.Y., Tsai M.T., Ong S.C., Chen C.H. and Lin C.S., 2009. Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration. *Bioresource Technology* 100(2): 833-838.

[24] de Morais M.G. and J.A.V. Costa. 2007. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Conversion Management* 48: 2169–2173.

[25] Chiu S.Y, Tsai M.T, Kao C.Y, Ong S.C, Chen C.H. and Lin C.S., 2009. The air-lift photobioreactors with flow patterning for a high-density culture of microalgae and carbon dioxide removal. *Eng Life Sci* 9: 254-260.

[26] Mandeno G., Craggs R., Tanner C., Sukias J. and Webster-Brown J., 2005. Potential biogas scrubbing using a high rate pond. *Water Science Technology* 51: 253-256.

[27] Klinthong W., Yang Y.-H, Huang C.-H. and Tan C.-S., 2015. A review: microalgae and their applications in CO_2 capture and renewable energy. *Aerosol and Air Quality Research* 15: 712–742.

[28] Murakami M. and M. Ikenouchi, 1997. The biological CO_2 fixation and utilization project by RITE (2): screening and breeding of microalgae with high capability in fixing CO_2. *Energy Conversion Management* 38: S493–S497.

[29] Iwasaki I., Hu Q., Kurano N., and Miyachi S., 1998. Effect of extremely high-CO_2 stress on energy distribution between photosystem I and photosystem II in a 'High-CO_2' tolerant green alga, *Chlorococcum littorale* and the intolerant green alga *Stichococcus bacillaris*. *J. Photochem. Photobiol* 44: 184–190.

[30] de Morais M.G. and J.A.V. Costa. 2007. Carbon dioxide fixation by *Chlorella kessleri*, *C. vulgaris*, *Scenedesmus obliquus* and *Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors. *Biotechnology Letters* 29: 1349–1352.

[31] Sakai N., Sakamoto Y., Kishimoto N., Chihara M. and Karube I., 1995. Chlorella strains from hot springs tolerant to high temperature and high CO_2. *Energy Conversion Management* 36: 693–696.

[32] Chiu S.Y., Kao C.Y., Huang T.T., Lin C.J., Ong S.C. and Chen C.D., 2011. Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using *Chlorella* sp. cultures. *Bioresources Technology* 102: 9135–9142.

[33] Kishimoto M., Okakura T., Nagashima H., Minowa T., Yokoyama S.Y. and Yamaberi K., 1994. CO_2 fixation and oil production using micro-algae. *J. Ferment. Bioeng*. 78: 479–482.

[34] Huntley M.E. and D.J. Redalje. 2007. CO_2 mitigation and renewable oil from photosynthetic

microbes: a new appraisal. *Mitig. Adapt. Strateg. Glob. Change* 12: 573–608.

[35] Ho S., Chen S., Lee D. and Chang J., 2011. Perspective on microalgae CO_2 emission mitigation system. *Biotechnology Advances* 29(2): 189–198.

[36] Tang D., Han W., Li P., Miao X. and Zhong J., 2011. CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. *Bioresource Technology* 102(3): 3071–3076.

[37] Chisti Y., 2007. Biodiesel from microalgae. *Biotechnology Advances* 25(3): 294–306.

[38] Marta J., 2015. Influence of CO_2 on *Scenedesmus obliquus*, *Chlorella vulgaris* and *Chlorella protothecoides* microalgae growth and evaluation of its biomass. M.S. Biotechnology thesis. Instituto Superior Técnico, Universidade de Lisboa, Portugal.

[39] Ahmad F., Khan A.U. and Yasar A., 2013. The potential of *Chlorella Vulgaris* for wastewater treatment and biodiesel production. *Pak. J. Bot.* 45(S1): 461-465.

[40] Wang L., Min M., Li Y., Chen P., Chen Y., Liu Y., Wang Y. and Ruan R., 2010. Cultivation of green algae *Chlorella sp*. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology* 162:1174-1186.

[41] El-Ibiari N.N., El-Ardy O., Salem Olfat M.A. and Abdelrahman A.M., 2015. Kinetic study for growth of *Phormedium* Sp. and *Chlorella Vulgaris*. *International Journal of ChemTech Research* 8(9): 284-289.

[42] Kabir M., Hoseini S.A., Ghorbani R. and Kashiri H., 2017. Performance of microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* in wastewater treatment of Gomishan (Golestan-Iran) shrimp farms. *AACL Bioflux* 10(3): 622-632.

[43] Lohman E.J., Gardner R.D., Pedersen T., Peyton B.M., Cooksey K.E. and Gerlach R., 2015. Optimized inorganic carbon regime for enhanced growth and lipid accumulation in *Chlorella vulgaris*. *Biotechnology for Biofuels* 8(82): 1-13.