CARBON CAPTURE AND STORAGE WITH LIPID PRODUCTION IN INTEGRATED SYSTEM OF AQUEOUS AMMONIA WITH MARINE MUTANT *SYNECHOCOCCUS* PCC 7002 IIUM01

AZLIN SUHAIDA AZMI[1*], MOHAMED ANWAR AWAN[1], AZURA AMID[2], NOOR ILLI MOHAMAD PUAD[1], FATHILAH BINTI ALI[1]

[1]*Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia(IIUM), P.O. Box 10, 50728 Kuala Lumpur, Malaysia*
[2]*International Institute for Halal Research and Training, International Islamic University Malaysia (IIUM), Jalan Gombak, Malaysia*

**Corresponding author: azlinsu76@iium.edu.my*

ABSTRACT: Carbon capture and storage (CCS) involves capturing, transporting and storing CO_2 geologically underground permanently. Carbon capture using solvent such as amine and aqueous ammonia has been extensively studied by many researchers. However, this capture technology for CCS scheme is costly. As an alternative, CO_2 emission can be cost-effectively captured and stored by utilizing the well-understood natural photosynthetic process of marine cyanobacteria. In contrast, the capturing process using cyanobacteria is very slow compared to the chemical absorption mentioned prior. Hence, this study aimed to investigate carbon capturing and storing process using integrated aqueous ammonia and mutated marine cyanobacteria (*Synechococcus* PCC 7002 IIUM01). The conditions that can maximize CO_2 reduction under various conditions; CO_2 flow rate (Lpm), absorption temperature (°C) and aqueous ammonia concentrations (% (w/v)) were to be identified. The effectiveness of the mutant cyanobacteria was quantified by measuring the cell concentration, percentage reduction in CO_2 concentration and lipid content. *Synechococcus* PCC 7002 IIUM01 showed it robustness by growing in aqueous ammonia solution at the concentration of 0.5 to 1% (w/v) at which the parent strain was not able to tolerate. The best conditions in maximizing CO_2 capture and storage while sustaining growth optimally and being a potential biofuel source was observed at 0.5 Lpm of 15% CO_2 gas flow rate, 0.75% (w/v) of ammonia concentration and 33°C of absorption temperature. At this specified condition, around 68% of CO_2 removal was achieved with 9% (w/w) yield of lipid and more than 13% (w/v) of cell concentration obtained.

KEY WORDS: *Ammonia, Carbon dioxide, Cyanobacteria, Lipid, Mutant, Synechococcus PCC 7002.*

1. INTRODUCTION

Since the industrial revolution period to the present times, energy consumption has led to a significant elevation in the level of atmospheric greenhouse gases (GHGs), predominantly carbon dioxide (CO_2). CO_2 emission is known to intensify the atmosphere's capacity to hold heat. To date, the atmospheric CO_2 concentration has surpassed 400 ppm which is significantly higher than the preindustrial emission level [1]. The emission stems globally, from fuel combustion, fugitive emission from fuel, cement production and other sources such as waste incineration. Reducing GHGs emission is no longer a solution to the

current state of the atmosphere; it will only help to delay the inevitable. Therefore, efforts should be made to eradicate the GHGs presence in our atmosphere. Researchers are making rigorous attempts to find sustainable methods of capturing and sequestering CO_2 [2] and curtailing the emission of not only CO_2, but other GHGs as well [3,4].

Among the effort to curb CO_2 emission is carbon dioxide capture. Carbon dioxide capture implies separating the CO_2 from its point source, for example industrial flue gases, instead of releasing the CO_2 directly into the atmosphere. Various CO_2 capture strategies have been investigated, which can be generally classified into three categories associated with different combustion processes: post-combustion, pre-combustion and oxyfuel combustion [4]. Currently, sophisticated conceptions are under research to attain cost-effective solutions. These include application of plasma for decomposition of coal before combustion, use of nano-composites for selective absorption of CO_2, and nano-catalysis to enhance the reaction rate of CO_2 with other chemicals and thus help the removal of CO_2 from the atmosphere [5].

Despite all these interesting and novel methods, the most feasible solution is chemical reaction-based CO_2 capture. This incorporates, but is not limited to, cyclic carbonation/de-carbonation reactions, gas-absorption process [2,6,7] and chemical absorption with various solvents [8] . The chemical reaction-based for carbon capture and storage (CCS) schemes such as cyclic carbonation/ decarbonation reactions, gas-absorption process and chemical absorption with monoethanolamine (MEA), diethanolamine (DEA), methyl diethanolamine (MDEA) or aqueous ammonia solvent - characteristically consist of three stages, namely separation, transportation, and sequestration. However, exercising these methods to capture CO_2 and CO_2 storage led the whole process to be comparatively costly and energy-consuming; hence the mitigation benefits become marginal. For that reason, it is necessary to develop more cost-effective and sustainable options to curb the rising emissions [9]. More specifically, in absorption process itself, ammine scrubbing has few shortcomings. Amines can be degraded by SO_2, NO_x, oxygen and particulates. Additionally, the solvent is expensive and the process requires high energy consumption. As an alternative, ammonia-based scrubbing is used to overcome the shortcomings. The overall reactions of CO_2 absorption into aqueous ammonia can be described as in Eqs. (1) and (2) and primary product is composed of NH_4HCO_3 crystal formed in the CO_2 scrubber [6].

$$CO_2\ (g) + NH_3\ (l) + H_2O\ (l) \rightarrow NH_4HCO_3\ (s) \quad (1)$$

$$CO_2\ (g) + 2NH_3\ (l) + H_2O\ (l) \rightarrow (NH_4)_2HCO_3\ (s) \quad (2)$$

The principal challenge concerning CO_2 capture technology is to reduce overall cost by decreasing both energy and capital cost requirements. One of the strategies includes combining chemical absorption with cyanobacteria or microalgae for CO_2 removal and bio-productions (*i.e.* biofuel). With the biological approach, CO_2 can be first converted into algal biomass and later be turned into value-added products such as proteins, vitamins, food, and biodiesel. On the contrary, procedures for flue gas clean-ups incorporating biological approach are still under development and possess problems such as low biomass productivity and inefficient CO_2 utilization [10]. Cyanobacteria and microalgae can play a pivotal role in providing carbon sinks and reducing atmospheric CO_2 by covering separation and sequestration in one system [11], which subsequently reduce the effects of global warming.

Cyanobacteria, the blue-green algae, are aquatic photosynthetic bacteria that can fix CO_2 efficiently from several sources. It is known for the CO_2-concentrating mechanism (CCM) which enables accumulation of inorganic carbon (*i.e.* HCO_3^- and CO_2) within the

cell [12,13]. Mutation of cyanobacteria has shown enhancement in the CO_2 fixation and improvement in cell biomass productivity [12,13]. Additionally, cyanobacteria are also a promising source for bioenergy generation mainly because they contain considerable amounts of lipids which are present in the thylakoid membranes. Cyanobacteria have higher photosynthetic levels and growth rates compared to other algae or higher plants, grow easily with basic nutritional requirements and the cultivation is relatively simple and inexpensive. Generally, the accumulation of lipids in cyanobacteria occurs when the organisms are under stress, *e.g.* nutrient deprivation and in their stationary growth phase. Furthermore, cyanobacteria have high optimal temperature of 38°C, compared to other species and this property has made the organism to be a great potential for use in CO_2 mitigation process enhanced by aqueous ammonia. The CO_2 absorption may generate heat and ability to withstand heat generated is an absolute advantage for cyanobacteria usage in the process. The marine strain of *Synechococcus* has an optimal pH of 8.5 (with pH range of 6.5 to 9.5), thus again making it a favorable option for the process since the process uses aqueous ammonia which is known to be alkaline in nature.

Taking into consideration the advantages offered by both chemical and biological approaches, it is worth to investigate the effects of their combination on CCS. Chemical absorption of CO_2 using solvents is advantageous time-wise, and can absorb more than 90% of CO_2 from flue gas [14], thus this study aims to incorporate aqueous ammonia solution in enhancing cyanobacterial fixation of CO_2. The ammonia can chemically absorb the CO_2, resulting in CO_2-rich aqueous ammonia which will then be utilized by mutant cyanobacteria for carbon sequestration and the resulting biomass for use in bio-productions.

2. MATERIALS AND METHODS

2.1. Cyanobacteria strain and chemical mutation

Synechococcus sp. PCC 7002 was purchased from American Type Tissue Culture (ATCC) and grown in ATCC medium 957 with 20 μg/L vitamin B12. Mutant strain of *Synechococcus* sp. PCC 7002 IIUM01 was chemically mutated based on Prices and Badger [15] with modification [16]. About 1 mL of cells from the log phase was combined with 1 mL of phosphate buffer containing 0.01 M ethyl methyl sulphonate (EMS). The cells were mixed and incubated for 45 min in the dark at 37°C. Then 10 mL of sodium thiosulphate (pH 8) was added to inactivate the mutagen. After the inactivation of mutagen, the mixture was vortexed. The suspension was then centrifuged at 4000 rpm for 5 minutes and the cell (pellet) was collected. The cells were suspended in 1 mL of ATCC medium before re-centrifuged to totally remove mutagen and inactivate the mutation. After second wash, the mutated cells were grown in culture medium (ATCC medium 957) at 5% CO_2 concentration which was purged two times per day for 30 minutes in the presence of ampicillin and light for few days until green color appeared. Aliquots of the mutagenized cultures were spread onto ATCC medium 957 solidified with 1% agar (Bacto™ agar) in petri dishes. Two mutant strains were successfully isolated from the screening step and one of the mutants which is IIUM01 was used in this study.

2.2. Experimental setup and analysis

Fig. 1 illustrates the experimental setup used in this study. A 5% of inoculum size was inoculated into media with aqueous ammonia (NH_3) in ATCC medium 957 at the specified concentration based on the experimental design described in Section 2.3. Pure CO_2 was mixed with the air using an air pump and was then aerated into 100 mL of media at 15% of in-flowing CO_2 concentration for 60 minutes each day for a period of 7 days. At the end of

each day, two responses (change in CO_2 concentration and cell dry weight (CDW)) were measured and recorded. Change in CO_2 concentration was captured and recorded using K-33 BLG CO_2 sensors (CO_2 meter) with Data Acquisition System (DAS) software. The percentage difference of CO_2 concentration (i.e. percent of CO_2 removal) was calculated based on Eq. (3). At the end of the 7-day period, the lipid content was measured using modified Bligh and Dyer Method [16].

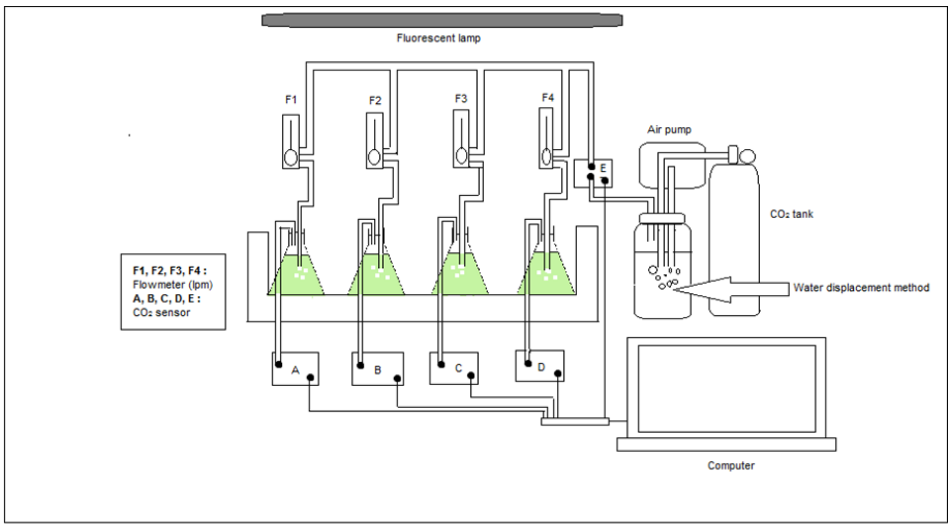

Fig. 1. Schematic diagram of the experimental setup.

$$\Delta CO_2\,(\%) = \frac{\text{Average Inlet Concentration} - \text{Average Outlet Concentration}}{\text{Average Inlet Concentration}} \times 100\% \quad (3)$$

CDW of *Synechococcus* sp. PCC 7002 IIUM01 was determined using gravimetric method and cell concentration was calculated as per Eq. (4). An empty centrifuge tube was dried in the oven at the temperature of 80˚C for 24 hours. After it achieved a constant weight, the weight was recorded as initial weight (A). 1 mL (V) sample was withdrawn and then centrifuged at 4000 rpm for 5 minutes. Then, the supernatant was separated from the pellet and dried at the temperature of 80˚C for 24 hours. The tube was weighed to get its final weight (B).

$$X\left({}^{mg}/_{mL}\right) = \frac{(B-A)g}{V\,mL} \times \left(\frac{1000\,mg}{1\,g}\right) \quad (4)$$

Lipid extraction was conducted by taking 1 mL of sample (*V*) and pipetted into a pre-weighed 15-mL centrifuge tube (*P*). A 3.75 mL of a chloroform-methanol mixture was then added in the ratio of 1:2 and then vortexed. Subsequently, 1.25 mL of chloroform was added again and vortexed well. A volume of 1.25 mL distilled water was then added and vortexed to ensure proper mixing. Later, the sample was centrifuged at 1000 rpm for 5 min and two layers were formed in the tube. The top clear liquid layer was discarded and the bottom white viscous liquid layer containing the lipids was retained. The sample was left to dry in a fume hood. The final weight of the centrifuge tube was then measured (*R*). The lipid content was calculated using Eq. (5).

$$Lipid\ content\ (mg/mL) = \frac{(R-P)g}{V\,mL} \times \left(\frac{1000\,mg}{1\,g}\right) \quad (5)$$

2.3. Experimental design

This study consists of three sets of experiment. The first set was to determine the CO_2 removal at different concentrations of aqueous ammonia (2% - 4%), CO_2 gas flow rate (0.5 Lpm - 2 Lpm) and temperature (27˚C to 35˚C) without *Synechococcus* sp. PCC 7002 IIUM01. The second set of experiment was conducted by varying only the rate of 15% CO_2 gas (0.5 Lpm - 2.5 Lpm), to determine CO_2 removal from the culture of *Synechococcus* sp. PCC 7002 IIUM01 with aqueous ammonia. Cell growth and lipid content of the cell were also observed. These two sets of experiment were conducted using one-factor-at-a-time (OFAT) method. Model for OFAT experiments can be either linear, quadratic or cubic model. The third set of experiment was based on Face Centered Central Composite Design (FCCCD) of Response Surface Methodology (RSM) with five center points. Table 1 shows coded and actual value experimental value used in FCCCD.

Table 1: Coded and actual value experimental value used in FCCCD

		Level	
Variables	**Component**	**-1**	**+1**
A	Temperature (˚C)	28	38
B	Concentration of aqueous ammonia (% (w/v))	0.5	1.0

The predictive model that describes the relationship between response and factor variables is elucidated by second-order polynomial of Eq. (6).

$$Y = \beta_o + \beta_a A + \beta_b B + \beta_c C + \beta_{ab} AB + \beta_{ac} AC + \beta_{bc} BC + \beta_{aa} A^2 + \beta_{bb} B^2 + \beta_{cc} C^2 \quad (6)$$

where Y is the response (dependent variables), while A, B and C are the factors (independent variables), β_o is an intercept term; β_a, β_b and β_c are linear coefficients; β_{ab}, β_{ac} and β_{bc} are the interaction coefficients; and β_{aa}, β_{bb} and β_{cc} are the quadratic coefficients. The Design of Experiment (DoE) and data analysis were derived using Design Expert V6.0.8. Additional experiment was then conducted to confirm the validity of the statistical experimental design.

3. RESULTS AND DISCUSSION

3.1. CO_2 absorption in aqueous ammonia in ATCC medium 957

This first set of experiment was conducted to observe the removal of 15% (v/v) CO_2 via ATCC medium 957 with aqueous NH_3 concentration varied from 2 to 4% (w/v) in the absence of mutant cells at 27˚C with gas flow rate of 0.5 Lpm for 60 minutes. CO_2 removal increased as the concentration of NH_3 increased (Fig. 2(a)). The same trend was also observed by [14] and [17]. Both studies reported that the concentration of NH_3 greatly influenced the absorption. In this current study, CO_2 removal was observed in the range of 80% to 95% with specified conditions (*i.e.* Fig. 2(a)). Yeh and Bai [17] observed maximum CO_2 removal of nearly 50% achieved at 7% (w/w) (~4.9% (w/v)) NH_3 concentration at 25˚C and CO_2 inlet concentration of 16% (v/v) purged at 2 Lpm gas flow rate. The observation was different in this study, however disparity in gas flow rate (*i.e.* 2 Lpm, equivalent to 10 vvm) used in Yeh and Bai [17] research should be well noted. The flow rate in the previous study [17] was twice the rate used of this study (*i.e.* 0.5 Lpm equivalent to 5 vvm). This has led to a deduction that gas flow rate has an inverse effect on CO_2 removal. The inverse relationship between gas flow rate and gas removal was similarly observed in Yeh and Bai [17] and this current study (Fig. 2(b)).

Furthermore, the gas flow rate has a massive influence in CO_2 removal process, at which higher flow rate reduces removal efficiency. This is agreed with a report by [14] which revealed that low concentration of ammonia led to a significant increase in CO_2 absorption. Thus, as a trade-off between CO_2 absorption and cyanobacterial growth, a new variable of lower range ammonia concentration between 2-4% (w/v) was introduced in the study and high CO_2 absorption was achieved as a result of the action.

Darde et al. [18] reported that chilled aqua ammonia process was proven to be more efficient than the ambient process. However, despite the report, since current study utilized cyanobacteria; hence ambient process was preferred over chilled process. The optimum temperature for cyanobacteria growth is approximately 29°C and the organism can grow well up to 35°C [19]. Taking this fact into consideration, the operational temperatures ranging from 27°C to 40°C were chosen to be studied and the data is presented in Fig. 2(c). From the figure, it is clear to derive that temperature has little influence in the absorption process as CO_2 removal efficiency reduces only slightly as the temperature increased. At low NH_3 concentration, reaction between CO_2 and NH_3 is exothermic in nature. Exothermal reaction favors lower temperature for higher reaction rate. Nevertheless, at higher concentration the reaction would demonstrate endo-exothermal reaction as described by [17].

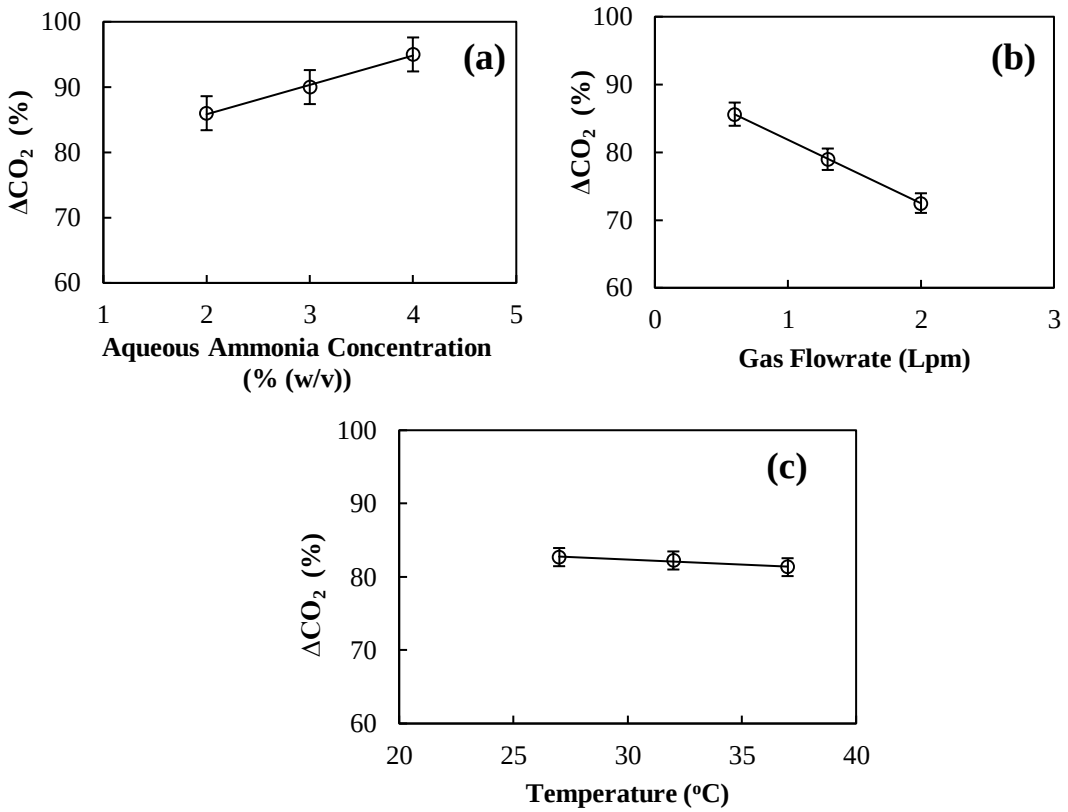

Fig. 2. Percent of CO_2 removal affected by (a) aqueous ammonia concentration (at 25°C and 0.5 Lpm), (b) gas flow rate (at 25°C and 2% (w/v) NH_3) and (c) temperature (at 0.5 Lpm and 2% (w/v) NH_3) of the system.

3.2. CO_2 absorption in the presence of *Synechoccocus* sp. PCC 7002 IIUM01

The culture of mutant *Synechoccocus* sp. PCC7002 IIUM01 was pale green in color compared to the parent strain as shown in Fig. 3. Further study using mutant cyanobacteria

culture shows that growth of the species is highly reduced at 2% (w/v) ammonia concentration (data not shown), proving that the concentration is sensitive to this mutant [20]. At the high concentration, the mutant culture may also suffer from ammonia toxicity. For this reason, the experiment proceeded with investigating influence of flow rate on CO_2 absorption at ammonia concentration of 0.5% to 1% in the presence of *Synechoccocus* sp. PCC 7002 IIUM01. Additionally, the parent strain could not grow at these concentrations. The study was performed based on OFAT experiment by varying CO_2 gas flow rate from 0.5 Lpm to 2.5 Lpm. Thereafter, optimization study was conducted using Face Centered Central Composite Design (FCCCD) to see the interaction between ammonia concentration and temperature (Table 1) for maximum CO_2 absorption, cell growth and lipid production.

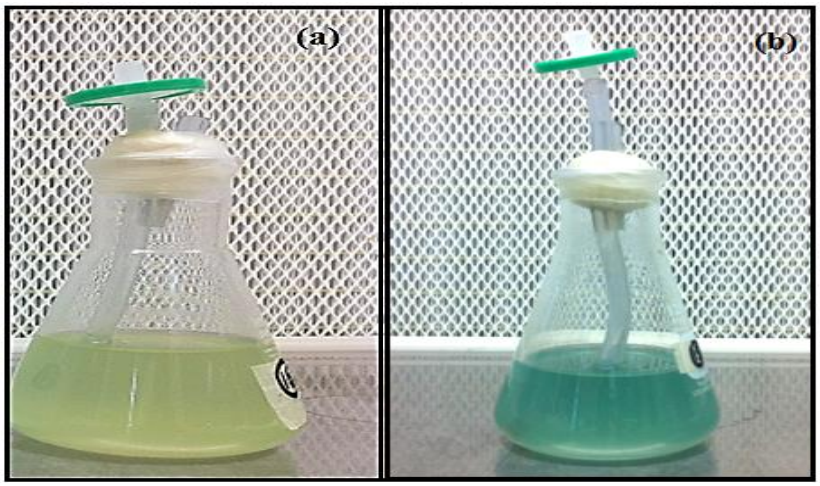

Fig. 3. (a) Mutant strain and (b) parent strain after a 7 days of culture period.

3.2.1. Effect of gas flow rate on CO_2 removal, lipid yield and cell growth

The study continues with evaluating CO_2 absorption using aqueous ammonia in the presence of the cyanobacteria. The experiment was performed at 1% of ammonia concentration with variation of flow rate from 0.5 to 2.5 Lpm at 27°C. Three responses namely the percent of CO_2 removal, lipid yield and CDW were observed and recorded. The analyses of variance (ANOVA) for each response are shown in Table 2 while Fig. 4 shows graphical results of the responses. ANOVA analyses (Table 2) show that all models are significant with non-significant of lack of fit. The predicted R^2 are in reasonable agreement with adjusted R^2 for all three responses.

A negative correlation between CO_2 absorption and gas flow rate is shown in Fig. 4(a), which is also represented by predictive model of Eq. (7) in coded factors. The maximum CO_2 absorption was around 80% at the flow rate of 0.5 Lpm. The CO_2 absorption or removal reduced with the increasing flow rate to its minimum value of about 43% at 2.5 Lpm. This could be caused by the short contact time between cyanobacteria and CO_2 at very high flow rates as the gas does not stay in the vessel for the same duration (lower resident time) as the lower flow rates. Furthermore, at very high flow rates, there could be saturation of HCO^{3-} in the vessel for CO_2 absorption capacity (of the ammonia solution) and has reached its maximum. Thus, a low flow rate is preferred since it offers longer contact time between CO_2 and solvent molecules. Taking into account the fact that cyanobacterial and microalga utilization rates of CO_2 is much slower than the chemical absorption rates, the lower the operation flow rate of CO_2 would be favorable.

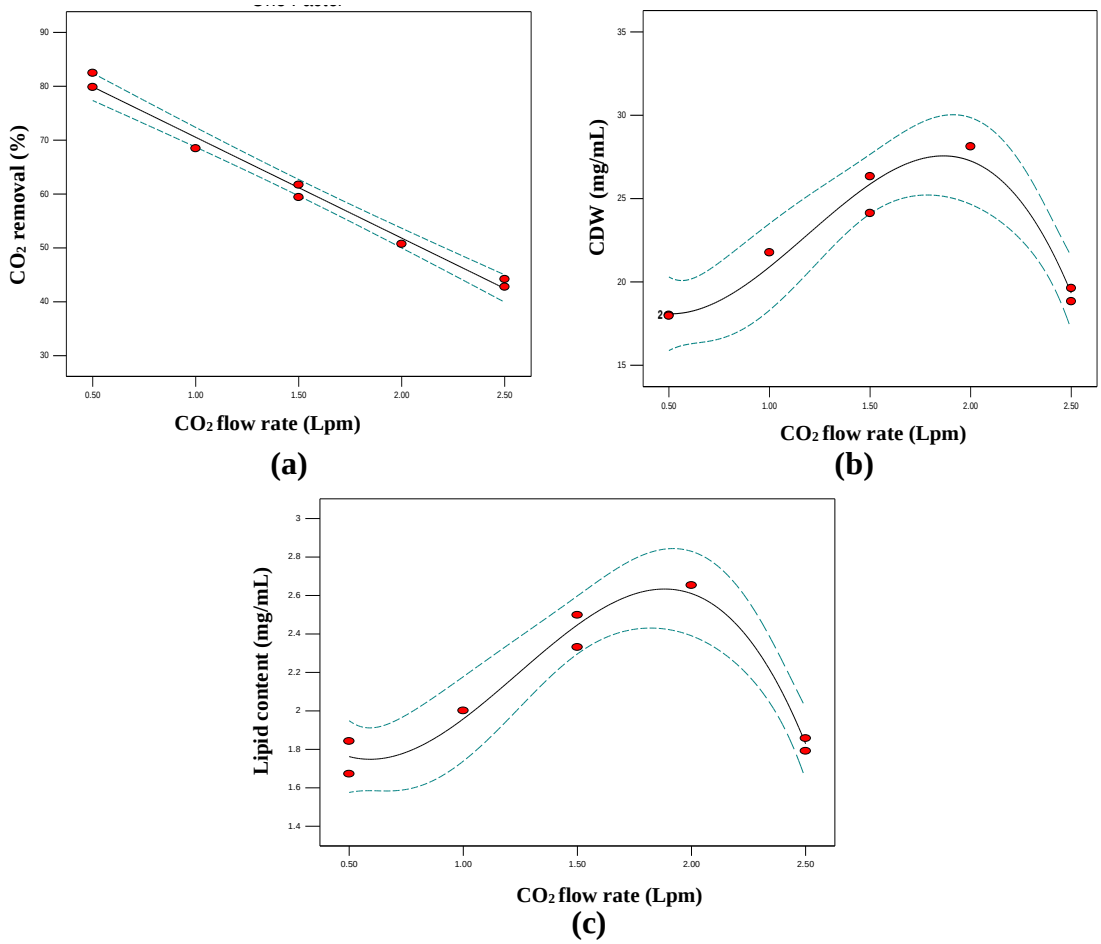

Fig. 4. Effects of various carbon dioxide flow rates on (a) %CO_2 removal; (b) CDW; and (c) lipid concentration of *Synechoccocus* sp. PCC 7002 IIUM01.

The correlation between the effect of CO_2 flow rate with the growth of cyanobacteria and lipid production is another vital point to note from this study. Growth of cyanobacteria is the key to success of the process since ammonia is only used as a solvent to enhance CO_2 absorption by cyanobacteria. Therefore, if the cyanobacteria do not grow successfully then the process conditions are considered not feasible. Hence, the maximum accumulative cell growth was observed and recorded as shown in Fig. 4(b). Eq. (8) is the predictive model of CDW in coded factors which is based on the flow rate and taking the form of cubic equation. The graph shows the highest growth was obtained at a flow rate of 2.0 Lpm. An increase in CO_2 flow rate beyond 2.0 Lpm did not enhance the cell growth. This is due to the fact that CO_2 is the main carbon source for the cyanobacteria. Thus, increasing the carbon source level will increase the growth up to a certain concentration at which the cyanobacteria are consuming maximum amount of carbon. Further increase in CO_2 flow rate will not increase the growth due to uptake saturation.

Fig. 4(c) illustrates the effect of gas (mixture of CO_2 and air) flow rate on the lipid content of *Synechococcus* PCC 7002 IIUM01 measured after one week. Eq. (9) represents the predictive model of lipid content (LC) in coded factors which is taking the form of cubic equation. It shows that the highest lipid content was observed at 2.0 Lpm, followed by 1.5 Lpm and lastly 1.0 Lpm. The lowest lipid content was observed at the flow rates of 0.5 Lpm and 2.5 Lpm. This is in accordance to the results of the cell growth where increasing carbon source will increase the growth and metabolism. This will consequently lead to the increase concentration of biomass and eventually increase lipid content of the biomass. The results

are similar to the findings by [21] which reported similar increase of lipid content to an optimum level. In Fig. 5, the result shows that lipid content is calculated at about 9.51% of the CDW. It is much lower than the value obtained from [22] who observed up to 47% lipid content derived from wild species of *Synechococcocus* sp. incubated at a longer time period (*i.e.* more than 15 days).

$$\%CO_2 = +61.19 - 18.71X_1 \quad (7)$$

$$CDW = +25.87 + 8.27X_1 - 7.16X_1^2 - 7.65X_1^3 \quad (8)$$

$$LC = +2.45 + 0.86X_1 - 0.65X_1^2 - 0.82X_1^3 \quad (9)$$

where, $\%CO_2$, *CDW*, *LC* and X_1 represent percent of CO_2 removal, cell dry weight, lipid content and gas flow rate, respectively.

Table 2: ANOVA analyses for CO_2 removal, lipid content and CDW with respect to single factor of CO_2 gas flow rate (X_1)

ANOVA for Response Surface Linear Model of CO_2 removal

Source	Sum of squares	df	Mean square	*F* Value	*p*-value Prob > *F*	
Model	1575.92	1	1575.92	508.25	< 0.0001	significant
X_1	1575.92	1	1575.92	508.25	< 0.0001	
Residual	18.60	6	3.10			
Lack of Fit	11.53	3	3.84	1.63	0.3489	not significant
Pure Error	7.07	3	2.36			
Cor Total	1594.53	7				

R^2 = 0.9883 , Adj R^2 = 0.9864, Pred R^2 = 0.9779, Adeq Precision = 42.510

ANOVA for Response Surface Cubic Model for lipid content

Source	Sum of squares	df	Mean square	*F* Value	*p*-value Prob > *F*	
Model	0.89	3	0.30	32.63	0.0029	significant
X_1	0.21	1	0.21	22.55	0.0090	
X_1^2	0.68	1	0.68	74.07	0.0010	
X_1^3	0.17	1	0.17	18.65	0.0125	
Residual	0.036	4	9.115E-03			
Lack of Fit	5.888E-003	1	5.888E-03	0.58	0.5025	not significant
Pure Error	0.031	3	0.010			
Cor Total	0.93	7				

R^2 = 0.9607 , Adj R^2 = 0.9313, Pred R^2 = 0.8511, Adeq Precision = 12.556

ANOVA for Response Surface Cubic Model for CDW

Source	Sum of squares	df	Mean square	*F* Value	*p*-value Prob > *F*	
Model	103.40	3	34.47	26.98	0.0041	significant
X_1	19.10	1	19.10	14.95	0.0180	
X_1^2	81.64	1	81.64	63.90	0.0013	
X_1^3	14.64	1	14.64	11.46	0.0276	
Residual	5.11	4	1.28			
Lack of Fit	2.36	1	2.36	2.56	0.2076	not significant
Pure Error	2.76	3	0.92			
Cor Total	108.51					

R^2 = 0.9529, Adj R^2 = 0.9176, Pred R^2 = 0.7827, Adeq Precision = 11.470

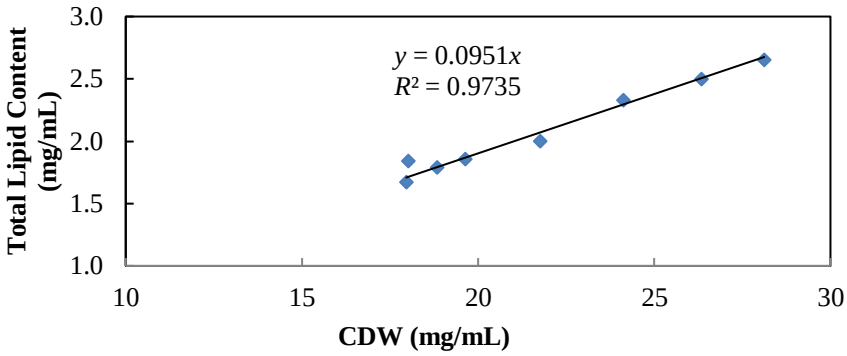

Fig. 5. Graph of total lipid content vs. CDW with the slope representing $Y_{p/x}$ of 0.0951 g/g.

3.2.2. Combined effects of aqueous ammonia concentration and culture temperature on CO_2 removal, lipid yield and cell growth

The experimental design studies were designed using FCCCD of RSM. The design factors were the culture temperature which ranges from 28°C to 38°C, and the ammonia concentration bearing values from 0.5% to 1.0% (w/v) (Table 3). The gas flow rate was fixed at 0.5 Lpm for all experimental runs based on OFAT result (Section 3.2.1). The experiment consisted of 13 experimental runs. The main objective of the optimization studies was to identify the optimum temperature and ammonia concentration for maximum CO_2 removal and lipid production at acceptable growth rate.

Table 3: FCCCD design matrix for two factors optimization with three responses

Run	Factor *A* Temp. (°C)	Factor *B* Ammonia conc. (% (w/v))	Response 1 ΔCO_2 (%)	Response 2 LC (mg/mL)	Response 3 CDW (mg/mL)
1	33.00	0.75	69.63	1.42	14.64
2	33.00	0.75	71.12	1.49	15.24
3	38.00	1.00	58.18	0.60	6.20
4	28.00	1.00	82.19	0.45	0.291
5	28.00	0.75	73.12	0.90	9.24
6	33.00	0.75	67.31	1.28	13.72
7	38.00	0.75	50.09	0.79	8.60
8	33.00	0.75	66.22	1.51	15.36
9	33.00	1.00	76.45	1.10	10.96
10	38.00	0.50	46.68	1.19	12.20
11	33.00	0.50	53.61	1.68	16.68
12	33.00	0.75	68.71	1.43	14.60
13	28.00	0.50	61.32	0.77	7.68

ANOVA for all three responses (*i.e.* percent of CO_2 removal, lipid content and CDW) are summarized in Table 4. The ANOVA results depict that all three models for the three responses are significant ($p < 0.0001$) and the lack of fit is not significant. Overall, the predicted R^2 are in reasonable agreement with the adjusted R^2 (*i.e.* both values are within 0.2 with each other) and adequate precision are all over 4. These indicate that the model provides good predictions for average outcomes which the data obtained are best fit to quadratic model for CO_2 removal and reduced quadratic model for lipid and CDW. The

regression equations obtained are represented by Eqs. (10) to (12) for CO_2 removal, lipid content and CDW, respectively.

$$\%CO_2 = +68.04 - 10.28A + 9.20B - 2.34B - 5.04A_2 - 1.61B_2 \quad (8)$$

$$LC = +1.43 + 0.077A - 0.25B - 0.61A_2 - 0.063B_2 \quad (9)$$

$$(CDW)^{1.41} = +44.65 + 4.50A - 10.35B - 23.58A_2 - 4.40B_2 \quad (10)$$

Where, $\%CO_2$, CDW, LC, *A* and *B* represent percent of CO_2 removal, cell dry weight, lipid content, temperature and aqueous ammonia concentration, respectively.

Table 4: ANOVA analysis for response surface of CO_2 removal, lipid content and CDW

ANOVA for Response Surface Quadratic Model for CO_2 percent removal

Source	Sum of squares	*df*	Mean Square	*F* Value	*p*-value Prob > *F*	
Model	1274.29	5	254.86	39.27	< 0.0001	significant
***A*-Temperature**	634.07	1	634.07	97.69	< 0.0001	
***B*-Ammonium concentration**	508.02	1	508.02	78.27	< 0.0001	
AB	21.95	1	21.95	3.38	0.1085	
A^2	70.02	1	70.02	10.79	0.0134	
B^2	7.16	1	7.16	1.10	0.3285	
Residual	45.43	7	6.49			
Lack of Fit	30.68	3	10.23	2.77	0.1748	not significant
Pure Error	14.75	4	3.69			
Cor Total	1319.72	12				

R^2 = 0.9656, Adj R^2 = 0.9410, Pred R^2 = 0.7759, Adeq Precision= 22.512

ANOVA for Response Surface Reduced Quadratic Model for lipid content

Source	Sum of squares	*df*	Mean of square	*F* Value	*p*-value Prob > *F*	
Model	2879.29	4	719.82	41.74	< 0.0001	significant
***A*-Temperature**	121.66	1	121.66	7.05	0.0290	
***B*-Ammonium concentration**	642.59	1	642.59	37.26	0.0003	
A^2	1536.00	1	1536.00	89.06	< 0.0001	
B^2	53.56	1	53.56	3.11	0.1161	
Residual	137.98	8	17.25			
Lack of Fit	107.61	4	26.90	3.54	0.1240	not significant
Pure Error	30.37	4	7.59			
Cor Total	3017.27	12				

R^2 = 0.9394, Adj R^2 = 0.9091, Pred R^2 = 0.7904, Adeq. Precision = 16.236

ANOVA for Response Surface Reduced Quadratic Model for CDW

Source	Sum of squares	df	Mean of square	*F* Value	*p*-value Prob > *F*	
Model	2879.29	4	719.82	41.74	< 0.0001	significant
***A*-Temperature**	121.66	1	121.66	7.05	0.0290	
***B*-Ammonium concentration**	642.59	1	642.59	37.26	0.0003	
A^2	1536.00	1	1536.00	89.06	< 0.0001	
B^2	53.56	1	53.56	3.11	0.1161	
Residual	137.98	8	17.25			
Lack of Fit	107.61	4	26.90	3.54	0.1240	not significant
Pure Error	30.37	4	7.59			
Cor Total	3017.27	12				

R^2= 0.9543, Adj R^2 = 0.9314, Pred R^2 = 0.8362, Adeq. Precision = 18.941

Fig. 6(a) shows a 3D surface plot of percent of CO_2 removal against both temperature and ammonia concentration. It indicates that CO_2 removal is more pronounced at the lower temperature and higher ammonia concentration. However, cell growth and lipid content (Fig. 6(b) and (c)) prefer lower ammonia concentration at the culture temperature around 34°C. This has been expected since preliminary experiments identified that 2% (w/v) ammonia greatly reduced growth rate and is toxic to the cyanobacteria. Similarly, many researches have reported that nitrogen limitation can lead to increase of lipid content in oleaginous microorganism [9,23-25].

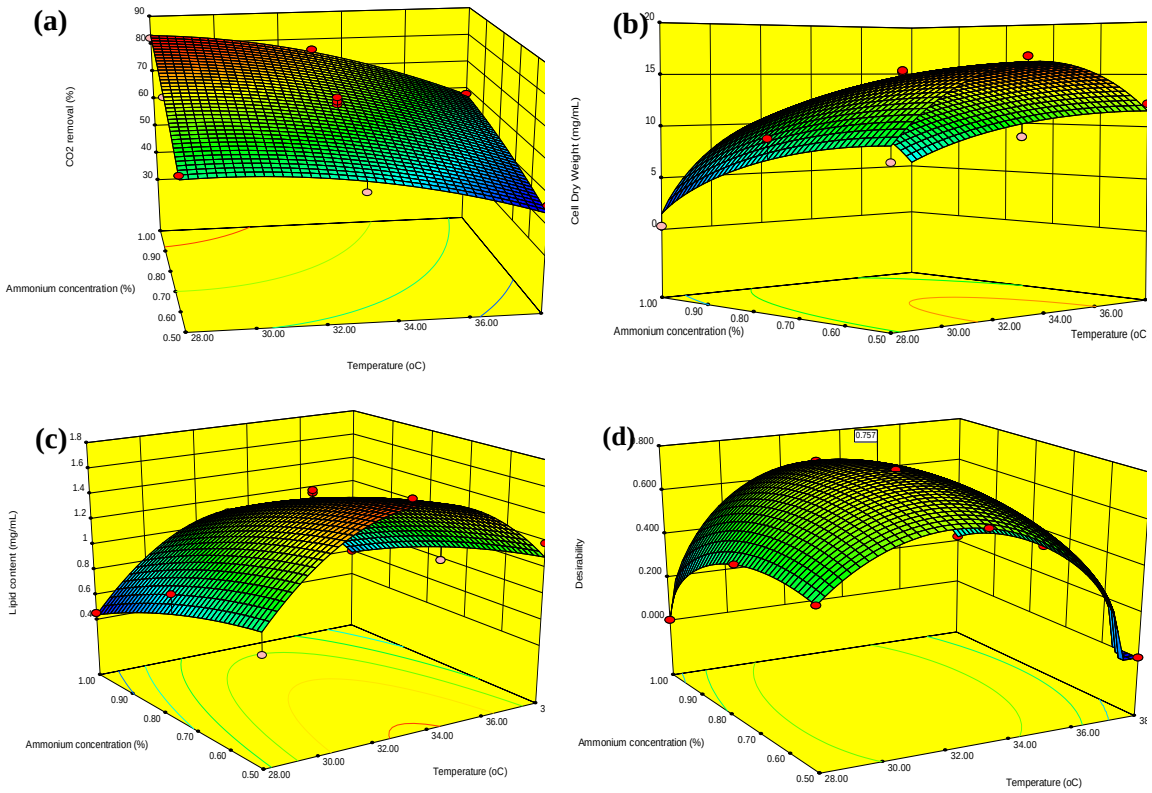

Fig. 6. Interactive effects of temperature and ammonia concentration on (a) %CO_2 removal, (b) growth rate (CDW), (c) lipid content and (d) desirability when optimizing all three responses at the same time.

Interactive effect of temperature and ammonia concentration was evaluated for individual responses. The optimum condition maximizing both CO_2 absorption and lipid production is shown in Fig. 6(d). Based on the desirability (where 1 is the most desired), the optimum condition maximizing the two responses (*i.e.* maximum %CO_2 removal and lipid content) while letting CDW within the range can be achieved at 32°C and 0.75% (w/v) of ammonia and the gas flow rate at 0.5 Lpm. Validation experiment at these conditions was conducted and the results indicated that the experiment is repeatable and reliable (Table 5) with less than 10% differences.

Table 5: Validation data for optimization studies

Response	Predicted	Experimental	% difference
Percentage of ΔCO_2 (%)	72.64	68.60	5.56
CDW (mg/mL)	13.52	14.71	8.80

Lipid Content (mg/mL)	1.32	1.42	7.57

4. CONCLUSION

CO_2 absorption that is based solely in aqueous ammonia has shown preference to high ammonia concentration and lower gas flow rate and temperature. However, maximum ammonia concentration has to be set below 2% (w/v), since 2% (w/v) and above will greatly hinder the growth of *Synechococcus* PCC 7002 IIUM01. In conclusion, the study proceeds at lower ammonia concentration (*i.e.* 0.5 to 1.0%) with the temperature ranging from 28°C to 38°C, subject to 15% CO_2 concentration introduced every day for 60 minutes. The optimum condition set by maximizing percent of CO_2 removal, growth rate and lipid content was obtained at ammonia concentration of 0.75 % (w/v), temperature of 32°C and at gas flow rate of 0.5 Lpm.

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