Chlorella vulgaris LOGISTIC GROWTH KINETICS MODEL IN HIGH CONCENTRATIONS OF AQUEOUS AMMONIA

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ABSTRACT: The ability of microalgae to utilize CO_2 during photosynthesis and grow rapidly shows their potential in CO_2 bio-fixation to capture and store the gas. However, CO_2 capture by this biological approach is very slow compared to chemical reaction-based processes such as absorption using amine or aqueous ammonia. Integration between chemical (aqueous ammonia) and biological (microalgae) aspects might enhance the capturing process and at the same time the microalgae can assimilate CO_2 for beneficial bioproduct formation. Thus, it is important to assess the growth of the microalgae in various concentrations of ammonia with CO_2 supply. Hence, the main objective of this study is to investigate *Chlorella vulgaris* growth and its kinetics in aqueous ammonia. To achieve that, *C. vulgaris* was cultivated in various concentrations of aqueous ammonia between 0 to 1920 mg/L at room temperature (i.e. 27 °C) and supplied with 15% (v/v) of CO_2 under illumination of 3500 lux of white fluorescent light. Result shows that the maximum growth capacity (X_{max}) of *C. vulgaris* is deteriorating from 1.820 Au to 0.245 Au as the concentration of aqueous ammonia increased. However, no significant change in maximum specific growth rate (μ_{max}) was observed. The growth data was then fitted into the logistic growth model. The model coefficient of determination (R^2) is decreasing, which suggests modification of the model is required.

ABSTRAK: Keupayaan alga-mikro untuk menggunakan CO_2 semasa proses fotosintesis dan pembiakannya yang pesat menunjukkan potensi dalam penggunaan dan penyimpanan gas ketetapan-biologi. Walau bagaimanapun, penggunaan CO_2 melalui cara ini adalah sangat perlahan berbanding proses tindak balas kimia melalui penyerapan amina ataupun cecair ammonia. Percampuran antara tindak balas kimia (cecair ammonia) dan tindak balas biologi, memungkinkan penambahan proses percampuran dan pada masa sama alga-mikro akan menyerap CO_2 bagi kepentingan pembentukan hasil biologi. Dengan itu, adalah sangat penting untuk mengawasi pertumbuhan alga-mikro dalam pelbagai ketumpatan ammonia bersama kandungan CO_2. Oleh itu, objektif utama penyelidikan ini adalah untuk menyiasat pertumbuhan *Chlorella vulgaris* dan proses kinetik dalam cecair ammonia. Bagi memperoleh hasil tersebut, *C. vulgaris* telah dikulturkan pada ketumpatan cecair berbeza antara 0 ke 1920 mg/L pada suhu bilik (iaitu 27 °C) dan dibekalkan dengan 15% (v/v) CO_2 di bawah cahaya putih flurosen 3500 lux.

Keputusan menunjukkan kapasiti pertumbuhan terbanyak (X_{max}) *C. vulgaris* telah berkurang daripada 1.820 Au kepada 0.245 Au apabila ketumpatan cecair ammonia dikurangkan. Walau bagaimanapun, tiada perubahan ketara pada kadar pertumbuhan (μ_{max}) dapat dilihat. Data kadar pertumbuhan kemudiannya dikemas kini pada model pertumbuhan logistik. Model pekali penentu (R^2) telah direndahkan di mana cadangan untuk mengubah model adalah diperlukan.

KEYWORDS: *aqueous ammonia; Chlorella vulgaris; growth kinetics; logistic growth model*

1. INTRODUCTION

Climate change and global warming issues related to excessive emission of CO_2 have been deliberatively discussed over years. To address this problem, Malaysia has participated and pledged to reduce 40% of CO_2 emission intensity by 2020 and re-pledged for 45% of CO_2 reduction by year 2030 [1,2]. To achieve the goal, CO_2 mitigation is part of the crucial areas identified by The Paris Agreement [1]. Microalgae and cyanobacteria have potential in mitigating CO_2 levels in the atmosphere as well as capturing directly from industrial plant flue gas [3-5]. The growth of photosynthetic microalgae is directly related to the rate of CO_2 fixation from the atmosphere. Their ability to grow rapidly and undergo photosynthesis makes them strong candidates for use in mitigating CO_2 levels [6]. However, the removal rate is still slower and the fixation capacity is lower compared to the chemical solvent absorption process using amine or aqueous ammonia [7,8].

Integrating chemical and biological systems to enhance the removal while simultaneously fixing or sequestrating CO_2 for its bioproducts, such as biofuel [9, 10], animal feed, or stable isotopes biochemical compound [11] derived from the microalgae, seems appealing. Previous studies show that *Chlorella vulgaris* a green microalga, was able to grow in wastewater containing a high level of ammonia [12,13]. The growth varied with the ammonia concentration at the respective process conditions, which is mostly no aeration or supply of CO_2, and the growth was limited by the toxicity of the free ammonia present in the culture. Thus, it is important to assess and understand the growth of *C. vulgaris* in the presence of aqueous ammonia and model the growth.

Generally, like any other microorganisms, there are six phases of *C. vulgaris* growth in a batch culture which are lag, acceleration, growth or exponential, decline, stationary, and death phases (Fig. 1). A general growth model is represented by the exponential growth model of Eq. (1) [14]. This mathematical model equation, when plotted, will produce a J-shape curve where the exponential growth is represented by μ_{max}, whereas, the typical growth curve shown in Fig. 1, has an S-shape (lag phase to stationary phase). The S-shape growth can be better represented by the logistic growth model of Eq. (2) which was originally proposed by Verhulst in the eighteenth century [15]. The model provides carrying capacity (X_{max}) as a moderating force in the growth rate [15] which is influenced by substrate depletion as well as toxic compounds in the environment [16]. The integral form of Eq. (2) is shown by Eq. (3).

$$\frac{dX}{dt} = \mu_{max} X \tag{1}$$

$$\frac{dX}{dt} = \mu_{max} \left(\frac{X_{max} - X}{X_{max}} \right) X \tag{2}$$

$$X(t) = \frac{X_{max}}{1 + \left(\frac{X_{max}}{X_o} + 1\right) e^{-\mu_{max} t}} \quad (3)$$

where μ_{max}, t, X_{max}, X_o and X represent the specific growth rate, time, maximum biomass concentration, initial biomass concentration and actual biomass concentration, respectively. The objective of this study is to assess *C. vulgaris* growth kinetics for its ability to grow in various concentrations of aqueous ammonia with periodically aerated CO_2 gas during the cultivation and model the growth based on the logistic growth equation.

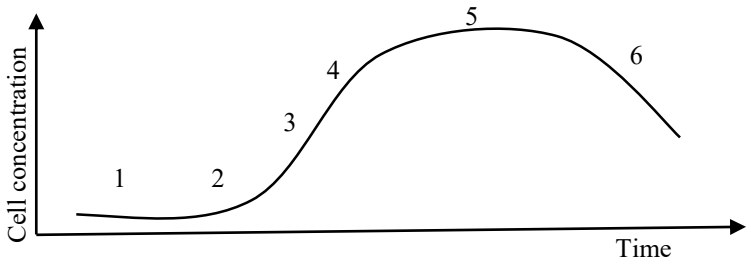

Fig. 1: Growth curve of microalgae culture in batch with phases;
(1) lag, (2) acceleration, (3) growth, (4) deceleration, (5) stationary, and (6) death.

2. MATERIALS AND METHODS

2.1 Microalgae Cultivation

Stock culture of *C. vulgaris* was purchased from the Institute of Ocean and Earth Sciences, University Malaya and was kept aseptically in a Bold Basal Medium (BBM). A 250 mL of culture solution was prepared in triplicates which contained 10% (v/v) of *C. vulgaris* in BBM medium with the addition of aqueous ammonia at different concentrations. Eight experimental runs were conducted at various aqueous ammonia concentrations i.e. 0 mg/L, 30 mg/L, 60 mg/L, 120 mg/L, 240 mg/L, 480 mg/L, 960 mg/L and 1920 mg/L. The culture was grown under illumination of 3500 lux from a white fluorescent lamp for 6 days with continuous (24 h) supply of filtered air at 0.5 Lpm. Pure CO_2 mixed with filtered air was supplied at a concentration of 15% (v/v) every day for 30 minutes continuously. The control of this experiment was conducted without aqueous ammonia (0 mg/L) with continuous supply of filtered air without extra CO_2 (i.e. culture acquired CO_2 from the air only). All experimental runs were conducted at room temperature (27 ^oC) in triplicate. Each flask was fitted with two silicon tubes (one for gas inlet and the other for gas outlet) and the culture was aerated for 24 hours with filtered air generated by air pump through a silicon tube. The sampling for each flask was carried out on a daily basis to measure *C. vulgaris* growth and the pH of the medium.

2.2 Determination of Growth Kinetic

Growth observation was based on the optical density (OD). The absorbance of *C. vulgaris* was determined by measuring the optical density of a 3 mL sample at a wavelength of 660 nm for every 24 hours using a UV-vis spectrophotometer. The data was then fitted nonlinearly using a nonlinear regression software, CurveExpert Professional software, version 2.4.0. The logistic equation model (Eq. 3) was selected to fit the data. Eq. (4) was used to calculate μ_{max} and compared it to μ_{max} obtained from the logistic growth model of Eq. (3). Doubling time was calculated based on Eq. (5).

$$\mu_{max} = \ln\left(\frac{x_{n-1}}{x_n}\right) \Big/ \left(\frac{t_{n-1}}{t_n}\right) \quad (4)$$

$$t_d = \frac{\ln 2}{\mu_{max}} \quad (5)$$

3. RESULTS AND DISCUSSION

3.1 Growth of C. vulgaris in BBM with air supply

Growth of *C. vulgaris* in BBM with the continuous air supply was studied before introducing aqueous ammonia and CO_2. This is to observe the typical growth kinetic and the stationary phase of *C. vulgaris* as illustrated in Fig. 1. Since the stationary phase of growth was observed as it entered the 4th day of cultivation, a subsequent cultivation run was conducted up to the 6th day after inoculation.

Figure 2(a) shows growth data in absorbance unit of optical density at 660 nm, while Fig. 2(b) is the same growth data in the form of natural-logarithms. Generally, the growth curve consists of six phases that have been discussed earlier. The same phases were also observed in *C. vulgaris* growth except for the death phase. This is due to a longer stationary phase (more than 12 days) of *C. vulgaris* which some have recorded being up to one month depending on the growth conditions [17,18]. In this study, the growth was monitored only for the duration of up to 12 days, thus the death phase was not perceived.

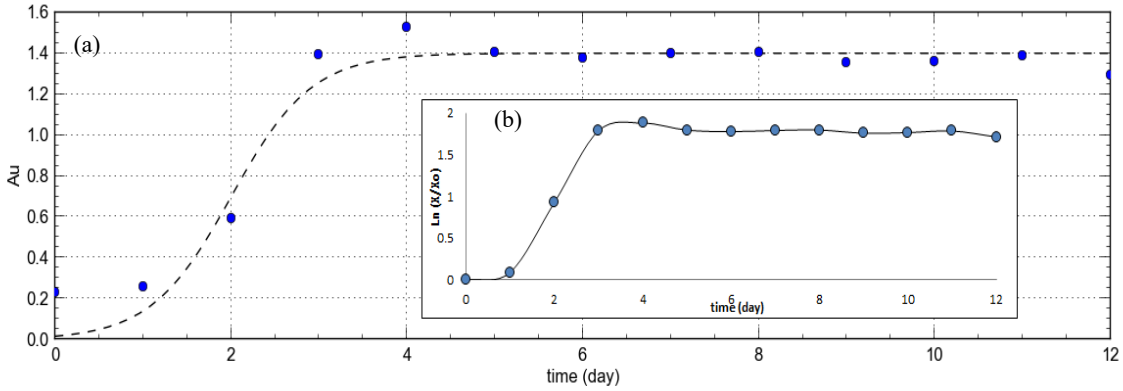

Fig. 2: The growth profile of *C. vulgaris* in BBM medium with (a) fitted model in dash line for circle symbol data and (b) growth curve in logarithms.

According to Doran [14], lag phase is best observed and confirmed in a logarithmic plotted curve as shown in Fig. 2(b). The figure shows that the cells required one day of adaptation (lag phase) to the new environment before entering the exponential (growth) phase. The exponential phase was started from day 2 to day 4 before entering the stationary phase (day 4 onwards). The maximum specific growth rate (μ_{max}) and doubling time (t_d) of the microalgae were 0.847 day^{-1} (0.036 h^{-1}) and 0.818 day (0.034 h^{-1}), respectively, as shown in Table 1. The μ_{max} obtained in this research is much lower compared to Lehana [19] (i.e. 0.24 h^{-1}) in a continuous culture but higher than Converti et al. [20] (i.e. 0.14 day^{-1}) grown in batch culture at about the same culture conditions as the current study. However, those studies used larger culture volumes of 3 L with 8% of CO_2 in air and 2 L with air only supplied to the culture, respectively whereas the current study is in a 250 mL culture medium with filtered air. Microalgae culture grown with aeration of

extra CO_2 as in Lehana [19] in continuous culture, has been shown to improve its growth rate compared to the culture grown with air only [4]. However, the lower maximum specific growth rate by Converti et al. [20] is due to the size of the culture, which in our study the air was supplied at flowrate of 2 vvm (0.5 Lpm). The growth of the current study was then modelled using the logistic growth equation with a coefficient of determination (R^2) of 0.95. However, the model predicted a very low rate of initial growth compared to the experimental data and this affected the values of other parameters shown in Table 1.

Table 1: Comparison of parameters obtained from logistic growth model fitting (Fig. 2(a)) and experimental data (Fig. 2(b)) for growth in BBM only.

Parameter	Value predicted by model	Value from experimental data
X_o (Au)	0.017	0.233
X_{max}(Au)	1.399	1.524
μ_{max}(day^{-1})	2.190	0.847
t_d (day)	0.317	0.818
R^2	0.95	1

3.1 Growth Kinetics of *C. vulgaris* at Different Concentrations of Ammonia

This experiment was conducted to understand how *C. vulgaris* responded to different concentrations of ammonia with the aeration rate of 0.5 Lpm for 15% (v/v) CO_2 in filtered air for 30 minutes every day under the illumination of 3500 lux. Since *C. vulgaris* growth reached the stationary phase in the beginning of day 5 (Fig. 2), subsequent observation of the microalgae growth was only recorded until day six in variation of ammonia concentrations (Fig. 3). The control run (0 mg/L ammonia with 15% CO_2) achieved the highest growth capacity (X_{max}) of 1.8 Au (Absorption unit) at OD_{660} (Fig. 3(a)). The growth was higher compared to the growth supplied with air only (Fig. 2). This shows that, even though 15% (v/v) of CO_2 was supplied for only 30 minutes every day, it had a positive influence on *C. vulgaris* growth (note that the initial cell density was maintained at around 0.2 Au for all runs). Many have reported how CO_2 influences the growth of photosynthetic microorganisms such as *C. vulgaris* [4,21,22] under illumination of light. The surrounding air, which contains only 0.03–0.06% CO_2, had limited *C. vulgaris* photosynthetic activity and slowed down its cell growth [23].

However, when aqueous ammonia was introduced in the culture at different concentrations, it is apparent that *C. vulgaris* growth was significantly influenced by the addition of aqueous ammonia. This is reflected by the decrease in the carrying capacity (X_{max}) values from both the model and the experimental data (Table 2). Nitrogen is a crucial nutrient for microalgae growth, which is assimilated in the form of nitrate (NO_3^-) or ammoniacal nitrogen (NH_4^+/NH_3). However, higher ammonia concentration can be toxic to the microalgae growth. This is especially depicted by Fig. 3 (g) and (h) where ammonia concentration of 960 mg/L and above showed that the growth of the microalgae was nearly inhibited. However, this is not the case for μ_{max}. Generally, μ_{max} (Table 2) obtained from experimental data (calculated based on Eq. (4)) are about the same, even though concentration of ammonia increased in a double amount of the previous concentration. The same was also observed by Tam and Wong [24]. A few papers suggested that μ_{max} is an important parameter in modelling microalgae or microbial population in batch production that is influenced by environmental factors [15,25]. In our

case, no significant change in μ_{max} was observed at various concentrations of ammonia. However, the X_{max} should also be considered as it is directly influenced by the environmental factor such as pH, temperature, nutrient, and others, which in this case is the ammonia toxicity.

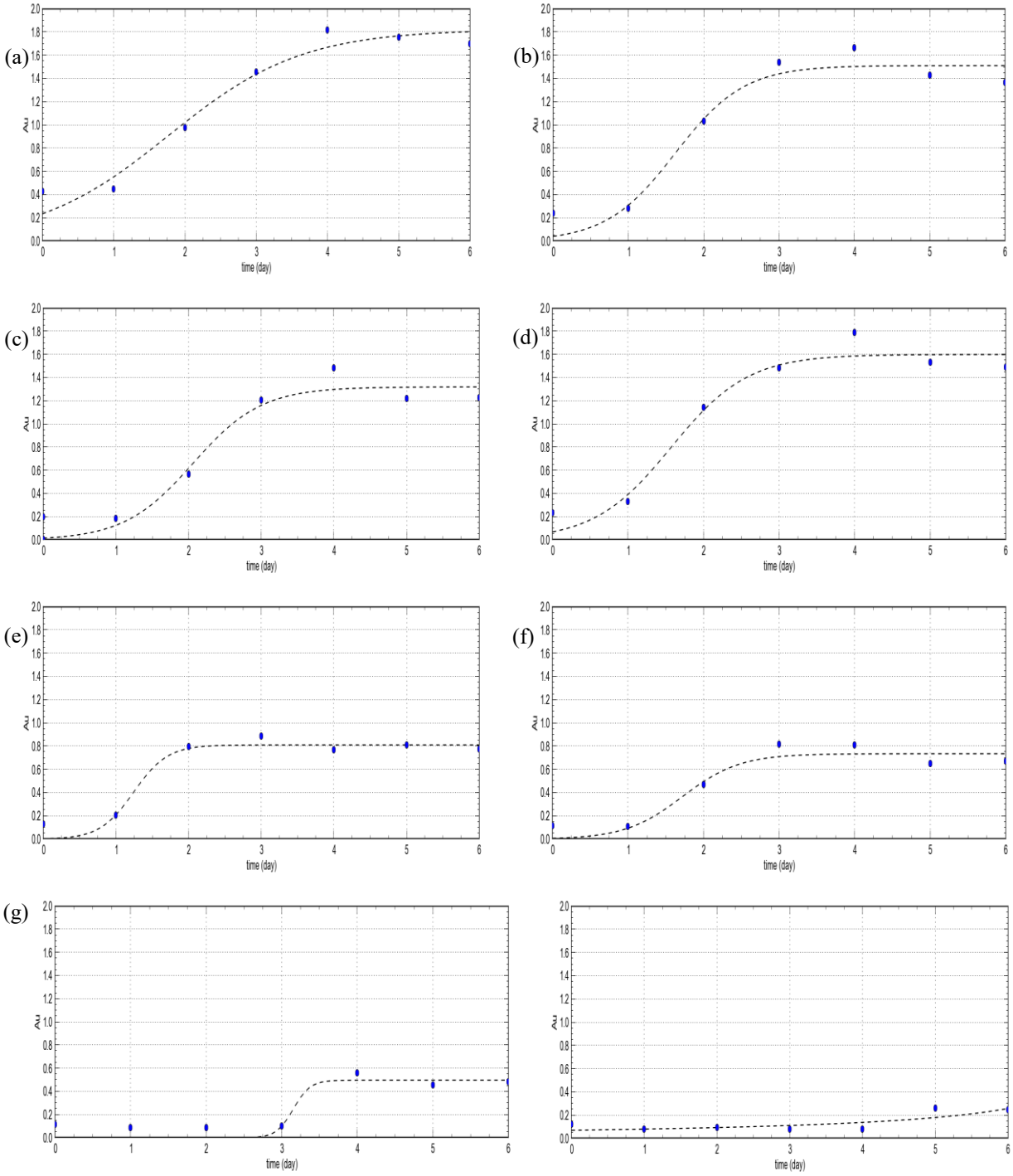

Fig. 3: Growth of *C. vulgaris* fitted in modified logistic equation at (a) 0 mg/L, (b) 30 mg/L, (c) 60 mg/L, (d) 120 mg/L, (e) 240 mg/L, (f) 480 mg/L, (g) 960 mg/L and (h) 1920 mg/L.

C. vulgaris growth is well fitted into the logistic growth kinetic model as depicted in Fig. 2 with R^2 between 0.88 to 1 (Table 2) except for the ammonia at the concentration of 1920 mg/L. The equation perfectly modelled the growth without ammonia with CO_2 supply. However, as the ammonia concentration increased, the R^2 was reduced and overestimated some of the parameters. This suggests that a model modification or application of another growth model that takes ammonia concentration and toxicity into account might be needed.

Table 2: Comparison of parameters obtained from logistic growth model and experimental data for variation of ammonia concentration.

NH_3 concentration ($mg \cdot L^{-1}$)	Parameters predicted by model				Parameter from experimental data		
	R^2	X_{max} (Au)	X_o (Au)	μ_{max} (day^{-1})	X_{max} (Au)	X_o (Au)	μ_{max} (day^{-1})
0	1.00	1.8200	0.4300	0.7787	1.8200	0.43	0.7787
30	0.95	1.5135	0.0433	2.1813	1.6660	0.239	1.2909
60	0.94	1.3217	0.0169	2.1137	1.4840	0.201	1.1075
120	0.96	1.6024	0.0704	1.9631	1.7890	0.231	1.2410
240	0.96	0.8128	0.0039	4.2902	0.8870	0.127	1.3481
480	0.92	0.7367	0.0080	2.6265	0.8170	0.115	1.4614
960	0.88	0.4997	0.0000	8.8764	0.5620	0.111	1.6968
1920	0.67	0.0001	0.0728	0.0001	0.2450	0.12	1.1670

The pH of the medium was originally adjusted to 6.8 with 1 N KOH before inoculation of the green microalgae. There were no significant changes in pH when ammonia was added in the medium until the ammonia concentration increased to 240 mg/L and above, the pH changed to 9.97 and higher as shown in Fig. 4. However when CO_2 was introduced in the culture medium, the pH reduced before it increased back as cell growth was observed. The increase of pH level as growth increased was also reported by others [26-28]. The growth of *C. vulgaris* in cultures containing ammonia is usually accompanied with a change in pH as the medium becomes acidic in the lag phase and then changes to alkaline when the cells are in the log or stationary phase.

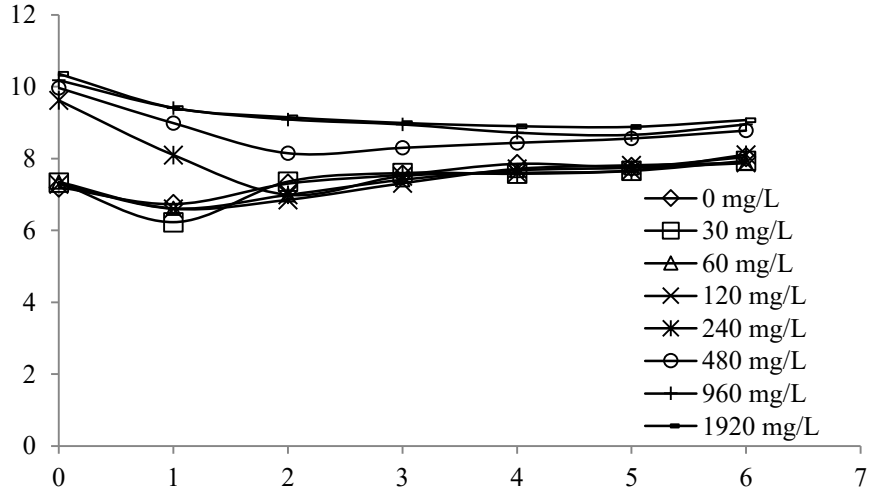

Fig. 4: Medium pH of *C. vulgaris* culture at different ammonia concentrations .

6. CONCLUSION

Growth of *C. vulgaris* in BBM with continuous air pumped into the culture was observed for a duration of 12 days to study the growth characteristic of *C. vulgaris* before introducing CO_2 and ammonia. The growth profile exhibits all phases (except death phase), with a significant extended stationary phase starting from day 4 onwards. Due to that, subsequent experimental runs were conducted for only six days.

Subsequently, eight runs were conducted at various concentrations of ammonia from 0 mg/L to 1920 mg/L with 15% (v/v) of CO_2 aerated at the volumetric flow rate of 0.5 Lpm under illumination at 3500 Lux (12 light:12 dark) at 27ºC. The highest growth was observed from the culture with 0 mg/L of aqueous ammonia aerated with CO_2. *C. vulgaris* growth capacity however declined with the increase of ammonia concentration. The data were also not well fitted into the logistic growth model as the concentration increased, suggesting modification of the model is required. The pH of the culture medium is influenced by growth of the cell in the presence of ammonia.

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REFERENCES

[1] Begum RA. (2017) Tackling Climate Change and Malaysia's Emission Reduction Target, In Scientific Malaysian. [http://magazine.scientificmalaysian.com/issue-13-2017/tackling-climate-change-malaysias-emission-reduction-target/]

[2] Lokman T. (2017) PM: Malaysia on course to reduce carbon emissions by 40 pct by 2020, In New Straits Times. [https://www.nst.com.my/news/nation/2017/12/310231/pm-malaysia-course-reduce-carbon-emissions-40-pct-2020]

[3] Benemann J, Pedroni PM, Davison J, Beckert H, Bergman P. (2003) Technology roadmap for biofixation of CO_2 and greenhouse gas abatement with microalgae, In Second National Conference on Carbon Sequestration. [https://www.netl.doe.gov/publications/proceedings/03/carbon-seq/PDFs/017.pdf]

[4] Azmi AS, Amid A, Puad NIM, Jamal P. (2017) The effect of CO_2 concentrations and the injection strategy on *Synechococcus* sp. PCC7002 culture, Asia Pacific of Molecular Biology and Biotechnology, 25:56-60.

[5] Fan LH, Zhang YT, Cheng LH, Zhang L, Tang DS, Chen HL. (2007) Optimization of carbon dioxide fixation by *Chlorella vulgaris* cultivated in a membrane-photobioreactor, Chemical Engineering & Technology, 30:1094-1099.

[6] Zhang X. (2015) Microalgae removal of CO_2 from flue gas. IEA Clean Coal Centre, UK.

[7] Clément-Larosière B, Lopes F, Gonçalves A, Taidi B, Benedetti M, Minier M, Pareau D. (2014) Carbon dioxide biofixation by *Chlorella vulgaris* at different CO_2 concentrations and light intensities. Engineering in Life Sciences 14:509-519.

[8] Yeh AC, Bai H. (1999) Comparison of ammonia and monoethanolamine solvents to reduce CO_2 greenhouse gas emissions. Science of the Total Environment, 228:121-133.

[9] Blinová L, Bartošová A, Gerulová K. (2015) Cultivation of Microalgae (*Chlorella vulgaris*) for Biodiesel Production. Research Papers Faculty of Materials Science and Technology Slovak University of Technology, 23:87-95.

[10] Ahmad A, Yasin NM, Derek C, Lim J. (2011) Microalgae as a sustainable energy source for biodiesel production: A review. Renewable and Sustainable Energy Reviews, 15:584-593.

[11] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. (2006) Commercial applications of microalgae. J. Bioscience and Bioengineering ,101:87-96.

[12] He P, Mao B, Shen C, Shao L, Lee D, Chang J. (2013) Cultivation of *Chlorella vulgaris* on wastewater containing high levels of ammonia for biodiesel production., Bioresource Technology, 129:177-181.
[13] Kim J, Lingaraju BP, Rheaume R, Lee J-Y, Siddiqui KF. (2010) Removal of ammonia from aastewater effluent by *Chlorella Vulgaris.* Tsinghua Science & Technology, 15:391-396.
[14] Doran PM. (1995) Homogeneous Reactions. In Bioprocess Engineering Principles, Academic press, London,Great Britain; pp257-296.
[15] Peleg M, Corradini MG, Normand MD. (2007) The logistic (Verhulst) model for sigmoid microbial growth curves revisited. Food Research International, 40:808-818.
[16] Escalante FM, Reyna-Angeles KA, Villafaña-Rojas J, Aguilar-Garnica E. (2017) Kinetic model selection to describe the growth curve of *Arthrospira* (*Spirulina*) *maxima* in autotrophic cultures. J. Chemical Technology and Biotechnology, 92:1406-1414.
[17] Blair MF, Kokabian B, Gude VG. (2014) Light and growth medium effect on *Chlorella vulgaris* biomass production. J. Environmental Chemical Engineering, 2:665-674.
[18] Sibi G. (2015) Low cost carbon and nitrogen sources for higher microalgal biomass and lipid production using agricultural wastes. J. Environmental Science and Technology, 8(3):113-121.
[19] Lehana M. (1990) Kinetic analysis of the growth of *Chlorella vulgaris*. Biotechnology and Bioengineering, 36:198-206.
[20] Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M. (2009) Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chemical Engineering and Processing: Process Intensification, 48:1146-1151.
[21] Singh S, Singh P. (2014) Effect of CO_2 concentration on algal growth: A review. Renewable and Sustainable Energy Reviews, 38:172-179.
[22] Anjos M, Fernandes BD, Vicente AA, Teixeira JA, Dragone, G. (2013) Optimization of CO_2 bio-mitigation by *Chlorella vulgaris*. Bioresource Technology, 139:149-154.
[23] Wang B, Li Y, Wu N, Lan C. (2008) CO_2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology, 79:707-718.
[24] Tam NFY, Wong YS. (1996) Effect of ammonia concentrations on growth of *Chlorella vulgaris* and nitrogen removal from media. Bioresource Technology, 57:45-50.
[25] Dalgaard P, Koutsoumanis K. (2001) Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models. J. Microbiological Methods, 43:183-196.
[26] Kim J, Lee J-Y, Keener T. (2009) Growth kinetic study of *Chlorella vulgaris*. AIChE Annual Meeting, American Institute of Chemical Engineers, Nashville, TN, United States; pp 1-6.
[27] Przytocka-Jusiak M, Duszota M, Matusiak K, Mycielski R. (1984) Intensive culture of *Chlorella vulgaris*/AA as the second stage of biological purification of nitrogen industry wastewaters. Water Research, 18:1-7.
[28] Santoso A. (2015) Increasing lipid accumulation of *Chlorella vulgaris* using *Spirulina platensis* in flat plate reactor for synthesizing biodiesel. Energy Procedia, 65:58-66.