COMPARISON OF CARBON FIXATION ABILITIES OF SELECTED MARINE MICROALGAE SPECIES

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Abstrak

Kaedah penyerapan gas karbon dioksida menggunakan mikroalga berfotosintesis merupakan salah satu kaedah alternatif diantara beberapa Teknoloji Penyerapan dan Pengasingan Karbon sedia ada. Selain daripada memanfaatkan proses semulajadi fotosintesis, kaedah ini juga berpotensi untuk memupuk perkembangan simbiosis diantara industri, dimana hasil buangan sesuatu industri akan menjadi satu input komoditi bagi satu industri yang lain. Kertas ini membincangkan dan melaporkan dapatan dari usaha awal dalam mengenalpasti spesis mikroalga marin yang berupaya untuk menyerap karbon dioksida dalam kadar yang tinggi. Spesis-spesis Tetraselmis dan Nannochloropsis Oculata telah digunakan dalam kerja ini dimana mereka telah dibiakkan menggunakan media f/2 di dalam pelbagai saiz kelalang Erlenmeyer sehingga 2 liter, dan dibiakkan selama 14 hari. Sumber gas karbon dioksida asli dan daripada udarakasa digunakan di dalam eksperimen ini. Suhu kultur dibiarkan pada takat suhu bilik dengan puratanya pada 26^{o}C. Pencahayaan dibekalkan menerusi tiub lampu fluoresen dengan kadar purata sebanyak 600 lux. pH, suhu, kemasinan dan oksijen terlarut disukat dan direkodkan dua kali sehari menggunakan alat jangka pelbagai guna EUTECH model PCD 650. Kadar penyerapan karbon dioksida dikira berpandukan daripada berat jisim mikroalga dan juga nisbah diantara karbon dan biojisim di dalam persamaan fotokimia yang lengkap. Keputusan mendapati mikroalga spesis Tetraselmis merupakan satu spesis yang mempunyai kadar penyerapan karbon yang lebih tinggi, iaitu sebanyak 0.03 – 0.04 g/hari berbanding dengan spesis Nannochloropsis Oculata. Keputusan ini juga penting untuk dijadikan sebagai salah satu perkiraan awal dalam menggunakan spesis mikroalga tempatan sebagai ejen penyerapan karbon dioksida dari industri.

Katakunci: karbon dioksida; mikroalga; fotosintesis; Tetraselmis sp.; Nannochloropsis Oculata

Abstract

Carbon fixation utilizing photosynthetic microalgae has been identified as an alternative means under various Carbon Capture Technologies. The appeal of using microalgae, apart from employing its natural process of photosynthesis, is it promotes towards an industrial symbiosis, where one's wastes becomes one's valuable input. This work makes a preliminary effort in assessing the abilities of some selected marine microalgae in fixing carbon. Two marine species; Tetraselmis sp. and Nannochloropsis Oculata were cultured using f/2 medium in multiple sizes of Erlenmeyer flasks, up to 2 liters, and up to 14 days. Carbonation was done by using both from air and pure CO_2. Temperature of culture was left to the room's average temperature of 26^{o}C. The skid's illumination was maintained at an average of 600 lux using fluorescent bulbs. pH, temperature, salinity and dissolved O_2 were recorded two times a day using EUTECH's PCD 650 multiparameter probe. Carbon fixation abilities among the species calculation was done empirically by comparing their gained dry weights between first and last days, taking into account the approximate biomass molecular weight in photosynthesis reaction. Results indicate Tetraselmis sp. is a better species to fix CO_2, in the range of 0.03 –

0.04 g/day, by the rate and percentage of CO_2 fixed and by its doubling time. Results shall be useful as an early consideration in utilizing local marine species in minimizing carbon dioxide from industries.

Keywords: carbon fixation; microalgae; photosynthesis; Tetraselmis sp; Nannochloropsis Oculata

INTRODUCTION

The task for a utility power company to continuously generate, transmit and distribute electricity cannot be done effortlessly without facing new challenges. Traditionally, parameters like reliability, efficiency, financial and safety have become ubiquitous considerations. Nowadays, with pressing concerns on environment and global warming, curbing CO_2 emissions has been a new agenda, internationally and nationally - upon our Prime Minister's pledge to reduce 40% carbon intensity, from 2005 level [1].

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In Malaysia, electricity and energy sector contributed 26% to the nation's CO_2 emission in 2006 [2], mainly due to use of fossil fuel. All this while, efforts in minimizing the statistics revolved by multiplying use of renewable and alternative fuels, and through increased practise in energy efficiency. A third means, dealing head-on with the carbon itself through capturing and fixation is considered a new and bold approach especially to local industries. Nevertheless, there are varieties of carbon capture technologies available with varying angles of approach and degrees of maturity [3]. Gasification for instance, falls under pre-combustion approaches, attempts to minimize the production of CO_2 by altering the process of combustion to a lower temperature through gasification to change matter's phase [4]. Oxy-combustion, on the other hand, separates O_2 from the air to be oxidized with fuel with combination of concentrated CO_2 from fuel gas, resulting in a high purity of CO_2 stream [5]. More mature approaches are technologies under post-combustion treatment, for example, chemical absorption using monoethanolamine (MEA), or its hybrid with other chemicals, or pressure swing adsorption, where most of installations were carried out with a high degree of CO2 purity captured [5].

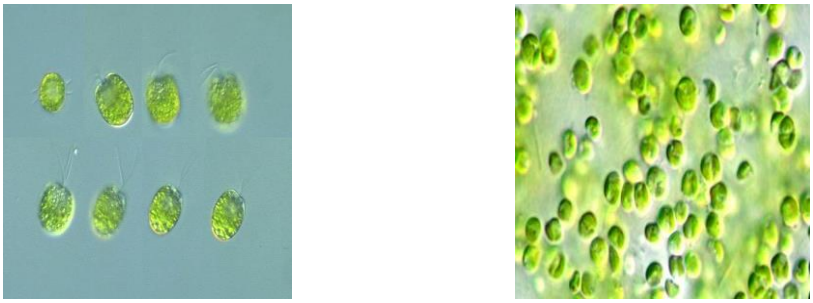
Notwithstanding to the above, biological fixation using microalgae is considered to be an alternative and more sustainable approach [6]. It has the potential to fulfil all the three pillars of sustainable development, namely the environment, social and economy. The approach utilizes the natural photosynthesis process converts (fixes) CO_2 (with the help of photon energy) into O_2 and organic matter in the form of C_x-H_y-O_z chain. This organic matter can in turn offers multitudes of value-added downstream products, which, with appropriate additional processes, can be converted into biomass, biofuel, nutrional diets, aquaculture food and fertilizers, to name a few [7].

In Malaysia, most of active activities attempting to harness the values from microalgae dealt with products and purposes for nutritional diets and aquaculture food [8] and to a certain extent, biodiesel production [9]. Note that these are considered downstream products, being the resultant of photosynthesis and carbon fixation process that takes place upstream. As there are almost scarce works done previously in this upstream process locally, this work sets a preliminary effort in studying microalgae species abilities to fix carbon, with the intention to bridge the gap between some knowledge readily acquired in the downstream products.

MATERIALS AND METHODS

Microalgae Species

Two marine microalgae species were selected – *Tetraselmis sp.* and *Nannochloropsis Oculata*. The former species was obtained from Universiti Selangor's (UNISEL) Faculty of Science and Biotechnology while the latter was obtained from Algaetech International Research and Development Centre. Marine type species were used in this work due to considerations of its resources readily available for coastal power plant's later application. Some particulars about these two species are tabulated in Figure 1 and Table 1 below [10], [11]:



(a) (b)

Figure 1. Images of (a) *Tetraselmis sp.* and (b) *Nannochloropsis Oculata*

Table 1. Characteristics of species understudy

	Tetraselmis sp.	***Nannochloropsis Oculata***
Description	has scaly and hair covered flagella	small, nonmotile spheres
Physical characteristics	ovoidal shape, 16-23μm diameter, actively in motion	2 μm diameter
Typical use	aquaculture diet	aquaculture diet

These two species are commonly cited for microalgae experiments and can be isolated locally [12], [13].

Culturing Conditions

The species were cultured using the standard f/2 media mixed with artificial sea water. The f/2 media consists of **Stock Solution** which consists of Sodium Nitrate ($NaNO_3$) and Sodium Dihydrogen Phosphate (NaH_2PO_4); **Trace Metals** which consists Disodium EDTA (Na_2EDTA) and Ferric Chloride Hexahydrate ($FeCl_3.6H_2O$); **Primary Trace Metals** which contain Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$), Zinc Sulfate Monohydrate ($ZnSO_4.7H_2O$), Cobalt (II) Chloride Hexahydrate ($CoCl_2.6H_2O$), Manganese Chloride Tetrahydrate ($MnCl_2.4H_2O$) and Sodium Molybdate Dehydrate, and **Vitamins** (Biotin, B12 and Thiamine).

The concentration of each of the chemicals in the f/2 media is as tabulated in Table 2 below:

Table 2. Concentrations of elements in f/2 media

Contents	Concentrations [g/L]
$NaNO_3$	75.0
NaH_2PO_4	5.0
Na_2EDTA	4.36
$FeCl_3.6H_2O$	3.15
Trace metal stock solution	1.0 mL/L
Vitamin stock solution	0.5 mL/L

The initial culture was started at 100-mL flask and was scaled up to 250 mL , 500 mL and 1-L flask, before final propagation was made to 2 L where the carbon fixation experiment be conducted. Each flask's duration was kept at about 14 days. The cultures' temperature was left to room's temperature at an average of 26^{o}C. The culturing flasks were kept on a boltless rack with three tiers and each tier was illuminated with 18W fluorescent bulb equipped with electronic ballast. The light's duration was automatically set to be switched on for only 12 hours with the help of a timer switch. Aeration was provided by an air pump (0.045 MPa compression, 150 l/min maximum capacity), distributed through plastic tubes attached with small valve at the end as to control the flow.

Carbon Fixation Experiment and Calculation

The survived cultures were then transferred to 2-L flask which is equipped with a 3-L pure CO_2 gas cylinder with an assembly of a regulator and a solenoid-operated valve. The pressure inside the cylinder was 65 barg and regulated to 2.4 barg at the outlet. The gas tube was further fitted with a one-way valve. Since there was no pH control in play, the discharge of the CO_2 gas was controlled, through a timer switch, to be available intermittently every 3 hours, and between 50 – 60 bubbles per minute. The weight of the cylinder was measured both before and after experiment, which was left to last up to 14 days. The experimental set-up is illustrated in Figure 2 below.

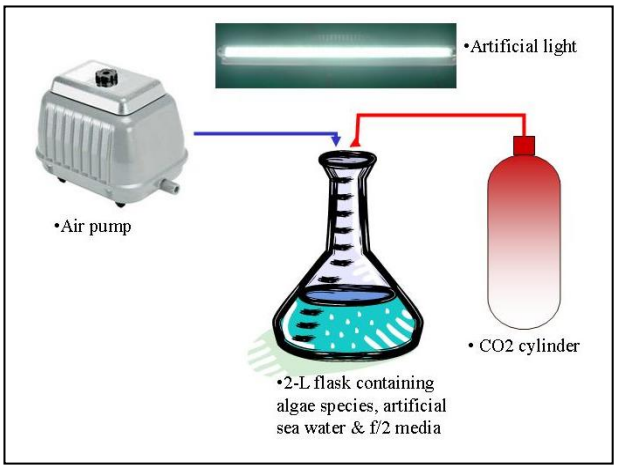


Figure 2. Simplified set-up for carbon fixation experiment

The experiment was run in duplicates. Similar set-up, but without pure CO_2 source were also done for aerated cultures in 500-mL and 2-L flasks.

30 mL from the culture was pipetted out from the flask and vacuum-filtered as to harvest its biomass. The filter paper used was of cellulose acetate of 0.2μm pore size. The sample was then dried-out at room temperature for a day before the dry weight was measured and recorded. The carbon fixation calculation was adopted by taking into considerations of the ratio between the moles of CO_2 and the moles of typical molecular formula of biomass [14]**.** Thus by taking into considerations of a balanced photosynthesis formula as in Equation 1 below :

$$\mathbf{4CO_2 + nutrient + H_2O + light \rightarrow 4CO_{0.48}H_{1.83}N_{0.11}P_{0.01} + 3(1/2)O_2} \qquad \textbf{(Eq. 1)}$$

the ratio of molecular weight of CO_2 and biomass is 1.882; i.e. about 1.8 g of CO_2 can be fixed by 1 g of microalgae. The growth rate, α is defined as the difference between the sample's maximum weight obtained throughout the experiment's and the sample's initial weight divided by the duration of experiment, as in Equation 2 below:

$$\alpha = \left(\frac{W_N - W_o}{t_N - t_o}\right) / 30mL \qquad \textbf{(Eq. 2)}$$

Where:

α	=	growth rate [g/mL.day]
W_N	=	maximum weight on Nth day [grams]
W_o	=	first day's weight [grams]
t_N	=	Nth day
t_o	=	first day

Though the anatomical construction of microalgae does not made-up entirely of carbon, any amount of carbon fixed during the Calvin cycle (light independent) will surely contribute to the mass of the whole cell. In the anticipation of small amount of biomass yield managed to be harvested from 2-L flask that fits for the minimum amount of the biomass elemental analysis, the above methodology by dry weight method is sufficient.

The percentage of CO_2 fixed, $\mathbf{\%CO_2}$, by each species is assessed as the following Equation 3:

$$\mathbf{\%CO_2 = \frac{\alpha * L * D}{Mc_N - Mc_o} * 100\%} \qquad \textbf{(Eq. 3)}$$

Where:

Mc_N	=	mass of CO_2 cylinder on the last day [grams]
Mco	=	mass of CO_2 cylinder on the last day [grams]
α	=	growth rate [g/mL.day]
L	=	Total culture volume [mL]
D	=	total number of days [days]

Doubling time of the microalgae cells tells how fast a species can double its initial population and was calculated from Monod's derived equation, Equation 4 [14]:

$$T_d = (t_2 \quad t_1) * \frac{\ln(2)}{\ln(N_t) - \ln(N_o)} \qquad \textbf{(Eq. 4)}$$

Where:

T_d	=	doubling time [day]
t_2	=	last day in the linear region of growth curve [day]
t_1	=	first day in the linear region of growth curve [day]
N_t	=	Number of cells on the last day
N_o	=	Number of cells on the first day

Parameter Measurements

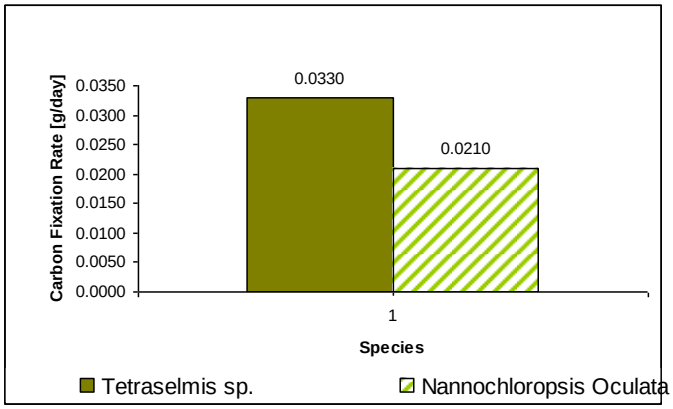
Table 3 below summarizes pertinent parameters' and its measurement tools throughout the experiment:

Table 3. Parameters and Measurement Tools

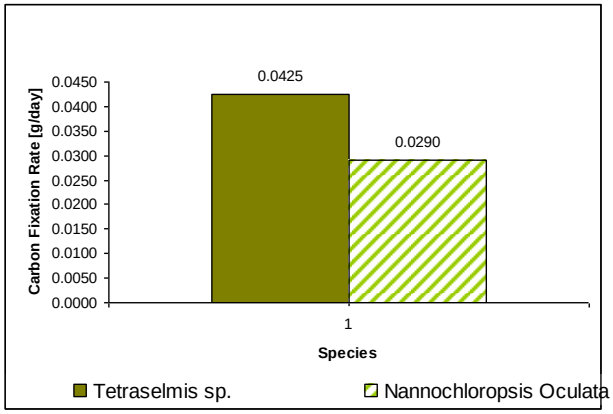
Parameters	Tools
pH	EUTECH's multiparameter portable probe model PCD650
Temperature [^oC]	
Salinity	
Light intensity [lux]	TES model 1335 portable lux meter
Optical Density [Abs]	HACH's DR4000U spectrophotometer
Cell Counts	OPTIKA's compound microscope DM series and Neubauer improved Haemacytometer set
Weight	▪ Biomass dry weight: A&D model GX400 analytical balance. 410 g maximum weight, three decimal places ▪ CO_2 cylinder weight: METTLER TOLEDO balance model IND425. 10 kg maximum weight, 3 decimal places

RESULTS AND DISCUSSIONS

From the flasks subjected to pure CO_2 gas, the amount of carbon fixed was superiorly performed by the microalgae species *Tetraselmis sp.* as evidenced in Figure 3 (a) and (b) below:



(a)



(b)

Figure 3. Comparison of the rate of carbon fixed for 2-L flasks charged with pure CO2 (a) First run (b) 2nd run

The amount carbon fixed for *Tetraselmis sp.* for the first run and second run were 0.033 g/day and 0.0425 g/day respectively, while for the *Nannochloropsis Oculata*, they were 0.0210 g/day and 0.0290 g/day respectively.

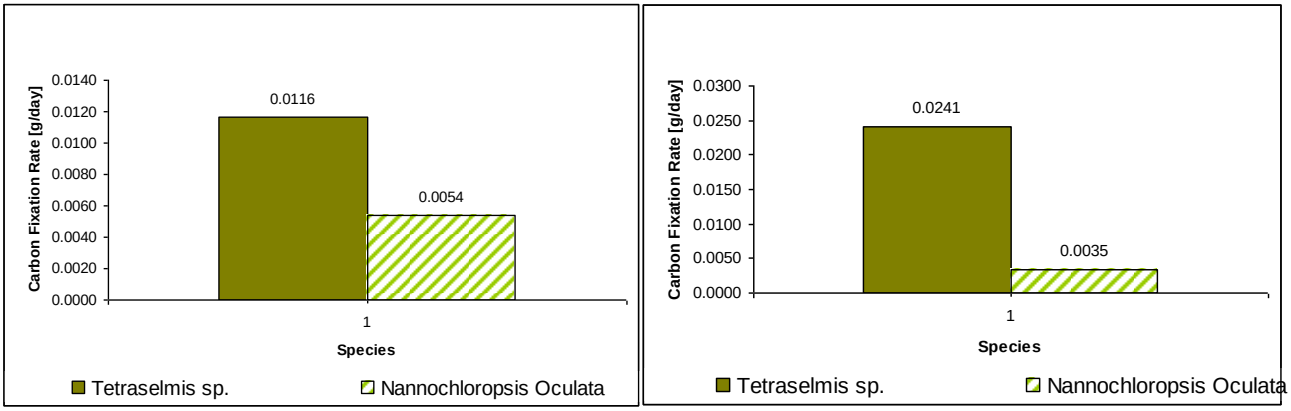
In actual case, some of CO_2 gases supplied are either remain dissolved in the culture solution or liberated to the ambient at the interface between the CO_2 gas bubble and the solution's surface in the flask. The percentage of CO_2 fixed as defined by Equation 3 above was used as a means to provide some normalized performance parameter of the carbon fixation abilities. The result is as tabulated in Table 4 below:

Table 4. Comparison of carbon fixation abilities in terms of percentage

	Tetraselmis sp.		***Nannochloropsis Oculata***	
	1st. Run	**2nd. Run**	**1st. Run**	**2nd. Run**
CO_2 fixation rate [g/day]	0.033	0.0425	0.0210	0.029
Total amount of CO_2 supplied [g]	70	80	85	120
% CO_2 fixed [%]	**0.717**	**0.531**	**0.295**	**0.314**

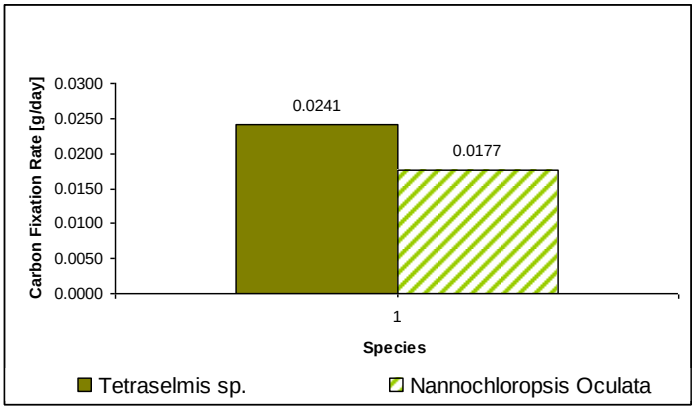
It is noted from Table 4 above that *Tetraselmis sp.* can fix the CO_2 better than Nannochloropsis Oculata by the highest percentage of CO_2 fixed in both runs.

Consistent results were also obtained from series of experiments where only diluted CO_2 from the air are supplied to the species, as further illustrated in Figure 4 (a), (b) and (c):



(a)

(b)



(c)

Figure 4. Comparison of the rate of carbon fixed for 500-mL flasks charged with air (a) First run (b) 2nd run, and for 2-L flask (c)

However, the fixation rate of *Tetraselmis sp.* established in this work is considered low when compared to other reports in the literature. When cultured in a proper column photobioreactor of 1,000 liter capacity outdoor, a biomass productivity of 0.42 g/(L.day) was cited [15], and after multiplying with 1.882 and 2 for the volume, as to equalize with the method employed in this work, gives a fixation rate of 1.6 g/day. Clearly, the volume and conducive environment play important roles for microalgae satisfactory growth.

Nevertheless, as the aim of this work is to provide a comparison of carbon fixation ability between these two species, the analysis continued to look at another growth parameter – doubling time. The doubling time will indicate the rate of growth of a species and in turn indicates the rate of CO_2 it consumed for the growth. Results as presented in Figure 5 and Table 5 below still support the superiority of *Tetrselmis sp*. as the better fixer of CO_2.

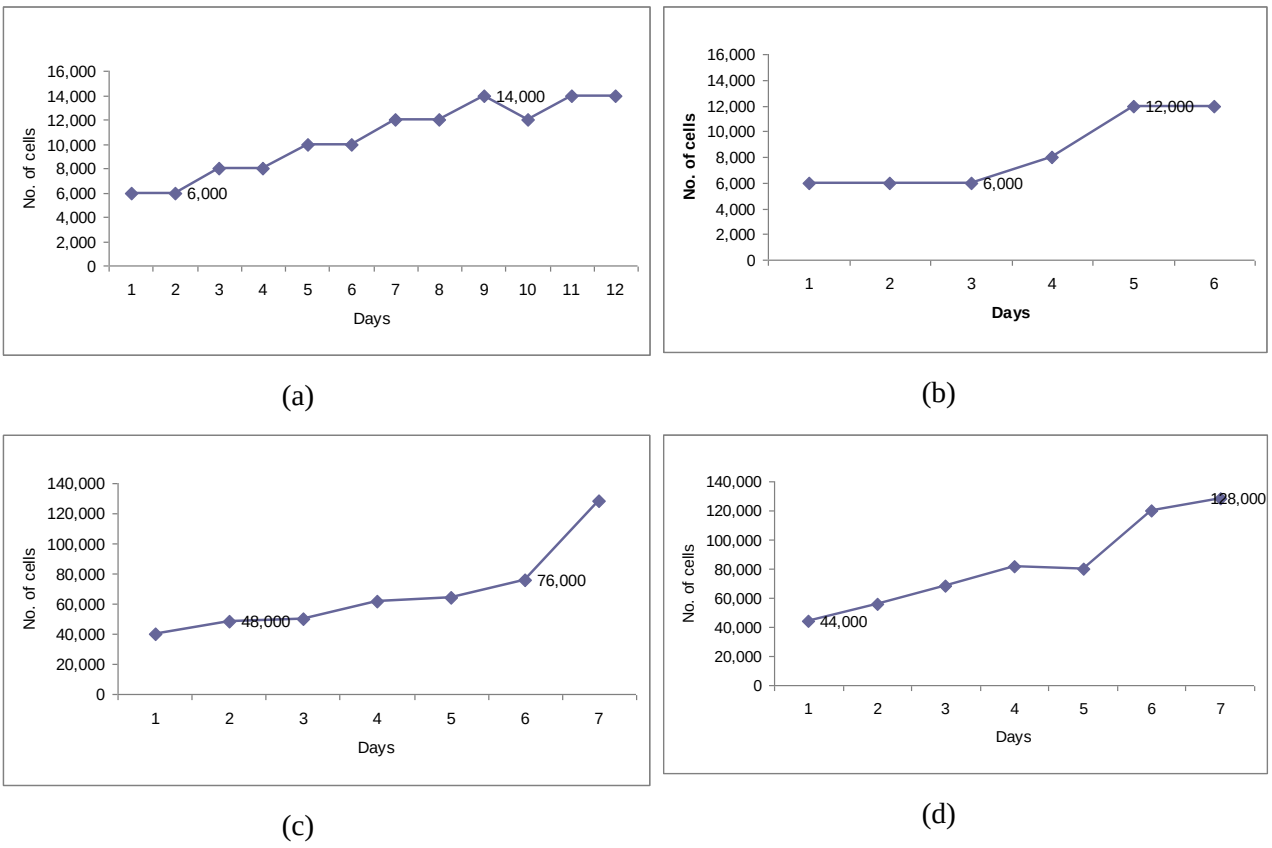


Figure 5. Number of cells profile for *Tetraselmis sp.* (a) 1st run (b) 2nd run and *Nannochloropsis Oculata* (c) 1st run (d) 2nd run

Table 5. Comparison of doubling time

	Tetraselmis sp.		*Nannochloropsis Oculata*	
	1st. Run	**2nd. Run**	**1st. Run**	**2nd. Run**
Doubling time [days]	5.7	2.0	6.8	3.7

CONCLUSIONS AND FURTHER WORKS

From basic carbonation experimentations described above, it is found out that *Tetraselmis sp.* is a better species to fix CO_2 than *Nannochloropsis Oculata*. The results are consistent whether pure or diluted CO_2 was applied, and whether small or large culture volumes were employed. The finding is further supported by the doubling time of the species which favors *Tetraselmis sp.*

The result form this work shall facilitate the task of identifying suitable species to fix CO_2 from power plants, and other industries. Downstream use of this species, for example aquaculture producers, could benefit from simultaneous upstream use of this species. This industrial symbiosis could enhance and realize sustainable development in its real sense by participation of key renowned industrial players.

One of key verifications from this work is that the carbon fixation rate by a microalgae species is proportional to its growth rate. Thus, further works could involve in improving the growth rate by culturing it in a suitable photobioreactor (PBR). Use of actual flue gas is also recommended as to assess any adverse effects on the species due to multiple gaseous entities. Optimization of PBR's operating parameter in obtaining highest CO_2 fixation is another area worth pursuing.

Indeed every creatures created have their own intended purpose. As stated in the Al-Quran's Surah Taha verse *53:*

"[It is He] who has made for you the earth as a bed [spread out] and inserted therein for you roadways and sent down from the sky, rain and produced thereby categories of various plants"

What men have to do are to capitalize the benefits and be His good servants.

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