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Original Article

Impact of hazardous components on CO_2 biofixation from synthetic flue gas using *Chlorella sp.* JPR-1 in a raceway pond photobioreactor

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Abstract

This work aimed to investigate the effects of hazardous compounds (NO and SO_2) in flue gas on *Chlorella* JPR-1 growth in a raceway pond photobioreactor at ambient temperature (30 °C) without pH control. *Chlorella* JPR-1 exhibited its tolerant to CO_2 content in the flue gas as high as 50%. The maximum carbon fixation rate (1.84 g CO_2/L.day) observed when the flue gas contained 10% CO_2. Although the specific growth rate was 25.88% lower than the control culture when cultivated with 50 ppm SO_2, *Chlorella* JPR-1 still could grow when cultured with 100 ppm SO_2 with slightly longer lag phase period. A growth rate reduction of 3.53% of the control culture was observed when *Chlorella* JPR-1 was cultured with flue gas containing 50 ppm NO. This study has shown that Chlorella JPR-1 has a high potential to be used for CO_2 fixation from flue gas.

Keywords: fixation, CO_2, SO_2, NO, microalgae, specific growth rate

1. Introduction

The increase of the atmospheric greenhouse gas (CO_2) concentration due to combustion is considered to be one of the main causes of global warming (e.g. Malhi *et al*., 2002). Within the last 10 years, the CO_2 gas emission in Indonesia has significantly increased from 100 to 380 Mt (Mega ton) CO_2 per year, and it is estimated that in 2020 the CO_2 emission will reach 684 Mt CO_2 (www.dirgantara-lapan.or.id). Therefore, the reduction of CO_2 emission becomes an inevitable issue. Many approaches have been adopted to limit and reduce CO_2 emissions. These include enhancing energy production efficiency, substituting carbon-rich fossil fuels such as coal and oil, with natural gas and other less carbon or carbon-free energy sources, and developing technologies to capture CO_2 in view of reutilization and/or sequestration. The CO_2 captures can be performed by physical and chemical absorption methods (Dugas and Rochelle, 2009), cryogenic and membrane separations, and biofixation (Wang *et al*., 2008).

The CO_2 biofixation approaches have drawn much attention because they lead to the production of biomass energy through photosynthesis by autotrophic organisms. Microalgae offer several advantages over the terrestrial energy crops, including higher photosynthetic efficiency, growth rates and biomass production (Gouveia and Oliveira, 2009). Microalgae can fix CO_2 from different sources, which can be categorized as CO_2 from the atmosphere, CO_2 from industrial and transportation exhaust gases, and fixed CO_2 in the form of soluble carbonates (Wang *et al*., 2008). One of the other advantages is that a number of microalgae strains have the ability to store large quantities of lipids, which varied from 1 to 26 % (Allard and Templier, 2000). This fact has attracted great attention from researchers around the world to investigate the potential of marine microalgae as the main oil source for biodiesel production (Gavrilescu and Chisti, 2005; Metzger and Largeau, 2005).

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Direct use of flue gas in microalgae culture may reduce the cost of pretreat-ment. However, the presence of algal growth inhibitors in the raw flue gas, such as SO_x and NO_x would be very critical as an addition to the high concentrations of CO_2 (Silva and Pirt, 1984). Yoshihara *et al*. (1996) found that at higher cell concentration in the culture, marine mi-croalga strain NOA-113 exhibited higher tolerance to NO. From their study, Yanagi *et al*. (1995) reported that *Chlorella* HA-1 was not inhibited by 50 ppm NO, but the strain could not grow with the presence of 50 ppm SO_2. Kurano *et al*. (1995) investigated the tolerances of three algal strains, C. *caldarium*, *Galdieria partita* and *Cyanidioschyzon melorae*, isolated from a hot water spring, to NO and SO_2, and reported that all of the strains showed good growth at 50 ppm NO aeration but only G. *partita* could proliferate under 50 ppm SO_2 aeration. Latter, Hauck *et al*. (1996) reported that microalga *Cyanidium caldarium* exhibited some growth in a sim-ulated flue gas containing about 200 ppm of SO_2 for the first 20 hrs, but the growth of *Chlorella vulgaris* was completely inhibited. This toxic effect may be due to either a lowering of the pH of the culture medium or direct inhibition by the SO_2 itself (Hauck *et al*., 1996). Considering that actual flue gas from industrial sources contains about 100-300 ppm NO_x and SO_x (Yoshihara *et al*., 1996), algal strains reported in the literature could not be used for direct CO_2 fixation from flue gas. Recently, Sung *et al*. (1998) isolated a fast growing alga in CO_2 enriched condition which could grow well in up to 20% CO_2. In the present study, the effects of SO_2 and NO on growth of *Chlorella* JPR-1 have been investigated to determine the feasibility for the direct CO_2 fixation process from actual flue gas.

2. Materials and Methods

2.1 Microorganism and growing medium

Chlorella JPR-1, a highly CO_2 tolerant and fast growing microalgae, isolated from marine water collected from Teluk Awur Marine Life Laboratory was used in this study. The strain was kept on Detmer agar plate, and cultured on a modified M4N medium. The composition of the medium and the preparation procedure was similar to that used by Sung *et al*. (1998). Although Hirata *et al*. (1996) reported that *C. vulgaris* is best grown at 30°C and pH 5.5 to 6.0, the initial pH of the medium used in this work was 5.5, with no further adjustment was made. This is because it is possible to cultivate algae using wastewater nutrients and the CO_2 present in the flue gases without buffering or pH control (Yun *et al*., 1996), and the fact that buffering is not a practical option for pH control in large cultivation systems.

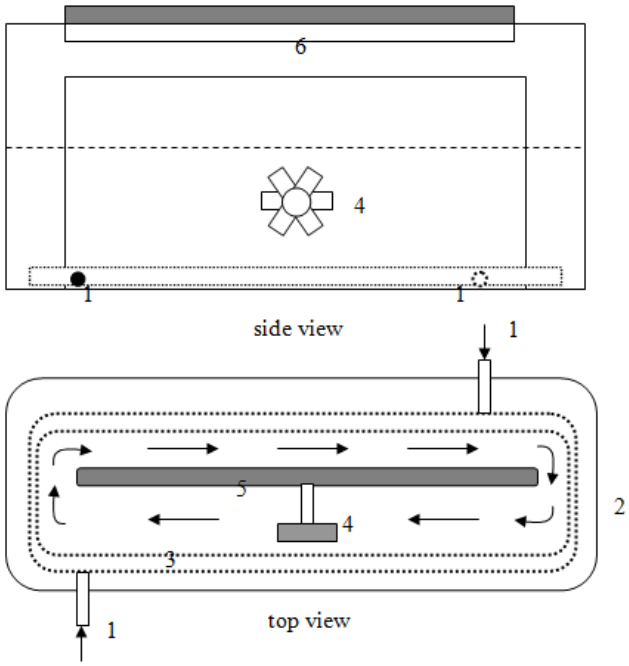
2.2 Culture experiments

The microalgae culture experiments were conducted to determine the tolerances of *Chlorella* JPR-1 to CO_2, SO_2 and NO in various gas mixtures using a 0.4 m^3 raceway pond bioreactor (Figure 1). Typical flue gas emitted from a boiler using low-sulfur heavy oil as fuel contains 10-15% CO_2 and 100-300 ppm NO_x and SO_x (Sung *et al*., 1998). Therefore, several synthetic gas mixtures containing various concentrations of CO_2, SO_2, and NO, were used for the experiments to evaluate the effects of the inhibitory compounds on growth of *Chlorella* JPR-1. The growth rates were monitored with different gas mixtures which contained different concentrations of CO_2, SO_2 and NO. The seed culture was centrifuged and washed before inoculation. Samples were removed intermittently from the vessels to determine the cell concentration for further algal growth calculation. The temperature of the culture media was maintained at local ambient temperature (30°C). The gas flow rate was measured with a flow meter (Dewyer, U.S.A.) and fixed to 0.5 volume gas/volume liquid/min. Air-grown cells were inoculated into the medium to obtain the initial cell concentration specified in the experimental results.

2.3 Microalgal carbon content, cell counting, and dry weight analysis

Biomass carbon content was determined using a Perkin-Elmer 2400 CHNS (carbon, hydrogen, nitrogen, and sulfur) element analyzer calibrated to the 100% value using a certified cystine standard (Perkin-Elmer, U.S.A.) (de Morais and Costa, 2007).

A direct microscopic count (cells mL^{-1}) was performed on a sample of microalgal suspension using a Bürker-Türk



1. flue gas inlet 2. bioreactor 3. gas sparger
4. electric driven paddle 5. baffle 6. lamps

Figure 1. Raceway pond bioreactor used for *Chlorella sp.* JPR-1 culture.

counting chamber (Brand, Germany) and a Nikon Eclipse 80i microscope (Nikon Corporation, Japan). Optical density of the microalgal suspension was measured by absorbance at 550 nm (*A*550) in an HP 8452 UV/Visible Spectrophotometer. The spectrophotometer was blanked with each medium. At the end of the experiments, 100 mL of the culture broth was removed from every flask, respectively. The samples were filtered through glass microfibre discs (Sartorius stedim biotech, Göttingen, Germany) and the dry weights of pellets were measured after drying at 105°C for 2 hrs.

2.4 Microalgae growth rate

The algal growth rate was determined in the linear growth phase because most of the algal growth occurred during this phase (Yun *et al*., 1997). The specific growth rate, *m*, (1/day), which is a measure of the number of generations (the number of doublings) that occur per unit of time in an exponentially growing culture of the *Chlorella* JPR-1 was then evaluated using the following equation (Lee and Shen, 2004):

$$\mu = \frac{\ln(x_2) - \ln(x_1)}{t_2 - t_1} \quad (1)$$

where x_2 and x_1 are biomass concentrations (g/L) after t_2 and t_1 day cultivation, respectively.

2.5 Carbon dioxide fixation rate

The CO_2 fixation rate, R_C, (g/L.day), was calculated from an elemental analysis of the algal biomass, as shown in Equation 2 (Zheng *et al*., 2011):

$$R_c = C_c \times \left(\frac{x_2 - x_1}{t_2 - t_1} \right) \times \frac{MCO_2}{MC} \quad (2)$$

Where C_c is the carbon content of the biomass and MCO_2 and MC are the molecular weight of CO_2 and carbon, respectively.

3. Results and Discussion

The effects of CO_2 concentrations on growth of *Chlorella* JPR-1 are illustrated in Figure 2 and 3. *Chlorella* JPR-1 was cultured under continuous illumination with different concentrations of CO_2 in the flue gas ranging from 10 to 50% (v/v). The curves of microalgae cells concentration in the cultivation pond presented in Figure 2 show that the concentration of CO_2 in the flue gas affects the growth rate of *Chlorella* JPR-1. This finding is similar to the growth of C. *vulgaris* reported by Yun *et al*. (1996). The fastest growth was achieved in the culture medium with 10% CO_2 concentration in the flue gas supply. However, the cells still can survive till CO_2 concentration in the flue gas up to 50%. The maximum biomass concentration reached by introduction of 10% CO_2 was 5.8 g/L, and it decreased significantly by increasing the CO_2 concentration. This result is in accordance with Sung *et al*. (1998) who grew *Chlorella sp.* JKR-1 in a culture with different concentrations of CO_2 ranging from air-level to 70% and obtained optimum growth rate at 10% CO_2. They also reported that *Chlorella sp.* JKR-1 maintained high growth rates and cell concentrations at high CO_2 concentrations of 30 % and 50 %, but the growth rate became remarkably low at 70% CO_2. These results suggest that *Chlorella* JPR-1 has an excellent tolerance to high CO_2 concentration in the flue gas, and therefore it is recommended as suitable species for CO_2 biofixation and producing high density biomass.

From Figure 2, the specific growth rate (m) of *Chlorella* JPR-1 at each value of CO_2 content can be obtained and then be depicted in Figure 3, which shows that the specific growth rate of *Chlorella* JPR-1 decreases with

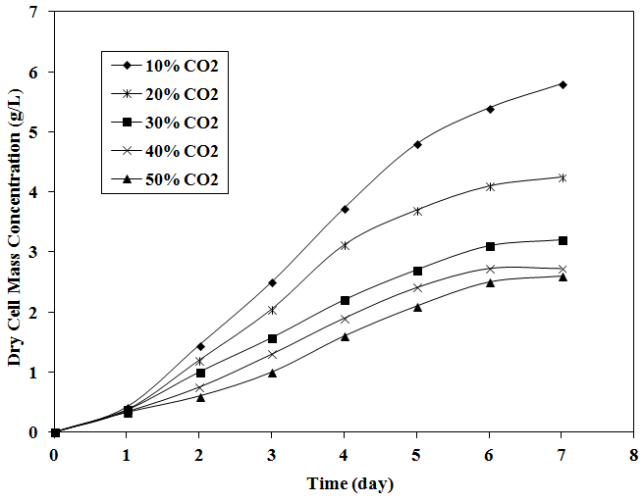


Figure 2. Effect of CO_2 concentrations on *Chlorella sp.* JPR-1 culture.

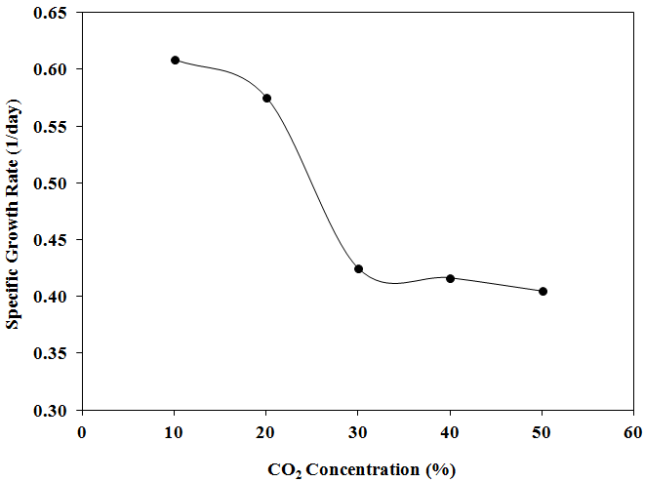


Figure 3. Effect of CO_2 concentrations on *Chlorella sp.* JPR-1 specific growth rate.

increasing CO_2 concentration in the flue gas feed. The highest specific growth rate was found to be 0.61 1/day when the CO_2 content in the gas feed was 10%. This specific growth rate is almost two times of that of C. *vulgaris* and C. *kessleri* cultured by de Morais and Costa (2007) in a 4 L vertical tubular photobioreactor (VTP) using flue gas containing 6, 12 and 18% CO_2, which were 0.31 and 0.38 1/day, respectively. However, the specific growth rate found in this work is still lower than that of *Chlorella* JKR-1 cultured with the same CO_2 supply reported by Sung *et al*. (1998), which was 0.67 g/L/day. In addition, this specific growth rate value is also below the specific growth rate of *Chlorella* sp cultured under controlled pH at 5.5 by Devgoswami *et al*. (2011) using CO_2 supply of 4.758 ppm, which was 0.70 g/L/day. The specific growth rate decreased almost linear when the CO_2 concentration in the feed increased from 10 to 30%. A further increase in CO_2 concentration gradually reduces the specific growth rate of *Chlorella* JPR-1.

The carbon contents and carbon fixation rates of *Chlorella* JPR-1 cultured using flue gases with various CO_2 concentrations are tabulated in Table 1. Table 1 shows that the carbon content of the algal cells closely ranged from 0.45 to 0.46 g C/g biomass for five different CO_2 concentrations and indicated that CO_2 concentration does not affect the cell carbon content. Similar results were reported by Zheng *et al*. (2011) who studied the cultivation of *T. subcordiformis* using CO_2 concentration ranged from 1.63-18.37 % and found that the cell carbon contents were between 0.44-0.47 g C/g biomass. In contrast to carbon content, the CO_2 concentration strongly affected the carbon fixation rate. The carbon fixation rate decreased from 1.84 to 0.91 g CO_2/L.day as the CO_2 concentration in the flue gas increased from 10 to 30%. As expected, further increase in CO_2 concentration was found to gradually reduce the carbon fixation rate due to low specific growth rate. Yun *et al*. (1997) observed that C. *vulgaris* previously adapted to 5% v/v CO_2 and cultivated in wastewater without pH control under 15% v/v CO_2, performed CO_2 fixation rate of 26.0 g CO_2/m^3.h or equal to 0.62 g CO_2/L.day. Therefore for high efficiency biofixation process, the CO_2 content in the flue gas must be less than 30%, where the microalgae can grow at reasonable speed.

Since CO_2 is the main component in the flue gas, it will almost remain constant during the course of the experiment. In fact, sulfur oxides are also present in the flue gas, mainly as sulfur dioxide (SO_2). In this experiment, the SO_2 variation was 50-150 ppm, while the concentration of CO_2 gas was kept constant at 20%. The effects of SO_2 concentrations on growth of *Chlorella* JPR-1 are depicted in Figure 4. As SO_2 concentration in the synthetic flue gas increased, the specific growth rate and the maximum cell concentration of *Chlorella* JPR-1 decreased significantly. The specific growth rate of the culture aerated with the synthetic flue gas containing 100 ppm SO_2 was 0.5756 1/day, which is only 38.66% of the control culture. The maximum cell concentration of the culture with 100 ppm SO_2 was 3.15 g/L which is 74.12% of the control culture. Growth of *Chlorella* JPR-1 was totally inhibited with 150 ppm SO_2 where reduction of cells concentration found after three days of cultivation. Without investigating the effect of SO_2 addition at lower concentrations, Westerhoff *et al*. (2010) reported that *Scenedesmus*, *Chlorella* and a mixture of these two cultures died almost immediately upon gaseous addition of 313 ppm SO_2 in 20% CO_2 (balance of gas blend was N_2), which was simultaneously followed by a drop in pH from 6.2 to 2.6. They also mentioned that since SO_2 is highly soluble in water, it readily partitions from the gas phase and decreases the solution pH.

The carbon content and carbon fixation rate of *Chlorella* JPR-1 cultured using flue gases containing 20% CO_2 and various concentrations of SO_2 are presented in Table 2. It is shown that the carbon content of the algal cells

Table 1. Carbon fixation rate of *Chlorella sp.* JPR-1 culture.

CO_2 concentration (%)	Carbon Content (g C/g biomass)	CO_2 fixation rate (g CO_2/L.day)
10	0.46	1.84
20	0.46	1.39
30	0.46	0.91
40	0.46	0.80
50	0.45	0.72

Table 2. Effect of SO_2 content in flue gas with 20% CO_2 on carbon fixation rate of *Chlorella sp.* JPR-1 culture.

SO_2 concentration (ppm)	Carbon Content (g C/g biomass)	CO_2 fixation rate (g CO_2/L.day)
0	0.46	1.39
50	0.46	0.98
100	0.45	0.56
150	0.44	0.26

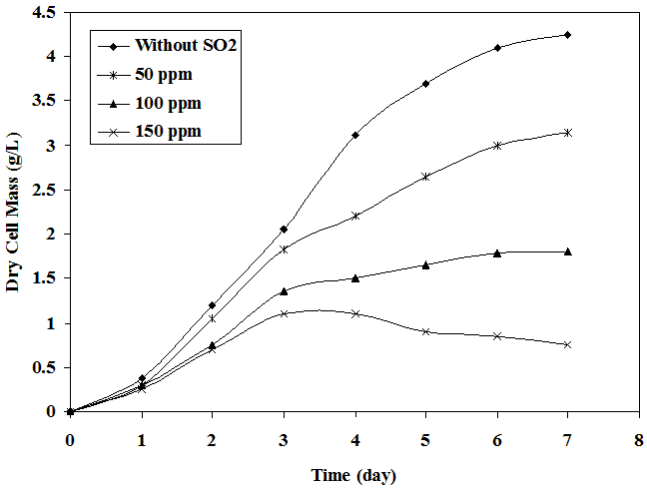


Figure 4. Effect of SO_2 concentrations on *Chlorella sp.* JPR-1 growth.

of four different SO_2 concentrations closely ranged from 0.44 to 0.46 g C/g biomass. The results indicated that SO_2 concentration almost has no effect on the cell carbon content. However, the SO_2 concentration strongly affected the carbon fixation rate. The carbon fixation rate decreased from 1.39 to 0.98 g CO_2/L.day as the SO_2 concentration in the flue gas increased from 0 to 50 ppm. As expected, further increase in SO_2 concentration was found to sharply reduce the carbon fixation rate to as low as 0.26 g CO_2/L.day when the SO_2 concentration in the flue gas was 150 ppm due to low specific growth rate.

Considering that growth of most algal strains reported in the literature was completely inhibited, when aerated with CO_2-air mixture containing SO_2 concentrations higher than 50 ppm (Hauck *et al*., 1996), *Chlorella* JPR-1 showed a remarkable excellent tolerance to SO_2. However, the reason for the high tolerance of *Chlorella* JPR-1 to SO_2 is not clear and due to its complexity, it is beyond the scope of this work.

Tolerances of *Chlorella* JPR-1 to NO are shown in Figure 5. When aerated with a gas mixture composed of 20% CO_2, 5% O_2, 50 ppm NO, and balance N_2, the growth of *Chlorella* JPR-1 was only slightly inhibited. The maximum cell concentration and the linear growth rate were almost the same as that obtained in the *Chlorella* JPR-1 cultured by 20% CO_2-air mixture. However, growth of *Chlorella* JPR-1 was completely retarded when aerated with the 20% CO_2 gas mixture containing 150 ppm NO. Westerhoff *et al.* (2010) cultured *Scenedesmus*, *Chlorella* and a mixture of the two with CO_2 supply from flue gas containing 325 ppm NO and observed a slightly longer lag phase than those cultured without nitrogen oxides, but all grew well and had similar growth rates. The presence of NO may lead to the formation of some nitrite (NO_2^-) in solution. At concentration of 0-50 mM, nitrite exhibited no inhibitory effect on the growth of algal cultures, but 250 and 500 mM nitrite caused algae to die. Fortunately, NO is only sparingly soluble in water and, thus, very high nitrite concentrations are unlikely to occur.

Table 3 shows the carbon content and carbon fixation rate of *Chlorella* JPR-1 cultured using flue gases containing 20% CO_2 and various concentrations of NO. It is shown that the carbon content of the algal cells of four different NO concentrations were ranged between 0.45-0.46 g C/g biomass. Similar to the CO_2 concentrations, the NO concentrations also do not affect the cell carbon content. Fortunately, the NO concentration only mildly affected the carbon fixation rate. The carbon fixation rate decreased from 1.39 to 0.97 g CO_2/L.day as the NO concentration in the flue gas increased from 0 to 100 ppm. However, further increase in NO concentration was found to sharply reduce the carbon fixation rate due to low specific growth rate.

Although *Chlorella* JPR-1 showed the limited tolerance to the high concentrations of NO, it could be still applicable for direct CO_2 fixation from LNG flue gas because the flue gas contains low concentrations of NO_x, less than 100 ppm, and almost no SO_x (Hauck *et al*., 1996). Increasing the inoculating cell con-centration was likely to be helpful to enhance the tolerances of *Chlorella* JPR-1 to the toxic compounds.

4. Conclusions

The results of this study show that *Chlorella* JPR-1 exhibited a tolerance to CO_2 content in the flue gas as high as 50%. The *Chlorella* JPR-1 growth was found to be decelerated by the presence of both SO_2 and NO. When *Chlorella* JPR-1 was cultured with the simulated flue gas containing 50 ppm SO_2, the specific growth rate was 3.15 1/day, which is about 25.88% lower than that of the control culture aerated with the gas mixture containing no toxic compounds, SO_2 and NO. The *Chlorella* JPR-1 could grow even with the synthetic flue gas containing 100 ppm SO_2 and its specific growth rate was 1.8 1/day. A milder effect was observed with a specific growth rate reduction of only 3.53% of the control culture when *Chlorella* JPR-1 was cultured with the simulated gas containing 50 ppm NO. The period for lag phase was slightly increased with increasing of SO_2 and NO concentration resulting in the decrease of the specific growth rate and the maximum cell concentration. These results indicated that *Chlorella* JPR-1 may be applied for

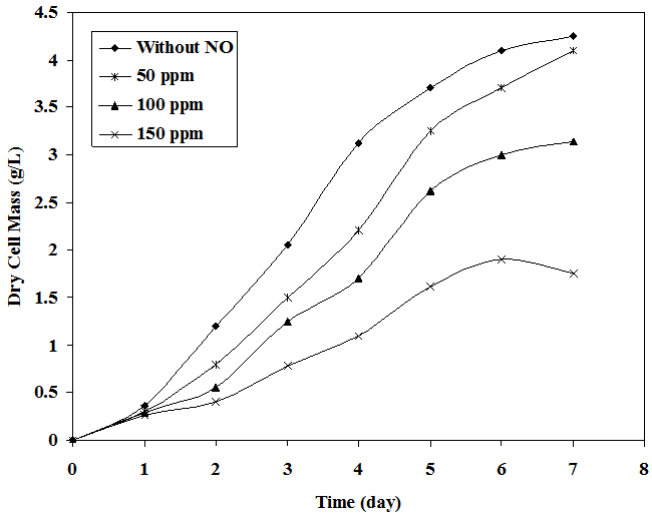


Figure 5. Effect of NO concentrations on *Chlorella sp.* JPR-1 growth.

Table 3. Effect of NO content in flue gas with 20% CO_2 on carbon fixation rate of *Chlorella sp.* JPR-1 culture.

NO concentration (ppm)	Carbon Content (g C/g biomass)	CO_2 fixation rate (g CO_2/L.day)
0	0.46	1.39
50	0.46	1.23
100	0.46	0.97
150	0.45	0.56

direct CO_2 fixation from actual flue gas at ambient temperature.

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