Impact of hazardous components on the CO$_2$ biofixation from synthetic flue gas using *Chlorella* sp. JPR-1 in a raceway pond photobioreactor

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Abstract

This work was 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 high potential to be used for CO$_2$ fixation from flue gas.

Key words: fixation, CO$_2$, SO$_2$, NO, microalgae, specific growth rate.

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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 (Malhi et al., 2002). Within last 10 years, the CO$_2$ gas emission in Indonesia has significantly increased from 100 to 380 mega ton CO$_2$ per year, and it is estimated that in 2020 the CO$_2$ emission will reach 684 mega ton 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).

Direct use of flue gas in microalgae culture may reduce the cost of pretreatment. However, the presence of algal growth inhibitors in the raw flue gas, such as SO\textsubscript{x} and NO\textsubscript{x} would be very critical as an addition to the high concentrations of CO\textsubscript{2} (Silva and Pirt, 1984). Yoshihara \textit{et al.} (1996) found that at higher cell concentration in the culture, marine microalga strain NOA-113 exhibited higher tolerance to NO. From their study, Yanagi \textit{et al.} (1995) reported that \textit{Chlorella} HA-1 was not inhibited by 50 ppm NO, but the strain could not grow with the presence of 50 ppm SO\textsubscript{2}. Kurano \textit{et al.} (1995) investigated the tolerances of three algal strains, \textit{C. caldarium}, \textit{Galdieria partita} and \textit{Cyanidioschyzon melora}, isolated from a hot water spring, to NO and SO\textsubscript{2}, and reported that all of the strains showed good growth at 50 ppm NO aeration but only \textit{G. partita} could proliferate under 50 ppm SO\textsubscript{2} aeration. Latter, Hauck \textit{et al.} (1996) reported that microalga \textit{Cyanidium caldarium} exhibited some growth in a simulated flue gas containing about 200 ppm of SO\textsubscript{2} for the first 20 h, but the growth of \textit{Chlorella vulgaris} was completely inhibited. This toxic effect may be due to either lowering of the pH of the culture medium or direct inhibition by the SO\textsubscript{2} itself (Hauck \textit{et al.}, 1996). Considering that actual flue gas from industrial sources contains about 100-300 ppm NO\textsubscript{x} and SO\textsubscript{x} (Yoshihara \textit{et al.}, 1996), algal strains reported in the literature could not be used for direct CO\textsubscript{2} fixation from flue gas. Recently, (Sung \textit{et al.}, 1998) isolated a fast growing alga in CO\textsubscript{2} enriched condition which could grow well up to 20% CO\textsubscript{2}. In the present study, the effects of SO\textsubscript{2} and NO on growth of \textit{Chlorella} JPR-1 have been investigated to determine the feasibility for the direct CO\textsubscript{2} fixation process from actual flue gas.
2. Materials and Methods

2.1 Microorganism and growing medium

Chlorella JPR-1, a highly CO\textsubscript{2} tolerant and fast growing microalga, 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 \textit{et al.} (1998). Although Hirata \textit{et al.} (1996) reported that \textit{C. vulgaris} is best grown at 30\textdegree 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\textsubscript{2} present in the flue gases without buffering or pH control (Yun \textit{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 microalga culture experiments were conducted to determine the tolerances of \textit{Chlorella} JPR-1 to CO\textsubscript{2}, SO\textsubscript{2} and NO in various gas mixtures using a 0.4 m\textsuperscript{3} raceway pond bioreactor (Figure 1). Typical flue gas emitted from a boiler using low-sulfur heavy oil as fuel contains 10-15\% CO\textsubscript{2} and 100-300 ppm NO\textsubscript{x} and SO\textsubscript{x} Sung \textit{et al.} (1998). Therefore, several synthetic gas mixtures containing various concentrations of CO\textsubscript{2}, SO\textsubscript{2} and NO, were used for the experiments to evaluate the effects of the inhibitory compounds on growth of \textit{Chlorella} JPR-1. The growth rates were monitored with different gas mixtures which contained different concentrations of CO\textsubscript{2}, SO\textsubscript{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, USA) 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, USA) (de Morais and Costa, 2007).

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

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 (\(\mu\), (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 \textit{Chlorella JPR-1} under studied was then evaluated using the following equation (Lee and Shen, 2004):

\[
\mu = \frac{\ln(x_f) - \ln(x_i)}{t_f - t_i}
\]
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 \((R_c)\)

The CO\(_2\) fixation rate \((R_c, \text{ (g/L.day)})\) was calculated from an elemental analysis of the algal biomass, as shown in Equation 2 (Zheng et al., 2011):

\[
R_c = C_c \times \left(\frac{x_2 - x_1}{t_2 - t_1}\right) \times \frac{MCO_2}{MC} 
\]

(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 in Figure 2 show that concentration of CO\(_2\) in the flue gas affects the growth rate of Chlorella JPR-1. This finding is similar to the growth of \( \text{C. vulgaris} \) 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 \( \text{sp.} \) 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 \( \text{sp.} \) 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% CO₂. These results suggest that *Chlorella JPR-1* has an excellent tolerant to high CO₂ concentration in the flue gas, and therefore it is recommended as suitable species for CO₂ biofixation and producing high density biomass.

From Figure 2, the specific growth rate ($\mu$) of *Chlorella JPR-1* at each value of CO₂ content can be obtained and then be depicted in Figure 3. Figure 3 shows that the specific growth rate of *Chlorella JPR-1* decreases by increasing CO₂ concentration in the flue gas feed. The highest specific growth rate was found to be 0.61 (1/day) when the CO₂ 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% CO₂, 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 CO₂ 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 CO₂ supply of 4758 ppm, which was 0.70 (g/L/day). The specific growth rate decreased almost linear when the CO₂ concentration in the feed increased from 10 to 30%. Further increased in CO₂ concentration gradually reduces the specific growth rate of *Chlorella JPR-1*.

The carbon content and carbon fixation rate of *Chlorella JPR-1* cultured using flue gases with various CO₂ concentration 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 CO₂ concentrations and indicated that CO₂ 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 microalgaes 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 3 days 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 p$H$ 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 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% CO₂-air mixture. However, growth of Chlorella JPR-1 was completely retarded when aerated with the 20% CO₂ gas mixture containing 150 ppm NO. Westerhoff et al (2010) cultured Scenedesmus, Chlorella and a mixture of the two with CO₂ 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 (NO₂⁻) 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% CO₂ 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 CO₂ concentrations, the NO concentrations also do not affect the cells 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 CO₂/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 CO₂ fixation from LNG flue gas because the flue gas contains low concentrations of NOₓ, less than 100 ppm, and almost no SOₓ (Hauck et al., 1996). Increasing the inoculating cell concentration was
likely to be helpful to enhance the tolerances of Chlorella JPR-1 to the toxic compounds.

Conclusions

The results of this study show that Chlorella JPR-1 exhibited its tolerant 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). Milder effect was observed with 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 direct CO$_2$ fixation from actual flue gas at ambient temperature.

Acknowledgements

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Table 3. Effect of NO content in flue gas with 20% CO₂ on carbon fixation rate of *Chlorella sp.* JPR-1 culture.

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

<table>
<thead>
<tr>
<th>CO₂ concentration (%)</th>
<th>Carbon Content (g C/g biomass)</th>
<th>CO₂ fixation rate (g CO₂/L·day)</th>
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<tr>
<td>10</td>
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<td>1.84</td>
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<td>20</td>
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<td>30</td>
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<td>50</td>
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</table>
Table 2. Effect of SO₂ content in flue gas with 20% CO₂ on carbon fixation rate of *Chlorella sp.* JPR-1 culture.

<table>
<thead>
<tr>
<th>SO₂ concentration (ppm)</th>
<th>Carbon Content (g C/g biomass)</th>
<th>CO₂ fixation rate (g CO₂/L.day)</th>
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<tbody>
<tr>
<td>0</td>
<td>0.46</td>
<td>1.39</td>
</tr>
<tr>
<td>50</td>
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<td>150</td>
<td>0.44</td>
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Table 3. Effect of NO content in flue gas with 20% CO₂ on carbon fixation rate of *Chlorella sp.* JPR-1 culture.

<table>
<thead>
<tr>
<th>NO concentration (ppm)</th>
<th>Carbon Content (g C/g biomass)</th>
<th>CO₂ fixation rate (g CO₂/L.day)</th>
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<td>0</td>
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<tr>
<td>150</td>
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<td>0.56</td>
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</table>
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Figure 1. Raceway pond bioreactor used for Chlorella sp. JPR-1 culture.

Figure 2. Effect of CO₂ concentrations on Chlorella sp. JPR-1 culture.

Figure 3. Effect of CO₂ concentrations on Chlorella sp. JPR-1 specific growth rate.

Figure 4. Effect of SO₂ concentrations on Chlorella sp. JPR-1 growth.

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

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

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