Effect of membrane on carbonation and carbon dioxide uptake of *Chlorella* sp.

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Abstract Recent studies showed that as low as 5% CO_2 increased microalgae growth. However, common bioreactor operation resulted in low carbonation due to poor CO_2 mass transfer and this inhibited CO_2 uptake of microalgae. Although bubbling increases mass transfer of CO_2-O_2 exchange, preserving high dissolved CO_2 remains the most challenging of microalgae cultivation in bioreactor. In order to increase high dissolved CO_2 and CO_2-O_2 exchange, this study employed two types of membrane; hollow-fibre membrane for carbonation and hydrophobic membrane for deoxygenation. It was found that membrane increased carbonation from 20 % to 75 % when operated at control CO_2 concentration. The hollow-fibre membrane capable of creating as small as 2 mm bubble which effective for high carbonation. At the same time, it increased CO_2 uptake up to 85% in bioreactor. The hydrophobic membrane removed 43% O_2 from the bioreactor. Both membranes increased mass transfer of CO_2-O_2 exchange in bioreactor which stimulated microalgae growth.

Keywords: Membrane; microalgae; carbonation; deoxygenation; *Chlorella* sp.

1 Introduction

Carbonation of microalgae in bioreactor has reported since 1990s [1,2]. However, study of hollow fibre membrane rarely discussed although it increases CO_2 uptake [3-5]. Microalgae has potential to produce sufficient biomass if cultivated under CO_2 with a mean to remove photosynthetic O_2 [6]. Microalgae growth associated with CO_2 uptake, which increased under carbonation.

In general, bubbling provides aeration to microalgae and this resulted in low CO_2 mass transfer because of bubble formation [7-10]. This causes most CO_2 released to the bioreactor headspace and lessen its potential for microalgae growth. Microalgae lack in vascular tissue and an internal means to capture CO_2 outside watery media [11,12]. Thus, carbonation by membrane ensures CO_2 entrapped in the watery media. In addition, membrane capable to scatter CO_2 in the media and this increases potential use of CO_2 [13-15].

Another challenge of microalgae cultivation in bioreactor is high O_2 concentration. Microalgae produced O_2 as a side product of photosynthesis, which occurring simultaneously with CO_2 uptake. Oxygen reduces efficiency of continuous photosynthesis and thus interfere the CO_2 uptake in bioreactor [16,17]. Common cultivation uses aeration to remove O_2. However, aeration removes CO_2 and decreases carbonation efficiency due to unstable bubbling created during aeration. An optimum CO_2 – O_2 exchange required at least two membranes each acts as CO_2 carrier and O_2 removal. Therefore, this study discussed hydrophobic hollow fibre membranes as O_2 removal in bioreactor and its effect on microalgae growth.

Carbonation affects media pH and microalgae tolerance on pH level varies among species [18,19]. This study has employed *Chlorella* sp. which known has high tolerance on pH level. The tolerance of *Chlorella* sp. was emphasize further in this study.

2 Materials and Methods

2.1 *Chlorella* sp and cultural condition

This study employed *Chlorella* sp from Borneo (found at 6 °N and 116 °E). In this study, the *Chlorella* was cultivated under lessened carbon source in an adjusted standard Jaworski medium. Thus, the medium solution has no contents of $NaHCO_3$, Cyanocobalamin, Thiamine HCl and Biotin. About 0.0401 kg/m^3 (40 ppm) of *Chlorella* presents in the medium and used as a basis analysis on the *Chlorella* growth. The CO_2 gas enters Membrane 1 at controlled flowrates and microalgal media enter Membrane 2 for O_2 removal.

2.2 Membrane bioreactor set up

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So far, no membrane can add and remove two different gases simultaneously. Thus, this study employed a hydrophobic-membrane with a model number UFS220 for carbonation and labelled as Membrane 1. The other membrane, 2 X 6 Radial Flow type superphobic membrane supplied by Liqui-Cel for O_2 removal and labelled as Membrane 2. Table 1 shows feature of Membrane 1 and Membrane 2 whereas others feature was published in previous work [10].

Figure 1 shows membranes position in the experimental setup. Membrane 1 connected to bioreactor 1 (BR1) and Membrane 2 connected to both bioreactors for O_2 removal. Two fluorescence white cool lamps lit up the bioreactors at 296 µE/m^{2}s and a Lux meter with a model number LX-101 used to measure the light intensity.

Table 1. Characteristic of membranes

	Membrane 1	**Membrane 2**
Number of fibres	2400	3550
Fibre pore size (µm)	0.01 to 0.1	0.03 to 0.06
Length (mm)	495	146
Total surface area (m^2)	0.80	0.50
Shell diametre (mm)	60	55.60
Capacity (m^3/s) x 10^{-5}	2.7	0.16 to 1.6
Fibre thickness (mm)	0.65	N/A
Shell side volume (m^3) x 10^{-3}	0.43	0.08
Volume occupies by hollow fibre (m^3) x 10^{-3}	2.5	N/A
Housing material	Polyvinyl chloride	Polyethylene
Membrane material	Polysulfone	Polypropylene

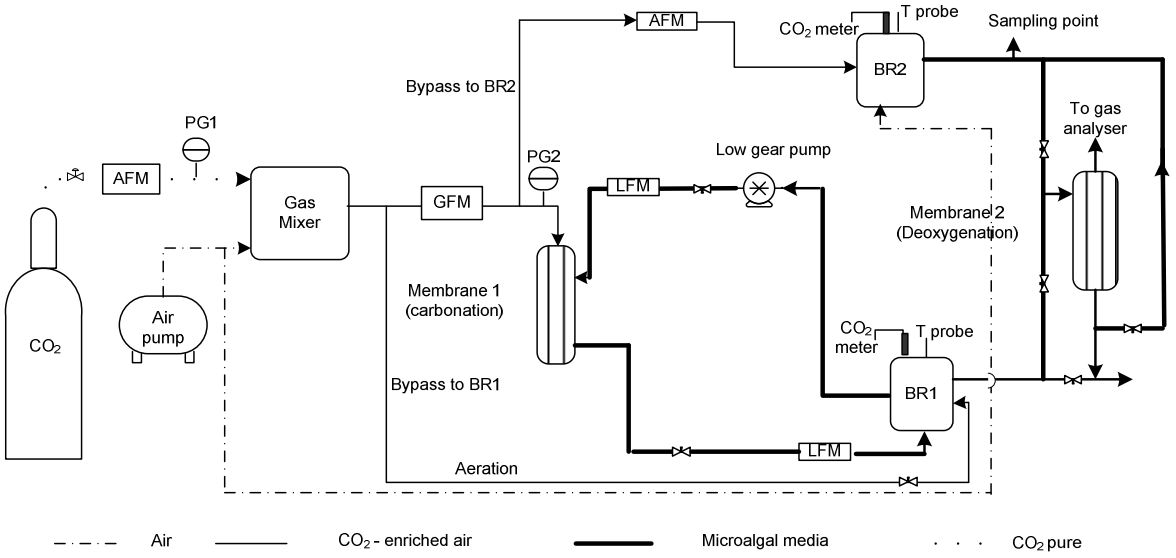


Fig.1. Membrane bioreactor set up

2.3 Carbonation and bubble measurement

The CO_2 feed was controlled from 0.49 x 10^{-3} kg/m^3 to 3.11 x 10^{-3} kg/m^3 for each experiment. The concentration of CO_2 in media and at the headspace of bioreactor was measured according to previous work [10]. The carbonic acid in media was estimated based on the total fraction of hydrogen ion to hydroxyl ions, which represented as pH reading [20].

The membrane created small bubbles in bioreactor, which was estimated according to Rodrigues and Rubio [21] method. The tubular bioreactor was labelled with numerical estimation from 0 cm to 100 cm as aid to the bubble estimation.

2.4 Oxygen removal by Membrane 2

Membrane 2 acted as O_2 removal prior to CO_2 depletion in the bioreactor. Membrane 2 is a superphobic membrane, which have high resistance to water and microalgae attachment at the membrane walls.

2.5 CO_2 uptake and microalgae growth

The CO_2 uptake by microalgae is a measured of (a) dissolved CO_2, (b) carbonic acid in media, (c) CO_2 at the bioreactor headspace and (d) CO_2 in the membrane. The CO_2 meter measures CO_2 concentration from 1.49 x 10^{-3} kg/m^3 to 1490 x 10^{-3} kg/m^3 (1 ppm to 1490 ppm) in media and 0.1 % to 100 % in the bioreactor headspace. At the same time, a reading of microalgae growth, pH, and dissolved O_2 was recorded daily for three weeks. A UV-Vis spectrophotometer with UV-Vis bandwidth of 2.0 nm was used to measure growth of microalgae. The absorbance was recorded at a wavelength of 657 nm.

3 Results and Discussion

3.1 Effect of membrane integration

Figure 2a shows dissolved CO_2 in a bioreactor with and without membrane. At 0.49 x 10^{-3} kg/m^3, the dissolved CO_2 was 0.1 x 10^{-3} kg/m^3 in bioreactor without membrane. This equivalent to 20% of CO_2 feed. In comparison, the dissolved CO_2 in membrane integrated-bioreactor was 0.4 x 10^{-3} kg/m^3. This equivalent to 82% of CO_2 feed and 4 times higher than in bioreactor without membrane. At 3.11 x 10^{-3} kg/m^3, about 42 % and 29 % of CO_2 was dissolved in media for integrated and non-integrated bioreactor, respectively. Thus, further increase in CO_2 feed was considered as ineffective beyond 3.11 x 10^{-3} kg/m^3.

The experimental result suggests that up to 82 % of CO_2 feed was delivered by membrane to the bioreactor and entrapped within the microalgae culture. Nearly 18 % was released into the headspace of the bioreactor, piled up within the membrane and converted to carbonic acid. From the perspective of Henry's law, the highest CO_2 could dissolve in water was approximated about 83 % of total supply (dissolved CO_2 = 0.8317 x CO_2 inlet) [22]. This means that 83 % of CO_2 was dissolved in water at atmospheric pressure and room temperature (28 ^oC). However, the equilibrium of CO_2 dissolves with CO_2 feed which is the solubility of CO_2 at natural conditions takes longer period to achieve compared with membrane-integrated bioreactor. This was a reason for drawback in microalgae cultivation with added CO_2. During carbonation, pH level was controlled at tolerable conditions for microalgae growth.

The acidic level of media in bioreactor was indicated by pH reading. From Figure 2b, pH level decreased as dissolved CO_2 increased. The natural conversion of CO_2 into carbonic acid in the presences of water caused the final reading of pH in the media as low as 4.82 [23]. It is desired to have high dissolved CO_2 compared to carbonic acid for microalgae cultivation. Microalgae ability that can use dissolved CO_2 without depending on carbon concentrating mechanism (CCM) increased CO_2 uptake in bioreactor. In comparison, carbonic acid conversion to useable carbon such as carbonate required CCM activity. Some microalgae have low CCM activity thus incapable to convert carbonic acid to useable carbon for growth. Microalgae can survive if less than 5% of carbonic acid in media [24,25]. Therefore, a small fraction of carbonic acid resulted from the reaction with water is sufficient for growth. Cultivation with more than 5% carbonic acid can cause acidic to the media.
Figure 2b shows that allowed CO_2 feed is less than 0.5 x 10^{-3} kg/m^3 CO_2 to prevent extreme acidic condition. In overall, Figure 2 shows that carbonic acid increased with CO_2 feed. It was found that CO_2 conversion to carbonic acid agreed with Henry's law statement whereas the equilibrium constant of carbonic acid in water is approximated at 1.7 x 10^{-3} at room temperature [26]. Most of the CO_2 gas remains as CO_2 in water. The remaining CO_2 was scattered to another part of the membrane bioreactor. Thus, further study was conducted to evaluate the CO_2 distribution.

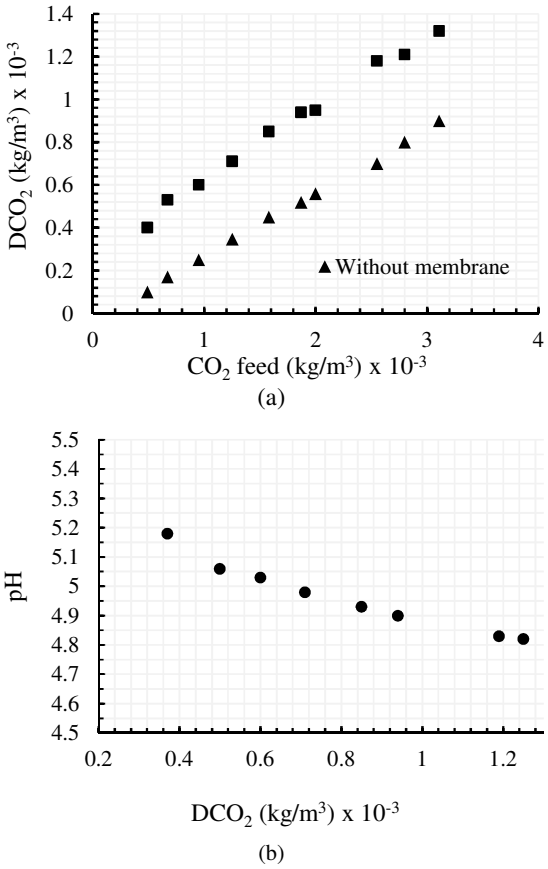


Fig.2. (a) Comparison of dissolved CO_2 in bioreactor; (b) pH

3.2 Distribution of CO_2 in bioreactor during carbonation

Carbon dioxide distributed in bioreactor mainly as dissolved gas in media, gas at the bioreactor headspace, gas piled up within the membrane and chemically converted to carbonic acid. Figure 3a, Figure 3b, Figure 3c and Figure 3d show the fraction of this CO_2, respectively. The membrane increased CO_2 delivery to media from 40 % to 80 % of CO_2 feed and thus reduced CO_2 at the bioreactor headspace up to 18 %. About 14 % amassed in the membrane and the smallest fraction, less than 1 % converted as carbonic acid in media.

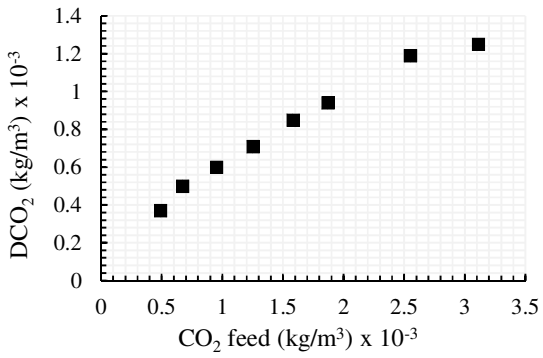
In Figure 3a, dissolved CO_2 increased as the CO_2 feed increased. However, low increment of dissolved CO_2 was found when fed with CO_2 above 2.5 x 10^{-3} kg/m^3. This suggests that CO_2 should not feed beyond 2.5 x 10^{-3} kg/m^3. There are three main reasons for the loss which were presented in Figure 3b, Figure 3c and Figure 3d.

In Figure 3b, the accumulated CO_2 within the membrane was 0.02 x 10^{-3} kg/m^3 to 0.42 x 10^{-3} kg/m^3 compared to the CO_2 feed which was 0.49 x 10^{-3} to 3.11 x 10^{-3} kg/m^3. This equivalent to 4 % to 14 % of CO_2 feed. This shows that the higher the CO_2 feed, the higher possibility of the CO_2 accumulated within the membrane. This considered as a loss to the cultivation of microalgae. However, a simple flushing by air at the final stage of the carbonation lessens the gas build-up in the membrane.

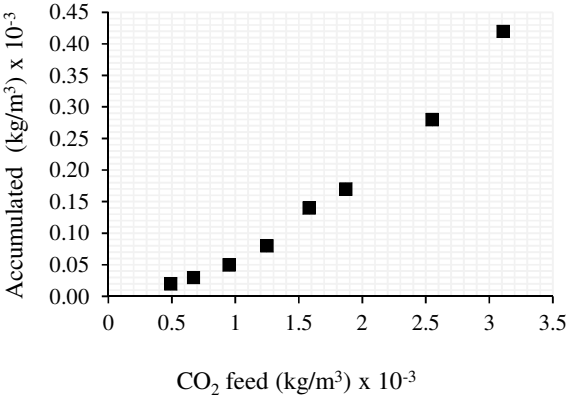
The remaining CO_2 was stored as gas at the bioreactor headspace. In Figure 3c, up to 1.42 x 10^{-3} kg/m^3 of CO_2 feed was found in the bioreactor headspace. This equivalent to 0.46 % of CO_2 feed. The stability of bubble formation during carbonation has affected gas concentration at the bioreactor headspace although it lower than CO_2 piled-up in the membrane. The CO_2 at the headspace lessen the possibility of CO_2 uptake by *Chlorella*. It is possible to use CO_2 outside the watery media but takes longer period to achieve equilibrium between the gas and liquid phase. This affects microalgae growth. Thus, CO_2 dissolved into media have more benefit for microalgae growth compared to CO_2 outside the media.

The smallest fraction of the CO_2 feed was found as carbonic acid. The carbonic formation in microalgal-media has a slight difference compared with water. The converted CO_2 was in the range of 0.01 x 10^{-3} kg/m^3 to 0.03 x 10^{-3} kg/m^3 as shown in Figure 3d. This equivalent to 0.006 % to 0.02 % of the CO_2 feed. However, this amount caused acidic in microalgal media. Some microalgae species have high tolerance to the acidic environment. In this study *Chlorella* sp. showed no decrease in microalgae growth at low pH which suggested that *Chlorella* sp. might has high tolerance on low pH. Adding alkaline such as NaOH probably can reduce the effect of acidic condition in media. However, the effect of alkaline on pH was not evaluated in this study. In overall, the use of membrane to aid carbonation lower CO_2 amount at the headspace and within the membrane. It increased carbonation and ease CO_2 addition into the *Chlorella* sp. culture. The key to high increase in carbonation with the aid of membrane is the ability of membrane to control CO_2 feed and thus control over the bubble formation.

The bubbling is the apparent reason for low carbonation in bioreactor which aerated without membrane. Some study has reported that carbonation of microalgae culture better achieved by bubbling column than common aeration [3,27]. However, bubbling promotes low carbonation in bioreactor. The use of bubbling column increases microalgae growth up to 0.63 per day [28]. Thus, this study presents discussion on bubbling by membrane.



(a)



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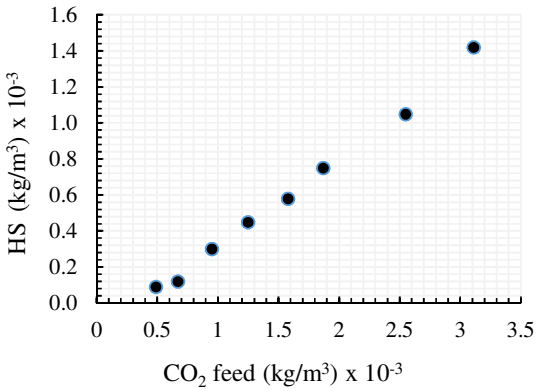

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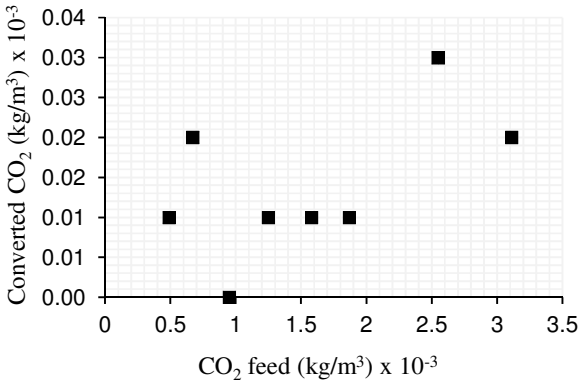

(d)

Fig.3. (a) Dissolved CO_2; (b) Amount of CO_2 in bioreactor headspace; (c) Accumulated CO_2 within the membrane; (d) Converted to carbonic acid

3.3 Relationship of carbonation and bubbling

Membrane distributes CO_2 in bioreactor and the key for achieving high dissolved CO_2 depends on membrane ability to control the size of the bubble. The bubble in bioreactor without membrane and aerated with standard air composition was found in the range of 5 mm to 8 mm in diameter. The bubble was in spherical shape. At constant flow rate, the bubble size was remained constant. The use of membrane resulted in small bubble ranges from 1 mm to 2 mm. Figure 4 shows bubble size compared with dissolved CO_2. Figure 4 shows that the

bigger the bubble size, the lower amount of CO_2 dissolved. Flow rate also was found affected the bubble formation. Less than 2 mm bubbles observed at 0.3 x 10^{-5} m^3/s. The dissolved CO_2 was found 70 % of the CO_2 feed. The bubble size increased with the gas feed. From 0.3 x 10^{-5} m^3/s to 0.7 x 10^{-5} m^3/s the bubble size was found from 1 mm to 2 mm and the dissolved CO_2 decreased from 70 % to 60 %.

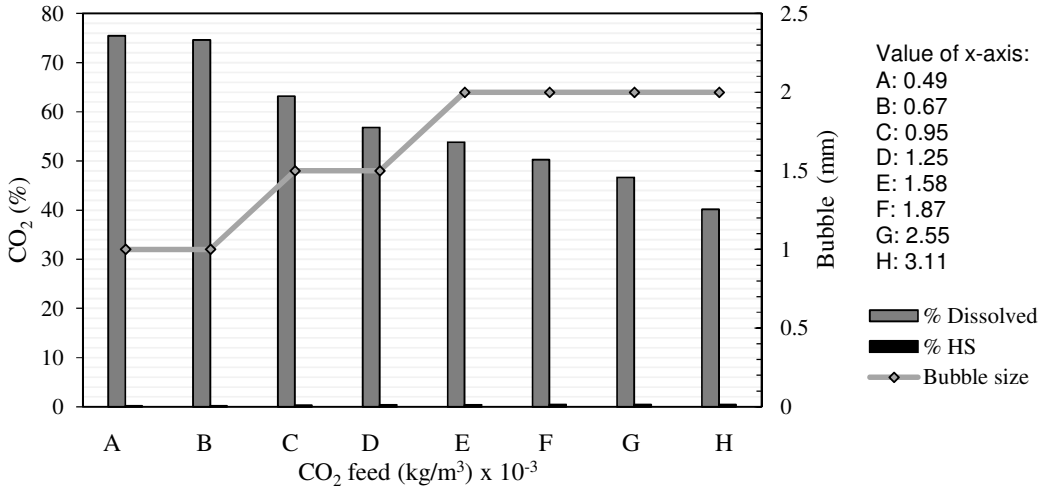

Fig.4. Effect of bubble on CO_2 concentration in media and bioreactor headspace

In continuous carbonation and cultivation, the bubble size decreased as the inlet media flow rate increased. At constant gas flow rate, which is 1.3 x 10^{-5} m^3/s, the bubble size decreased from 2 mm to barely seen by eyes as a side effect of increasing liquid flowrate. The effect of liquid and gas flow rate in bioreactor was discussed in previous work [10].

As conclusion, the bubble size affected CO_2 in media and bubble created between 1 mm to 2 mm resulted in 70 % dissolved CO_2. When bubbled at 5 mm and above, the dissolved CO_2 was found less than 30 %. The large bubble created without membrane indicates that membrane plays a major effect on the bubbling formation. In addition, the inlet flow rate affected size of bubble. This result agreed with findings in bubbling column [29-31]. The instability of inlet flow rate and high Reynolds number creates large bubbles. The large bubble fetched up the CO_2 gas and escaped into the bioreactor headspace. Logically, microalgae only uses CO_2 that is in the water because of its cell phycology that does not have vascular tissue [32]. Thus, the CO_2 released into the headspace lessened the potential of CO_2 uptake by microalgae. The relation of carbonation and CO_2 uptake was measured further in this study.

3.4 Membrane carbonation and CO_2 uptake

The purpose of this study is to examine the range of tolerable carbonation or dissolved CO_2 by *Chlorella* sp. This work also aims to find a way to increase CO_2 uptake of *Chlorella* sp. Preliminary study shows that CO_2 uptake was in the range of 20 % to 85 % regardless of CO_2 feed. The highest CO_2 uptake was recorded at 2 mm bubble, followed by 1 mm. The lowest CO_2 uptake was when the cultivation was bubbled at 1 mm. It shows that at 1 mm, *Chlorella* sp. used only 20 % of the total CO_2 feed. At the same time, the specific growth was recorded as 0.17 per day, which is the lowest among bubble size. However, this value higher compared to fast-growing grass which in the range of 0.09 d^{-1} to 0.59 d^{-1} [33].

The uneven distribution of CO_2 in media was identified as a main reason for low CO_2 uptake. The distribution of CO_2 bubbled at 1 mm was evaluated with the pH measure of the media. The pH was measured at the bottom, middle and surface of the media. Less than 0.2 % of CO_2 feed at the bioreactor headspace shows that most CO_2 entraps in the media. The *Chlorella* sp. growth which is 0.17 per day was due to insufficient CO_2 in the bioreactor. In overall, the CO_2 uptake by microalgae is best achieved when carbonized with bubble size approximately 2 mm where the CO_2 uptake was up to 85 %. This resulted in *Chlorella* sp. specific growth of 0.21 d^{-1}. This show that 2 mm was effective for both biomass and CO_2 uptake.

The CO_2 uptake in bioreactor associated with O_2 produced in the bioreactor. The total O_2 produced in bioreactor was 30 % and the dissolved O_2 was 18 %. The higher the growth rate, the higher the O_2 produced in the bioreactor. The O_2 generation also indicates the efficiency of CO_2 uptake. In ideal photosynthetic theory, each molecule of CO_2 produces one molecule of O_2. The O_2 in bioreactor headspace is in balance with the dissolved O_2. The presence of dissolved O_2 in the media causes photorespiration, thus preventing the photosynthetic enzyme to drive CO_2 uptake by microalgae. As low as 20 % of O_2 in media enough to hinder the photosynthesis cycle [14]. The O_2 reacts with RubisCO and produces phosphoglycolate, which inhibits the enzymes of photosynthesis.

The main factor of affecting CO_2 uptake was the bubbling within the bioreactor and the preliminary microalgae density in media. The density of microalgae affects light penetration inside the bioreactor. In extremely low microalgae density, light was easy to reach to each microalgae cell. However, the CO_2 uptake was found less compared to denser *Chlorella* sp. concentration. In addition, at extremely dense microalgae cell, more O_2 was produced. The produced O_2 inhibits the continuous photosynthesis cycle. Large bubbles in media capable of removing the dissolved O_2. Thus, the O_2 generated does not prevent the photosynthesis of *Chlorella* sp. as most of the dissolved O_2 released into the bioreactor headspace. The average ratio of dissolved O_2 to O_2 in bioreactor headspace was 4:1. Other means of removing O_2 without creating large bubble is applying hydrophobic membrane instead of aeration. Thus, preliminary effect of secondary membrane was discussed in this study.

3.5 Effect of secondary membrane to O_2 removal and *Chlorella* sp.growth

The Membrane 2 lessened O_2 in media from 8.5 x 10^{-3} kg/m^3 to 4.9 x 10^{-3} kg/m^3 at third week of cultivation. Another set of experiment which ran simultaneous also shows similar result. The BR1 which integrated with Membrane 1 was bubbled at 1 mm to 2 mm and BR2 without Membrane 1 ran at 5 mm to 10 mm. Both bioreactor were equipped with Membrane 2. About 41 x 10^{-3} kg/m^3 of CO_2 was depleted in BR1. This equivalent to 89 % of the CO_2 feed. The overall dissolved O_2 and O_2 at the bioreactor headspace was about 24 % and 12 %, respectively. In comparison, BR2 shows 5 % and 21 % depletion of dissolved O_2 and O_2, respectively. The large bubble size in BR2 has aided the release of O_2 from the media. However, this resulted in low growth of *Chlorella* sp. which was only up to 0.19 per day (specific growth rate).

As a conclusion, the secondary membrane increased CO_2 uptake of *Chlorella* sp. The secondary membrane has no direct effect on the CO_2 uptake. However, both membranes have improved CO_2 uptake and O_2 removal from the bioreactor, thus, increased the *Chlorella* sp. growth.

4. Conclusion

This study presents an evaluation of membranes to increase carbonation of microalgae media. About 82 % of CO_2 feed was successfully delivered to the media with the aid of Membrane 1 and this resulted in low pH of media. The membrane also distributed CO_2 as dissolved CO_2 in media, piled up in membrane, accumulated at headspace and converted to carbonic acid. This study suggested that 2 mm bubble size is optimum for high carbonation. Finally, the secondary membrane has indirectly aided in increasing the CO_2 uptake by removing O_2 from the media.

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