Biodiesel production by microalgae cultivated using permeate from membrane bioreactors in continuous system

Siok Ling Low, Say Leong Ong and How Yong Ng

ABSTRACT

Microalgae in three submerged ceramic membrane photobioreactors (SCMPBRs) with different hydraulic retention times (HRTs) were fed with permeate of a submerged ceramic membrane bioreactor for a period of 3 months to investigate the lipid content and also the biodiesel quality produced at different HRTs. The lipid content, lipid productivity and fatty acid compositions for all three SCMPBRs were not significantly different at the 95% confidence level. These results suggested that insignificant change in the amount of fatty acids was observed at different HRTs that supplied varying concentration of nitrate in the medium. Among the fatty acids, palmitic acid, palmitoleic acid, oleic acid and linoleic acid were the main components, whereas stearic acid was a minor fatty acid. Since there was insignificant effect of HRT on lipid content, lipid productivity and fatty acid compositions, the optimum HRT for SCMPBRs can then be designed based on optimum nutrient removal performance and low membrane fouling propensity.

Key words | biodiesel, *Chlorella vulgaris*, microalgae, submerged ceramic membrane photobioreactor (SCMPBR), submerged membrane bioreactor (SMBR)

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INTRODUCTION

Microalgae, having the photoautotrophic ability of plants and also immense growth rates, have been cited as the next generation feedstock for biodiesel production. However, microalgal biofuel production was limited by the cultivation methods of microalgae and contamination of microalgal culture with native species (Sheehan *et al.* 1998). Besides a feedstock for biodiesel production, microalgae have been used as a nutrient removal agent in wastewater treatment for the past 50 years (Hoffmann 1998). Although using microalgae to remove nutrients from wastewater is relatively cheaper and microalgal biomass produced can be reused, the productivity and the quality of desired products from microalgae can be significantly affected if there is any contamination by microorganisms. Membrane bioreactor (MBR) permeate, which has a low bacteria concentration, appears to be a feasible culture medium to produce microalgae in large quantity. This approach is possible since the MBR is increasingly being used in many countries due to its good performance characteristics and compactness (Visvanathan *et al.* 2000). For example, in Singapore, the MBR has been used for domestic wastewater treatment. Microalgal biomass produced should

have minimal bacterial contamination and can be harvested as a feedstock for biodiesel production that, in turn, could be used for generating electricity for treatment plant usage. This combination creates a win–win situation, which may improve the economic feasibility of microalgal biodiesel production while potentially gaining a cost saving for the MBR stage (i.e. a lower degree of treatment can be provided by the MBR as residual compounds could be taken up by the downstream microalgal system).

The effectiveness of *Chlorella vulgaris* to remove nutrients from MBR permeate and its ability to accumulate biomass were demonstrated in the previous study conducted by Low *et al.* (2011). Among all species of microalgae, *C. vulgaris* is one of the most widely studied species in nutrient removal processes because it is robust to the wastewater environment and able to uptake nutrients efficiently. It also has an immense growth rate due to its simple structure (Li *et al.* 2008; Converti *et al.* 2009) and is able to produce significant lipid content (up to 38% of dry weight) in comparison with other freshwater and seawater microalgal strains (Widjaja *et al.* 2009). However, the lipid content and biodiesel quality of microalgal biomass produced from

MBR permeate are yet to be investigated. Lipid content and biodiesel quality are sensitive to operational conditions and critical to the economics of large-scale biodiesel technologies. Since hydraulic retention time (HRT) generally controls nutrient loading, a good understanding of the influences of HRT on lipid content and biodiesel quality is essential for optimizing biodiesel production. Thus the influences of HRT should be investigated to facilitate the implementation of a microalgal system for larger-scale applications.

For lipid production, research has been focused on identifying cultivation conditions that will provide the greatest lipid productivities by inducing high accumulation of neutral lipids (particularly triacylglycerol) in the microalgal cells, such as nutrient stress associated with nitrogen (N) or phosphorus (P) limitation (Hu *et al.* 2008; Converti *et al.* 2009; Griffiths & Harrison 2009; Dean *et al.* 2010; Pittman *et al.* 2011). However, very high accumulation of lipids induced by nutrient-stressed condition is often coupled with low biomass productivity, and hence the lipid productivity will be lower (Pittman *et al.* 2011). Therefore, there is growing interest in understanding the culture conditions, such as growing microalgae in wastewater or treated wastewater, that are able to provide high biomass productivity for achieving high lipid productivities. Furthermore, the studies conducted on lipid production induced by nutrient-stressed conditions were on batch cultivation systems to trigger the conditions of nutrient limitation. Operating a batch system cannot really represent the real situation when the full-scale system is intended to operate on a continuous-flow arrangement for a prolonged period. In view of the above, the objectives of this study are to investigate the lipid content and also the biodiesel quality produced at different HRTs or different nutrient loading levels in continuously operated submerged ceramic membrane photobioreactors (SCMPBRs). Three SCMPBRs with different HRTs were operated in parallel and fed with the permeate of a submerged ceramic membrane bioreactor (SCMBR) for a period of 3 months. The lipid content and the quality of biodiesel produced at different HRTs were analyzed.

MATERIALS AND METHODS

Algal strain and subculture

C. vulgaris CCAP 211/11B was obtained from the Culture Collection of Algae and Protozoa, Argyll, UK, and was cultured under continuous illumination (7,500 lux) at 25 °C. The microalgae were cultured in an artificial culture medium, *Euglena gracilis* medium (EG medium), with ammonia-N as the N source. A volume of 10% of microalgal seed (in an exponential growth phase) was inoculated into 100 mL of culture media. The sub-cultured cells were used as an inoculum in the study of microalgal cultivation in the SCMPBRs. Microalgal cells from the culture medium were harvested by centrifugation at 6,500 rpm, 4 °C for 5 min. The supernatant was decanted and cell pellets were washed twice with autoclaved distilled water to remove the culture medium from the microalgal cells. The washed cell pellets were re-suspended with sterilized distilled water for inoculation.

Experimental set-up for aerobic SCMBRs and SCMPBRs

One 7 L SCMBR was operated at a HRT of 3 h and a solids retention time (SRT) of 20 d to produce enough membrane permeate for the three SCMPBRs. Previous work (Low *et al.* 2011) had shown good nutrient removal performance and microalgal growth for an MBR operated at a shorter HRT. Furthermore, operating the SCMBR at a shorter HRT would reduce the combined HRT of both the SCMBR and SCMPBR. The SCMBR was started up with identical seeding mixed liquor collected from the aeration basin of a local wastewater treatment plant. Domestic wastewater collected from the same plant was pre-filtered with a 1 mm sieve and fed continuously to the SCMBR. The SCMBR was operated for 60 d (i.e. three times of SRT) for sludge acclimatization before commencing experiments on the SCMPBRs. To facilitate a meaningful comparison of performance between each SCMPBR, the SCMBR permeates produced were fed to a well-mixed equalization tank and distributed to the three 7 L SCMPBRs simultaneously to grow *C. vulgaris*. The SCMBR was equipped with two 200 nm pore-sized flat-sheet ceramic membrane modules (ITN, Germany), each with 2 L permeate channels, mounted between two baffle plates. Each module was located above a diffuser with an effective aeration rate of 3 L of air per minute per module. The dimensions of the flat sheet membrane were $6.5 \times 110 \times 330$ mm. These membranes were made of fine layers of $ZrO_2/TiO_2/\alpha\text{-}Al_2O_3$ with nominal sizes of 200 nm established on $\alpha\text{-}Al_2O_3$ porous support. The initial permeability of the new membrane was about 1,450 L/(h·m²·bar) based on de-ionized water tested at 20 °C.

The three SCMPBRs are denoted as SCMPBR$_{72h}$, SCMPBR$_{24h}$ and SCMPBR$_{6.5h}$, which referred to the

SCMPBR operated at 72, 24 and 6.5 h HRT, respectively (see Table 1). One membrane module identical with that in the SCMBR was used in each SCMPBR.

The SCMBR and SCMPBRs were operated with a similar intermittent suction cycle of 8 min on and 2 min off for the relaxation of the membrane. All experiments were performed under ambient condition (26–32 °C) during the entire experimental period. The SCMBRs were operated at a pH of 7.2 ± 0.2, controlled using an alkaline-dosing (0.2 M Na_2CO_3) pump. For the SCMPBRs, the pH in each reactor was found to be stable naturally within the range of 7.0 ± 0.2, and hence no pH control was required. Light was provided to each SCMPBR by cool-white biological fluorescent lights (from both the longer sides of the SCMPBR) at 7,500 lux, measured with a digital light meter (LX 802). Ambient air was supplied continuously through an air diffuser at a flow rate of 3.5 L/min (0.5 L of air/L of water/min) with the addition of 6% CO_2. Previous studies reported highest biomass productivities when cultivating microalgae with air containing 6% of

CO2 (Morais & Costa 2007; Chinnasamy *et al.* 2010). The SCMPBRs were inoculated with 1.4 L of microalgal culture with an initial biomass concentration of 2 g/L. The configuration of the complete SCMBR and SCMPBR system is shown in Figure 1.

Lipid extraction and fatty acids methyl esters analysis

The biomass concentration (mg/L) of the *C. vulgaris* was determined by filtering the microalgal culture sample through a GF/C Whatman filter paper and oven-drying the filter paper (with microalgae on it) at 103–105 °C for 1 h. The lipids stored in the microalgal cells were then extracted using a dry lipid extraction method developed by Zhu *et al.* (2002) (Widjaja *et al.* 2009).

The solvent phase of the samples, obtained from the filtration for determining the lipid content, was evaporated in a rotary evaporator under vacuum condition at 60 °C. The samples for fatty acid methyl esters (FAME) analysis were prepared according to the method specified in the EU regulation (European Commission 1991). The dried lipid of each sample was dissolved in hexane and 2N potassium hydroxide in a methanol solution (11.2 g KOH in 100 mL CH_3OH). About 10 mL of hexane and 100 µL KOH-CH_3OH solution were used for every 100 mg of dried lipid. The mixture was then vortexed for 30 s and centrifuged for 10 min at 9,000 rpm. The clear supernatant was transferred into a 2 mL auto-sampler vial for FAME analysis. The fatty acid composition of the biodiesel samples was determined using an Agilent 6890 gas chromatograph equipped with a flame ionization detector.

Table 1 | Operating conditions of the SCMPBRs operated in parallel

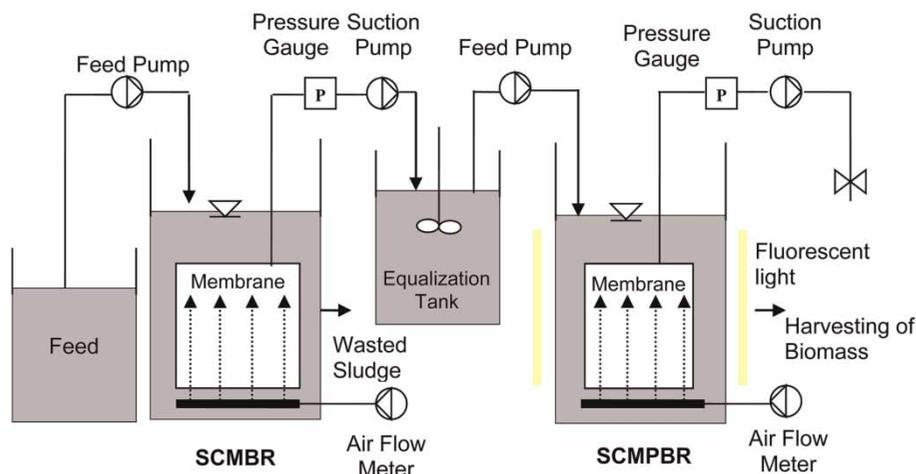| Parameter	Value
HRT (h)	72, 24, 6.5
SRT (d)	10
Working volume (L)	7
Influent NO_3^-–N (mg/L)	55.45 ± 6.38 (4,000 µmol N/L)
Flux (L/(m²·h))	1.52, 4.56, 16.83
Aeration rate (L air/min)	3.5
pH	7.0 ± 0.2

Figure 1 | Experimental set-up of SCMBR and SCMPBR for microalgal cultivation.

RESULTS AND DISCUSSION

Lipid content of *C. vulgaris* in all three SCMPBRs

Table 2 shows the biomass productivity, lipid content and total lipid productivity for all three SCMPBRs. All these parameters increased slightly with decreasing HRT. However, the lipid content and total lipid productivity values for all three SCMPBRs were not significantly different at the 95% confidence level. This phenomenon will be discussed in the next paragraph.

It was reported that supplying a high N content to microalgae would inhibit the lipid accumulation. Johnson & Wen (2010) reported relatively low total fatty acid (TFA) content in *Chlorella* sp. (8.5–10.7% of dry biomass) due to the high N content (517 ± 94 mg/L TN) in dairy manure wastewater, which may inhibit the algal lipid/fatty acids synthesis. Their low fatty acid yield may also be attributed to the contamination or competition from undesired algae and other microorganisms that affect the lipid productivity significantly. Such decrease in TFAs was not observed in this study as the N content in the SCMBR permeate was moderate (at 55.45 ± 6.38 mg/L NO_3^-–N). There have also been studies reporting the effect of nutrient starvation on lipid content accumulation when the microalgae was subjected to nutrient starvation. Converti *et al.* (2009) reported an increase of lipid fractions of *C. vulgaris* from 5.90 to 16.41% when the N concentration was reduced by 75% of the initial N concentration in the medium (247 mg/L NO_3^-–N). Piorreck *et al.* (1984) noted that at a low N level (0.0003% KNO_3), *C. vulgaris* contained high percentages of total lipids (58% of the biomass); however, at a high N level (0.1% KNO_3), the percentage of total lipids dropped to 22.6%. This drastic change of lipid content occurred only at very low N concentrations, 0.0003 to 0.1% KNO_3, which was equivalent to 300 to 600 µmol N/L (Piorreck *et al.* 1984). However, Richardson *et al.* (1969) grew two unicellular algae (*Chlorella sorokiniana* and *Oocystis*

polymorpha) at rather high N concentrations (3,000–20,000 µmol N/L) in continuous culture, and did not observe modifications in the lipids. The average influent N concentration observed in this current study was 55.45 mg N/L, which was equivalent to 3,961 µmol N/L, within the range tested in the study of Richardson *et al.* (1969). Therefore, it could be concluded from different findings obtained by previous studies that the effect of N concentration on lipid content of microalgal biomass occurred only at either very high or very low N concentrations. The effect of N on lipid content was not observed within the range of N content used in this current study. Another possibility for the different observations would be the operating modes of the microalgal system. Piorreck *et al.* (1984) and Converti *et al.* (2009) operated their microalgal systems in batch mode, and the accumulation of high lipids in the microalgal cells appeared to be triggered during conditions of nitrate limitation. During these stressful periods, the metabolism shifted primarily to triacylglycerol accumulation in the cytoplasm for the purpose of energy and carbon storage (Sheehan *et al.* 1998; Hu *et al.* 2008; Beer *et al.* 2009; Wiley *et al.* 2011). For the continuous system used in this current study, on the other hand, nutrient was not limited and *C. vulgaris* was not under starvation, and hence the phenomenon of accumulation of high lipids was not observed.

Biodiesel quality of all three SCMPBRs

Palmitic, stearic, oleic, and linolenic acids are recognized as the most common fatty acids contained in biodiesel (Knothe 2008). The TFA compositions, as shown in Table 3, indicated the presence of palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2). Among the fatty acids, palmitic acid, palmitoleic acid, oleic acid and linoleic acid were estimated as the main components, which ranged from 13 to 44 wt% of TFAs in *C. vulgaris*, whereas stearic acid existed as a minor fatty acid.

The results obtained in this study are in agreement with the findings of Yoo *et al.* (2010). Yoo *et al.* (2010) reported that the most abundant fatty acids were palmitic acid, oleic acid and linoleic acid with an average relative percentage of 24.0, 24.8 and 47.8%, respectively. Slightly higher oleic acid content (24.8%, w/w TFA) was reported by Yoo *et al.* (2010), who cultivated *C. vulgaris* using commercial culture medium, BG11 medium, with 10% CO_2 for 14 days. Cultivation of microalgae using commercial culture medium in the presence of higher concentration of CO_2

Table 2 | Biomass productivity, lipid content and total lipid productivity for all the SCMPBRs

Parameters	SCMPBR$_{72h}$	SCMPBR$_{24h}$	SCMPBR$_{6.5h}$
Biomass productivity (mg/(L·d))	161.7 ± 6.5	175.4 ± 12.9	176.2 ± 9.7
Lipid content (%)	17.95 ± 2.51	20.22 ± 2.48	22.36 ± 4.71
Total lipid productivity (mg/(L·d))	29.11 ± 1.30	35.44 ± 2.34	39.51 ± 2.26

Table 3 | TFA composition (%) of *C. vulgaris* for all the SCMPBRs

Name	Parameters (%, w/w)	SCMPBR$_{72h}$	SCMPBR$_{24h}$	SCMPBR$_{6.5h}$
Palmitic acid	C16:0	20.84 ± 1.31	22.26 ± 3.85	22.67 ± 1.45
Palmitoleic acid	C16:1	19.04 ± 9.78	16.93 ± 9.88	16.07 ± 9.86
Stearic acid	C18:0	0.90 ± 0.32	1.15 ± 0.48	1.69 ± 1.39
Oleic acid	C18:1	13.37 ± 6.24	14.11 ± 7.57	14.78 ± 9.08
Linoleic acid	C18:2	44.77 ± 2.13	43.26 ± 9.26	43.70 ± 6.57

might have contributed to the higher oleic acid content. Another research work conducted by Lee *et al.* (2010), the same research group as Yoo *et al.* (2010), observed rather different fatty acid compositions in their studies. Using the same strain of *C. vulgaris* as Yoo *et al.* (2010), cultivated using the same commercial culture medium and extracted using a similar lipid extraction method except without the addition of CO_2, Lee *et al.* (2010) reported the amount of fatty acids for stearic acid, oleic acid and linoleic acid in *C. vulgaris* as 0.85, 4.07 and 19.79 mg/g dry weight, respectively. This would correspond to relative compositions of 3.4, 16.3 and 79.4%, respectively. Palmitic acid, which is one of the commonly reported fatty acids in *C. vulgaris*, was not detected in the study of Lee *et al.* (2010). The findings of having different fatty acid compositions from both studies indicated that the fatty acid composition can vary significantly across the same strain of microalgae cultivated using different CO_2 concentrations in the cultivation medium.

For Johnson & Wen (2010), linoleic acid was less than 10%, while oleic acid was relatively higher in the composition. The relative percentages of fatty acid for palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid and others were 20.29, 7.35, 16.29, 32.28, 8.34 and 15.44%, respectively. These results are in contrast with the results reported by Yoo *et al.* (2010) and Lee *et al.* (2010), whereby linoleic acid was not the major fatty acid as observed in the study of Johnson & Wen (2010). This difference could be due to different microalgal species cultivated in their studies. The microalga used in the study of Johnson & Wen (2010) was *Chlorella* sp. isolated from a local dairy wastewater treatment lagoon. Using *Desmodesmus communis* isolated from an artificial freshwater pond and an algal consortium isolated from the tertiary treatment sedimentation pond of the wastewater reclamation facility, Samori *et al.* (2013) reported almost similar fatty acid composition as that observed in the study of Johnson & Wen (2010). The most abundant fatty acids were palmitic acid, oleic *cis* and oleic *trans* acids and linoleic acid with a relative

percentage of 24.5, 22.4, 20.4 and 12.3%, respectively (Samori *et al.* 2013). Samori *et al.* (2013) and Johnson & Wen (2010) found a much lower linoleic acid and a slightly higher oleic acid produced by the microalgae compared to those obtained in this current study and the studies of Yoo *et al.* (2010) and Lee *et al.* (2010). These conflicting findings on fatty acid composition might be due to the different microalgal communities. Studies of Samori *et al.* (2013) and Johnson & Wen (2010) shared one similarity: their microalgal culture used was an indigenous strain and isolated from natural wastewater or a freshwater environment, while other studies used microalgal culture supplied or purchased from a culture collection centre with strain data.

Although different studies showed different TFA relative compositions, the fatty acid compositions for the three SCMPBRs used in this current study were not significantly different at the 95% confidence level. This finding is in agreement with those of the study of Samori *et al.* (2013). In the study of Samori *et al.* (2013), the TFA relative composition did not show any significant difference in each culture condition when the culture condition was varied in terms of nutrient concentrations. In this current study, different nutrient concentrations due to different operating HRTs did not affect the fatty acid composition. However, this finding contradicts those of the study of Piorreck *et al.* (1984). Piorreck *et al.* (1984) found that at low N levels (0.0003% KNO$_3$), 66.4% of the lipids of *C. vulgaris* were neutral lipids such as triacylglycerols (containing mainly 16:0 and 18:1 fatty acids), and at high N levels (0.1% KNO$_3$) the predominant lipids (77.4%) were polar lipids containing polyunsaturated C16 and C18 fatty acids. The conflicting findings could be attributed to the different N concentrations present in the cultivation medium. Richardson *et al.* (1969) grew two unicellular algae (*Chlorella sorokiniana* and *Oocystis polymorpha*) at rather high N concentrations (3,000–20,000 μmol N/L) and did not observe modifications in the fatty acids. The average influent N concentration observed in this current study was 55.45 mg N/L, which was

equivalent to 3,961 μmol N/L, within the range tested in the study of Richardson *et al.* (1969). The changes in lipid and fatty acids in the study of Piorreck *et al.* (1984) occurred only at a lower N concentration (in the range of 300 to 600 μmol N/L), much lower than the N concentration used in this current study and also the study of Richardson *et al.* (1969).

Given the lipid content, total lipid productivity and fatty acid compositions were insignificantly different at different HRTs, the optimum HRT of the SCMPBRs could then be designed or chosen based on the desired nutrient removal performance and low membrane fouling propensity.

CONCLUSIONS

(1) The lipid content, lipid productivity and fatty acid compositions for all three SCMPBRs were insignificantly different at the 95% confidence level.
(2) Among the fatty acids, palmitic acid, palmitoleic acid, oleic acid and linoleic acid were the main components, which ranged from 13 to 44 wt% of TFAs in *C. vulgaris*, whereas stearic acid existed as a minor fatty acid.
(3) Since there was no significant effect of HRT on lipid content, lipid productivity and fatty acid compositions, the optimum HRT for the SCMPBRs can then be designed based on optimum nutrient removal performance and low membrane fouling propensity.

ACKNOWLEDGEMENT

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