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The impact of using recycled culture medium to grow *Chlorella vulgaris* in a sequential flow system: Evaluation on growth, carbon removal, and biochemical compositions

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ABSTRACT

Excessive of carbon dioxide (CO_2) emission and water pollution have been identified as the two primary challenges to humans and environment. Hence, biological carbon sequestration by microalgae is recommended as an environmentally friendly approach to capture and convert this CO_2 into value-added products. However, research related to the development of efficient system to concurrently overcome low CO_2 solubility in water and reduction of water footprint in microalgae cultivation is still limited in the literature. In this study, the CO_2 capture by *Chlorella vulgaris* in a recycled cultivation medium was exploited using a sequential flow photobioreactor system. The study revealed that nutrient replenished recycled medium did not significantly affect the growth performance and lipid content of *C. vulgaris*. It was also observed that the CO_2 capture efficiency and protein content were gradually increased from the first (SFB-RWN1) to the third (SFB-RWN3) cycle of cultivation due to the increment of carbon and nitrogen content in the microalgae cell. Besides, the lipid profile of *C. vulgaris* cultivated in the recycled medium comprised of high concentration of saturated (up to 32.41%) and polyunsaturated (up to 43.21%) fatty acid methyl ester (FAME). The present study suggested that growing *C. vulgaris* in a recycled medium is a feasible solution to fix CO_2 from the atmosphere and help to reduce water footprint in the microalgae cultivation system.

1. Introduction

In developing countries, greater emphasis is given to economic growth, which comes at the expense of environmental sustainability [1]. The high energy demand for rapid urbanization and industrialization in developing countries is primarily accomplished by petroleum, coal and natural gas sources [2]. High dependency on these non-renewable resources has resulted to several serious environmental issues that we are facing now in this 21st century [3]. The unregulated combustion of fossil fuels caused rapid increment of carbon dioxide (CO_2) concentration in the atmosphere since the 18th century, which directly contributing to climate changes and global warming [4]. Although COVID-19 pandemic lockdowns have resulted in the decline of CO_2 emission in 2020, the impact is likely to be temporary [5,6]. Therefore, continuous efforts are required to maintain the global carbon balance. Carbon capture and storage (CCS) and carbon capture and utilization (CCU) are the two most promising methods to stabilize the current atmospheric CO_2 concentration [7]. However, the risk of secondary pollution and high operation cost have made CCS less attractive than the CCU technologies [8].

Among the CCU technologies, biological conversion of CO_2 into value-added products through photosynthetic microalgae is a plausible approach to mitigate CO_2 emission and to promote circular bioeconomy

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[9,10]. Microalgae fix CO_2 via the Calvin cycle and convert the CO_2 into beneficial cellular compounds, such as lipid, carbohydrate, protein, and pigments [11,12]. Besides, microalgae exhibit higher photosynthetic efficiency and could accumulate substantial amount of lipid within their cells (~20–50 wt%) for potential biodiesel production [3]. However, high operational cost due to enormous water demand during microalgae cultivation has further limited the commercialization potential of microalgae-based bioproducts [13]. According to Yang et al. [14], the production of 1 kg of microalgae biodiesel in an open system requires about 3726 kg of fresh water. Furthermore, these requirements may vary depending on the microalgae cultivation system (e.g., open pond, bubble column, and tubular) [15]. Thus, reuse of wastewater from aquaculture industry [16], including fish [17] and shrimp farm [18] to grow microalgae have been identified as an effective way to improve economic and environmental sustainability of microalgae cultivation industry [19]. Accordingly, recycle use of microalgae cultivation medium for multiple cycles is a sustainable approach to reduce the operating cost and water footprint of microalgae biomass production [20,21].

Nevertheless, the use of recycled culture medium is not commonly practiced due to the accumulation of extracellular substances (e.g., polysaccharides, lipids, proteins, and organic acids) released during growing phase of microalgae [20]. Several studies had reported on reduction of microalgae biomass productivity and lipid content due to the presence of "aging" cell walls and polysaccharides in the recycled cultivation medium [22,23]. Besides, organic matters (e.g., polysaccharides) that released during cell division caused formation of aggregates and thereby reduced nutrient and light absorption by microalgae cells to attain high biomass productivity [22]. On the other hand, a study conducted by Farooq et al. [21] had demonstrated increment in biomass and lipid productivity of *C. vulgaris* when cultivated in recycled medium. Nevertheless, research information are still limited in the literature on the effect of using recycled cultivation medium on the biochemical composition of microalgae.

In our previous work [24], we had examined the feasibility to grow *C. vulgaris* in sequential flow photobioreactor system. Meanwhile, the present work was aimed to extend the study on the growth behaviour, carbon capture efficiency and lipid accumulation by *C. vulgaris* when cultivated in nutrient replenished recycled culture medium. This approach is more applicable in industry to reduce the overall water footprint of microalgae cultivation process. Besides, the composition of fatty acid methyl ester (FAME) was assessed to determine the quality of produced biodiesel.

2. Material and methods

2.1. C. vulgaris feedstock

Green microalgae *C. vulgaris* strain was procured from the Centre for Biofuel and Biochemical Research, Universiti Teknologi PETRONAS (CBBR). The microalgae strain was cultured in Bold's Basal Medium (BBM) with a constant light intensity of 60–70 μmol $m^{-2}s^{-1}$ (Philip TL-D 36 W-965, cool-white) at 25 °C to 28 °C in a 5 L Duran bottle. The inoculum pH was adjusted to 6.8 value and continuously sparged with atmospheric air [25].

2.2. Experimental setup and operation

Experiments were conducted in a sequential flow cultivation system (SFB-PBR) (Fig. 1) which consisted of five photobioreactors (PBR) connected in series. The PBRs are made up of borosilicate glass with a working volume of approximately 5 L each (33.5 cm in height and 18.2 cm in diameter). Each PBR in the sequential system was numbered consecutively as PBR1, PBR2, PBR3, PBR4 and PBR5. Microalgae cultures in all five PBRs in series were aerated with continuous flow of atmospheric air (9 L min^{-1}). Fresh medium (FM) was prepared by mixing 400 mL of C. *vulgaris* seed culture with 4200 mL of water and 400 mL of organic fertilizer (TANI Organic Brand, a product by Nilai Landscape Sdn. Bhd., Malaysia) containing all essential plant nutrients (Table 1) to support their growth [24]. The pH of the cultivation medium was adjusted to 3 with either sulfuric acid (H_2SO_4, 1 M) or sodium hydroxide (NaOH, 1 M) to avoid contamination of culture by invasive fungi [24]. Meanwhile, the SFB-PBR was continuously irradiated from one side using cool-white fluorescent light (Philip TL-D 36W-865, resulting in light intensity of 60–70 μmol m^{-2} s^{-1}) at a temperature of 25 ± 5 °C.

The microalgae biomass was collected after 14 days of cultivation through gravity sedimentation method, in which nearly 90% of the cultivation medium was recovered for subsequent use. The recovered cultivation medium (supernatant) was then replenished with 400 mL of organic fertilizer (TANI Organic Brand, a product by Nilai Landscape Sdn. Bhd., Malaysia) and reused in the next batch of the cultivation cycle. The nutrient replenished recycled medium (RWN) was

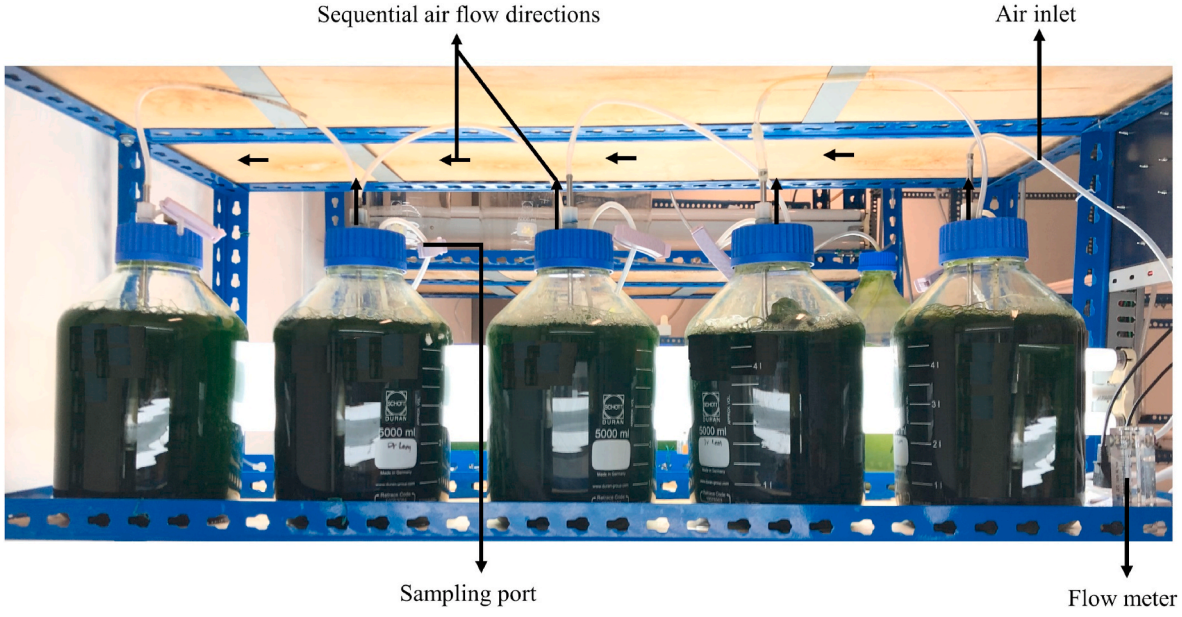

Fig. 1. Schematic representation of sequential flow setup used for CO_2 capture and culture medium recycling.

Table 1
Composition of macronutrients in organic compost medium (TANI) [24].

Test Descriptions	Amount	Method or Equipment used
Nitrogen (as N) (wt%)	5.9	MS417: Part 3: 1994[a]
Phosphorus (as P_2O_5) (wt%)	1.2	MS417: Part 4: 1994[a]
Potassium (as K_2O) (wt %)	11.0	MS417: Part 5: 1994[a]
Calcium (as CaO) (wt%)	15.1	MS417: Part 5: 1994[a]
Magnesium (as MgO) (wt%)	2.9	MS417: Part 6: 1994[a]
Sulphur (as S) (wt%)	ND < 0.1	AOAC 980.02, 17th Ed[b]
Boron (as B_2O_3) (wt%)	0.3	AOAC 980.01, 17th Ed[b]
Chloride (as Cl^-) (wt%)	12.9	MY-STP-043 based on Methrohm Manual Method 21 D 3[c]
Iron (as Fe) (wt%)	0.2	AOAC 965.09, 17th Ed[b]
Zinc (as Zn) (mg.kg^{-1})	218.3	AOAC 965.09, 17th Ed[b]
Manganese (as Mn) (mg.kg^{-1})	368.6	AOAC 965.09, 17th Ed[b]
Copper (as Cu) (mg.kg^{-1})	76.8	AOAC 965.09, 17th Ed[b]

[a] SIRIM Malaysian standard, www.msonline.gov.my/catalog.php?find=ms&sort_mode=effective_date_desc (2019).
[b] Official Methods of Analysis (2005) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method 980.01/02 and 965.09, www.aoac.org/(2019).
[c] EPA Method 21, Determination of Volatile Organic Compound Leaks, CFR40, Part 60.

reintroduced into the SFB-PBR system with similar cultivation conditions as the FM. The supernatant was recycled up to three times (RWN1, RWN2, and RWN3) with an identical parameter setup to evaluate their performances. The water lost due to evaporation was replenished daily to maintain the cultivation volume at constant value. The mass flow (%) of microalgae cultivation system using fresh and recycle medium is illustrated in Fig. 2 (Sankey diagram).

2.3. C. vulgaris growth performances and carbon fixation analysis

The growth of *C. vulgaris* in FM and RWN medium was determined based on the optical density (OD) at 688 nm using UV–Vis Spectrophotometer (UV-2600 Shimadzu). A linear regression relationship was established to determine the biomass concentration (X, g.L^{-1}) using OD_{688} (Eq. (1)):

$$X = 0.532(OD_{688}) + 0.0333,\ R^2 = 0.972 \quad (1)$$

Specific growth rate (μ, day^{-1}) of *C. vulgaris* was determined according to Eq. (2):

$$\mu = \frac{(lnX_2 - lnX_1)}{t_{2-t_1}} \quad (2)$$

where X_1 and X_2 are the biomass concentration (g.L^{-1}) of *C. vulgaris* at the beginning (t_1) and end (t_2) of the logarithmic growth phase (day).

The CO_2 fixation rate by *C. vulgaris* in FM and RWN medium was calculated based on Eq. (3):

$$CO_2\ fixation\ rate\ \left(gL^{-1}day^{-1}\right) = BP \times C_{biomass} \times \frac{M_{CO_2}}{M_C} \quad (3)$$

where *BP* is the biomass productivity of *C. vulgaris* (g.L^{-1}.day^{-1}), $C_{biomass}$ is the carbon content in *C. vulgaris* determined using PerkinElmer 2400 CHNS element analyzer and M_{CO_2} and M_C are the molar mass of CO_2 and carbon.

Meanwhile, the carbon fixation efficiency (%) [26] by microalgae was determined based on Eq. (4):

$$Carbon\ fixation\ efficiency\ (\%) = \frac{CO_{2\ input} - CO_{2\ output}}{CO_{2\ input}} \times 100\% \quad (4)$$

where, $CO_{2\ input}$ and $CO_{2\ output}$ (g.L^{-1}) are the initial and output concentration of total CO_2 at the end of *C. vulgaris* cultivation, respectively.

2.4. Protein content analysis

The protein accumulation in *Chlorella vulgaris* was estimated based on Eq. (5) below:

$$Protein\ Content\ (\%) = N\ (\%) \times 6.25 \quad (5)$$

where N (%) is the total nitrogen content determined using CHNS analyzer (PerkinElmer Model 2400) [27].

2.5. Lipid content determination

The total lipid was extracted from dried microalgae biomass via the Soxhlet extraction method. This method was partially adapted from a previously published work, in which 0.2 g of microalgae dried biomass

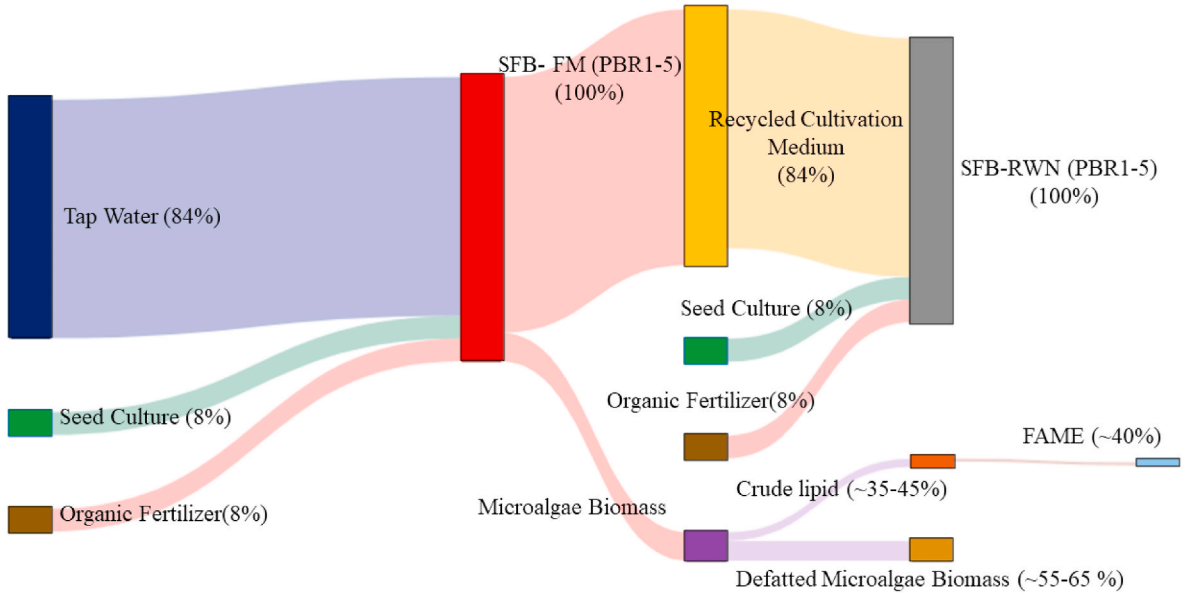

Fig. 2. Sankey diagrams showing the microalgae mass flows (%) to the FAME for the fresh and recycled cultivation medium.

were placed in a cellulose extraction thimble along with methanol, chloroform and water (2:1:0.25 vol ratio). The solvent mixture was then refluxed for 8 h at 75 °C and the resulting crude lipid extract was recovered using rotary evaporator. The crude lipid was oven-dried at 105 °C for 24 h to remove the remaining moisture content [28,29]. Lipid content (Y, %) and productivity (P_{lipid}, $gL^{-1}day^{-1}$) was calculated using Eqs. (6) and (7):

$$Y(\%) = \frac{W_L}{W_{DA}} \times 100\ \% \tag{6}$$

$$P_{lipid}\left(gL^{-1}day^{-1}\right) = \frac{Y(\%) \times X_f}{Cultivation\ time\ (day)} \tag{7}$$

where W_L, and W_{DA} are the weights of the extracted lipids (g), the initial weight of dry microalgae biomass (g), respectively. Meanwhile, X_f is the final biomass concentration ($g.L^{-1}$), respectively [24].

2.6. Transesterification and fatty acid profile analysis

The crude lipid (3 mg) extracted from *C. vulgaris* were dissolved in 3 mL of methanol containing 10 μL of concentrated sulfuric acid (H_2SO_4) and agitated in an incubator shaker (200 rpm) at 60 °C for 6 h. In order to purify the reaction mixture, 3 mL of each potassium chloride solution (KCl, 10%) and water were added to the cooled reaction mixture, followed by centrifugation for 10 min at 4000 rpm. Meanwhile, 3 mL of hexane containing internal standard (0.76 mg of heptadecanoate in 1 mL of hexane) was added to the reacted mixture for FAME composition quantification analysis. The top layer containing hexane and fatty acid methyl esters (FAME) was transferred into another vial for fatty acid profile analysis by gas chromatography (Shimadzu GC-2010, Japan). The quantity of individual FAMEs, C_{FAME} (%) were calculated based on Eq. (8):

$$C_{FAME}\ (\%) = \frac{A_{comp}}{A_T - A_{I.S}} \tag{8}$$

where A_T and $A_{I.S}$ are the total peak area from C14 to C24 and peak area of internal standard (methyl heptadecanoate), respectively; A_{comp} is the peak area of individual component exist in the FAME profile [24].

3. Results and discussion

3.1. Biomass production and CO_2 fixation efficiency of C. vulgaris in recycled culture medium

Fig. 3a and b shows the effect of recycled culture medium on the growth behaviour of *C. vulgaris*. As shown in Fig. 3a, the growth trend of microalgae in RWN was similar to those obtained in FM, indicating that RWN was able to support the growth of *C. vulgaris* without any pre-treatment or purification process. Besides, no lag phases were observed for *C. vulgaris* grown in all three RWN (1–3) mediums due to the remaining microalgae cells that had been adapted well in the cultivation medium [30]. Based on Fig. 3a, the highest biomass concentration of 1.494 g L^{-1} was attained for *C. vulgaris* that grown in SFB-RWN2-PBR1, which was 3.57% higher than that of the SFB-FM-PBR1. Meanwhile, the lowest microalgae biomass (1.224 g L^{-1}) produced in SFB-RWN3-PBR5 was probably caused by the accumulation of organic matter (e.g., polysaccharides), thus inhibited the microalgae growth [31]. Despite their high biomass concentration, the growth rate in RWN (1–3) mediums was way lower than in the FM (Fig. 3b). This was due to the remained microalgae cells from earlier cultivation cycle that resulted in variation of initial cell composition (higher initial cell density), thus contributed to the low growth rates in the RWN (1–3) mediums [31].

Fig. 4 demonstrates variation in the CO_2 fixation rate and efficiency by microalgae with regards to the number of recycle. The maximum CO_2 fixation of 58.1 ± 0.2% was obtained for *C. vulgaris* cultivated in FM medium. Meanwhile, in the RWN1 medium, the CO_2 fixation efficiency declined by 10.03% in comparison to FM, and the percentage started to gradually increase to 54.79 ± 0.11% for the RWN3 medium. Similar trends were observed in term of carbon fixation rate, in which the values of fixation rate in RWN3 increased by 13.98 wt% compared to the RWN1 medium. The differences in the CO_2 fixation efficiency were closely

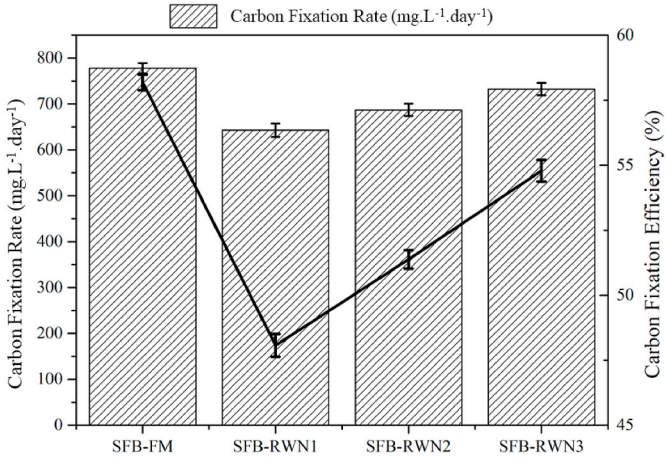

Fig. 4. Effect of recycling culture medium on carbon fixation efficiency by *C. vulgaris*.

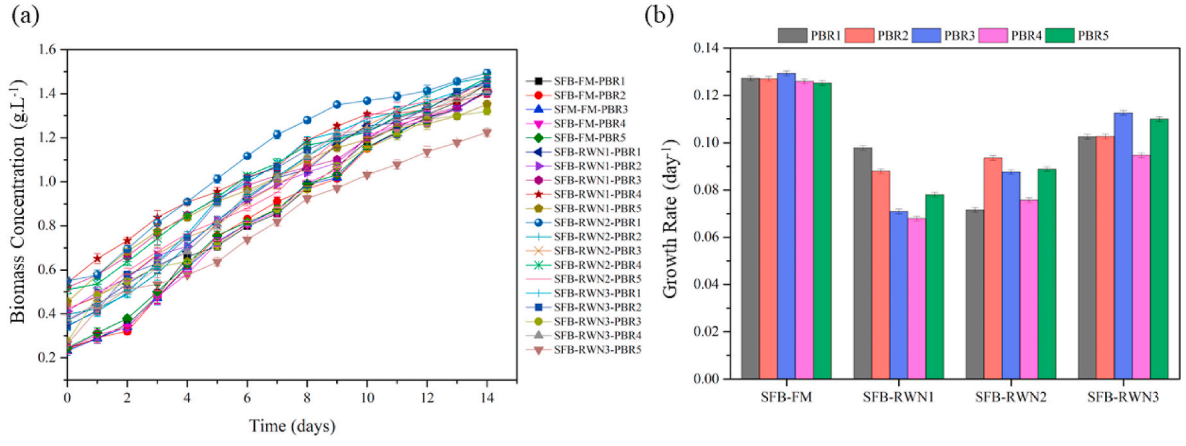

Fig. 3. (a) Biomass concentration ($g.L^{-1}$) and (b) growth rate (day^{-1}) of *C. vulgaris* grown in fresh and nutrient repleted recycled culture medium.

related to the variation of biomass productivity and carbon content of *C. vulgaris* cell (Eq. (4)). Although all the RWN mediums recorded low biomass productivity, the increment of carbon content in the microalgae resulted to the improvement of CO_2 fixation efficiency. Furthermore, the carbon fixation rate of *C. vulgaris* obtained in this study (640–780 mg L^{-1}.day^{-1}) was among the highest as compared to the literature [32–34].

3.2. Lipid accumulation in recycled culture medium

Fig. 5 summarizes the lipid content of *C. vulgaris* grown in fresh and nutrient replenished recycled medium. As shown in Fig. 5, the total lipid content of *C. vulgaris* gradually decreased from FM to RWN3 with an increasing number of culture medium recycles. Previous studies showed that nitrate limited conditions induced lipid accumulation in microalgae cell (e.g. *Chlamydomonas reinhardtii*, *Scenedesmus subspicatus, Chlorella vulgaris* and *Scenedesmus bijugus*) [35–38]; meanwhile, in some cases, lipid accumulation could be induced by excessive nitrate level in some microalgae species (e.g. *Isochrysis zhangjiangensis* and *Tetraselmis* sp.) [39,40]. However, in the present study, lipid content of *C. vulgaris* showed a declining trend, although the RWN medium was supplemented with equal nutrient concentration as the FM. This result was in accordance with that of Nigam et al. [41], where the lipid content of *Chlorella pyrenoidosa* was observed to reduce when the nitrate concentration was doubled.

Furthermore, the presence of high nitrogen concentration in the supernatant was found to transform the organic matter of *Microcystis aeruginosa* and *Scenedesmus obliquus* into protein instead of lipid [42,43]. The highest lipid content and productivity were attained for microalgae cultivated in the FM-PBR1 and slowly decreased in the subsequent four PBRs in the series due to the reduction in the carbon concentration in the inlet air [44]. Fan et al. [45] demonstrated that key metabolic factor which controlled the lipid and starch accumulation rate in *Chlamydomonas* was carbon supply, thus carbon limited conditions could inhibit microalgae product biosynthesis. Although the obtained lipid content could be slightly overestimated due to the present of impurities, however, the amount of non-lipid materials were insignificant as according to the standard extraction method used in the present work [29]. In fact, the total lipid contents determined in the present work were consistent with the available literature (Fig. 5) [39,46–52].

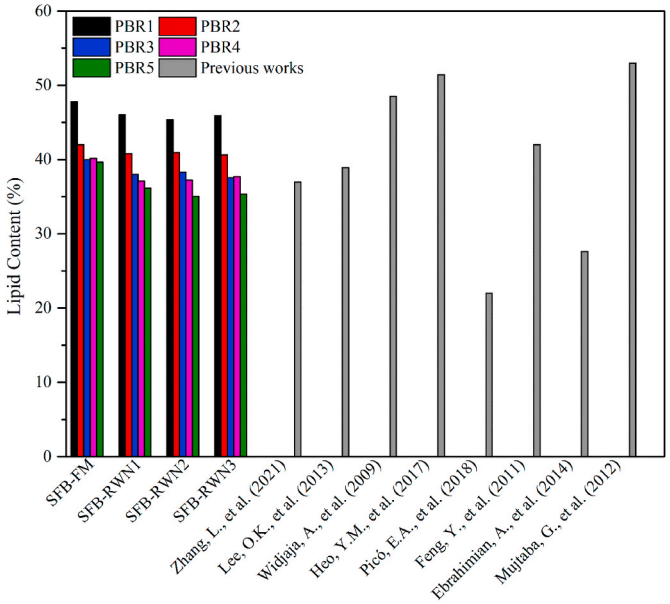

Fig. 5. Comparison of *Chlorella vulgaris* lipid content values from literature with the present work.

3.3. Protein content analysis

The protein content within *C. vulgaris* in repetitive recycling of cultivation medium (RWN) is illustrated in Fig. 6. The results indicated that protein content of *C. vulgaris* gradually increases in the repeated recycled medium (RWN 1 to 3), with the highest protein content of 61.63% was attained in PBR1 of RWN3. As reported in the literature that almost 55% of essential nutrients (e.g., nitrate and phosphate) are likely left unused at the end of the cultivation cycle [14]. Thus, the increase in protein content in the current study could be linked to the high nitrate concentration accumulated in repeated recycled mediums. Nevertheless, the protein content in the third recycled medium (RWN3) was reduced due to the limited ability of microalgae to convert nitrogen in the medium into cellular proteins [53]. A similar observation was reported by Xie et al. [54], in which higher nitrogen concentration in the medium (more than 1.25 g L^{-1}) were found to lower protein content in microalgae cells. On the other hand, the protein content was observed to reduce in the consecutive PBRs in series. According to Tan et al. (2020), sugar produced during the conversion of CO_2 and water via photosynthetic activity is an essential source of energy for protein synthesis; thus, the reduction in protein accumulation was observed from PBR2 to PBR5 as a result of low CO_2 availability [55]. Accordingly, the present finding suggested that an optimal supply of carbon and nitrogen source could promote the accumulation of cellular protein content in *C. vulgaris*.

3.4. Fatty acid profile analysis

Fig. 7a and b illustrate the FAME composition of produced biodiesel from the fresh and recycled medium. As shown in Fig. 7a, biodiesel produced from microalgae consisted of C16:0 (palmitic), C16:1 (palmitoleate), C18:0 (stearic), C18:1 (oleic), C18:2 (linoleic), and C18:3 (linolenic) fatty acids. *C. vulgaris* grown in FM medium had shown lipid composition of oleic acid (27.53–40.13 ± 0.3%), linoleic acid (14.48–28.83 ± 0.18%), palmitic acid (17.49–19.20 ± 0.11%) and linolenic acid (7.44–14.47 ± 0.23%) as the major contributing fatty acids. Meanwhile, for microalgae in RWN (1–3) medium, the major fatty acids were palmitic acid (21.48–32.21 ± 0.12%), palmitoleate acid (7.43–14.91 ± 0.22%) and linoleic acid (26.93–31.81 ± 0.32%). The results indicated that recycling culture medium resulted in significant increment in palmitic, palmitoleic, and linoleic acid composition. On the other hand, the oleic acid composition was observed to decrease from FM to RWN3 by 3.5-fold. It was reported that FAME composition varied according to nutrient concentration in culture media [56]. Particularly,

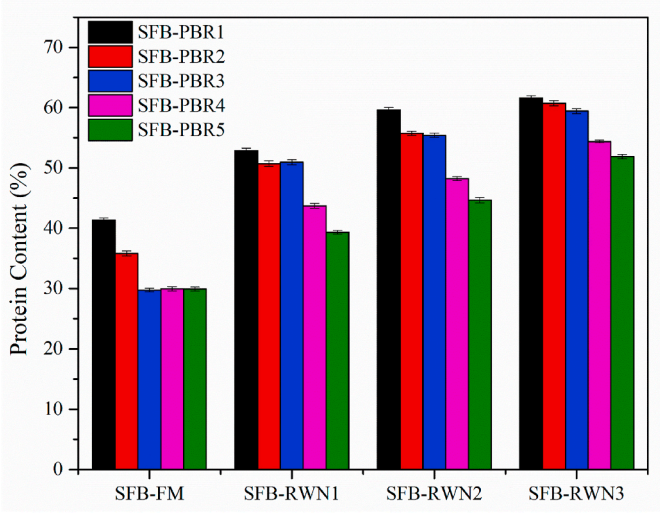

Fig. 6. Protein content of *C. vulgaris* grown in fresh and nutrient repleted recycled culture medium.

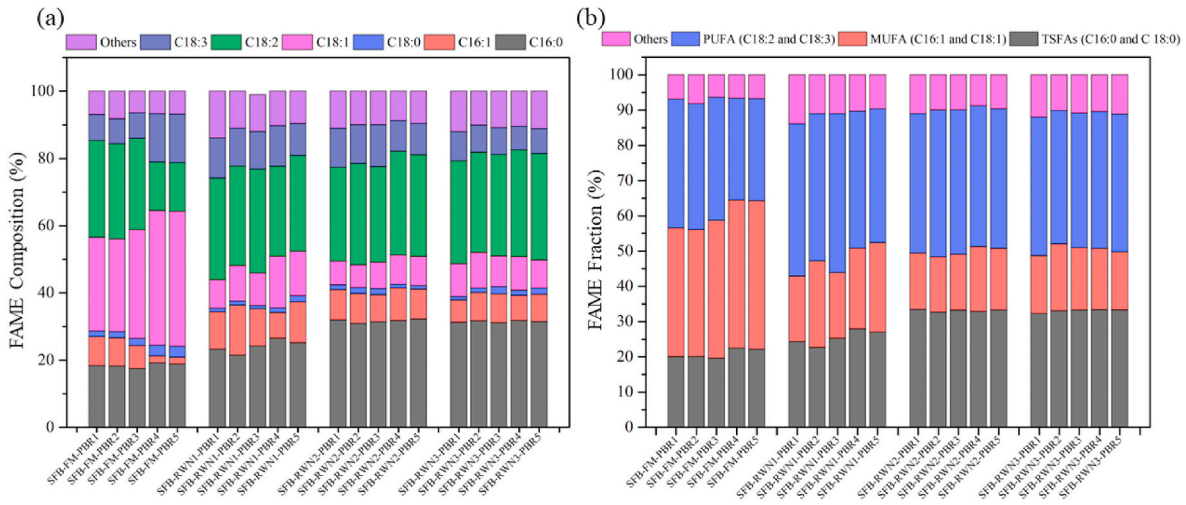

Fig. 7. (a) Fatty acid composition and (b) degree of saturation of *C. vulgaris* grown in fresh and nutrient repleted recycled culture medium.

under nitrate-rich cultivation conditions, *C. vulgaris* was found to favour the accumulation of palmitic acid rather than oleic acid [57]. Similar observations were reported for two other microalgae strains, including *V. stellate* and *E. vischeri,* wherein nitrate repleted cultivation conditions demonstrated a reduction in oleic acid compositions compared to nitrate limited environment [58,59]. However, the composition of stearic acid (1.01–3.25 ± 0.2%) did not vary significantly when the cultivation conditions changed.

Generally, the quality of microalgae biodiesel is evaluated based on the percentage ratio of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids [60]. Present analysis (Fig. 7a and b) showed the presence of PUFAs (37.90–43.21 ± 0.11%) and SFA (22.65–32.41 ± 0.12%) for *C. vulgaris* cultivated in the recycled medium. Furthermore, the saturation degree of FAME composition derived from the recycled medium was found to increase from RWN1 to RWN3 by 7.53 ± 0.3%. The ratio of saturated to unsaturated fatty acids of *C. vulgaris* also dropped significantly with an increase in the number of recycles. On the contrary, the FM had shown a high amount of PUFA (34–37.6 ± 0.22%) and MUFA (35–42 ± 0.12%), but lower SFA (19–22.44 ± 0.33%) composition. Evidently, the composition of PUFA was sensitive to the changes in nitrate concentration owing to its role in maintaining the stability of the organelle membrane by minimizing oxidative stress [61]. Therefore, increment of SFAs (palmitic acid) and PUFAs (linoleic acid) compositions could be due to the stress imposed by the presence of an extracellular substance in the recycled medium, thereby influenced the partitioning of fatty acid composition [23,62]. Similar patterns were observed in other studies, in which high percentage of PUFA values were recorded for microalgae cultivated in recycled medium (Table 2). A higher percentage of PUFA from *C. vulgaris* lipids grown in RWN medium could enhance the lubricity and cold flow properties of produced biodiesel by delaying crystallization process in cold weather [40,63]. In addition, the high SFA concentration could result in high oxidative stability and cetane number of biodiesel [39,60,64,65]. Besides, high cetane number was found to be beneficial for good ignition quality and combustion properties [66]. Accordingly, the presence of a high percentage of SFA and PUFA in the RWN medium indicated good quality of the produced biodiesel.

4. Conclusion

The present study demonstrated the feasibility of growing *C. vulgaris* in recycled culture medium under nutrient-repleted conditions. It was found that biomass concentration was maintained at around 1.3–1.5 g. L^{-1}, but the growth rate reduced with the increase in the number of recycles. The accumulation of elemental carbon content of *C. vulgaris* improved the CO_2 capture efficiency in the recycled medium. Meanwhile, lipid accumulation of microalgae in recycled medium seemed to be affected by the high concentration of nutrients (e.g., nitrate). The nutrient-rich environment in the recycled medium was observed to significantly alter the fatty acid composition of *C. vulgaris*, in which the microalgae in RWN (1–3) tended to produce PUFA and SFA as compared to the FM. Overall, the results suggested that cultivation of microalgae in the recycled medium represents a viable option to reduce the operating cost and water consumption of microalgae biorefinery. However, the main challenge of long-term utilization of recycle medium is potential accumulation of toxic metabolites which can halt the microalgae growth. Future exploration into the biochemical composition of microalgae as a function of nutrient concentration in the recycled medium could be helpful to understand the metabolic pathway of microalgae under nutrient repleted and depleted conditions.

Authors contribution

Yaleeni Kanna Dasan: Investigation, Conceptualization, Writing -

Table 2
FAME profile of microalgae cultivated in recycled medium reported in previous work.

Microalgae Species	Cultivation condition	Catalyst	FAME Composition			Reference
			SFA	MUFA	PUFA	
S. quadricauda	Heterotrophic	–	13.82	42.84	43.32	[67]
T. suecica	Mixotrophic	–	30.58	22.83	46.59	[67]
Chlorella vulgaris	Autotrophic (50x dilution)	H_2SO_4	26.6	11.9	61.5	[68]
Chlorella vulgaris	Autotrophic (100x dilution)	H_2SO_4	29.5	9.0	61.5	[68]
Chlorella vulgaris	Autotrophic (200x dilution)	H_2SO_4	22.9	10.0	67.1	[68]
Chlorella sp.	Autotrophic (Autoclaved centrate)	H_2SO_4	37.90	25.36	36.73	[69]
Chlorella sp.	Autotrophic (Raw centrate)	H_2SO_4	37.73	19.33	42.94	[69]
Chlorella vulgaris	Autotrophic	H_2SO_4	21.6–24.6	23.7–34.1	41.3–54.5	[70]

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