Comparative Performances of Microalgal-Bacterial Co-Cultivation to Bioremediate Synthetic and Municipal Wastewaters Whilst Producing Biodiesel Sustainably

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Keywords: biodiesel, lipid, Biomass, wastewater treatment, microalgal-bacterial cultures

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Abstract: The potentiality of a microalgal-bacterial culture system was explored in bioremediating wastewater while generating biomass for biodiesel production. A pre-determined optimal activated sludge and microalgal ratio was adopted and cultivation performance was evaluated in both synthetic and municipal wastewater media for nitrogen removal along with biomass and lipid generation for biodiesel production. The microalgal-bacterial consortium grown in the municipal wastewater medium produced higher biomass and lipid yields than those in the synthetic wastewater medium. The presence of trace elements in the municipal wastewater medium, e.g., iron and copper, contributed to the upsurge of biomass, thereby leading to higher lipid productivity. Both the microbial cultures in the synthetic and municipal wastewater media demonstrated similar total nitrogen removal efficiencies above 97%. However, the nitrification and assimilation rates were relatively higher for the microbial culture in the municipal wastewater medium, corresponding to the higher microbial biomass growth. Accordingly, the feasibility of the microalgal-bacterial consortium for bioremediating real municipal wastewaters was attested in this study by virtue of higher biomass and lipid production. The assessment of fatty acid methyl esters (FAME) composition showed the mixed microbial biomasses comprised 80–93% C16 to C18 FAME species, signifying efficient fuel combustion properties for quality biodiesel requirements.

Keywords: microalgal-bacterial cultures; wastewater treatment; biomass; lipid; biodiesel

1. Introduction

Since the conceptualization of peak oil theory in the 1950s, social unease about fuel insecurity and drastic climate change has led to the paradigm shift from fossil fuels towards renewables [1].

Despite late widespread use of the "biomass energy" phrase, biomass energy represents the main renewable already harnessed by combustion throughout several millennia, starting with the Stone Age to the period before the Industrial Revolution. Among the thermochemical conversion approaches that convert biomass to energy, biomass combustion is disputed for its unrestrained discharge of gaseous pollutants (e.g., NO_x, SO_2, CO, and CO_2) [2], while liquefaction, pyrolysis, and gasification are discouraged because of their high energy requirements due to elevated reaction temperatures (>300 °C) [3]. In contrast to thermochemical conversions, biological conversions offer a milder temperature range and thus a lower energy necessity in processing biomass to biofuels (e.g., H_2 and CH_4) or biodiesel precursor (microbial lipids) [4].

Oleaginous microorganisms, especially microalgae, garnered attention as a potential feedstock for sustainable biofuel production due to the high cell lipid content coupled with high growth tendencies [5]. Nonetheless, the commercialization of microalgal biodiesel is still hazy due to the high energy input requirements, namely, associated with cultivation (artificial lighting and aeration), separation (filtration, centrifugation, flotation, etc.), and drying stages [6]. On the other hand, in a conventional activated sludge process, the nitrifiers are only capable of oxidizing ammonia (NH_3) into nitrate (NO_3^-) and nitrite (NO_2^-), and the supplementation of external carbon substrate is often required to support the growth of denitrifiers for the reduction of NO_3^- and NO_2^- to N_2 [7]. For wastewater bioremediation, the integration of microalgae into activated sludge treatments is beneficial in terms of diversified abatement ability for various organic pollutants [8] and inorganic nitrogen species (NH_3, NO_3^-, and NO_2^-) [4]. Moreover, organic nutrients (such as nitrogen and phosphorus) found in wastewater will nourish the microalgae for rapid growth and enhanced metabolism rate, respectively [9]. Therefore, the co-cultivation of microalgae and activated sludge offers both economic and environmental prospects, employing the valuable microalgae feedstock for biodiesel production while bioremediating wastewater for compliance of discharge thresholds.

Recently, the microalgal-bacterial system had been touted as a promising alternative wastewater bioremediation approach by tapping into the symbiotic relationship between microalgae and bacteria [10,11]. Microalgal-bacterial symbiosis is often utilized in promoting the growth and production of microalgal biomass in the photobioreactor cultivation system [12,13]. The surge of biomass growth is attributed to the simultaneous exchange of organic and inorganic nutrients between the photosynthesis and respiration processes performed by the mixed microalgal-bacterial consortium [14]. In this study, municipal wastewater was used as the cultivation medium, alluded to its prevalence regardless of geographical area and relevance to household activities. Furthermore, simulated wastewater is often used for wastewater bioremediation studies, owing to possible batch and source variation of wastewater parameters; however, the presence of minerals is frequently disregarded given the difficulty of replication. Henceforth, this study aimed to examine the implications of a microalgal-bacterial system employed for the bioremediation of simulated and real municipal wastewaters. Accordingly, the nutrient removal efficiency as well as microbial biomass and lipid generation were compared in tandem thus, providing a clear understanding between the nutrient uptake or removal from wastewaters, often correlated with microbial population growth dynamics which are still limited to simulated studies and not extensively studied on real wastewater applications. Therefore, this research assessed microalgal-bacterial culture performance in degrading real municipal wastewater to attest to the feasibility of the co-cultivation system for possible application in actual wastewater treatment facilities. Finally, a quality assessment of microalgal biodiesel was conducted to compare the performance of the microalgal-bacterial co-cultivation system for biodiesel production from simulated and real municipal wastewaters.

2. Materials and Methods

2.1. Municipal Wastewater Source

The municipal wastewater source was collected from the primary clarifier at a sewage treatment facility situated in Silibin, Perak Darul Ridzuan, Malaysia. Characterisation of the wastewater sample

was undertaken according to the Standard Methods for the Examination of Water and Wastewater [15] while trace elements analysis was done via a microwave plasma atomic emission spectrometer (Agilent 4210 MP-AES). The determined compositions of the municipal wastewater sample are presented in Table 1 together with a modified municipal wastewater medium [16] deemed as synthetic wastewater medium to be used in this study. The components in the synthetic wastewater medium were as follows (in mg/L): $CaCl_2$ (42), $FeCl_3 \cdot 6H_2O$ (10), $MgSO_4$ (49), $NaHCO_3$ (354), NaH_2PO_4 (35), Na_2HPO_4 (180), $(NH_4)_2SO_4$ (226) and sucrose (109).

Table 1. Compositions of the synthetic and municipal wastewaters.

Parameter	Unit	Synthetic Wastewater	Municipal Wastewater
pH	-	8.1	7.8
Chemical oxygen demand, COD	mg/L	135	160–200
Ammonium-nitrogen, NH_4^+-N	mg/L	48	43–46
Nitrite-nitrogen, NO_2^-N	mg/L	-	-
Nitrate-nitrogen, NO_3^--N	mg/L	-	1–3
Arsenic	mg/L	-	0.02
Cadmium	mg/L	-	0.03
Copper	mg/L	-	0.65
Iron	mg/L	1.99	5.51
Lead	mg/L	-	0.17
Manganese	mg/L	-	0.04
Nickel	mg/L	-	0.02
Zinc	mg/L	-	0.37

2.2. Activated Sludge and Chlorella vulgaris Culture

Samples of activated sludge were obtained from the local municipal sewage treatment facility and inoculated for acclimation to the synthetic wastewater medium in an 18 L working volume sequencing batch reactor (SBR). The operation of the SBR was set at a 24 h cycle with the respective sequencing periods as follows: instant FILL (0 h); aerobic REACT (10 h); SETTLE, (1.5 h); DRAW (1 h) and IDLE (11.5 h). During the instant FILL period, approximately 14 L of the synthetic wastewater medium was introduced into the SBR whereas the same volume of treated effluent was drawn off during the DRAW period for each operational cycle. The sludge age was maintained at 40 days by discarding the surplus activated sludge biomass from the SBR.

The freshwater microalgal species (500 mL) i.e., *Chlorella vulgaris*, obtained from the culture collections belonging to the Centre for Biofuel and Biochemical Research (CBBR), Universiti Teknologi PETRONAS, was inoculated in a 5-L bottle with 4.5 L of the synthetic wastewater medium as the cultivation medium. The photobioreactor was illuminated with white light-emitting diode (LED) light at the light intensity of 1200 lux and aerated with compressed air, continuously. The initial microalgal inoculation pH was regulated to 7.1 ± 0.1.

2.3. Co-Cultivation Setup in Synthetic and Municipal Wastewater Media

The activated sludge and microalgal cultures acclimated to the synthetic wastewater medium were used for the co-cultivation performance investigation. A pre-determined optimal inoculation ratio of 1:0.75 (activated sludge to microalgae) [7] was selected as the initial inoculation culture ratio and the culture performance in terms of microbial biomass growth and nutrient removal efficiencies were assessed in two different cultivation media, namely, the synthetic and municipal wastewater media. Approximately 900 mL of each medium was separately mixed with a 100 mL of mixed microbial culture loaded with 100 mg activated sludge and 75 mg microalgae in a 1-L Erlenmeyer flask. The bioreactors were illuminated with white LED light at the light intensity of 1200 lux and aerated with compressed air, continuously. The cultivation temperature and pH of the bioreactors were maintained at 27 ± 1 °C and 7.1 ± 0.1, respectively until the stationary growth phase was attained indicating experimental termination. Samplings were taken on a daily basis for analysing the concentrations of chemical

oxygen demand (COD), nitrogen species (NH_4^+-N, NO_2^--N and NO_3^--N) for effluent analysis and the microbial biomass concentrations for growth analysis. Once the stationary growth phase was attained, the microbial biomasses from the experimental co-cultivation bioreactors were harvested via gravitational sedimentation and washed with distilled water twice before being oven-dried at 105 °C for further analyses. All experimental runs were conducted in duplicate.

2.4. Microbial Biomass Lipid Extraction and Transesterification

The harvested microbial biomasses were subjected to lipid extraction with a methanol and chloroform mixture at the ratio of 2:1 following Bligh and Dyer [17]. The extraction process was repeated twice, and the extracted lipid was subjected to solvent evaporation (air-dried) before being oven-dried at 105 °C.

Prior to transesterification process, 1 mL of tetrahydrofuran was introduced into the vial containing the extracted lipid to promote sample mixing. A 2 mL of methanol and 0.1 mL of concentrated sulfuric acid was added then, the transesterification process was reacted in an incubator shaker operated at 60 °C and 200 rpm for 3 h. The product was allowed to cool to room temperature and was added with 3 mL of 10 wt.% sodium chloride solution, 3 mL of hexane containing internal standard (0.6 mg C17:0/mL hexane) and 3 mL of distilled water. The mixture was vortexed to ensure homogenous mixing followed by centrifugation at 6000 rpm for 5 min. The upper layer containing the mixed fatty acid methyl esters (FAME) and hexane was drawn out into a separate vial for FAME analysis by gas chromatography (Shimadzu GC-2010) equipped with a flame ionization detector (FID) and a capillary column (BPX-BD20) using helium as the carrier gas. The operating conditions was set at initial temperature of 150 °C and programmed to increase to 240 °C with a ramping rate of 15 °C/min whereas the temperature for FID and injector was set at 250 °C.

2.5. Analytical Procedures and Calculations

The microbial biomass concentration was analysed gravimetrically by collecting 10 mL of sample followed by centrifugation at 2800 rpm for 20 min. The supernatant was collected for effluent characterization of concentrations of COD and nitrogen species following the Standard Methods [15] and the residual biomass was oven-dried at 105 °C to the constant weight.

The kinetics of the microbial biomass growth was modelled using the Verhulst logistic kinetic model as expressed by Equation (1):

$$\frac{\delta X(t)}{\delta(t)} = kX(t)\left[1 - \frac{X(t)}{X_{max}}\right] \tag{1}$$

Integrating Equation (1) will yield Equation (2):

$$X = \frac{X_{max}}{1 + Ae^{-kt}}; A = \frac{X_{max} - X_0}{X_0} \tag{2}$$

where X is the microbial biomass concentration (g/L) at arbitrary time, X_{max} is the maximum microbial biomass concentration (g/L), X_0 is the initial microbial biomass concentration (g/L), A is the overall biomass growth constant, k is the specific growth rate (1/d), and t is the time (d). The kinetics of the microbial biomass growth were simulated with the mathematical software tool, MATLAB R2020a, to obtain the values of X_{max}, k and A.

The total microbial lipid was determined gravimetrically while the lipid content (%) of the microbial biomass recovered was calculated using Equation (3):

$$Lipid\ content\ (\%) = \left(\frac{M_L}{M_B}\right) \times 100\%, \tag{3}$$

where M_L and M_B are the weights of the extracted lipids and the dry microbial biomass, respectively.

The FAME composition, C_{FAME} (%) was calculated using Equation (4):

$$C_{FAME}(\%) = \left(\frac{A_{comp}}{A_r - A_{I.S}}\right) \times 100\% \quad (4)$$

where A_r is the total peak area from C6 to C24, $A_{I.S}$ is the peak area of the internal standard (methyl heptadeconatoate) and A_{comp} is the peak area of the individual component exist in the FAME profile.

3. Results and Discussion

3.1. Microbial Biomass Growth and Kinetics

The microbial biomass growth patterns cultivated under synthetic and municipal wastewater media are shown in Figure 1. The microbial culture in the municipal wastewater medium observed a relatively plateaud initial lag phase as evidenced by the relatively little biomass growth from day 0 to day 3 as compared to the culture cultivated in the synthetic wastewater medium. The microbial culture in the synthetic wastewater medium experienced an initial upsurge in biomass growth (days 0–2) accompanied by a short lag phase (days 2–4). Such differences exhibited between the cultures between the two wastewater cultivation media were due to the presence of refractory organic carbon compounds detected in the municipal wastewater composition which were tougher to degrade as compared to those in the synthetic wastewater medium which contains a readily biodegradable carbon source from sucrose. The residual COD concentrations detected in the municipal wastewater medium of about 80.30 mg/L ± 4.76 mg/L further attested to the presence of incomplete degradation of the said refractory organic compounds, proving the difficulty to be degraded completely. Thus, the microbial consortium would require a timely acclimation period to adapt to the municipal wastewater medium from the presence of the refractory organic compounds as evidenced by the initial plateau and longer lag phase as exhibited by the microbial culture in the municipal wastewater medium (Figure 1). Even so, the remaining COD concentrations in the municipal wastewater medium still obeyed the safe effluent discharge standards (<125 mg/L) based on the European Union effluent discharge standards. When compared with the cultivation in synthetic wastewater medium, the said biodegradable COD sources were completely depleted within the first hour of aerobic activity, resulting in the initial upsurge in biomass concentrations due to immediate assimilation of available carbon sources into the microbial biomasses. Any trace of COD concentrations remained undetected throughout the entire cultivation period after that.

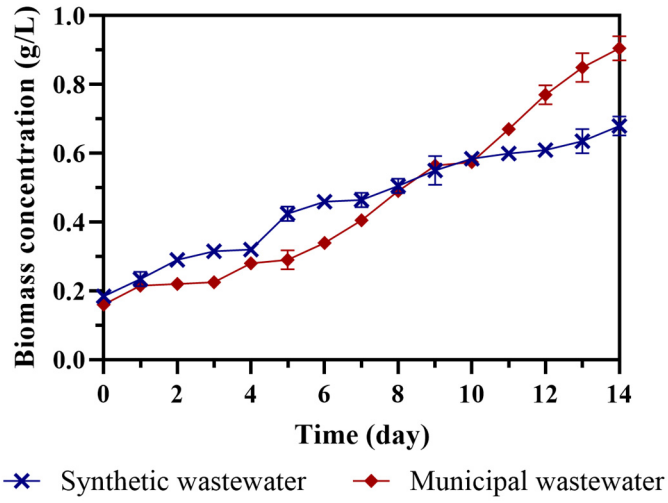

Figure 1. Microbial biomasses time course profile cultivated in synthetic and municipal wastewaters.

Eventually, the microbial culture under municipal wastewater medium experienced a swift growth rate from day 3 onwards; perhaps it had been acclimatized to the wastewater medium. The growth then surpassed the microbial culture under synthetic wastewater medium between days 10 and 14 (Figure 1). In this regard, the final biomass yields of 0.93 g/L ± 0.03 g/L were achieved under cultivation with municipal wastewater medium as opposed to those cultivated using synthetic wastewater medium with the final biomass yields of merely 0.68 g/L ± 0.02 g/L. The higher biomass production observed in the microbial consortium cultivated using municipal wastewater medium was likely to be caused by the availability of trace elements in the cultivation medium, especially iron (5.51 mg/L) and copper (0.65 mg/L) concentrations in the municipal wastewater medium composition (Table 1). These trace elements were proven to have crucial roles in cellular metabolic activities, hence affecting the uptake rate of main nutrients in the microalgal cells. For instance, the iron trace element is required in synthesizing chlorophyll pigments and carbon dioxide (CO_2) fixation which are both vital processes for cell growth via photosynthesis. Meanwhile, the copper trace element is responsible for CO_2 absorption in the photosynthesis process [18].

The kinetics of the microbial biomass growth were subsequently modelled using the Verhulst logistic kinetic model to predict the mixed microbial biomasses growth performances in the synthetic and municipal wastewater media (Table 2). Cultivation using the municipal wastewater medium recorded a higher specific growth rate (k) of 0.26/d ± 0.01/d and maximum biomass production (X_{max}) of 1.00 g/L ± 0.03 g/L when compared with the use of synthetic wastewater medium which only recorded 0.23/d ± 0.01/d (k) and 0.73 g/L ± 0.05 g/L (X_{max}). These reflected values were in conformity with the higher biomass yields obtainable by the mixed microbial consortium cultivated in the municipal wastewater medium than in the synthetic wastewater medium. On justifying the reliability of the Verhulst model employed in the study, the R^2 values recorded for both the variables were all above 95%, indicating possible model reproducibility. The application of microalgal-bacterial co-cultivation in real municipal wastewaters seemed feasible concerning the dynamics of microbial biomass growth performance in the wastewater media.

Table 2. Biomass growth kinetics of the microbial biomasses cultivated in synthetic and municipal wastewaters.

Microbial Biomass Cultivation	Specific Growth Rate, k (1/d)	Maximum Biomass Production, X_{max} (g/L)	Overall Biomass Growth Constant, A	R^2 Value
Synthetic wastewater	0.23 ± 0.01	0.73 ± 0.05	2.67 ± 0.01	0.98
Municipal wastewater	0.26 ± 0.01	1.00 ± 0.03	4.66 ± 0.18	0.96

3.2. *Synergistic Nitrogen Removal*

Nutrient removal in a microalgal-bacterial co-culture stems from the synergistic associations between the bacterial and microalgal consortia, leading to higher cellular metabolism rates and efficient exchange of both organic and inorganic nutrients in the microbial consortium. With emphasis on the nitrogen nutrient, the mechanism behind the synergistic associations in the microalgal-bacterial cultivation was revealed to be from the concurrent nitrification and assimilation processes, resulting in a more efficient nitrogen uptake for useful biomass generation or removal of nutrients via bioremediation. Accordingly, the bacteria (activated sludge) were capable of converting the available NH_4^+-N in wastewater media into the oxidized form of nitrogen species (NO_2^--N and NO_3^--N) via a nitrification process and these oxidized forms of nitrogen would, in turn, be assimilated into the microalgal biomasses at a much higher uptake rate due to the preferential uptake of nitrogen species [7]. Moreover, the prior nitrification of NH_4^+-N into oxidized forms of nitrogen would prevent the occurrence of free ammonia or ammonia toxicity at alkaline conditions which may be detrimental to the microbial consortiums in bioremediating the nutrients in the wastewater [19,20]. The profiles of nitrogen removal of the microbial biomasses cultivated in the synthetic and municipal wastewater media were depicted in Figure 2. Overall, both the microbial cultures cultivated either in synthetic or municipal wastewater recorded identical total nitrogen removal rates where all the nitrogen species were depleted by day 12

thus achieving above 97% of total nitrogen removal efficiencies, which was also in conformity with the optimal activated sludge and microalgal co-cultivation ratio determined in a previous study [7].

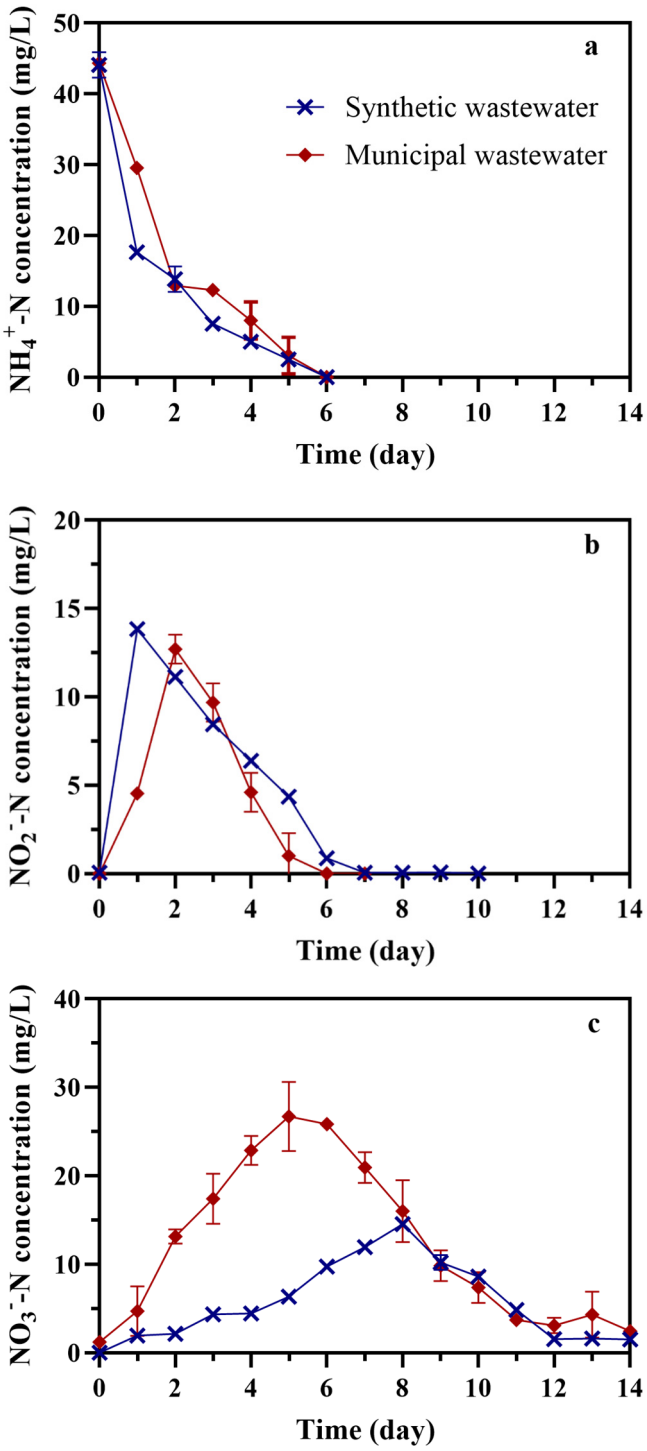

Figure 2. Nitrogen removal profiles of the microbial biomasses cultivated in synthetic and municipal wastewaters. (**a**) NH_4^+-N; (**b**) NO_2^--N; (**c**) NO_3^--N.

While the overall total nitrogen removal performances of both the microbial cultures in the synthetic and municipal wastewater media were similar, the nitrogen removal rates in terms of specific nitrogen species (NH_4^+-N, NO_2^--N and NO_3^--N) exhibited by the cultures were different. The microbial culture in the municipal wastewater medium experienced a slower initial NH_4^+-N

removal rate as compared to that cultivated in synthetic wastewater medium (days 0–2) (Figure 2a). As with the microbial biomass growth behavioral patterns, the microbial culture in the municipal wastewater medium had a much longer acclimatization period as compared to that cultivated using synthetic wastewater medium due to the difference in degradability of the available biodegradable COD sources. Nevertheless, the microbial culture in the municipal wastewater eventually removed all the NH_4^+-N species by day 6 which was the same as synthetic wastewater medium. However, the NH_4^+-N removal profile alone would not be able to give a clear insight into the differences when it comes to nitrification and assimilation processes in the mixed microbial consortium.

On the other hand, the NO_2^--N and NO_3^--N removal profiles of the microbial consortium would be able to reflect on the nitrification and assimilation processes in the cultures. The similar NO_2^--N profiles exhibited by both of the cultures (Figure 2b) indicated that the nitritation rates were almost similar in the sense that both accumulated up to 12–13 mg/L of NO_2^--N concentrations (days 2–3), followed by a continuous reduction leading to a complete removal by days 6–7. As for nitratationrates which were reflected by the NO_3^--N profiles (Figure 2c), the microbial culture in the municipal wastewater medium accumulated up to 26 mg/L of NO_3^--N concentration by day 5 which was higher than the NO_3^--N concentration accumulated in the synthetic wastewater (14 mg/L by day 8). The higher NO_3^--N concentrations accumulated in the municipal wastewater medium indicated higher nitratationrate which also led to a higher NO_3^--N assimilation rate as the microalgal consortia in the mixed microbial consortium would have more NO_3^--N concentrations available to be assimilated into their biomasses. Again, the symbiotic metabolisms in the microalgal-bacterial culture revolved around the concurrent nitrification and assimilation activities by the bacteria (activated sludge) and microalgal consortium, respectively, by which the NH_4^+-N was converted into oxidized forms of nitrogen species, i.e., NO_2^--N and NO_3^--N. These oxidized nitrogen species made it relatively easier to be assimilated into the microalgal biomasses hence, resulting in a more effective and higher nitrogen removal efficiencies. With the higher amount of NO_3^--N concentrations available in the municipal wastewater medium, the microbial culture eventually experienced a swift biomass growth from day 3 onwards (Figure 1) as the accumulated NO_3^--N concentrations were rapidly assimilated into the microalgal biomasses; corresponding to the decrease or removal in NO_3^--N concentrations in the medium (Figure 2c). Therefore, this led to a higher maximum microbial biomass attainability from the municipal wastewater cultivation medium.

3.3. Lipid Production and Fatty Acid Methyl Esters (FAME) Profile

The microbial lipid recovered from the microbial consortium under municipal wastewater medium generated higher lipid yields at 0.23 g/L ± 0.02 g/L as compared to the microbial lipid yields attained from the synthetic wastewater medium which was only at 0.13 g/L ± 0.01 g/L. The higher lipid yield recovery from the microbial culture in municipal wastewater medium corresponded to the higher biomass yield recorded using municipal wastewater medium of 0.93 g/L ± 0.03 g/L as opposed to biomass yield from synthetic wastewater medium (0.68 g/L ± 0.02 g/L). Moreover, the overall lipid content obtained in the microbial culture in the municipal wastewater medium was much higher as well at 22.68% ± 1.48% than that in the synthetic wastewater medium (19.56% ± 0.01%). The higher lipid content observed in the microbial culture under municipal wastewater medium was attributed to the higher NO_3^--N accumulation in the municipal wastewater medium (Figure 2c). This possibly led to an increase in acetyl-CoA carboxylase activity or other key enzymes which increased the lipid production in microalgal cells [21]. Also, the higher lipid content may be plausible due to the availability of trace elements in the cultivation medium, e.g., iron in the municipal wastewater composition which spurred the cell lipid accumulation, resulting in higher lipid attainability without compromising the biomass generation as well. The role of the iron trace element in inducing cell lipid accumulation was further supported by Liu et al. [22] whereby optimized iron concentrations could elevate the cell lipid content up to 56.6%, a 3–7 times higher lipid content in *Chlorella vulgaris* cultures.

The FAME compositions of the microbial consortium cultivated in the synthetic and municipal wastewaters are summarized in Table 3. The table includes saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and others. The dominant FAME composition in the mixed microbial consortium was determined to be C16:0 (palmitic), followed by C18:1 (oleate), C18:2 (linoleate) and C18:3 (linolenate), comprising about 80–93% by FAME content. The composition was as well in conformity with the requirements of quality biodiesel production [23]. The presence of C16 to C18 FAME species in the biodiesel would induce kinetic viscosity (C16:0; C18:0 and C18:1) and promote fuel–air mixing (C18:2 and C18:3) thus enhancing the combustion properties of the biodiesel [24,25]. Interestingly, there were other FAME components present in miniscule amounts for the microbial consortium cultivated in municipal wastewater medium which were absent in synthetic wastewater medium. The FAME components were C6:0 (hexanoate), C8:0 (octanoate), C11:0 (undecanoate), C12:0 (dodecanoate), C20:0 (arachidate), C14:1 (myristoleate), C16:1 (palmitoleate) and C22:2 (*cis*-13,16-docosadienoate). The nutrient compositions in the municipal wastewater most likely contributed to the presence of these other FAME components in the mixed microbial consortium, particularly the trace elements, which may have slightly altered the FAME compositions but not with a significant effect on the overall biodiesel quality. The percentage of SFAs in the microbial consortium comprised the largest FAME content of 43–45%, followed by PUFAs (27–28%) and MUFAs (16–18%). Overall, the biodiesel quality derived from the mixed microbial cultures presented a balanced composition among the total saturated fatty acids and unsaturated fatty acids (MUFAs and PUFAs). When compared with axenic microalgal cultures, the percentage of SFAs in the microbial cultures of bacterial activated sludge and microalgal *Chlorella vulgaris* was nearly double of what was reported in microalgal cultures alone, ranging at about 20–26% [26,27]. Accordingly, the biodiesel with higher fractions of SFAs were deemed to be preferable as this renders low hazardous gas emissions, e.g., carbon monoxide, nitrogen monoxide, hydrocarbon and smoke [28,29].

Table 3. The fatty acid methyl esters (FAME) compositions derived from mixed microbial biomasses cultivated in synthetic and municipal wastewaters.

Carbon Type	FAME Species	FAME Content (%)	
		Synthetic Wastewater	Municipal Wastewater
Saturated FAME			
C6:0	M. hexanoate	-	0.68
C8:0	M. octanoate	-	0.21
C11:0	M. undecanoate	-	0.42
C12:0	M. dodecanoate	-	0.23
C14:0	M. tetradecanoate	0.57	0.87
C15:0	M. pentadecanoate	1.23	0.84
C16:0	M. palmitate	41.34	36.96
C18:0	M. stearate	2.83	3.08
C20:0	M. arachidate	-	0.24
Total		45.98	43.54
Monounsaturated FAME			
C14:1	M. myristoleate	-	0.13
C16:1	M. palmitoleate	-	0.54
C18:1	M. oleate	18.33	16.13
Total		18.33	16.81
Polyunsaturated FAME			
C18:2	M. linoleate	9.41	17.6
C18:3	M. linolenate	8.76	19.4
C22:2	M. *cis*-13,16-docosadienoate	-	0.36
Total		27.02	28.53
Total saturated and unsaturated FAME		91.34	88.88
Others		8.67	11.12

4. Conclusions

A comparison study evaluating the microalgal-bacterial culture performances in bioremediating synthetic and municipal wastewater, respectively, revealed that the microbial culture from the municipal wastewater medium yielded higher biomass and lipid productions of 0.93 g/L ± 0.03 g/L and 0.23 g/L ± 0.02 g/L, respectively, that those cultivated in the synthetic wastewater medium (0.68 g/L ± 0.02 g/L and 0.13 g/L ± 0.01 g/L, respectively). The higher biomass yields observed in the microbial consortium in the municipal wastewater medium were likely to be generated by the availability of trace elements in the cultivation medium, especially iron and copper in the municipal wastewater composition thus leading to higher lipid attainability from the microbial consortium cultivated in the municipal wastewater medium. The biomass growth kinetics showed higher biomass generated from the microbial consortium in the municipal wastewater medium with values of a specific growth rate of 0.26/d ± 0.01/d and maximum biomass production of 1.00 g/L ± 0.03 g/L as opposed to the synthetic wastewater medium (0.23/d ± 0.01/d and 0.73 g/L ± 0.05 g/L, respectively). Both the microbial cultures recorded total nitrogen removal efficiencies of above 97% at the end of the cultivation. However, the microbial cultivation under municipal wastewater medium observed more rapid nitrogen nitrification and assimilation rates due to the higher NO_3^--N concentration accumulation in the medium thus giving rise to the higher microbial biomass yields due to more efficient nitrogen uptake rates. Meanwhile, the FAME compositions derived from the microbial consortium of bacterial-activated sludge and microalgal *Chlorella vulgaris* are valued as requirements of quality biodiesel. The biodiesels comprised 83–93% in C16 to C18 FAME species for efficient fuel combustion properties. The microalgal-bacterial culture was proven to be feasible for application in the bioremediation of real municipal wastewaters if not better for generating useful biomass for sustainable biodiesel production.

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References

1. Renewable Energy: All You Need to Know. Available online: https://www.environmentalscience.org/renewable-energy (accessed on 2 October 2020).
2. Ge, S.; Yek, P.N.Y.; Cheng, Y.W.; Xia, C.; Mahari, W.A.W.; Liew, R.K.; Peng, W.; Yuan, T.-Q.; Tabatabaei, M.; Aghbashlo, M.; et al. Progress in microwave pyrolysis conversion of agricultural waste to value-added biofuels: A batch to continuous approach. *Renew. Sustain. Energy Rev.* **2021**, *135*, 110148. [CrossRef]
3. Cheng, W.; Chong, C.C.; Lee, P.; Lim, J.W.; Wu, T.Y.; Cheng, C.K. Syngas from palm oil mill effluent (POME) steam reforming over lanthanum cobaltite: Effects of net-basicity. *Renew. Energy* **2020**, *148*, 349–362. [CrossRef]
4. Wang, Y.; Cheng, C.; Kee, M.; Hong, W.; Fatt, L.; Yusup, S.; Setiabudi, H.D.; Tang, Y.; Lim, J.W. Identification of microbial inhibitions and mitigation strategies towards cleaner bioconversions of palm oil mill effluent (POME): A review. *J. Clean. Prod.* **2021**, *280*, 12434. [CrossRef]
5. Lam, M.K.; Lee, K.T. Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnol. Adv.* **2012**, *30*, 673–690. [CrossRef] [PubMed]
6. Rashid, N.; Ur Rehman, M.S.; Sadiq, M.; Mahmood, T.; Han, J.I. Current status, issues and developments in microalgae derived biodiesel production. *Renew. Sustain. Energy Rev.* **2014**, *40*, 760–778. [CrossRef]

7. Leong, W.H.; Lim, J.W.; Lam, M.K.; Uemura, Y.; Ho, C.D.; Ho, Y.C. Co-cultivation of activated sludge and microalgae for the simultaneous enhancements of nitrogen-rich wastewater bioremediation and lipid production. *J. Taiwan Inst. Chem. Eng.* **2018**, *87*, 216–224. [CrossRef]
8. Li, Y.; Chen, Y.F.; Chen, P.; Min, M.; Zhou, W.; Martinez, B.; Zhu, J.; Ruan, R. Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresour. Technol.* **2011**, *102*, 5138–5144. [CrossRef]
9. Leong, W.H.; Lim, J.W.; Lam, M.K.; Uemura, Y.; Ho, Y.C. Third generation biofuels: A nutritional perspective in enhancing microbial lipid production. *Renew. Sustain. Energy Rev.* **2018**, *91*, 950–961. [CrossRef]
10. Lee, C.S.; Lee, S.-A.; Ko, S.-R.; Oh, H.-M.; Ahn, C.-Y. Effects of photoperiod on nutrient removal, biomass production, and algal-bacterial population dynamics in lab-scale photobioreactors treating municipal wastewater. *Water. Res.* **2015**, *68*, 680–691. [CrossRef]
11. Liu, L.; Fan, H.; Liu, Y.; Liu, C.; Huang, X. Development of algae-bacteria granular consortia in photo-sequencing batch reactor. *Bioresour. Technol.* **2017**, *232*, 64–71. [CrossRef]
12. Ma, X.; Zheng, H.; Addy, M.; Anderson, E.; Liu, Y.; Chen, P.; Ruan, R. Cultivation of *Chlorella vulgaris* in wastewater with waste glycerol: Strategies for improving nutrients removal and enhancing lipid production. *Bioresour. Technol.* **2016**, *207*, 252–261. [CrossRef]
13. Uggetti, E.; Sialve, B.; Latrille, E.; Steyer, J.P. Anaerobic digestate as substrate for microalgae culture: The role of ammonium concentration on the microalgae productivity. *Bioresour. Technol.* **2014**, *152*, 437–443. [CrossRef] [PubMed]
14. Ramanan, R.; Kim, B.H.; Cho, D.H.; Oh, H.M.; Kim, H.S. Algae-bacteria interactions: Evolution, ecology and emerging applications. *Biotechnol. Adv.* **2016**, *34*, 14–29. [CrossRef]
15. Rice, E.W.; Baird, R.B.; Eaton, A.D.; Clesceri, L.S. *Standard Methods for the Examination of Water and Wastewater*, 22nd ed.; American Public Health Association: Washington, DC, USA, 2012.
16. Lim, J.W.; Lim, P.E.; Seng, C.E.; Adnan, R. Alternative solid carbon source from dried attached-growth biomass for nitrogen removal enhancement in intermittently aerated moving bed sequencing batch reactor. *Environ. Sci. Pollut. Res.* **2014**, *21*, 485–494. [CrossRef]
17. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef] [PubMed]
18. Quigg, A. Micronutrients. In *The Physiology of Microalgae*; Borowitzka, M.A., Beardall, J., Raven, J.A., Eds.; Springer: Cham, Switzerland, 2016; pp. 211–231. [CrossRef]
19. Liu, Y.; Ngo, H.H.; Guo, W.; Peng, L.; Wang, D.; Ni, B. The roles of free ammonia (FA) in biological wastewater treatment processes: A review. *Environ. Int.* **2019**, *123*, 10–19. [CrossRef] [PubMed]
20. Tan, X.B.; Zhang, Y.L.; Yang, L.B.; Chu, H.Q.; Guo, J. Outdoor cultures of *Chlorella pyrenoidosa* in the effluent of anaerobically digested activated sludge: The effects of pH and free ammonia. *Bioresour. Technol.* **2016**, *200*, 606–615. [CrossRef] [PubMed]
21. Sánchez-García, D.; Resendiz-Isidro, A.; Villegas-Garrido, T.L.; Flores-Ortiz, C.M.; Chávez-Gómez, B.; Cristiani-Urbina, E. Effect of nitrate on lipid production by *T. suecica*, *M. contortum*, and *C. minutissima*. *Cent. Eur. J. Biol.* **2013**, *8*, 578–590. [CrossRef]
22. Liu, Z.Y.; Wang, G.C.; Zhou, B.C. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresour. Technol.* **2008**, *99*, 4717–4722. [CrossRef]
23. Nayak, M.; Suh, W.I.; Lee, B.; Chang, Y.K. Enhanced carbon utilization efficiency and FAME production of *Chlorella* sp. HS2 through combined supplementation of bicarbonate and carbon dioxide. *Energy Convers. Manag.* **2018**, *156*, 45–52. [CrossRef]
24. Fernández-Linares, L.C.; Guerrero Barajas, C.; Durán Páramo, E.; Badillo Corona, J.A. Assessment of *Chlorella vulgaris* and indigenous microalgae biomass with treated wastewater as growth culture medium. *Bioresour. Technol.* **2017**, *244*, 400–406. [CrossRef]
25. Cheah, W.Y.; Show, P.L.; Juan, J.C.; Chang, J.S.; Ling, T.C. Microalgae cultivation in palm oil mill effluent (POME) for lipid production and pollutants removal. *Energy Convers. Manag.* **2018**, *174*, 430–438. [CrossRef]
26. Dasan, Y.K.; Lam, M.K.; Yusup, S.; Lim, J.W.; Show, P.L.; Tan, I.S.; Lee, K.T. Cultivation of *Chlorella vulgaris* using sequential-flow bubble column photobioreactor: A stress-inducing strategy for lipid accumulation and carbon dioxide fixation. *J. CO_2 Util.* **2020**, *41*, 101226. [CrossRef]

27. Rosli, S.S.; Lim, J.W.; Jumbri, K.; Lam, M.K.; Uemura, Y.; Ho, C.D.; Tan, W.N.; Cheng, C.K.; Kadir, W.N.A. Modeling to enhance attached microalgal biomass growth onto fluidized beds packed in nutrients-rich wastewater whilst simultaneously biofixing CO_2 into lipid for biodiesel production. *Energy Convers. Manag.* **2019**, *185*, 1–10. [CrossRef]
28. Hernández, D.; Riaño, B.; Coca, M.; Solana, M.; Bertucco, A.; García-González, M.C. Microalgae cultivation in high rate algal ponds using slaughterhouse wastewater for biofuel applications. *Chem. Eng. J.* **2016**, *285*, 449–458. [CrossRef]
29. Omidvarborna, H.; Kumar, A.; Kim, D.S. A laboratory investigation on the effects of unsaturated bonds and chain lengths of different biodiesel feedstocks on carbon dioxide, carbon monoxide, and methane emissions under low-temperature combustion. *J. Environ. Chem. Eng.* **2016**, *4*, 4769–4775. [CrossRef]