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ORIGINAL PAPER

Improved production of lipid contents by cultivating *Chlorella pyrenoidosa* in heterogeneous organic substrates

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Abstract

The study is aimed to enhance the productivity of microalgal culture by varying the organic and inorganic components during wastewater treatment. A model organism *Chlorella pyrenoidosa* (*C. pyrenoidosa*) was grown in four different sources of wastewater namely piggery, palm oil mill effluent (POME), mixed-kitchen, and domestic wastes. The growth efficacy of *C. pyrenoidosa* on POME was tested for their ability to remove nutrients. It was observed that POME showed the highest chemical oxygen demand of 700 mg L^{-1}. Meanwhile, the piggery waste had the highest amount of total nitrogen of 590 mg L^{-1}. *C. pyrenoidosa* species were reported to grow well with different nutrient sources and produce high levels of lipids. The highest content of chlorophyll *a* was obtained with POME (3 mg L^{-1}) and domestic wastes (2.5 mg L^{-1}). The optimum growth rate of *C. pyrenoidosa* was reported for POME as a substrate. Also, the results indicated the lipid content for POME (182 mg L^{-1}), domestic sample (148 mg L^{-1}), piggery (0.99 mg L^{-1}), and mixed-kitchen wastes (117 mg L^{-1}). The results above revealed that among the tested substrates, POME could be the best alternative for *C. pyrenoidosa* to improve the yield of lipids and ultimately, biofuels production. Therefore, the treatment of POME in wastewater using *C. pyrenoidosa* can boost clean technology and energy generation. In future studies, the screening of other waste effluents is needed to cultivate the microalgae and enhance biomass production to meet increasing energy demands and waste treatment applications.

Keywords *Chlorella pyrenoidosa* · Lipid content · Organic substrate · Wastewater

Introduction

In the last century, a great deal of research and development as well as applications has been devoted to waste management (Wu et al. 2009). A wide range of conventional and emerging approaches has been established to effectively reduce waste generation constitutes to an acceptable level to discharge the treated wastewater for reuse (Goh et al. 2019). The public demand for pollutant-free waste discharge to receiving waters has

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made decontamination of industrial wastewaters a top priority (Crini and Lichtfouse 2019). Wastewater treatment facilities are considered as factories (bio-refineries), where wastewater purification is carried out (Bertanza et al. 2018). Therefore, it is necessary to apply appropriate treatments (Avagyan 2008) for all water sectors with biomass production (Rezania et al. 2016).

Palm oil mill effluent (POME) is a viscous and thick brownish liquid, containing voluminous colloidal organic and inorganic particles (Hossain et al. 2019). It was proven that palm oil mills generate several environmentally harmful by-products (Zainal et al. 2018). This waste discharge called POME from the Malaysian palm oil industry is an affluent source of nutrients and carbon for exploitation in microalgae cultivation (Kamyab et al. 2016a). Palm oil and other industries in Malaysia generate significant amounts of wastewater every year (Tan and Lim 2019). According to the Department of Environment (DOE) of Malaysia, effluent produced by industries does not meet the standards and is not properly treated before being released into rivers and lakes (Resdi et al. 2016). To halt this serious issue in the industrial sectors, several studies were conducted recently (Kamarudin et al. 2015). However, a few reports have explored the lipid contents of microalgae grown in agro-wastewater such as POME (Kamyab et al. 2016b).

C. pyrenoidosa was once cultivated under photoautotrophic, mixotrophic, and cyclic light-autotrophic/dark heterotrophic conditions (Yang et al. 2000). As discussed by Rai et al. (2013), there was a remarkable increase in lipid content and lipid productivity from *Chlorella* sp. under mixotrophic cultures. Therefore, enhanced biomass productivity was achieved by using carbon sources (Rai et al. 2013). Microalgae could be used as an active agent to uptake nutrients (Kamyab et al. 2018a) and some toxic chemicals discharged from industrial sections into rivers or lakes (Avagyan 2011). The chemical composition of algal biomass varies from one species to another (Avagyan 2018). The lipid contents of algal cells ranging from 1 to 70% are novel raw materials for energy production (Avagyan 2018). Simultaneously, the lipid content in microalgae cultivated in artificial media solutions was reported to exceed 80% with different lipid classes (Chisti 2007). However, the phototrophic growth of microalgae alone was unable to ensure positive environmental impact, but must take into consideration the total greenhouse gas (GHG) emission of microalgal biofuel production and application (Avagyan 2018). Lipid contents from different sources of nutrients implied that microalgae bio-recycling coupled with cost-effective biomass production has unlimited potential that remains unused (Avagyan 2011). Growing microalgae in different wastewaters, such as artificial wastewater (Vo et al. 2019), municipal wastewater (Osundeko et al. 2019), agricultural wastewater (Shelef 2018), domestic waste water (El Asli et al. 2019), POME (Kamyab et al. 2016a), piggery (Raeisossadati et al. 2019), food waste (Ende and Noke 2019), have been investigated for more than half a century. Previously, it was shown that mixotrophic cultivation of microalgae *Chlorella pyrenoidosa* could be used for waste treatment; e.g. for second treatment process of piggery waste (Wang et al. 2012).

Microalgal biomass is helpful in producing different types of biofuels, including bioalcohols, biohydrogen, and biogas (Zabed et al. 2019). However, not much attention is dedicated to the evaluation of different microalgal cultivation systems in wastewater for biofuel/bioenergy and phytoremediation applications (Kamyab et al. 2016b). The idea and main purpose of this study was initiated and presented by Kamyab et al. (2018b) at the international conference on urban drainage modelling. This current paper is to fulfil and extend the work on microalgae cultivation with high lipid content by using different organic substrates. Therefore, the aim of this study is to analyse the biomass production, growth rate, and lipid content of mixotrophic growth of microalgae *C. pyrenoidosa* in various organic substrates such as POME, piggery, domestic, and mixed-kitchen wastes. Sample collection, OD, COD, chlorophyll *a* content, MLSS, MLVSS CDW, TN, and biomass production, lipid content are discussed in detail. Furthermore, the biomasses of *C. pyrenoidosa* grown in piggery, POME, mixed-kitchen, and domestic wastes were investigated with respect to lipid content of the cultured microalgae, *C. pyrenoidosa* growth rate, and biomass production.

Materials and methods

For comprehensive understanding, besides the nutrient removal efficiency, microalgal growth, biomass production, and lipid content were also monitored during cultivation in the various organic substrates, such as POME, piggery, domestic, and mixed-kitchen waste. This research is unique such that it involved a set of physicochemical approaches that allow for a holistic view of microalgal cultivation, from growth monitoring and lipid production for the different sources of wastes as nutrients.

Sample collection

Researchers gained the POME from various ponds at Felda's palm oil mill, Kulai, Malaysia. Before the test, POME was kept at normal room temperature. The raw piggery, mixed-kitchen, and domestic wastes were obtained from the piggery industry at Universiti Teknologi Malaysia (UTM). The Desa Bakti River was chosen for collecting the domestic waste of a stabilisation pond (Rezania et al. 2016). In addition, random domestic restaurants within UTM were also

selected to collect the mixed-kitchen wastes (Kamyab et al. 2015). All specimens were assimilated in 3-month intervals and kept at 4 °C for a month to prevent any contamination. Sludge and debris were isolated using a vacuum filtered through 1-µm glass fibre filters, followed by 0.45-µm nylon membrane filters (Teknokroma, Barcelona, Spain) for an hour (Kamyab et al. 2016b).

Microalgae and cultivation procedure

The cells of *Chlorella pyrenoidosa* (University of Texas, Austin, 1230) were grown in Bold's basal medium (BBM) as observed in Table 1. Stock cultures that were kept in a 1-L glass photobioreactors were aerated through 0.2-µm air filter and then stirred to be well mixed (Beuckels et al. 2015). By adding a Tris buffer, the culture was buffered at pH 8. These cultures were irradiated with daylight fluorescent tubes from one side, with the intensity of light being 150 mol m^{-2} s^{-1} at the surface of the Erlenmeyer flasks (Kamyab et al. 2016a). The structures of the original BBM were altered in order to increase the growth rate as well as prevent nutrient limitations (Chen et al. 2011).

Preceding each test, inoculums for cultivation were set up by transferring *C. pyrenoidosa* aseptically from agar plates to a 250-mL container, including BBM of 100 mL. Then, *C. pyrenoidosa* was incubated in a shaker under light of 150 µmol/m^2 s at 28 °C and 100 rpm for 2 days. Inoculums of *C. pyrenoidosa* cells were collected in the exponential point from the container after a 2500 rpm centrifugation for 10 min, and it was suspended again in BBM of 1.5 L to accomplish a cell with 10^6 cell/mL concentration (Kamyab et al. 2016c). Next, the 1.5 L *C. pyrenoidosa* was put in a bigger flask and *C. pyrenoidosa* was acceptable to acclimatise the culture medium over 12 h. Optical density, chlorophyll *a*, pH, temperature, and content were recorded each day for 20 days (Yoon et al. 2008). Culture time lasted about 20 days till the stationary phase was attained, i.e. when the concentration of the cell remained constant (Chen et al. 2011). Different cultivation indicators, such as chemical oxygen demand (COD), optical density (OD_{600}), mixed liquor volatile suspended solid (MLVSS), mixed liquor suspended solid (MLSS), chlorophyll *a* content, cell dry weight (CDW), and total nitrogen (TN), were specified to introduce the appropriate substrates for the high content of lipids at *C. pyrenoidosa* cultivation (Órpez et al. 2009).

Table 1 Bold's basal medium preparation methods. *Source*: Kamyab et al. (2016a)

No	Stock	Stock solution (g L^{-1})	mL L^{-1}
1	KH_2PO_4	17.5	10
2	$CaCl_2.2H_2O$	2.5	10
3	$MgSO_4{\cdot}7H_2O$	7.5	10
4	$NaNO_3$	25	10
5	K_2HPO_4	7.5	10
6	NaCl	2.5	10
7	Na_2EDTA	10	1
	KOH*	6.2	
8	$FeSO_4{\cdot}7H_2O$	4.98	1
	H_2SO_4 (conc.)	1 mL/L	
9.1	H_3BO_3	2.86	1
9.2	$MnCl_2{\cdot}4H_2O$	1.81	
9.3	$ZnSO_4{\cdot}7H_2O$	0.222	
9.4	$Na\ MoO_4{\cdot}5H_2O$	0.390	
9.5	$CuSO_4{\cdot}5H_2O$	0.079	
9.6	$Co(NO_3)_2{\cdot}6H_2O$	0.0494	
10	H_3BO_3	11.5	0.7

Optical density (OD), chemical oxygen demand (COD), and chlorophyll *a* content

Four mL of *C. pyrenoidosa* cells was harvested using a centrifuge tube at 5000 rpm for 5 min, and the supernatant was shifted into the glass cuvettes. The cuvettes were placed in the spectrophotometer (Shimadzu 160 A) to assess the optical intensity at 600-nm wavelength (APHA 1995). The standard protocol for measuring COD was performed to examine the water and wastewater (Clesceri et al. 1989). The aim of this experiment was to determine the *C. pyrenoidosa* potential in decreasing the COD of the wastewater. The cells of the algal were homogenised first via a vortex and then added to the COD vials. The samples were mixed and examined using a HACH DR 5000 spectrophotometer by applying the 5220 B method (Clesceri et al. 1989).

Eight mL of *C. pyrenoidosa* cells was harvested by centrifugation at 5000 rpm for 5 min. A pellet was put in a sonicator for a minute and resuspended in 1 mL of acetone of 90% aqueous solution (Clesceri et al. 1989). Subsequently, the pellet was separated by a 5-min centrifugation at 5000 rpm. In the end, the pellet was shifted to a cuvette to be measured. The standard method of 10200 H was used for analysis (Clesceri et al. 1989). Chlorophyll *a* content was estimated based on the equation below:

$$\text{Chlorophyll } a\ (\text{mg L}^{-1}) = \frac{26.7(64_b - 665_a) \times v_1}{v_2 \times L} \quad (1)$$

Determination of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS)

Biomass was attained with a pre-dried 0.45-µm Whatman paper filtration for an hour at 103 °C. Suspended volatile solids were gained by incinerating dried solids in the oven at 550 °C

for 15 min. The analysis was based on the standard methods of 2540.D; E for MLSS and MLVSS (Clesceri et al. 1989). Formulae that were employed to determine the volume of the samples are:

$$\text{MLSS}\,(\text{mg L}^{-1})\left(\frac{(A-B)\times 1000}{\text{Sample volume (mL)}}\right) \quad (2)$$

$$\text{MLVSS}\,(\text{mg L}^{-1})\left(\frac{(C-D)\times 1000}{\text{Sample volume (mL)}}\right) \quad (3)$$

A is the filter weight + dried residue (mg), B is the filter weight (mg), C is residue weight + pre-ignition dish (mg), and D is the residue weight + post-ignition dish or filter (mg).

Cell dry weight (CDW) and total nitrogen (TN) for *C. pyrenoidosa*

Ten mL culture of *C. pyrenoidosa* was taken and centrifuged at 4000 rpm for 15 min. The algal pellet was washed with distilled water. Biomass productivity is defined by Rao et al. (2007) as mg per L of dry biomass; the dry *C. pyrenoidosa* biomass weight was specified by gravimetrical method. Biomass productivity was calculated based on the following equations:

$$\text{Cell dry weight (mg)} = W_2 - W_1 \quad (4)$$

$$\text{Biomass productivity}\,(\text{mg L}^{-1}) = 1/t \cdot \frac{(W_2 - W_1)\,\text{mg}}{\text{volume}\,(L)} \quad (5)$$

W_1 is the mere weight of the tube of the centrifuge without the weight of the empty tube of the centrifuge (mg), W_2 is the weight of the tube of the centrifuge after it is freeze-dried (mg), while t is the experiment time (day) (Kamyab et al. 2016b).

An amount of 2 mL of *C. pyrenoidosa* suspension was taken and centrifuged at 4000 rpm for 10 min, and the supernatants were diluted and analysed for the TN concentration using a spectrophotometer, HACH DR 5000 (Kamyab et al. 2016a). The variations in nutrients and TN from the experiment were used to determine the *C. pyrenoidosa* growth.

Determination of lipid content

During cultivation, 10 mL samples were dewatered by centrifugation at 6000 rpm (Hermle Z206A, Germany) for 10 min and then dried at 70 °C for 24 h to obtain the dry biomass (Huang et al. 2015). Intracellular lipid determination and extraction were done based on the standard protocols from the literature (Bligh and Dyer 1959). The freeze-dried algal biomass (50–100 mg) was treated thrice with 10% methanol in DMSO and stirring for 50 min. After that, for 10 min, the mixture was centrifuged, and then the remnants were taken out. The remnants were treated again for 30 min twice with a mixture of diethyl ether and hexane (ratio of volume of 1:1). Then, water and hexane were added to the remnants to reach the ratio of 1:1:1:1 for each solvent. Then, for another 10 min, the mixture was shaken and centrifuged and afterwards 250 mg of the organic layer at the top was collected. After the water was treated twice with a mixture of diethyl ether and hexane (ratio of volume of 1:1), the organic phases were merged and vaporised to dry. After that, it was dissolved again with a little amount of hexane. The lipid solution was transferred to a pre-weighed vial and evaporated in a 30 °C water bath in the beginning by a rotatory evaporator and was later dried by a high vacuum (Liu et al. 2011). These dried remnants were kept under gaseous nitrogen and then weighed by an electronic microbalance. Each experiment was performed twice. The specimens were collected every 20 days for verification and investigation of the lipids produced by *C. pyrenoidosa*. Light intensity and temperature were constant throughout all the experiments. Concentration of biomass (g L^{-1}) was analysed from the dry weight of the produced microalgal biomass per one litre of culture. Lipid content was calculated as percentage mass in the dry biomass culture (Kamyab et al. 2016b).

Results and discussion

Growth performance/chemical oxygen demand (COD) and biomass production

The optical densities (OD_{600}) of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic waste are shown in Fig. 1, where a similar trend can be observed for both POME and domestic waste with maximum of 0.7

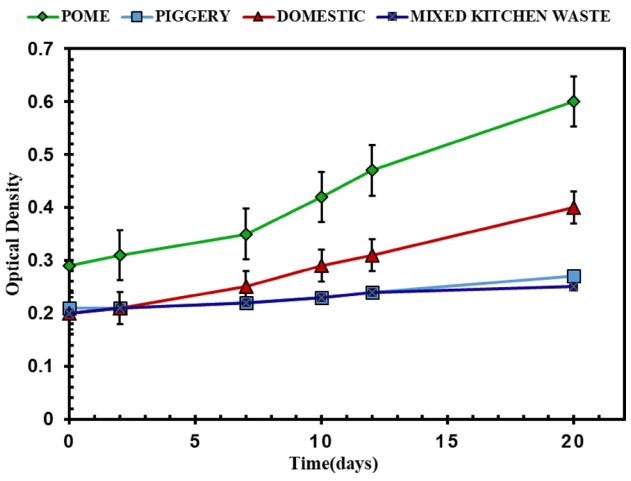

Fig. 1 Optical density (OD_{600}) versus time of microalgae cultivated in piggery, POME, mixed-kitchen, and domestic wastes as different organic substrates

OD_{600} and 0.4 OD_{600}, respectively. The results presented that the microalgae of the domestic wastes with POME were still in the exponential phase. In addition, the gradual growth in piggery with the final OD_{600} after 20 days was more than the OD_{600} value for mixed-kitchen waste, which showed that microalgae in piggery sample were in the stationary phase. The OD_{600} growth also demonstrated that the *C. pyrenoidosa* on the POME had the highest rate of growth. Microalgae can utilise nutrients from wastewater, while photosynthetically fixing carbon dioxide, a greenhouse gas, for growth. This indicates the potential of microalgae-based treatment as an economically (Yu et al. 2019). As discussed by Cheirsilp and Torpee (2012), the growth of *Chlorella* sp. and *Nannochloropsis* sp. was developed when they were cultivated under mixotrophic condition.

Figure 2 illustrates the chemical oxygen demand (COD) for each organic substrate through microalgae *C. pyrenoidosa* cultivation in piggery, POME, mixed-kitchen, and domestic wastes. The initial soluble COD concentration was observed at 180 mg L^{-1} for the mixed-kitchen waste. Moreover, piggery and domestic waste corresponded to 220 mg L^{-1} and 380 mg L^{-1}, respectively. POME had the largest COD of around 700 mg L^{-1} at the beginning of the experiment. After 20 days, COD values for POME, mixed-kitchen, and domestic wastes decreased; however, the COD of piggery remained constant. The higher COD value of POME showed that there was higher concentration of nutrients with more compounds available in POME than other substrates, which led to a rich cultivation of microalgae and high lipid content. However, domestic waste was the second alternative. Diluted piggery wastewater with 1900 mg L^{-1} COD provided an optimal nutrient concentration for *C. zofingiensis* cultivation (Zhu et al. 2013). Organic materials may function as an accessory growth factor or a vital organic nutrient (Kamyab et al. 2016a). Also, microalgal species such as mixotrophic species can also use light and inorganic substances for energy. Mixotrophy allows some algal species to tap organic nutrient pools and function at multiple trophic levels (Cloern and Dufford 2005). Lau et al. (1995) reported that nutrient uptake by microalgae had little effect on the removal of COD.

The values of COD for the biomass of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic wastes are shown in Fig. 3. POME had a higher biomass COD than the other waste samples. POME showed increased COD biomass during cultivation and a COD biomass production of 340 mg L^{-1}. In the case of piggery waste, a gradual increase can be seen until Day 20. In addition, mixed-kitchen and domestic wastes showed a similar increasing trend with an endpoint near to piggery waste of 130 mg L^{-1}. Mixed-kitchen and domestic samples showed a significant increase in biomass production COD from Day 0 to Day 6 and achieved a constant rate after Day 10. Research showed that the green microalgae *Chlorella* sp. cultivation eliminated 50.9–83.0% of COD in different municipal wastewaters (Wang et al. 2010). In contrast, bacteria could reduce 95% and 71% of COD in petroleum wastewater and POME (Neoh et al. 2016), respectively. Lalucat et al. (1984) reported that *Chlorella* sp. could grow autotrophically, heterotrophically, and mixotrophically on a variety of organic substrates.

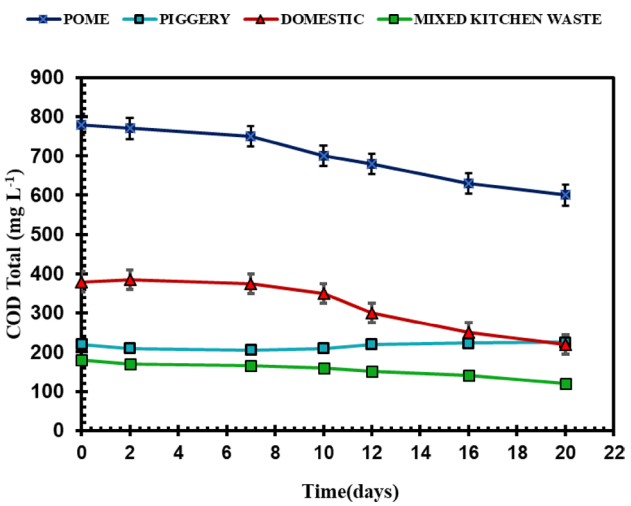

Fig. 2 Chemical oxygen demand (COD) total versus time for *C. pyrenoidosa* cultivation in piggery, POME, mixed-kitchen, and domestic wastes

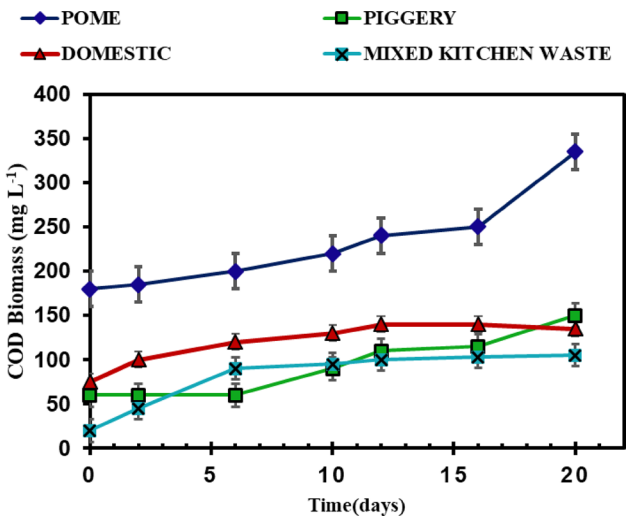

Fig. 3 Chemical oxygen demand (COD) biomass versus time of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic wastes as substrate

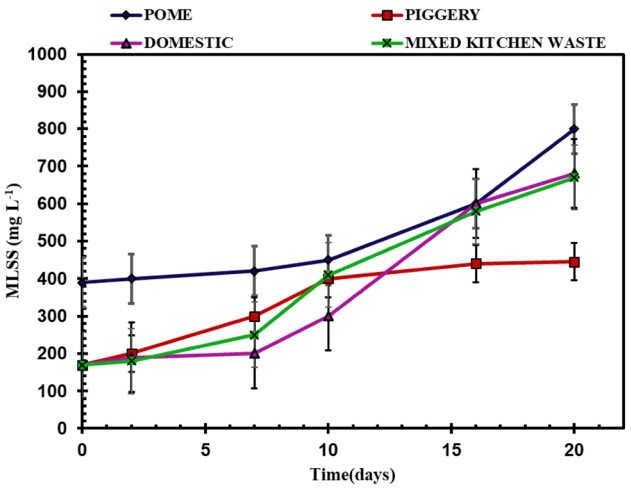

Fig. 4 Mixed liquor suspended solid (MLSS) versus time of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic wastes

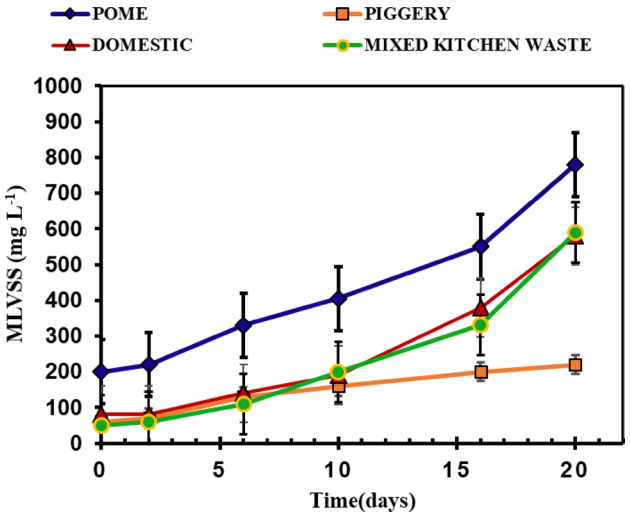

Fig. 5 Mixed liquor volatile suspended solid (MLVSS) versus time of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic wastes

Mixed liquor suspended solid (MLSS)/mixed liquor volatile suspended solid (MLVSS) and chlorophyll *a* content

Figure 4 illustrates the mixed liquor suspended solids (MLSS) of *C. pyrenoidosa* cultivated in piggery, POME, mixed-kitchen, and domestic wastes. Figure 4 shows that POME contained the largest MLSS of about 820 mg L^{-1} but the least rate of growth. On the other hand, domestic waste had the highest rate of growth. Meanwhile, mixed-kitchen waste rose to about 700 mg L^{-1}; the MLSS of *C. pyrenoidosa* cultured in piggery waste, however, rose only a bit from Day 0 to Day 10 but declined a little after, and then remained unchanged till Day 20, meaning that the microalgae growth in piggery waste achieved a stationary phase. After cultivation, the microalgae in mixed-kitchen waste had also reached the stationary phase of growth. Exponential growth rates of cultured *C. pyrenoidosa* in POME and domestic wastes were observed. Performance comparisons pertaining to the ratio of MLVSS/MLSS and lipid content of *C. pyrenoidosa* cultivated in POME were also found (Kamyab et al. 2016a). The comparison between lipid content and MLVSS/MLSS ratio was specified for POME, which showed that the content of lipids raised with the ratio of MLVSS/MLSS. The augmentation was caused by substrate consumption by *C. pyrenoidosa* that affected lipid accumulation and storage.

Figure 5 shows the MLVSS for piggery, POME, mixed-kitchen, and domestic wastes of cultured *C. pyrenoidosa* substrates. The results presented that POME got the most MLVSS in comparison with the other three substrates. This proved that the best nutrient for cultivating *C. pyrenoidosa* was indeed POME with the maximum MLVSS of around 750 mg L^{-1}. On the other hand, the least rate of growth was shown by the piggery waste. The low amount of *C. pyrenoidosa* in piggery waste meant that the waste was inert and unsuitable for microalgae cultivation. Ji et al. (2013) reported that the growth rate of *C. vulgaris* increased with a decrease in concentration of piggery wastewater in the culture media regardless of the diluent type.

Chlorophyll *a* and MLVSS contents are the most significant microalgae growth elements for determining whether microalgae could live in a specific medium of cultivation (Kamyab et al. 2016b). Figure 6 displays chlorophyll *a* content of *C. pyrenoidosa* cultivated in piggery, POME, mixed-kitchen, and domestic wastes. According to Fig. 6, an increasing trend can be observed for chlorophyll *a*

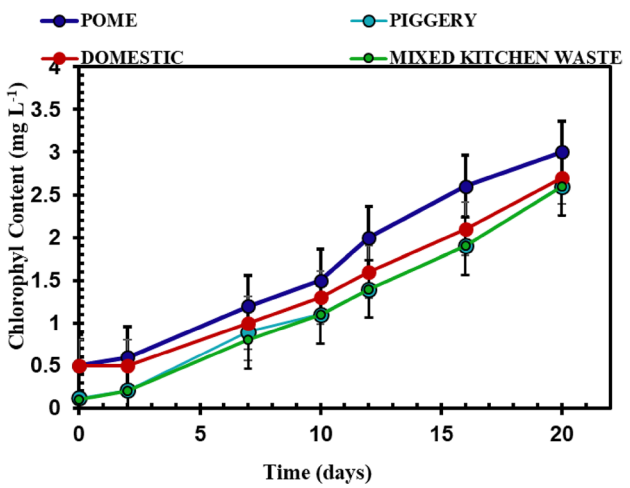

Fig. 6 Chlorophyll *a* content versus time of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic wastes

content for all four types of substrates. After treatment, POME and domestic waste had the first (3 mg L^{-1}) and second (2.5 mg L^{-1}) highest chlorophyll *a* content. This may be due to the addition of POME or domestic waste samples to microalgae before cultivation. Data for the piggery sample were almost the same as the mixed-kitchen waste. In a study conducted by Kamyab et al. (2016b), all microalgae used various types of carbon to achieve maximum chlorophyll *a* content. *C. sorokiniana* produced chlorophyll *a* of 0.59 mg L^{-1} day^{-1}, with a consumption of carbon in 43 mg L^{-1} day^{-1}.

Total nitrogen

Nitrogen is a vital nutrient to produce microalgae biomass. Availability of the nutrient has a great impact on biochemical composition of microalgae (Beuckels et al. 2015). Biomass nitrogen level may vary from 1 to 10%, depending on the amount, accessibility, or nitrogen source (Costa et al. 2001). Figure 7 shows that the TN concentration for the piggery sample was higher for other substrates. Yet, during the first 20 days, an overall decrease for all samples was observed. The highest TN concentration of 590–350 mg L^{-1} was observed for the piggery substrate, which was much more than the second highest TN concentration in substrate (POME) of 210–100 mg L^{-1}. In addition, the trends for the domestic substrate were similar to that of piggery from Day 0 to Day 20, but the TN concentration for the domestic sample was much lower than that of piggery sample with 53–200 mg L^{-1}. Both samples showed a significant decrease in TN concentration from day 6 to 12. Furthermore, the mixed-kitchen waste has the lowest nitrogen concentration of 114 mg L^{-1}, which dropped to 9 mg L^{-1}. The highest nitrogen removal was achieved by *C. mexicana* in piggery wastewater (Abou-Shanab et al. 2013). After 20 days of microalgae growth, TN concentration dropped from 53 mg L^{-1} (control) to 49 mg L^{-1}. Kothari et al. (2012) found that 80–85% of total phosphorus (TP) and 60–80% of total nitrogen (TN) could be removed by *C. pyrenoidosa* from the dairy wastewater. In contrast with organic carbon sources, microalgae were shown to adapt almost all forms of nitrogen to satisfy its nitrogen requirements (Li et al. 2011). It is figured out that at the nutrient limitation on microalgae was found to first accumulate carbohydrates and later lipids (Procházková et al. 2014).

Cell dry weight (CDW) and lipid content

Figure 8 illustrates the CDW of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic wastes. According to Fig. 8, the increasing trends of CDW for all four substrates were observed. It was clear that CDW in POME was the highest with 300 mg L^{-1} and 500 mg L^{-1} before and after 20 days of *C. pyrenoidosa* cultivation. This may be due to the nutrient concentration available in the sample. Domestic waste was the second highest with 400 mg L^{-1}, followed by mixed-kitchen waste and piggery with 310 mg L^{-1} and 290 mg L^{-1}, respectively. Figure 8 also shows the direct relationship between the type of substrate for cultivation and number of days for culturing *C. pyrenoidosa* in the specific substrate. The production of *C. pyrenoidosa* for biomass and lipid generation was optimised using a 5-L batch of microalgae culture for 2 weeks, where biomass reached 0.68 g $CDWL^{1}$ d^{-1} (Kamyab et al. 2016a). But a 250 mL batch of microalgae culture for 4 weeks produced a maximum of 0.009 g L^{-1} day^{-1} mg CDW^{-1} biomass.

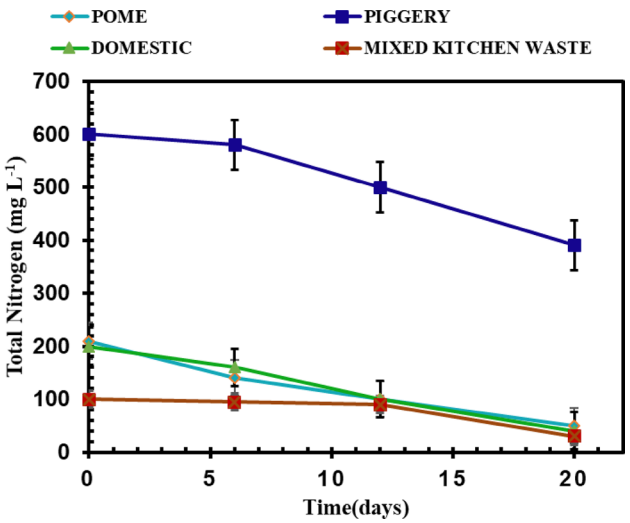

Fig. 7 Total nitrogen (TN) versus time for cultivation of microalgae in piggery, POME, mixed-kitchen, and domestic waste substrates

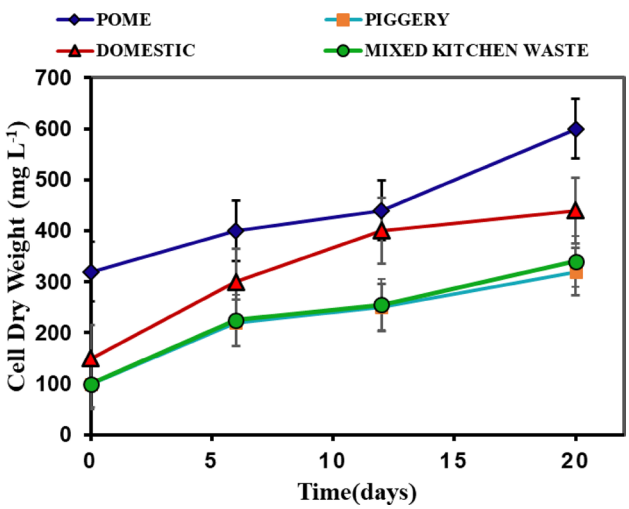

Fig. 8 Cell dry weight (CDW) versus time of cultivated microalgae in piggery, POME, mixed-kitchen, and domestic wastes as substrate

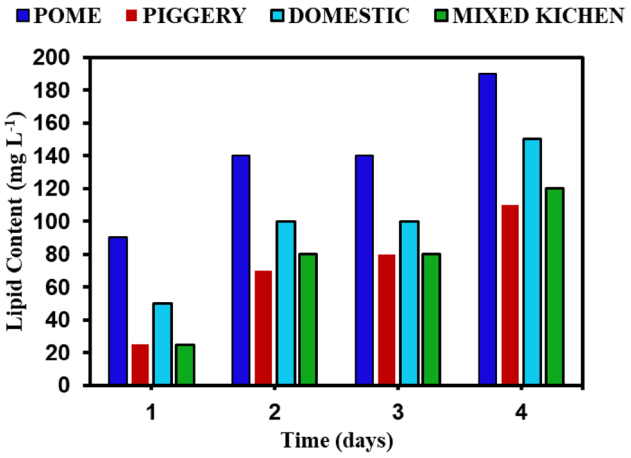

Fig. 9 Lipid content versus time of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic wastes as substrate

Cheirsilp and Torpee (2012) found that high concentration of glucose at the beginning negatively impacted the accumulation of lipid of the microalgae in mixotrophic condition. Liu et al. (2011) discovered below 40% of lipid content in the dry biomass with enriched nutrient contents; on the other hand, more lipid content could be accumulated if the microalgae were cultured in nitrogen deficiency conditions.

The content of lipid production of cultivated *C. pyrenoidosa* in piggery, POME, mixed-kitchen, and domestic wastes is shown in Fig. 9. According to Fig. 9, lipids were produced by *C. pyrenoidosa* in all the wastes. POME showed an increasing trend with lipid content ranging from 88 to 182 mg L^{-1}, meaning that POME is the best substrate with the most production of lipid in comparison with the other organic substrates. In the case of domestic waste, there was a rise from 46 to 148 mg L^{-1}, which meant that domestic waste is the next best option for high lipid production of *C. pyrenoidosa*. The raising trend of production of lipid in piggery and mixed-kitchen wastes was similar between 25 and 117 mg L^{-1}, and as such, piggery and mixed-kitchen wastes are the next best options for cultivation of *C. pyrenoidosa* with high lipid content. According to Kamyab et al. (2016b), the most and the least content of lipid for cultivated *C. sorokiniana* and *C. pyrenoidosa* microalgae in POME were 2.68 and 0.74 mg/mg CDW, respectively. A drop in the content of the nutrient was acknowledged from increased lipid concentrations (Converti et al. 2009). Moreover, ecological stresses like nitrogen limitation caused cell division inhibition with no major decline in oil or lipid generation (Widjaja et al. 2009). *C. pyrenoidosa* was the best species in the POME cultivation. In addition, *C. pyrenoidosa* produced the most lipid at Day 20 with 56 mg/L content (Kamyab et al. 2017). Cheirsilp and Torpee (2012) also found that the highest production of lipids was in mixotrophic culture that corresponds to the most biomass in the culture. It was reported in a previous study that green microalgae *C. pyrenoidosa* was one of the best oil producers based on their investigations, with the total lipid content of 51% of dry biomass (Liu et al. 2011).

Conclusion

Environmental pollution, especially water pollution, is at an alarming state all over the world. Microalgae growth and production of lipid were distinguished by a local microalgae applied to piggery, POME, mixed-kitchen, and domestic wastes. The results revealed that POME had the most COD in the beginning of the experiment (700 mg L^{-1}). Over 20 days, COD values for POME, mixed-kitchen, and domestic wastes dropped; however, the COD in piggery remained constant. The TN concentration of piggery was the highest at 590 mg L^{-1}. The alga *Chlorella* produced the most chlorophyll *a* when grown with POME and domestic waste, with 3 mg L^{-1} and 2.5 mg L^{-1}, respectively. It was concluded that the best rate of growth for *C. pyrenoidosa* was with POME. This study confirmed that the highest levels of CDW were observed in POME samples at 500 mg L^{-1} after cultivation for 20 days. The CDW levels for domestic, piggery, and mixed-kitchen waste were 400 mg L^{-1}, 290 mg L^{-1}, and 310 mg L^{-1}, respectively. POME exhibited the highest MLSS and MLVSS values among all substrates at 820 mg L^{-1} and 750 mg L^{-1}. Moreover, the least rate of growth was for the piggery waste. Little increase in the *C. pyrenoidosa* from the piggery waste suggested an inert substrate that was not viable for microalgae cultivation. The maximum value for the content of lipid was 182 mg L^{-1} for POME. Therefore, POME was the best substrate with the highest rate of lipid production among the four substrates due to the nutrient content in the samples. This was followed by domestic samples with 148 mg L^{-1}, making it a good second choice for lipid production. A similar trend for production of lipid was demonstrated by piggery and mixed-kitchen wastes with 25 mg L^{-1} and 117 mg L^{-1}, respectively. The results showed that POME is an ideal media for the growth of microalgae *C. pyrenoidosa* as it gave maximum lipid to produce biofuel. Algal biomass can be utilised to create a few types of biofuels; however, there are critical technological boundaries related to energetic equalisation and cost-competitiveness. Thus, microalgae are the real instruments for life improvement around the globe, and at present, it can be accepted as significant producers of atmospheric oxygen, just as it is the regular cleaner of the world's sea waters. It is the decision of nature.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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