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Screening and cultivation of oleaginous microalgae in industrial wastewater: Possible applications for wastewater treatment and feedstock production

Jarucha Yeesang[1]

[1]Department of Microbiology, Faculty of Science and Technology,
Nakhon Pathom Rajabhat University, Nakhon Pathom 73000, Thailand

Abstract

The aim of this research is to isolate local oleaginous microalgae from the Nakhon Chai Si and Thajeen river basins (Nakhon Pathom, Ratchaburi, and Samut Sakhon provinces) for potential use as feedstock for biodiesel fuel production. Colonies of microalgae were separated using a sterile micropipette washing method. Five strains of green microalgae (*Botryococcus s*p., *Chlorella* sp., *Scenedesmus* sp., *Volvax* sp. and *Dunaliella* sp.) and one of the cyanobacteria *Spirulina* sp. were isolated. The isolates were grown in selective media under photoautotrophic conditions in the laboratory. The results showed that *Chlorella* sp. yielded the highest amount of lipid production at 28.35% of dry cell weight. *Spirulina* sp. showed the lowest amount of lipid production at 7.75% of dry cell weight, but exhibited the highest specific growth rate (μ) at 0.412 d^{-1} when compared with other strains. The effects of varied physical-chemical conditions for cultivating *Chlorella* sp. in wastewater from an industrial plant were estimated. The results showed that wastewater from an instant food processing plant with a dilution rate with a basal medium at 1:10 (volume by volume), cultivated at light intensity 3,000 lux and 150 rpm agitation speed yielded a specific growth rate of 1.752 d^{-1} with 0.84 gL^{-1} of dry cell mass, 30.8% lipid production and yielded the highest lipid production rate at 53.96 $mgL^{-1}d^{-1}$. Moreover, the effectiveness of lipid production simultaneously with wastewater treatment by *Chlorella* sp. was also studied. The results demonstrated that when cultivating *Chlorella* sp. in a wastewater medium diluted with a basal medium at a dilution rate of 1:10 (volume by volume), *Chlorella* sp. could decrease the level of initial COD from 1,650 to 154 mgL^{-1} (90.67%) with a COD removal rate 195 $mgL^{-1}d^{-1}$. This set of conditions also yielded 1.4 gL^{-1} of dry cell mass with a specific growth rate 1.758 d^{-1} and 31.1% of lipid production, respectively. The fatty acids of lipid derived from isolated *Chlorella* sp. contains mostly palmitic acid (53.7%) and also contains similar fatty acids as those in plant oil. *Chlorella* sp. has potential for cultivation in wastewater from agricultural plants and could possibly serve as a model for better utilization of factory wastewater in the development of cost-effective biodiesel production in the future.

Keywords: oleaginous microalgae, wastewater treatment, lipid production

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1. Introduction

Approximately 80% of current global energy demand is met by the combustion of fossil fuels. However, intensive usage of fossil fuels has led to energy crises, global climate change and environmental pollution [1]. Many countries are thus turning their attention to the development of new, cleaner and more sustainable alternative energy sources. Among the various potential sources of renewable energy, biofuels are of high interest and these are expected to play a crucial role in the global energy infrastructure of the future. Microalgae are an effective intermediary that can convert carbon dioxide and solar energy into various forms of bio-energy (such as biodiesel, bio-ethanol, and bio-butanol) since they possess 20% higher photosynthetic efficiency rates compared to terrestrial plants [2]. On a per area basis, microalgae are already reported to produce 15–300 times more oil for biodiesel production than traditional crops [3]. Furthermore, compared with conventional crops that are usually harvested only once or twice a year, microalgae have very short harvest cycles (~1– 10 days, depending on the process and on species), allowing for multiple or even continuous harvests with significantly increased yields. The use of non-potable water and non-arable land for algal cultivation is also a viable option, as it uses far less water and avoids food crop displacement [4].

Wastewater from industrial plants contains a large amount of organic matter and nitrogenous compounds. It typically also contains highly concentrated pollutants, including suspended solids, organics and nutrients.

*E-mail: pond_rno@hotmail.com

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Due to its abundance of nutrients, wastewater can be used as a low-cost nutrient source for microalgae cultivation. Microalgae have high potential to remove nutrients from wastewater and to accumulate biomass for biofuel production [5, 6]. Algal treatment of wastewater offers a cheaper and more efficient means of removing nutrients and metals from wastewater than conventional tertiary treatment methods [7]. As it is the most cost-effective way to produce biofuel, microalgae cultivation in wastewater has been widely discussed by researchers worldwide. Many algal strains have the potential to grow in mass culture and are able to accumulate lipids when they are subjected to stress [8]. Some species are generally sensitive to different types of wastewater with imbalanced nutrient profiles, the presence of inhibiting pollutants in wastewater, and deficiencies in certain essential trace elements. Typically, *Chlorella* sp. and *Scenedesmus* sp. are more tolerant across a wide range of wastewater profiles than other strains. The efficiency of microalgal growth to assimilate nutrients is indicated by the microalgal biomass, lipid content, carbohydrate content, dry cell weight and biomass productivities. The performance of microalgae cells is strongly dependent on the microalgal species itself and the growth conditions. The following sections focus on the growth of microalgae cultivated in wastewater, as well as their pollutant removal efficiencies [9]. In this study, the microalgae were isolated from samples collected from different locations in the Nakhon Chai Si and Tha-jeen river basins of Thailand. Several algal species were purified to homogeneity using standard microbiological techniques. Isolated algae were cultivated in specified media and microalgae species were identified by morphological features. Growth and lipid production by isolated microalgal strains were investigated. The most suitable microalga for lipid production was cultivated in pre-treated effluent from industrial processing plants. The present study investigates the potential of six shortlisted algal isolates for biomass production and lipid accumulation in wastewater media for biodiesel feedstock production.

2. Materials and methods

2.1 Isolation and purification

Microalgae were collected from the Nakhon Chai Si and Tha-jeen river basins (Nakhon Pathom, Ratchaburi, and Samut Sakhon provinces) with a plankton net (10–12 µm x 7–9 µm in size). Colonies of microalgae were separated using a sterile micropipette washing method [10]. Microalgae were enriched and cultured in a specified medium for each strain, supplemented with antibiotic solution (tetracycline 50 µg mL^{-1}). The algae were subjected to purification by serial dilution followed by plating. The individual colonies were isolated and inoculated into specified medium and incubated at 30°C under 3,500 lux light intensity with 16:8 h light and dark cycles. The purity of the culture was ensured by repeated plating and by regular observation under microscope. The isolated microalgae were tentatively identified as belonging to a specific genus according to morphological properties. Microscopic identification was performed using standard morphological feature key [11].

2.2 Algal cultures

The isolates were grown in 50-mL Erlenmeyer flasks, each containing 20 mL of modified Chu 13 medium with 1.0 vvm aeration rate at 30°C under 3000 lux light intensity with 16:8 h light and dark cycles for 2 weeks. The dry biomass and lipid content were measured. All the experiments were replicated at least once to ensure accurate results. The specific growth rate was calculated as the slope of the following equation:

$$Ln\frac{C}{C_0} = \mu dt$$

where C_0 is the initial biomass concentration (g/L) and C is the biomass concentration (g/L) at any time t [12].

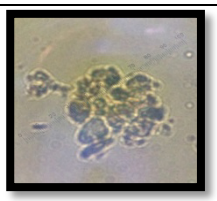
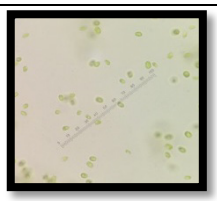
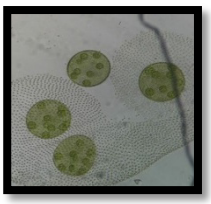
2.3 Wastewater from industrial plants

The wastewater used in this study was the primarily pretreated wastewater from a modified starch production factory (Sunflour Factory), poultry processing plant (KCF Factory), animal feed and fertilizer processing plant (Thai- food Factory) and a pickled ginger processing plant in Nakhon Pathom province. The pretreated wastewater was filtered through a mesh to discharge suspended solids and pH adjusted at 6.5 before sterilization in an autoclave at 121 °C for 15 min.

2.4 Analytical methods

Cells were harvested by centrifugation at 1500 g for 20 min, and pellets were dried at 108°C for 18 hours. The dry weight of the algal biomass was determined gravimetrically and growth was expressed in terms of dry weight. The total lipid content (dry weight) was measured by modified method of Lin and Wu [13]. After drying, the samples were pulverized by a homogenizer and then extracted using a chloroform–methanol mixture (1:2, v/ v). Approximately 15 mL of solvent was used per 50 mg of dried samples in each extraction step. After the samples were mixed using a vortex mixer for 1 min, they were ultrasonicated for 30 min and centrifuged at 3000 rpm for 10 min. The solid phases were carefully separated using Whatman No. 4 filter paper, and the solids were washed using 5 mL of chloroform. After this process, 9 mL of sterilized water was added to a solvent phase. The solvent was then mixed using a vortex mixer. The solvent phase was centrifuged at 3000 rpm for 10 minutes, and the chloroform layer was collected. After removing the solvent, the weight of the lipids was measured using a nitrogen blowing concentrator; the lipid content was then calculated.

Table 1 Morphological characteristics of isolated microalgae

Isolate	Morphology (40x)	Strain	Isolate	Morphology (40x)	Strain
1.		*Botryococcus* sp.	2.		*Chlorella* sp.
3.		*Spirulina* sp.	4.		*Scenedesmus* sp.
5.		*Volvox* sp.	6.		*Dunaliella* sp.

Fatty acid methyl esters (FAME) from extracted lipid involved the hydrolysis of the lipids followed by esterification [14]. The fatty acid composition of the FAME was analysed using a HP6850 Gas Chromatograph equipped with a cross-linked capillary FFAP column (by Aligent Technologies, Palo Alto, CA, USA: length 30 m, 0.32 mm I.D, 0.25 lm film thickness) and flame ionization detector. Operating conditions were as follows: inlet temperature 290 °C, initial oven temperature 210°C held for 12 min., then ramped to 250°C at 20°C C/min, hold 8 min and detector temperature was 300°C. Fatty acids were qualified by comparing their retention times with those of known standards.

The pretreated wastewater was characterized based on pH, chemical oxygen demand (COD), ammonia (phenate method), total nitrogen (Kjeldahl method), and nitrate concentration (cadmium reduction method) according to the standard methods [15]. Nitrate removal rate was calculated using the following equation:

$$R_i = -\frac{S_0 - S_t}{t_0 - t_t}.$$

2.5 Statistical analysis

The data was calculated with mean values, and standard deviation (mean±SD) was determined from at least duplicate trials. Statistical significance of the results was evaluated by one way ANOVA (analytical of variance) and Duncan's multiple range tests ($P < 0.05$) using SPSS 11 software.

3. Results and discussion

3.1 Morphology, growth and lipid content of isolated microalgae

Six strains of microalgae were isolated from the Nakhon Chai Si and Tha-jeen river basins, located in Nakhon Pathom, Ratchaburi and Samut Sakhon provinces of Thailand). The pH values at these sites were neutral (pH 6.2–7.6). Most sites had clear water with a slight blue tinge, with an optical density at 435 nm in the range of 0.074–0.126. These isolates were divided into 6 groups, *Botryococcus* sp., *Chlorella* sp., *Spirulina* sp., *Scenedesmus* sp., *Volvox* sp. and *Dunaliella* sp., based on the morphological characters. Colony morphologies of the six isolated microalgal species, as identified under light microscope, are provided in Table 1.

All of the isolated strains were cultured in specified media: Modified Chu medium for *Botryococcus* sp.; Zarrouk medium for *Spirulina* sp.; and basal medium for *Chlorella* sp. and *Dunaliella* sp.; and BG-11 medium for *the Scenedesmus* sp. and *Volvox* sp. strains. The growth and lipid content of the six isolates after 14 days of culture are shown in Table 2. Among the six strains, *Spirulina* sp. grew fastest and showed the highest specific growth rate (0.412 $d^{-1)}$, followed by *Chlorella* sp. (0.283 d^{-1}), *Scenedesmus* sp. (0.230 d^{-1}), *Dunaliella* sp. (0.218 d^{-1}) and *Volvox* sp. (0.195 d^{-1}). *Botryococcus* strain grew slowly and showed the lowest specific growth rate (0.154 d^{-1}). In its specified medium, *Chlorella* sp. showed the highest lipid content of 28.35% based on its dry biomass weight and also showed the highest lipid productivity of 80.23 mg $L^{-1}d^{-1}$ (Table 2).

Table 2 Growth, lipid content and productivity of six isolated strains

Strain	Specific growth rate (d^{-1})	Lipid content (%)	Lipid productivity (mg $L^{-1}d^{-1}$)	Medium	Source
Botryococcus sp.	0.154	26.10^{b}±0.14	40.19	Modified Chu	Nakhon Pathom
Chlorella sp.	0.283	28.35^{a}±0.49	80.23	Basal	Nakhon Pathom
Spirulina sp.	0.412	7.75^{f}±0.39	31.93	Zarrouk	Nakhon Pathom
Scenedesmus sp.	0.230	17.9^{c}±007	41.17	BG-11	Ratchaburi
Volvax sp.	0.195	12.55^{e}±0.64	24.47	BG-11	Samut Sakhon
Dunaliella sp.	0.218	14.10^{d}±0.28	30.74	Basal	Samut Sakhon

Notes: The lipid productivity was calculated as the maximum lipid content multiplied by the specific growth rate.
Different letters indicate significant difference between sources of wastewater in the same strain ($P < 0.05$).

Table 3 Lipid content of newly isolated *Chlorella* sp. strains in the present study and in previous reports

Strain	Lipid content(%)	Reference
Chlorella sp.	28.7	In Present study
Chlorella sp.	28	Chisti, [3]
Chlorella vulgaris	22	Danielo, [20]
C. vulgaris	14	Backer, [21]
Chlorella pyrenoidosa	14.57	Xu *et al*., [22]
C. pyrenoidosa	2	Backer, [21]
Chlorella protothecoides	55	Miao and Wu [18]
Chlorella sorokiniana CY1	43	Chen *et al*., [19]

Table 4 Characteristics of agro-industrial wastewater sources

Parameter	Starch modified	Poultry processing	Feed meal	Pickled ginger
pH	6.57	8.64	6.32	5.42
Dissolve oxygen (mgL^{-1})	3.43	4.90	4.87	6.03
Ammonium-nitrogen (mgL^{-1})	1.2	3.4	2.5	2.0
Nitrate-nitrogen (mgL^{-1})	0.5	4.0	2.4	0.5
Phosphate (mgL^{-1})	0.020	0.15	0.10	0.05
BOD (mgL^{-1})	7.26	13.4	8.47	6.85
COD (mgL^{-1})	11,600	12,500	6,700	4,570
Total soluble solid (mgL^{-1})	790	480	340	235
Salinity (ppt)	1.0	2.4	2.3	3.6

Although *Spirulina* sp. gave the highest specific growth rate, a lower lipid productivity of 31.93 mg $L^{-1}d^{-1}$ was obtained due to its lower lipid content (7.75%) compared with other isolated strains. Furthermore, *Chlorella* sp. was selected for further study in a subsequent experiment.

Several previous studies have shown that species of the genera *Chlorella* are the most widely employed for use as feedstock due to their high growth rates, high environmental tolerance, and high lipid/starch accumulation potentials [16, 17]. A comparison of lipid contents among newly-isolated *Chlorella* sp. strains in the present study with those reported in previous studies is shown in Table 3. Although the lipid content of a newly-isolated *Chlorella* sp. in the present study was lower than *Chlorella protothecoides* isolated by Miao and Wu, [18] and *Chlorella sorokiniana* CY1 isolated by Chen *et al*., [19], it was much higher than in all other strains. [20, 21, 22]. In addition, it would be useful to conduct further study on the locally isolated strains to investigate the feasibility of the mass production of biodiesel fuel using microalgae as feedstock.

3.2 Biomass and lipid production by *Chlorella* sp. strain in wastewater medium

Based on the results of the previous experiment, the isolated *Chlorella* sp. strain that showed the highest growth and lipid production rates was chosen to evaluate its cultivation potential in the effluent from various sources of wastewater. The biomass and lipid production by the isolated *Chlorella* sp. strain under mixotrophic conditions was studied. The characteristics of the effluents are shown in Table 4. Both atmospheric

Table 5 Specific growth rate, lipid content and lipid productivity of *Chlorella* sp. under different wastewater source media

Factory	Specific growth rate (d^{-1})	Lipid content (%)	Lipid productivity (mg $L^{-1}d^{-1}$)	Initial COD concentration (mg L^{-1})
Starch Modified	1.302	20.85^{c}±0.49	27.15	4,640
Poultry Processing	1.380	24.0^{a}±0.42	33.12	4,680
Feed Meal	1.323	22.90^{b}±0.14	30.29	4,466
Pickled Ginger	1.264	15.05^{d}±0.53	19.02	4,570

Note: The lipid productivity was calculated by the maximum hydrocarbon content multiplied by the specific growth rate.
Different letters indicate significant difference between sources of wastewater in the same strain ($P < 0.05$).

Table 6 Growth and lipid content and productivity of isolated *Chlorella* sp. under various conditions

Culture condition	Specific growth rate (d^{-1})	Maximum dry cell weight (g L^{-1})	Maximum lipid content (%)	Lipid productivity (mg $L^{-1}d^{-1}$)
Effect of dilution rate (Effluent: Basal medium)				
1:1	1.260	0.22	16.7^{c}±0.92	20.97
1:2	1.358	0.40	22.0^{ab}±0.90	29.89
1:10	1.361	0.48	23.8^{a}±0.57	32.39
1:20	1.243	0.35	20.4^{b}±1.02	25.29
Effect of light intensity (lux)				
2,000	1.361	0.48	23.8^{b}±0.57	32.39
3,000	1.506	0.62	28.0^{a}±0.92	42.17
4,000	1.474	0.53	25.9^{ab}±0.64	38.10
5,000	1.108	0.32	14.9^{c}±0.90	27.59
Effect of agitation rate (rpm)				
100	1.506	0.62	28.0^{b}±0.92	41.94
150	1.752	0.84	30.8^{a}±0.85	53.96
200	1.378	0.40	26.1^{b}±0.42	35.97
300	1.216	0.39	21.1^{c}±0.35	25.60

Notes: The lipid productivity was calculated as the maximum lipid content multiplied by the specific growth rate.
Different letters indicate significant difference between sources of wastewater in the same strain ($P < 0.05$).

CO_2 (normally 0.04% by volume) and complex organic carbon in the effluent were available as carbon sources in the culture. All wastewater was diluted until an initial COD in the range of 4,400-4,700 mg L^{-1} was achieved. The isolated *Chlorella* sp. strain could grow in all of the wastewater media under study, yielding similar specific growth rates in each (range 1.264-1.380 d^{-1}) (Table 5). However, it was found that the lipid content of microalgae significantly declined when cultivated in wastewater media, as compared to growth in a synthetic medium. This result may has been due to nutrient composition, organic concentrations and metal ions present in the wastewater such as iron, manganese when comparing with synthetic media, which are probably polluted by other toxic substances [23, 24].

The study revealed that a wastewater medium from a poultry processing plant was the most suitable source for cultivation of *Chlorella* sp. In addition, compared with other sources of wastewater the specific growth rates and lipid content of the selected *Chlorella* sp. in the poultry processing plant wastewater medium were shown to be the highest, at 1.380 d^{-1} and 24.0%, respectively. Many microalgae can grow well under ‘‘nutrient rich” conditions, rapidly converting nutrients (e.g., ammonium used as a nitrogen source) contained in agro-industrial wastewater to biomass [17].

3.3 Effects of chemical-physical conditions on the growth and lipid production of isolated *Chlorella* sp.

Based on the results from the previous experiment, the isolated *Chlorella* sp. strain that showed the highest growth and lipid production was chosen for evaluation of its cultivation potential in effluent from the poultry processing plant at varying conditions in terms of dilution rates (effluent diluted with basal medium at 1:1, 1:2, 1:10 and 1:20 concentrations),

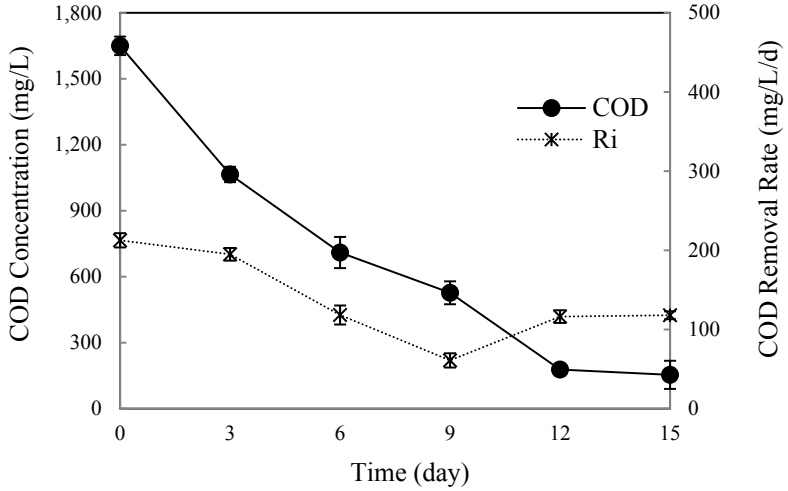


Figure 1 Batch cultivation of isolated *Chlorella* sp. in the effluent from the poultry processing plant: nitrate concentration (closed circle) and nitrate removal rate (star)

light intensity (2,000-5,000 lux) and agitation rates (100-300 rpm). The impact of these varied chemical-physical conditions on algal growth and lipid production are shown in Table 6.

The growth rate of isolated *Chlorella* sp. increased when the initial COD concentration was decreased (increase dilution rate from 1:1 to 1:10). Increasing the dilution rate further (1:20) did not enhance the growth of this strain. Dilution rates of 1:1 and 1:2 also showed the lower growth rates than the dilution rate 1:10. This could have been an effect of substrate inhibition. The results showed that at dilution rate of effluent per basal medium at 1:10 gave the highest specific growth rate as 1.316 and maximum lipid content (23.8%). Chen and Johns [25] have indicated that, compared with bacteria and yeasts, microalgae can tolerate only relatively low substrate concentrations. Algae can grow well only in wastewater containing relatively low COD (below 5000 mg L^{-1}) [17, 26]. However, algal cells have been shown to adapt to critical conditions. Adaptation ability depends on the individual strain [3].

In the light trail, the lipid contents increased with increasing light intensity from 2,000 to 3,000 lux, then decreased when the light intensity was increased up to 4,000 lux. From Table 6, it was observed that 3,000 lux light intensity yielded the highest lipid content. Previous studies have attempted to identify optimal irradiance levels to support growth and lipid production. High intensity light increases the carotenoid-to-chlorophyll ratio, and this affects the algal colonies [27]. The lower productivity of dark-adapted culture was due to high susceptibility to photoinhibition.

As for the effect of agitation rate on growth and lipid production of the isolated *Chlorella* sp. strain, it was shown that both increased slightly when the agitation rate was increased from 100 to 150 rpm. Agitation is necessary for microalgae cultivation. In addition to keeping the algae well mixed, it also helps to prevent cell precipitation [28]. Among the combinations, an optimum dilution rate with moderately high light intensity and an agitation rate of 150 rpm enhanced the lipid content in isolated *Chlorella* sp. strain up to the highest level observed at 30.8%.

3.4 Benefit and potential of the isolated *Chlorella* sp. strain

The isolated *Chlorella* sp. strain fulfilled the major requirements for lipid production, including high lipid content, yield and productivity in the growth medium. After growing in the effluent medium with the optimal levels of dilution, light intensity and agitation, the lipid content of isolated *Chlorella* sp. was observed to further increase from 24.0% to 30.8%. The results show that modification of culture conditions should be tailored to the specific demands of highly productive microalgae strains to attain a consistently good yield of lipid. Its higher lipid content has attracted attention to the possibility of this content being exploited as a renewable energy source. However, the commercial production of lipid from *Chlorella* sp. strain cultivation is not yet widely viable because its growth rate is not rapid in comparison with cyanobacteria, as well as high production costs [27, 29]. Although the isolated *Chlorella* sp. strain forms spectacular blooms in nature, today its culture in open ponds is difficult to control, especially due to competition with other fast-growing microalgae [29]. It could be conclude that, a co-process of wastewater treatment and lipid production in this study showed the potential to realistically satisfy commercial demands since the isolated *Chlorella* sp. was able to grow in diluted effluent from the poultry processing plant, where it reduced COD concentration in the effluent from 1,650 mgL^{-1} to 154 mgL^{-1}, approximately 90.67%,

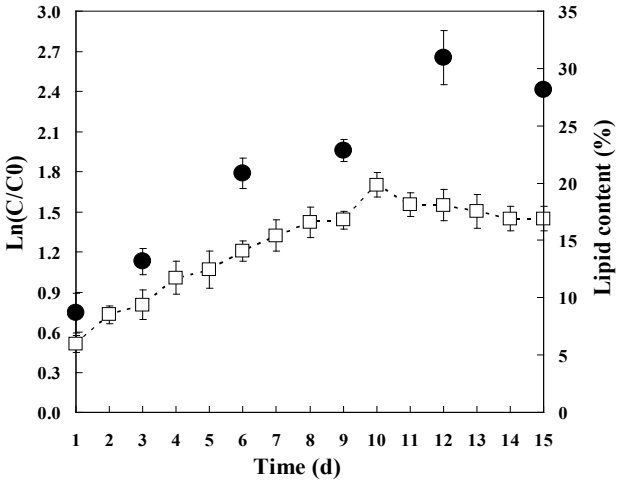


Figure 2 Specific growth rate and lipid content of isolated *Chlorella* sp. in poultry processing plant effluent LnC/C_0 (open square), lipid content (closed circle)

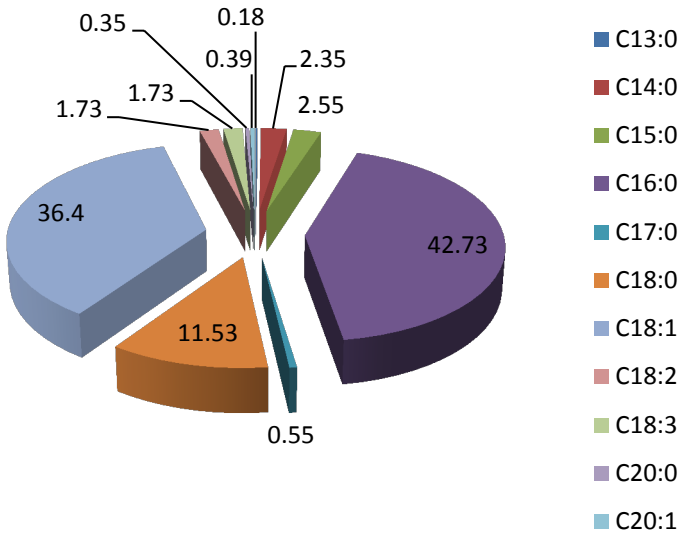


Figure 3 Fatty acid composition of biodiesel from the isolated *Chlorella* sp. lipid

with a COD removal rate of 195.0 $mgL^{-1}d^{-1}$, as shown in Figure 1. In this condition, this isolated strain produced lipid content at 31.1% with specific growth rate 1.758, as shown in Figure 2.

3.5 Fatty acid composition of biodiesel produced from microalgal lipids

The fatty acid of isolated *Chlorella* sp. was extracted and the biodiesel (fatty acid methyl esters) was produced. The fatty acid composition of the biodiesel obtained from this strain are shown in Figure 2. *Chlorella* sp. accumulated fatty acid profiles of C13:0 to C21:1. Palmitic and oleic acids were the major fatty acids, indicating good agreement with the results observed in the lipid production of *Chlorella vulgaris* [3]. The major monounsaturated fatty acid (MUFA) was oleic acid (18:1). Results indicated that *Chlorella* sp. accumulated a high proportion of saturated fatty acids (SFA) and MUFA, to 50% of the total lipid content. Compared to soybean oil commonly used as feedstock for biodiesel production in the US [23], the biodiesel derived from microalgae lipid in this study would be more saturated and provide a higher cetane number (CN), lower NOx emissions, shorter ignition delay time, and higher oxidative stability.

4. Conclusions

Comparison among six strains of isolated microalga, *Chlorella* sp. fulfils the major requirements for lipid production, including high lipid content, yield and productivity in the growth medium. Economical lipid production *Chlorella* sp. can growth in Poultry Processing effluent diluted with basal medium and

highest specific growth rate of 1.361 d^{-1} and lipid content of 23.4% were achieved. The combined trials of dilution rate at 1:10 (volume:volume) with 3,000 lux light intensity and 150 rpm agitation rate were preferred for lipid accumulation in *Chlorella* sp. strains. After growing in the optimum conditions, the lipid content of *Chlorella* sp. could be further increased to 31.1% with specific growth rate 1.758 d^{-1}. Besides the lipid accumulating ability, *Chlorella* sp. strain also removed 90.67% of COD in the effluent. Moreover, Palmitic and oleic acid were dominant fatty acid in lipid of isolated microalgal. This suggests that *Chlorella* sp. might be used as a potential feedstock for microalgal based biofuel production.

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