Nutrient removal from pickle industry wastewater by cultivation of *Chlorella pyrenoidosa* for lipid production

Liang Wan, Yixiao Wu, Xuemei Zhang and Weihao Zhang

ABSTRACT

The present research examined the feasibility of cultivating *Chlorella pyrenoidosa* in pickle industry wastewater for simultaneous nutrient removal and lipid production. The characteristics of microalgae growth, nutrient removal, lipid accumulation and composition of *C. pyrenoidosa* cultivated in pickle wastewater with different dilution ratios were investigated. The results showed the maximum algae biomass concentration of 1.57 ± 0.12 g L^{-1} was achieved in non-diluted pickle wastewater with the highest biomass productivity of 170.65 mg L^{-1} day^{-1}. Maximum nutrient removal efficiency was observed in 20.0% pickle wastewater with removal rates of chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN) and NH_4-N at 84.67%, 92.46%, 85.82% and 93.42%, respectively. The lipid content of *C. pyrenoidosa* growing in pickle wastewater ranged from 29.73% to 31.78%, with a highest lipid productivity of 57.23 mg L^{-1} day^{-1}. The relative content of triolefinic acids (C16:3 and C18:3) decreased while the monoenoic acids (C16:1 and C18:1) increased synchronously with the pickle wastewater concentration. Unsaturated fatty acid methyl esters were the main components, ranging from 73.04% to 77.6%. The biodiesel properties satisfied the major specifications in US and European biodiesel standards. The results indicated that *C. pyrenoidosa* is a promising species for nutrient removal together with lipid production in pickle industry wastewater.

Key words | lipids production, microalgae, nutrients removal, pickle industry wastewater

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INTRODUCTION

With the rapid development of the pickle industry, the annual pickle wastewater amount has increased to 3.5 million m^3 in Fuling District, Chongqing, China (Chai & Kang 2012). High salinity (3% to 6% salt) is the key problem that affects the traditional biological wastewater treatment processes of pickle industry wastewater. It has been shown that high salinity can decrease microbial activity, resulting in lower organic and nutrient removal rates, and moreover the dilution of wastewater to decrease salinity means low treatment load and high processing cost (Zhang *et al.* 2012). Besides salinity, the concentration of organic compounds up to 20 g L^{-1} mainly contains carbohydrate, pectin and cellulose, etc. (Kargi *et al.* 2000). When discharged into the environment, the high salinity and contaminant wastewater can cause serious water environment problems.

Renewable energy sources have attracted significant attention due to the world-wide energy crisis (Wan *et al.* 2014). Biodiesel is one of the attractive alternatives for renewable energy from nontoxic, biodegradable and renewable sources (Nasreen *et al.* 2015). Microalgae, with the strong capacity to accumulate lipids inside cells, are considered a viable feedstock for the production of biofuel with efficient photosynthesis by utilizing sunlight and CO_2 (Lam & Lee 2012). However, the cost of biofuel production from microalgae is higher than that from traditional crops, related to many factors, such as the harvesting and drying processes as well as the high cost of chemical usage during algae cultivation in order to get relatively high biomass productivity (Hu *et al.* 2012). The coupling of microalgae cultivation with wastewater treatment is an effective solution for wastewater disposal since nutrients in wastewater can be recycled in biomass cultivation system to save costs.

Many studies focused on the microalgae treatment of various forms of sewage aimed at lipid production have been published (Chen *et al.* 2015; Mennaa *et al.* 2015). However, studies on microalgae cultivation in wastewater with high salinity have rarely been reported. Generally, the lipid content of microalgae can be enhanced under stress

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cultivation conditions like light, nutrient, and salinity, etc (Li *et al.* 2011a; Ruangsomboon 2012; Wu *et al.* 2012). Salinity stress is a complicated mechanism for microalgae which can alter their metabolism to adapt to the extreme environment to improve lipid accumulation (Kalita *et al.* 2011; Yeesang & Cheirsilp 2011; Khatoon *et al.* 2014). In addition, microalgae have strong adaptability and tolerance to salinity, and can even grow with salinity up to 45 g L^{-1} (Church *et al.* 2017). Therefore, the major objective of this research is to investigate the performance of microalgae for treating the salinity of pickle industry wastewater. *C. pyrenoidosa* was chosen as the model species that is used for commercialized nutrient supplements and also for wastewater treatment (Spolaore *et al.* 2006).

To treat the high salinity and contaminant pickle industry wastewater, experiments in this research were employed to investigate the algal growth, nutrient removal, lipid production, fatty acid profile as well as the fuel properties of the algal lipids produced by *C. pyrenoidosa* in a batch cultivation system.

MATERIALS AND METHODS

Pretreatment and analysis methods

The pickle wastewater used in this research was obtained from a local pickle plant wastewater outlet (Wuhan, China). Prior to use for algae cultivation, the wastewater sample was filtered to remove the suspended solids and then stored at 4 °C. The wastewater physicochemical characteristics were analyzed using the methods described below, the chemical oxygen demand (COD) was measured using a Hach DR 1900 spectrophotometer (Li *et al.* 2011c), total nitrogen (TN) and total phosphorus (TP) were determined colorimetrically as nitrate and phosphate after the samples had been oxidized, and ammonia (NH_4-N) was measured using Nessler's reagent colorimetric method.

The physicochemical characteristics of the pickle wastewater can be found in Table 1. As shown in Table 1, pickle wastewater contained abundant nutrients which indicated there were sufficient nutrients for the growth of microalgae cultivated in pickle wastewater.

Microalgae strain pre-culture

The microalgae *C. pyrenoidosa* (FHACB-10) were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (Chinese Academy of Sciences, Wuhan, China). In pre-experiment, *C. pyrenoidosa* grew well in the salinity of pickle wastewater. *C. pyrenoidosa* was cultivated with 100 mL of sterilized BG11 medium in 250 mL conical flasks, placed in a light incubator (PGX-450A-3HM, LaiFu Technology Co. Limited, Ningbo, China). The cultivation conditions were as follows: light intensity = 4,800 lux, light/dark ratio = 12:12, temperature = 25 ± 1 °C. The BG11 medium consists of the following components: 1,500 mg L^{-1} $NaNO_3$, 40 mg L^{-1} K_2HPO_4, 75 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 36 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 6 mg L^{-1} citric acid, 6 mg L^{-1} ferric ammonium citrate, 0.1 mg L^{-1} EDTA-Na_2, 20 mg L^{-1} Na_2CO_3, 1 mL L^{-1} A5 solution (trace metal). The A5 solution contains 2.86 g L^{-1} H_3BO_3, 1.86 g L^{-1} $MnCl_2{\cdot}4H_2O$, 0.22 g L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.39 g L^{-1} $Na_2MoO_4{\cdot}2H_2O$, 0.08 g L^{-1} $CuSO_4{\cdot}5H_2O$, 0.05 g L^{-1} $Co(NO_3)_2{\cdot}6H_2O$. The pH of the BG11 medium was adjusted to 7.1 with 1.2 mol L^{-1} HCl before sterilization.

Table 1 | Compositions of the pre-treated salinity pickle wastewater before and after sterilization (121 °C, 20 min)

Parameter	Non-sterilized	Sterilized
pH	4.2	6.4[a]
COD (mg L^{-1})	8,500	6,366
TN (mg L^{-1})	388.8	404.5
NH_4-N (mg L^{-1})	101.0	94.3
TP (mg L^{-1})	47.9	14.5
Salinity (%)	2.37	2.35
Cl^- (g L^{-1})	11.34	11.30

[a]The pH of wastewater was adjusted to 7.0 before sterilization with 0.5 N NaOH.

Experimental procedures

The wastewater was diluted with deionized water (v:v) to a series of concentrations of 20%, 50% and 100%, respectively. The solutions were sterilized at 121 °C for 20 min in an autoclave before use. Batch experiments were performed in 500 mL conical flasks containing 200 mL sterilized pickle wastewater and the volume of the inoculum was 25 ml (initial cell density was 0.09 g L^{-1}). The BG11 medium used for cultivation of *C. pyrenoidosa* was a reference for comparison purposes. In all experimental groups, microalgae were cultivated for 9 days with artificial shaking three times a day. The cultivation conditions were as described above. All treatments were carried out in triplicates.

Determination of algal growth

A 10 mL culture solution was collected every day from each flask, and the liquid samples were centrifuged at 10,000 rpm,

4 °C for 10 min. The biomass pellets were washed in deionized water to remove salt and then dried in an oven at 60 °C to constant weight to measure the biomass dry weight.

The maximum specific growth rate μ in the exponential phase of algal growth was calculated by using Equation (1):

$$\mu(\text{day}^{-1}) = \ln(N_i/N_0)/(t_i - t_0) \quad (1)$$

where N_i and N_0 are defined as dry biomass (g L^{-1}) at time t_i and t_0, respectively.

The doubling time (t_d, days) was calculated as follows:

$$t_d(\text{days}) = \ln(2)/\mu \quad (2)$$

The biomass productivity (P) was calculated according to the following formula:

$$P = (DW_i - DW_0)/(t_1 - t_0) \quad (3)$$

where DW_i and DW_0 are dry biomass (g L^{-1}) at time t_1 and t_0 (initial time), respectively.

Nutrient analysis

The supernatants of the centrifuged culture solution were filtered and diluted appropriately for nutrient removal analysis. COD, NH_4-N, TN, and TP were measured according to the methods described above.

The percentage of removal rates was calculated using the following formula:

$$W\% = 100\% \times (C_0 - C_i)/C_0 \quad (4)$$

where C_0 and C_i are the values of nutrient concentration at initial time t_0 and time t_i, respectively.

Total lipid and fatty acid methyl ester content analysis

The algae were harvested after growing for 9 days, then washed with deionized water and dried in a freeze vacuum dryer before the lipid analysis. Total lipid content in microalgae was measured according to the ultrasound assisted extraction method based on the one-step extraction method (Folch *et al.* 1957). Briefly, around 20 mg dried algae powder was weighed accurately and mixed with 10 ml 2:1 chloroform/methanol (v/v) mixture. Oil was extracted in a water bath with ultrasound for 15 min, after which mixtures of organic solvent and algal residuals were separated through a centrifuge (10,000 rpm, 10 min). Algae powder was extracted three times and the supernatant organic solvent was transferred and removed using a nitrogen evaporator, and the bottom was dried in an oven followed by the gravimetrical measurement of the crude lipids.

Fatty acid content and composition were analyzed by two steps, including the preparation of fatty acid methyl ester (FAME) and gas chromatography–mass spectrometry (GC-MS) analysis. The content of FAME was analyzed following a one-step extraction–transesterification method with minor modification (Indarti *et al.* 2005; Luo *et al.* 2016). Dried algae samples (about 20 mg) were weighed into clean, 10 mL screw-top glass bottles, to which 5 mL mixtures of methanol, concentrated sulfuric acid, and chloroform (4.25:0.75:5, v/v/v) were added. Transesterifications were carried out in a 90 °C heating jacket using a Hach DBR200 for 90 min. Upon completion of the reaction, the mixtures were cooled down to room temperature, after which 1 mL of deionized water was added, then the mixtures were agitated vigorously for 1 min. After the formation of two phases, the lower chloroform phase containing FAME was transferred to a clean bottle with dried anhydrous Na_2SO_4. The anhydrous organic phase containing FAME was carefully collected and subjected to GC-MS analysis. The GC-MS (Agilent 7890-5975C, USA) was equipped with a flame ionization detector and a DB-5-MS capillary column. The oven temperature was set at 80 °C, held for 5 min, raised to 290 °C at 4 °C min^{-1}, and held at 290 °C for 5 min, and the temperature for injector and detector was set at 250 °C and 230 °C, respectively. The carrier gas (helium) was controlled at 1.2 mL min^{-1}. Chromatographic data were recorded and integrated using the built-in Agilent data analysis software. The compounds were identified in the NIST Mass Spectral Database.

Properties of algae biodiesel calculation

For evaluating the biodiesel properties, the average degree of unsaturation (ADU) was calculated as follows by Equation (5) (Redel-Macías *et al.* 2013):

$$ADU = (1\%_M + 2\%_D + 3\%_T)/100 \quad (5)$$

where $\%_M$, $\%_D$ and $\%_T$ are the percentages in weight of mono-unsaturated, di-unsaturated and tri-unsaturated methyl esters, respectively.

The relationships between ADU and kinematic viscosity (Y_1), specific gravity (Y_2), cloud point (Y_3), cetane number

(CN) (Y_4), iodine value (Y_5) and higher heating value (HHV) (Y_6) were calculated by the following Equations (6)–(11) (Song *et al.* 2013):

$$Y_1 = -0.6316ADU + 5.2065 \quad R^2 = 0.6704 \tag{6}$$

$$Y_2 = 0.0055ADU + 0.8726 \quad R^2 = 0.6644 \tag{7}$$

$$Y_3 = -13.356ADU + 19.994 \quad R^2 = 0.6809 \tag{8}$$

$$Y_4 = -6.6684ADU + 62.876 \quad R^2 = 0.8049 \tag{9}$$

$$Y_5 = 74.373ADU + 12.71 \quad R^2 = 0.9484 \tag{10}$$

$$Y_6 = 1.7601ADU + 38.534 \quad R^2 = 0.38 \tag{11}$$

RESULTS AND DISCUSSION

Microalgae growth and biomass production

The algal growth indicated by biomass concentration in the BG11 medium and three nutrient concentration levels (20%, 50% and 100%) were investigated. Generally, there are four phases during microalgae growth: lag, exponential, stationary and lysis (Li *et al.* 2011b). As shown in Figure 1, the lag time (about 1 day) for *C. pyrenoidosa* cultivated in 100% pickle wastewater was obviously observed while no significant lag phase was observed in 20% and 50% wastewater. Comparing between the serial dilutions, it can be found that the microalgal biomass concentration increased with the increasing of the initial pickle wastewater concentration during the 9 d cultivation, and the highest biomass production was observed in 100% pickle wastewater. The good growth of *C. pyrenoidosa* indicated that pickle wastewater can be used as a culture medium directly for microalgae, which means the pickle wastewater had no toxicity for microalgae. Subsequently, the microalgae growth reached a plateau in 20% and 50% pickle wastewater after 3 and 5 days due to exhaustion of the lesser amount of nutrients (Wang *et al.* 2010).

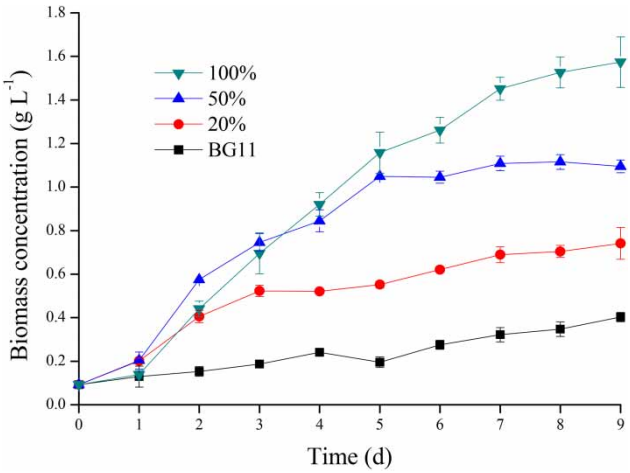

Figure 1 | Growth curves for *C. pyrenoidosa* in BG11 medium and pickle wastewater with different concentrations of salinity over 9 days.

As shown in Table 2, the specific growth rates in the exponential time under different culture conditions were 0.240 ± 0.018, 0.433 ± 0.025, 0.544 ± 0.014 and 0.581 ± 0.014 d^{-1}, respectively. The corresponding doubling times (d) of the cultivation were 2.90 ± 0.22, 1.60 ± 0.025, 1.25 ± 0.042 and 1.19 ± 0.029 days. The algal biomass concentration and productivity in 100% pickle wastewater were the highest, correspondingly up to 1.57 ± 0.12 g L^{-1} (dry weight) and 170.65 ± 12.9 mg L^{-1} day^{-1}, while the cultures in 50% and 20% pickle wastewater exhibited lower biomass concentration (1.095 ± 0.029 and 0.742 ± 0.073 g L^{-1}, respectively) and biomass productivities (111.42 ± 4.63 and 72.14 ± 9.96 mg L^{-1} day^{-1}, respectively). The biomass concentration and biomass productivity in this research were obviously higher than the result reported by Wang *et al.* (2012), in which the biomass concentration and biomass productivity achieved were around 0.3 g L^{-1} and 40 mg L^{-1} day^{-1}, respectively, when *C. pyrenoidosa* was cultivated in diluted primary piggery wastewater. The growth parameters in all pickle wastewater groups were superior than in BG11 medium, and increased synchronously with the nutrient concentration. The result also showed there

Table 2 | Growth parameters of C. *pyrenoidosa* in BG11 medium and pickle wastewater with different concentrations of salinity over 9 days

Cultivation medium	Biomass dry weight (g L^{-1})	Specific growth rate μ(day^{-1})	Doubling time (days)	Biomass productivity (mg L^{-1} day^{-1})
BG11 medium	0.403 ± 0.022	0.240 ± 0.018	2.90 ± 0.22	34.53 ± 2.40
20% wastewater	0.742 ± 0.073	0.433 ± 0.025	1.60 ± 0.025	72.14 ± 9.96
50% wastewater	1.095 ± 0.029	0.544 ± 0.014	1.25 ± 0.042	111.42 ± 4.63
100% wastewater	1.574 ± 0.116	0.581 ± 0.014	1.19 ± 0.029	170.65 ± 12.91

was a significant relation ($P < 0.05$) between the nutrient concentration, biomass dry weight and biomass productivity.

Nutrient removal efficiencies

The removal efficiencies of COD, NH_4-N, TN and TP in different dilution ratios of wastewater in the 9-day cultivation are shown in Figure 2(a)–2(d), respectively.

Carbon sources are the essential nutrient for the photosynthesis of plants. Figure 2(a) shows the COD removal effects varied greatly in different experimental groups. The COD removal efficiencies were correlated with the dilution ratio and the removal rates were 85.56%, 84.67% and 64.08% for the different diluted wastewaters. Diluted piggery wastewater was used to cultivate *C. vulgaris* and the similar result showed that COD removals increased from 20.6% to 88.0% while the initial COD concentration decreased from 1,000 to 250 mg L^{-1} (Travieso *et al.* 2006). In 20% wastewater, a 3-day exponential phase was observed first and a slow growth autotrophic phase followed, which can be attributed to the complete assimilation of the organic substrate (Martínez *et al.* 1997). The good growth of algae and considerable COD removal rates indicated that *C. pyrenoidosa* could effectively utilize organic substrates in pickle wastewater as carbon sources besides CO_2. Some studies have indicated that algal growth was inhibited in particular wastewater with high initial COD (Zhu *et al.* 2013). However, *C. pyrenoidosa* in this study has better adaptability to high COD concentration (6,366 mg L^{-1}) and thus obtains more biomass production.

Figure 2(b) shows the NH_4-N removal rates were 93.43%, 43.97% and 42.93% in the different dilutions of wastewater, respectively. Generally, there are two ways to remove NH_4-N from wastewater in algae cultivation: directly assimilation of NH_4-N and NH_3 stripping. However, NH_3 stripping occurs only under alkaline or high-temperature conditions. Since the temperature in this experiment was kept at 25 °C and the system was neutral, the removal of NH_4-N could be attributed to utilization by algal cells.

As shown in Figure 2(c), after 9 d cultivation, the TN removal efficiencies were 85.81%, 74.83% and 61.76% for the different dilutions of pickle wastewater, indicating that a low dilution ratio means a higher TN removal efficiency. Nitrogen is an essential macronutrient for algal cell growth which is necessary for the synthesis of various intracellular biological chemicals, such as proteins, enzymes and

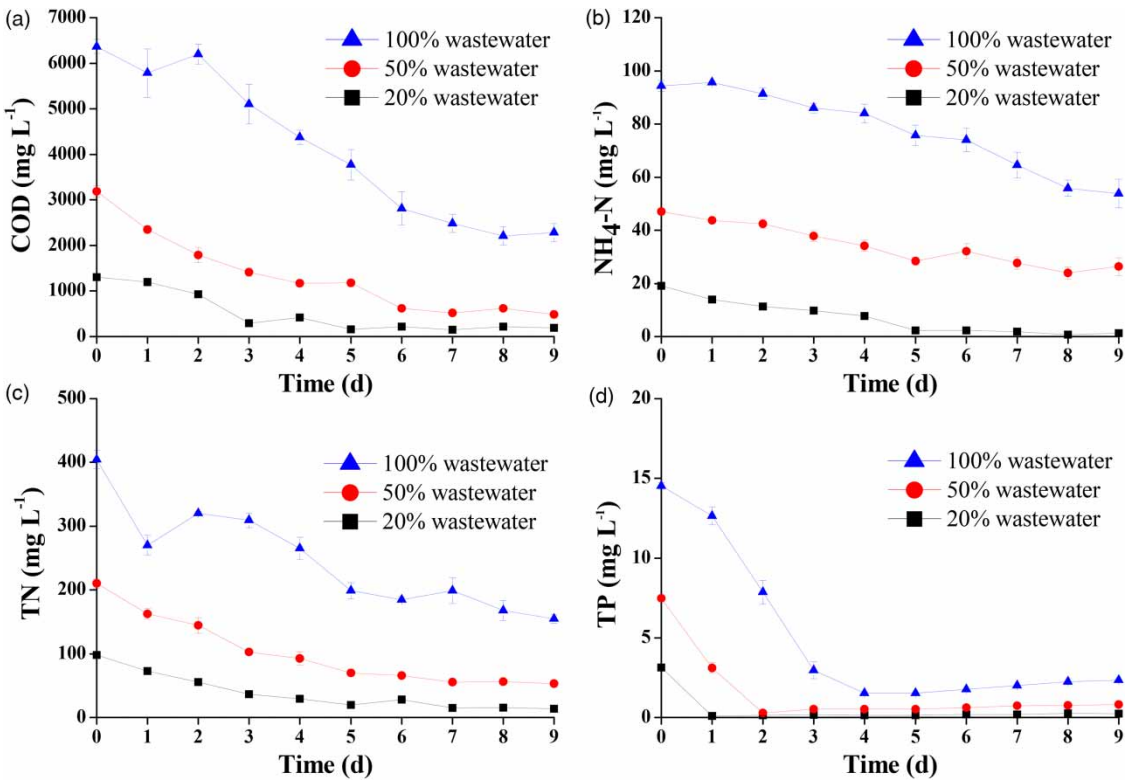

Figure 2 | Nutrient removal from different concentrations of pickle wastewater by *C. pyrenoidosa* over 9 days: (a) COD removal; (b) NH_4-N removal; (c) total nitrogen removal; (d) total phosphorus removal.

chlorophyll. The forms of inorganic nitrogen of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4-N) can be directly assimilated by algae. It has been reported that NH_4-N was directly involved in metabolism and was the main type of nitrogen for microalgae because less energy was required for its uptake (Converti *et al.* 2006; Ruiz *et al.* 2011). However, the TN removal amount in 50% and 100% pickle wastewater was 151.33 mg L^{-1} and 249.82 mg L^{-1}, while the NH_4-N removal amount was 20.67 mg L^{-1} and 40.50 mg L^{-1}, respectively. The result also showed that the TN removal amount was always higher than that of NH_4-N during 9 days cultivation. Li *et al.* (2011b) also reported NH_4-N removal capacity was about 80 mg L^{-1}, while the TN level was reduced to 119 mg L^{-1} after 14 d of cultivation of *Chlorella* sp. in concentrated municipal wastewater. TN in wastewater contains ammonia, nitrate nitrogen, and organic nitrogen. During the COD removal, ammonia was generated accompanied by organic nitrogen degradation. The TN removal rates were higher than for NH_4-N suggesting ammonium was not the exclusive nitrogen source for microalgae in pickle wastewater.

Unlike nitrogen assimilation, phosphorus uptake showed a consistently high removal efficiency. Figure 2(d) shows the TP removal rates were 92.44%, 89.22% and 83.83% for the different dilutions of pickle wastewater. The optimal mass ratio of N:P is 7.7:1 for algal growth because of the balanced supply of N and P (Chinnasamy *et al.* 2010). However, in sterilized pickle wastewater, the mass ratio of N:P was 27.86. In spite of the high mass ratio of N:P, *C. pyrenoidosa* grew well in pickle wastewater after the phosphorus was exhausted in a few days of cultivation which indicated that phosphorus was not the limiting factor for algae cultivation. It has been reported that in phosphorous-starvation cultivation, the algal biomass exhausted the phosphate and continued to grow and the more consumed phosphorus did not result in more biomass production (Wu *et al.* 2012). Therefore, the phosphorus should be appropriately limited to prevent wasting resources in commercial biomass production.

Lipid productivity and FAME compositions

The lipid content and productivity of *C. pyrenoidosa* cultivated in BG11 medium as well as pickle wastewater with different dilutions of salinity are shown in Figure 3. The lipid contents of *C. pyrenoidosa* in all experimental groups were roughly the same, ranging from 29.73% to 33.95%. And these were higher than cultivated *C. pyrenoidosa* in diluted piggery wastewater, in which lipid contents were lower than 25% (Wang *et al.* 2012). The lipid productivities varied with the dilution rates, and the highest lipid productivities were found in 100% wastewater, reaching 57.23 ± 2.20 mg L^{-1} day^{-1} as a result of the highest biomass production (Figure 1).

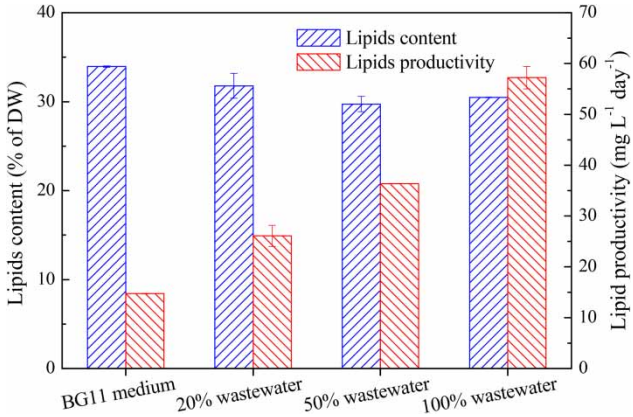

Figure 3 | Lipid contents and productivities for *C. pyrenoidosa* in BG11 medium and pickle wastewater with different concentrations of salinity over 9 days.

Besides lipid productivity, the fatty acid profile of lipids is an important characteristic affecting the quality of the biodiesel product. Fatty acid compositions of *Chlorella* sp. were 16:0, 16:1, 16:2, 16:3, 18:0, 18:1, 18:2, and α-18:3 under photoautotrophic and heterotrophic cultivation (Petkov & Garcia 2007). Table 3 shows the fatty acid profiles of *C. pyrenoidosa* cultivated in BG11 medium and different dilutions of pickle wastewater. The unsaturated fatty acid methyl esters were the main components in the FAME profile, accordingly accounting for a total of 76.37%, 73.04%, 77.6% and 75.29%, respectively. But the fatty acid compositions varied obviously with different experimental groups. Compared with BG11 medium, there were significant reductions of the relative content of hexadecatrienoic acid (C16:3) and linolenic acid (C18:3) in the wastewater. Hexadecenoic acid (C16:1) and oleinic acid (C18:1) were increased obviously in the pickle wastewater samples. Polyunsaturated fatty acids took a big proportion of total fatty acids in BG11 medium but dropped to 24.41% in 100% pickle wastewater. It is worthy of note that the content of linolenic acid methyl ester in biodiesel for vehicle use is limited to 12% (mol/mol) in the European Standard EN 14214. The linolenic acid of microalgal species (*S. obliquus* and *C. vulgaris*) is normally in the range 23–28% (Abou-Shanab *et al.* 2013), which means additional treatment is required to meet the standard. However, the remarkable reduction of linolenic acid (C18:3) in 100% pickle wastewater in this study leads to linolenic

Table 3 | Fatty acid compositions for *C. pyrenoidosa* in BG11 medium and different concentrations of pickle industry wastewater over 9 days

	Fatty acid percentage (%)			
Fatty acid profiles	**BG11 medium**	**20% wastewater**	**50% wastewater**	**100% wastewater**
C16:0	22.05 ± 0.61	23.53 ± 1.07	20.65 ± 0.55	21.87 ± 0.74
C16:1	4.55 ± 3.94	2.18 ± 3.77	8.43 ± 1.87	10.79 ± 0.25
C16:2	3.67 ± 0.11	4.78 ± 0.33	3.83 ± 0.32	3.81 ± 0.32
C16:3	16.07 ± 0.75	14.99 ± 1.24	13.20 ± 1.70	8.21 ± 0.57
C18:0	1.58 ± 0.37	3.43 ± 0.61	2.28 ± 0.27	2.84 ± 0.17
C18:1	1.04 ± 1.80	3.16 ± 5.47	16.08 ± 4.34	40.09 ± 1.93
C18:2	13.71 ± 0.19	15.73 ± 1.44	14.69 ± 1.68	12.39 ± 1.03
C18:3	37.34 ± 0.92	32.21 ± 5.58	20.84 ± 2.32	ND
SFA	23.63 ± 0.97	26.96 ± 1.38	22.94 ± 0.79	24.72 ± 0.80
MUFA	5.58 ± 2.14	5.33 ± 9.24	24.51 ± 6.19	50.88 ± 2.14
PUFA	70.79 ± 1.86	67.71 ± 8.43	52.56 ± 5.95	24.41 ± 1.72

SFA = saturated fatty acids (C16:0, C18:0), MUFA = mono-unsaturated fatty acids (C16:1, C18:1), and PUFA = polyunsaturated fatty acids (C16:2, C16:3, C18:2, C18:3).

acid obviously meeting the limitation to avoid additional treatment, which will save considerable cost.

Properties of biodiesel from algal lipids

In order to evaluate whether the oil produced from *C. pyrenoidosa* cultivated in pickle wastewater was suitable for biodiesel production, six different biodiesel properties were computed based on the relationships with ADU (Song *et al.* 2013). The biodiesel properties as well as the ADU of *C. pyrenoidosa* are shown in Table 4. The results show that an increase of wastewater concentration leads to higher cetane number and better oxidation stability, but reduces the cloud point. There is no explicit limited value of the cloud point because the low temperature performance varies according to the climate condition. According to the biodiesel quality standards in the USA and in Europe, the biodiesel properties of 100% wastewater satisfied the specifications. Bagul *et al.* (2017) reported the quality of biodiesel being almost as per the ASTM standards by cultivation of *Chlorella* sp. in secondary treated wastewater. Therefore, the microalgae species *C. pyrenoidosa* appears to be an appropriate species to treat pickle industry wastewater for lipid production.

CONCLUSION

The present study showed that microalgae *C. pyrenoidosa* was highly adaptable in high salinity pickle industry wastewater. Maximum nutrient removal was observed for 20% pickle wastewater, with removal rates of COD, TP, TN and NH_4-N at 84.67%, 92.46%, 85.82% and 93.42%, respectively. The highest biomass concentration and biomass productivity were 1.57 ± 0.12 mg L^{-1} and 170.65 ± 12.91 mg L^{-1} day^{-1}, respectively. The unsaturated

Table 4 | Properties of biodiesel from microalgae oil and biodiesel standards

Property	**BG11 medium**	**20% wastewater**	**50% wastewater**	**100% wastewater**	**US (ASTM D6751 − 08)**	**Europe (EN 14214)**
Kinematic viscosity (mm^2 s^{-1})	3.94 ± 0.02	4.02 ± 0.09	4.17 ± 0.06	4.52 ± 0.01	1.9–6.0	3.5–5.0
Specific gravity (kg L^{-1})	0.88 ± 0.00	0.88 ± 0.00	0.88 ± 0.00	0.88 ± 0.00	0.82–0.9	0.86–0.9
Cloud point (°C)	−6.79 ± 0.48	5.11 ± 1.93	−1.86 ± 1.30	5.58 ± 0.29		
Cetane number	49.50 ± 0.24	50.34 ± 0.96	51.96 ± 0.65	55.68 ± 0.15	≥51	≥47
Iodine value (gI_2 100 g^{-1})	161.88 ± 2.66	152.49 ± 10.74	134.43 ± 7.25	92.97 ± 1.63	51–120	
Higher heating value (MJ kg^{-1})	42.06 ± 0.06	41.84 ± 0.25	41.41 ± 0.17	40.43 ± 0.04		
ADU	2.01 ± 0.04	1.88 ± 0.14	1.64 ± 0.10	1.08 ± 0.02		

fatty acid methyl esters were the main lipid production ranging from 73.04% to 77.6%. The biodiesel properties satisfied the main specifications in US and European standards. The results indicated that cultivating microalgae with pickle industry wastewater provides a solution for high salinity wastewater treatment as well as nutrient recycling to get profitable production.

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