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Growing *Chlorella* sp. on meat processing wastewater for nutrient removal and biomass production

Qian Lu [a], Wenguang Zhou [a], Min Min [a], Xiaochen Ma [a], Ceria Chandra [a], Yen T.T. Doan [a,b], Yiwei Ma [a], Hongli Zheng [a,c], Sibo Cheng [a], Richard Griffith [a], Paul Chen [a], Chi Chen [a,d], Pedro E. Urriola [a,e], Gerald C. Shurson [a,e], Hans R. Gislerød [a,f], Roger Ruan [a,c,*]

[a] *Center for Biorefining and Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN, USA*
[b] *School of Environmental Science and Technology, Hanoi University of Science and Technology, Viet Nam*
[c] *MOE Biomass Energy Research Center and State Key Laboratory of Food Science, Nanchang University, Nanchang, Jiangxi, People's Republic of China*
[d] *Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA*
[e] *Department of Animal Science, University of Minnesota, St. Paul, MN, USA*
[f] *Norwegian University of Life Sciences, Norway*

HIGHLIGHTS

- *Chlorella* sp. (UM6151) is capable of utilizing nutrients in meat processing wastewater.
- Biomass yield of algae grown on mixed wastewater was improved to 0.675–1.538 g/L.
- NH_3–N and TN removal efficiencies in mixed wastewater were 68.75–90.38% and 30.06–50.94%.
- Protein content in algae grown on mixed wastewater was improved to 60.87–68.65%.
- Mixing wastewater balanced nutrient profiles and improved protein and biomass yield.

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ABSTRACT

In this work, *Chlorella* sp. (UM6151) was selected to treat meat processing wastewater for nutrient removal and biomass production. To balance the nutrient profile and improve biomass yield at low cost, an innovative algae cultivation model based on wastewater mixing was developed. The result showed that biomass yield (0.675–1.538 g/L) of algae grown on mixed wastewater was much higher than that on individual wastewater and artificial medium. Wastewater mixing eased the bottleneck for algae growth and contributed to the improved biomass yield. Furthermore, in mixed wastewater with sufficient nitrogen, ammonia nitrogen removal efficiencies (68.75–90.38%) and total nitrogen removal efficiencies (30.06–50.94%) were improved. Wastewater mixing also promoted the synthesis of protein in algal cells. Protein content of algae growing on mixed wastewater reached 60.87–68.65%, which is much higher than that of traditional protein source. Algae cultivation model based on wastewater mixing is an efficient and economical way to improve biomass yield.

1. Introduction

Microalgae have the potential to become an important protein and oil source for animal feeds, human diets, and fuels because of their high productivity (Vigani et al., 2015). Commercial large scale production of algae is expected to help address the worldwide food and energy shortage concerns. However, current algae technologies are mostly unsustainable and expensive. Most commercial algae cultivation systems use synthetic chemicals as nutrient source for algae growth. High price of synthetic chemicals is one of the critical factors which improved the production cost of algal biomass and limited its wide application in practice. Replacing the expensive synthetic chemicals with cheap resources as nutrient source is a promising way to reduce the cost of algae technologies.

Cultivation of algae on wastewater is considered a pathway to sustainable production of algal biomass because it reduces the production cost and generates environmental benefits by cleaning the

* Corresponding author at: University of Minnesota, St. Paul, MN 55108, USA. Tel.: +1 6126251710; fax: +1 6126243005.
E-mail address: ruanx001@umn.edu (R. Ruan).

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wastewater (Norsker et al., 2011). Previous studies showed that algae could grow on different types of wastewaters, including municipal wastewater, animal manure, and industrial wastewaters, which are available at no or very low cost (Su et al., 2012). The early interest in algae cultivation can be traced back to the use of algae to treat wastewater. The benefits of using algae to clean different wastewaters have been documented in numerous research reports (Christenson and Sims, 2011; El-Sikaily et al., 2007; Li et al., 2011).

One of the major concerns with using wastewaters for algae cultivation is the chemical composition of the wastewaters. Firstly, wastewaters often do not have balanced nutrient profiles to allow or sustain algae growth. For example, Chinnasamy et al. (2010) cultivated algae on carpet industry wastewater and the biomass yield was only 0.34 g/L. The main reason for the low biomass yield was that carpet industry wastewater contained low contents of necessary nutrients, including nitrogen and organic carbon, for algae growth. Zhou et al. (2011) reported that in concentrated municipal wastewater carbon source is insufficient to sustain algae growth. Such nutrient deficiency prevented the growth of algae and led to low biomass yield and poor nutrient removal efficiencies. Secondly, toxic compounds in some wastewaters prohibit or retard algae growth (Hughes and Poole, 1991) and may also be absorbed by algal biomass, which makes such algal biomass unsuited for animal or human consumption. Different from industrial and municipal wastewater, food service or processing wastewaters contain few toxic ingredients (Jacobsen et al., 2013), making them suitable for production of algal biomass for feed or food uses.

Cultivation of algae on food service and processing wastewaters such as those from cafeteria and dairy processing plants has been reported in the literature (Blier et al., 1995; Kern and Idler, 1999). However, currently no food processing plant has commercially implemented an algae based wastewater treatment process. One of the key challenges is that due to the low or imbalanced nutrient profile of wastewater, biomass yield of algae grown on many types of wastewater was not promising. For example, meat processing wastewater used in the research of Kern and Idler (1999) only contained 15.0 mg/L total phosphorous (TP) and 125.0 mg/L total nitrogen (TN) (Kern and Idler, 1999). To solve this problem, previous studies used acid digestion to release more nutrients in wastewater or added chemicals to balance the nutrient profile (Wang et al., 2013). However, these methods increased the production cost of microalgae due to the digestion treatment and addition of chemical.

The meat processing industry is one of the major food industries in Minnesota, USA. It was reported that a typical meat processing facility produced up to 10,000 m^3 wastewater each day (Bhamidimarri, 1991). Previous studies showed that in meat processing wastewater nutrients for algae growth (TN: 75–200 mg/L, TP: 20–40 mg/L; and COD: 800–2000 mg/L) (Thayalakumaran et al., 2003) were extremely low, not sufficient to support algae growth. To our knowledge, there was no research on the application of algae in the treatment of meat processing wastewater.

In this work, five types of wastewater from different processing steps in a meat processing plant were utilized to cultivate algae. The main aim was to determine the relationships between nutrient profile and algae growth and nutrient removal and develop a strategy to improve algal biomass yield and nutrient removal efficiency. The specific objectives were (1) to analyze the nutrient profile and metal profile of the meat processing wastewaters; (2) to identify algal strains that grew well on the wastewaters; (3) to measure the growth of microalgae on both non-mixed (individual) wastewaters and mixed wastewaters and test the nutrient removal efficiencies; (5) to analyze protein, lipid, and carbohydrate contents in microalgae grown under different conditions.

2. Methods

2.1. Materials and chemicals

Five types of wastewater, namely KILL, CUT, MPGP, REFINERY, and DS, were obtained from different processing steps in a meat processing plant in Minnesota, USA. Prior to use for algae cultivation, all wastewaters were centrifuged at 8000 RPM for 10 min to remove solid particles which could not be absorbed by algae and sterilized at 121 °C for 30 min. In commercial scale system, separated solids could be used for the production of fertilizer or animal feed. TAP medium which is commonly used for the cultivation of fresh water algae was used as a reference for comparison purpose. The TAP medium contained: NH_4Cl (0.375 g/L), $MgSO_4·7H_2O$ (0.1 g/L), $CaCl_2·2H_2O$ (0.05 g/L), Tris (2.42 g/L), K_2HPO_4 (0.11 g/L), KH_2PO_4 (0.06 g/L), $CuSO_4·5H_2O$ (1.5 mg/L), H_3BO_3 (11 mg/L), $(NH_4)_6Mo_7O_{24}·4H_2O$ (1 mg/L), $FeSO_4·7H_2O$ (5 mg/L), $ZnSO_4·7H_2O$ (22 mg/L), $MnCl_2·4H_2O$ (5 mg/L), $CoCl_2·6H_2O$ (1.5 mg/L), and acetic acid (1 mL/L). Chloroform and methanol were obtained from Sigma–Aldrich. Analysis kits for chemical oxygen demand (COD), ammonia nitrogen (NH_3–N), TN, and TP were obtained from Hach.

2.2. Algal strains screening

The algal strains used in this work were collected and identified from lakes and rivers of Minnesota in our previous study (Zhou et al., 2011) or purchased from UTEX. All strains were preserved on agar plate based on autotrophic (AC) medium under continuous light (120 μmol photons m^{-2} s^{-1}) at 25 °C.

Nutrients in AC medium include KH_2PO_4 (0.7 g/L), K_2HPO_4 (0.3 g/L), $MgSO_4·7H_2O$ (0.15 g/L), Glycine (5 g/L), H_3BO_3 (14.26 mg/L), $Na_2MoO_4·2H_2O$ (0.04 mg/L), $ZnSO_4·7H_2O$ (22.22 mg/L), $MnCl_2·4H_2O$ (5.87 mg/L), and $CuSO_4·5H_2O$ (0.07 mg/L), EDTA disodium salt (50 mg/L), $CoCl_2·6H_2O$ (1.61 mg/L), $CuSO_4·5H_2O$ (1.57 mg/L), $(NH_4)_6Mo_7O_{24}·4H_2O$ (1.10 mg/L), and $FeSO_4·7H_2O$ (4.99 mg/L) (Zhou et al., 2011). A total of eight strains were screened in this study. To examine the growth characteristics of these strains on individual wastewaters, each strain was inoculated onto an agar plate containing one type of wastewater (15 g agar in 1 L wastewater) and allowed to grow for six days. Algal strain which exhibited good growth on the agar plates of all five types of wastewater was in the rest of the experiments.

Growth of algae on agar plate was divided into three categories: (1) "growth": algal colony did not turn yellow but colony size had not change; (2) "good growth": algal colony was light green and colony size increased less than one time; and (3) "very good growth": algal colony turned dark green and colony size increased more than one time.

2.3. Experimental design

Experiments in this study were carried out by five steps. The first step was to measure the properties of meat processing wastewaters and screen robust algal strains. The second step was targeted at testing the growth of algae and nutrient removal in individual wastewaters. The third step was aiming at mixing wastewaters to balance the nutrient profiles if biomass yields of algae grown on individual wastewaters are low. The fourth step was cultivating algae on mixed wastewaters for the improvement of biomass yield and nutrient removal efficiency. The last step was targeted at comparing compositions, including protein and lipid, of algae grown on individual wastewaters and mixed wastewaters.

2.4. Growth and chemical analysis

2.4.1. Determination of algal growth

In this work, TVSSs (Total volatile suspend solids) were used to reflect the algal biomass concentration. TVSSs of wastewaters with algae were measured daily based on the standard method (Zhou et al., 2012). Before algae inoculation, TVSSs of wastewaters were measured and recorded. TVSSs of alga biomass were calculated by subtracting TVSSs of wastewaters from TVSSs of wastewaters with algae. The growth rate of microalgae was calculated according to Eq. (1)

$$R = (W_t - W_0)/(T_t - T_0) \quad (1)$$

where R is the growth rate of microalgae based on TVSS; T_0 and T_t are time on day 0 an day t; W_t and W_0 are the TVSS at day t and day 0, respectively.

To evaluate the effects of wastewater mixing on algae growth and nutrient removal, theoretical average biomass yield and nutrient removal efficiency were calculated based on the biomass yield and nutrient removal of algae grown on individual wastewaters. Eq. (2) was applied for the calculation of theoretical average biomass yield

$$T_b = (X_1 + X_2)/2 \quad (2)$$

where T_b is the theoretical average biomass yield in the mixture of two wastewaters; X_1 and X_2 are the biomass yield on two individual wastewaters.

Theoretical average nutrient removal efficiency was calculated according to Eq. (3)

$$T_n = (N_1 \times RE_1 + N_2 \times RE_2)/(N_1 + N_2) \quad (3)$$

where T_n (%) is the theoretical average nutrient removal efficiency; N_1 and N_2 are concentrations of certain nutrient in two individual wastewaters; RE_1 and RE_2 are nutrient removal efficiencies (%) in two individual wastewaters.

2.4.2. Nutrient analysis

COD, NH_3–N, TN, and TP were measured on Hach DR 5000 Spectrophotometer according to the method described by Li et al. (2011). Concentrations of nutrients were expressed as mg/L.

2.4.3. Protein content analysis

Protein content in microalgae biomass was determined according to the total nitrogen content in algae which was measured by using CE-440 elemental analyzer (Exeter Analytical Inc., Chelmsford, MA) according to the procedure described by Hu et al. (2013). Nitrogen-to-protein conversion factor (NTP) of 6.25 was used for calculating the protein content (Dominguez, 2013).

2.4.4. Total lipid content analysis

Harvested algae were dried in vacuum dryer before subjected to oil extraction. Total lipid in microalgae was measured according to the ultrasound assisted extraction method based on one-step extraction method described by Folch et al. (1957). Briefly, around 40 mg dried algae powder was weighted accurately and mixed with 2:1 chloroform/methanol (v/v) mixture. Oil was extracted in water bath with ultrasound for 15 min. Algae powder was extracted for two times. After the completion of extraction process, mixtures of organic solvent and algal residuals were separated through centrifuge. Organic solvent was removed using NEVAP Analytical Nitrogen Evaporator (Organomation Associates, Inc., USA) and lipid left behind in the bottom was weighted.

2.5. Statistical analysis

All experiments and tests in this study were carried out in triplicate. The average results were shown as means ± standard deviation values. Analysis of variation (ANOVA) was used to analyze the variance of these results.

3. Results and discussion

3.1. Nutrient and metal profiles of wastewater

Four main nutrient parameters, namely TN, TP, NH_3–N, and COD, in the wastewaters, were analyzed and compared with those in the reference TAP medium. The results (Table 1) show that the five wastewater samples varied in great deal and suffered deficiency in one or more nutrients when compared with TAP medium. Except KILL, other four wastewaters lacked TN, NH_3–N, and COD in comparison with TAP medium. The difference in the nutrient profiles of meat processing wastewater was caused by the different types and amounts of materials (manure, blood, hair, meat pieces, cooking ingredients, etc.) entering the wastewater streams during different stages of meat processing. It was reported that some slaughterhouse wastewater contained high content of COD (1500–11118 mg/L) while the content of TP was less than 20 mg/L (Johns, 1995; Sayed and de Zeeuw, 1988). The variabilities and deficiencies of certain necessary nutrients is a common problem existing in the wastewaters from meat industry.

Certain metallic elements, such as manganese, zinc, calcium, copper, etc., are significant to the growth of algae while some metallic elements, such as lead and aluminum, are toxic to algae (Gadd and Griffiths, 1977; Hughes and Poole, 1991). A deficiency in essential metallic elements or the presence of toxic metallic elements in wastewater may lead to unhealthy growth or death of algal cells. In addition, toxic metallic elements in wastewater may be absorbed by algal cells. As a result, harvested algal biomass will likely contain toxic metals is unsuitable for feed or food applications (Bulgariu and Bulgariu, 2012). Data in Table 2 indicates that meat processing wastewater contained most necessary trace metallic elements for algae growth but concentrations of some elements in wastewaters and TAP medium were very different. Some macro metallic elements, including Ca, Mg, and Na, in wastewater were higher than those in TAP medium. For example, concentrations of Na in DS, CUT, KILL, REFINRY, and MPGP were 21 times, 15 times, 7 times, 31 times, and 37 times more than the concentration of Na in TAP medium. Table 2 also indicates that concentrations of some toxic elements, such as Al and Pb, in wastewater were extremely low. Therefore, toxic metallic elements should not be a concern for both algae growth and use of harvested algae for feed or food applications. Based on the metal profile analysis,

Table 1
Nutrient profile of meat processing wastewater.

Wastewater	TN (mg/L)	TP (mg/L)	NH_3–N (mg/L)	COD (mg/L)
DS	76.5	10.2	11.1	734
CUT	64.8	45.9	8.2	1019
KILL	327.6	46.8	193.0	3560
REFINARY	117.5	5.6	101.7	1016
MPGP	91.3	32.9	2.2	2035
TAP medium	364.4	28.6	132.0	3870
DS + KILL	204.9	16.3	92.5	2100
CUT + KILL	212.0	29.7	102.1	2100
REFINARY + KILL	251.0	16.4	169.6	2340
MPGP + KILL	197.6	22.8	101.6	3020

" + " means the mixture of the wastewater; "TN" refers to total nitrogen; "TP" refers to total phosphorous; "NH_3–N" refers to ammonia nitrogen; and "COD" refers to chemical oxygen demand.

Table 2
Metal profiles of meat processing wastewater.

(Metal concentration) mg/L	DS	CUT	KILL	REFINERY	MPGP	TAP medium
B	0.10	0.06	0.05	0.08	0.06	2.02
Ca	22.58	17.10	13.47	13.23	61.26	13.60
Co	<0.01	<0.01	<0.01	<0.01	<0.01	0.40
Cu	0.79	<0.02	0.03	<0.02	<0.02	0.40
Fe	0.21	0.39	0.48	0.37	1.26	1.00
Mg	7.96	18.63	19.91	20.82	20.25	9.76
Mn	0.03	0.01	0.01	0.01	0.02	1.41
Mo	<0.01	<0.01	<0.01	<0.01	<0.01	0.60
Na	136.30	100.80	55.11	199.20	238.80	6.18
Zn	0.14	0.09	0.03	0.06	1.10	4.93
Al	<0.08	0.17	<0.08	<0.08	0.18	–
Pb	<0.18	<0.18	<0.18	<0.18	<0.18	–

meat processing wastewater could be used as medium alternatives for algae production.

The pH values of DS, CUT, KILL, and REFINERY are around 6.5 while that of MPGP is 3.4. In this work, MPGP was subjected to pH adjustment before use while other four types of wastewater were used without pH adjustment.

3.2. Screening of algal strains

Nutrient and metal profile analysis indicates that all five types of wastewater lacked some nutrients or metallic elements. These deficiencies might prevent the growth of some strains or even lead to the failure of algae cultivation. To select the robust algal strain for further experiments, eight algal strains from different sources were tested on agar plates containing the wastewaters. Performances of these algal strains were shown in Table 3.The result shows that all eight algal strains were able to grow on meat processing wastewaters. To ensure the high biomass yield, only algal strains having "very good growth" on all five types wastewater were used in further experiments. Table 3 shows that UM6151 (*Chlorella* sp.) exhibited very good growth on DS, CUT, KILL, and REFIERY as well on MPGP while other seven algal strains did not perform well. Therefore, UM6151 was considered the most robust algal strain for further experiments.

3.3. Growth of algae on individual wastewaters

3.3.1. Algal biomass yield

The algal biomass yields in individual (non-mixed) wastewaters and TAP medium are shown in Fig. 1. Biomass concentrations on KILL, MPGP, CUT, REFINERY reached peak values, 1.800 ± 0.126, 0.675 ± 0.018, 0.642 ± 0.080, 0.633 ± 0.063 g/L, respectively, on the 6th day while did not show any growth in DS. The most possible reason contributed to the failure of algae growth in DS is the high concentration of copper, which is toxic to algae. Previous studies showed that copper concentration higher than 0.5 mg/L would stop the growth of *Chlorella* sp. (Wong and Chang, 1991). In DS, the concentration of copper was 0.79 mg/L. In addition, low concentrations of necessary nutrients in DS also created an unfavorable environment for algae growth. Except for KILL, all other wastewaters were worse than TAP in which the highest biomass yield (on the 3rd day) was 1.100 ± 0.071 g/L. From 7th day, microalgae in these four types of wastewaters began to decrease. The possible reason for the decline in algae biomass after 7th day is that the nutrients had been exhausted. Therefore, the growth period of microalgae on wastewater was 6-days. Average growth rates of algae on KILL, MPGP, CUT, REFINERY, and DS by the 6th day were 0.256, 0.075, 0.057, 0.060, and 0 g/L/day, respectively. In terms of average growth rate, KILL was the most favorable for the growth of UM6151 while DS was the most unsuitable for the growth of UM6151. The main factor affecting the difference of algal biomass in five types of wastewaters was the difference in their nutrient profiles. The lack of one or two nutrients in wastewater became a bottleneck for algal growth. For instance, compared with TAP medium, MPGP had sufficient TN and COD while lacking in TN and NH_3–N. REFINERY had extremely low concentration of TP and CUT had insufficient TN, COD, and NH_3–N. Compared with TAP medium, DS lacked all four types of nutrients, TN, TP, NH_3–N and COD. Therefore, the lack of nutrients might be a possible reason for the low growth rate or no growth in these wastewaters.

3.3.2. Nutrients removal

Fig. 2 presents the daily changes in NH_3–N, TN, TP and COD of non-mixed wastewaters during cultivation. Due to the fact that algae did not have any growth in DS, nutrients removal efficiency in DS were 0. Data in Fig. 2(a) indicates that NH_3–N removal efficiencies in KILL, MPGP, CUT and REFINERY were 45.60%, 100.00%, 100.00% and 60.50% on 7th day, respectively. Data in Fig. 2(b) indicates that TN removal efficiencies in KILL, MPGP, CUT and REFINERY were 33.29%, 33.21%, 0%, and 32.74%, respectively. TP removal efficiencies in KILL, MPGP, CUT and REFINERY were 65.37%, 30.85%, 0%, and 100.00%, respectively (Fig. 2(c)). COD removal efficiencies in KILL, MPGP, CUT and REFINERY were 52.25%, 15.07%, 0%, and 39.63%, respectively (Fig. 2(d)).

There are various likely reasons for the difference in nutrient removal efficiency for the meat processing wastewaters. The first possible reason is that some nutrients in the wastewaters could not be utilized by algae efficiently. For instance, COD in KILL decreased by 52.25% after being treated by algae while that in CUT did not change. We hypothesized that organic carbon in KILL could be absorbed by algae efficiently while that in CUT could not be utilized by algae easily. It was reported that there are fine pores on algal cell walls and membrane for nutrient transportation. Nutrients in large particles could not be utilized by algal cells because of the restrict regulation on the transportation of large particles (Zemke-White et al., 2000). The second possible reason based on Liebig's Law of the Minimum is that the exhaustion of one or more nutrients prohibited the algae growth and the removal of other nutrients. For example, the COD removal efficiency in CUT was low although there was sufficient COD (1790 mg/L) in CUT for algae cultivation. Growth of algae might be prohibited when other nutrients, such as TN, TP and NH_3–N, in CUT were exhausted. As a result, metabolism in algal cells grown on CUT was restricted and

Table 3
Growth performance of microalgae on agar plate of wastewater.

Wastewater	UM4255	UM6151	UM665	UM669	UM667	UM785	UM7167	UTEX2229
DS	+	+++	+++	+	++	+++	++	+
CUT	+	+++	+	++	+	+	++	+
KILL	+++	+++	+++	++	+++	+++	+++	++
REFINERY	++	+++	+++	++	+++	++	+++	+
MPGP	+	++	++	+	+	++	++	+

"+" refers to "growth", "++" refers to "good growth", and "+++" refers to "very good growth".

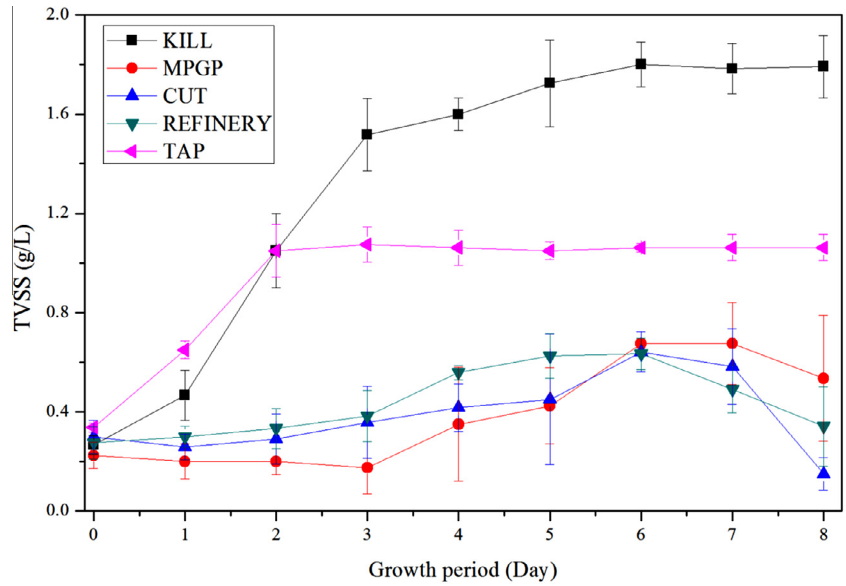

Fig. 1. Growth curve of UM6151cultivated on non-mixed wastewater and TAP medium.

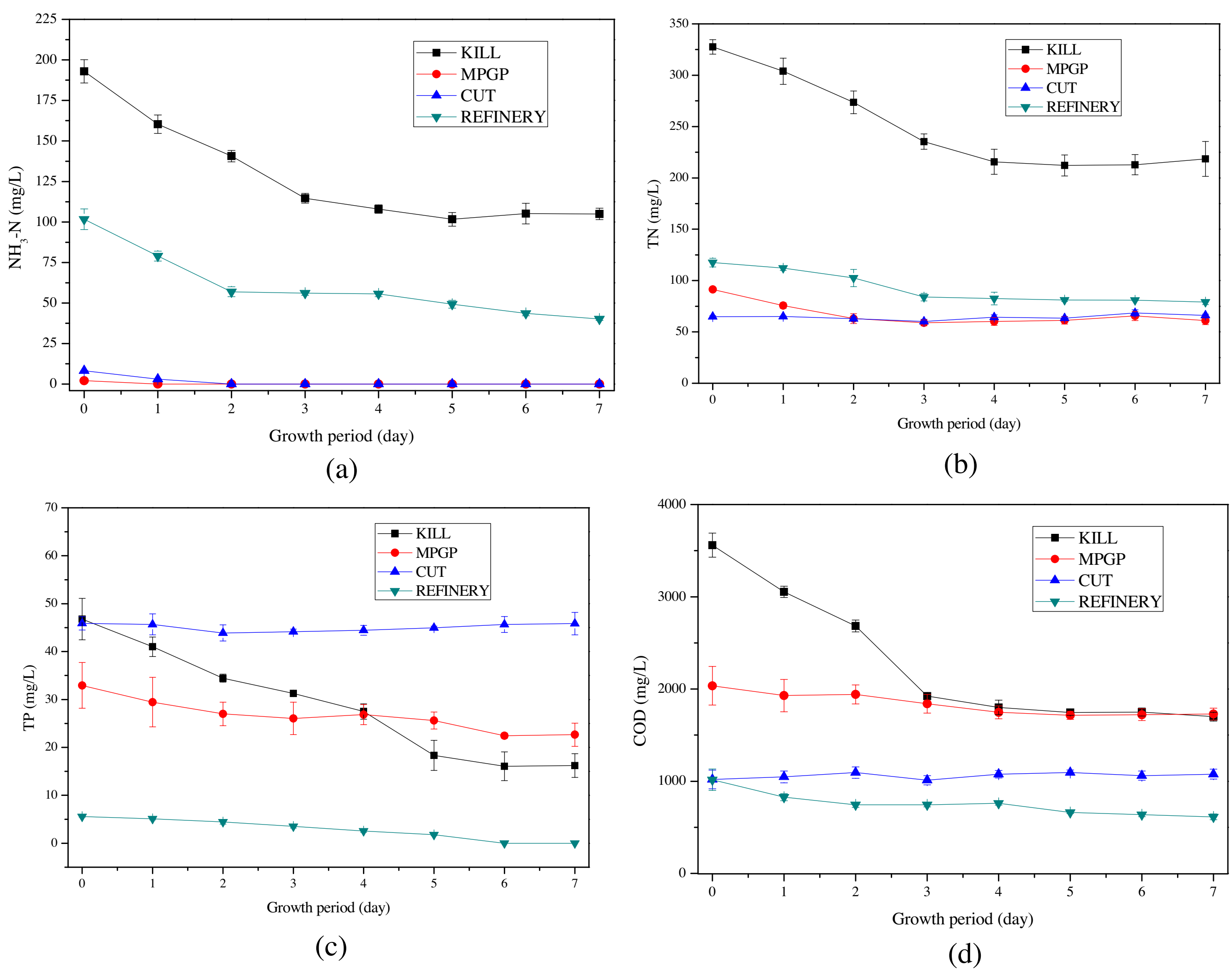

Fig. 2. Removal of nutrients in non-mixed wastewater (a) removal of NH_3–N in non-mixed wastewater; (b) removal of TN in non-mixed wastewater; (c) removal of TP in non-mixed wastewater; (d) removal of COD in non-mixed wastewater.

the removal of COD was also prevented. The last reason might be the high concentration of toxic metallic elements in some wastewaters. It was reported that aluminum could inhibit the activity of acid phosphatase in algae and lead to low biomass yield (Kong and Chen, 1995). So high concentrations of aluminum in CUT and MPGP might inhibit the metabolism of algae and lead to low nutrient removal efficiencies.

Our data show that nutrient removal efficiency was generally correlated with biomass yield. For example, algae on CUT did not remove TN, TP and COD efficiently. As a result, average growth rate of algae growing on CUT was just 0.057 g/L/day, which was lower than that of algae grown on KILL, REFINERY and MPGP. However, it was unclear which nutrients had most significant impact on biomass yield.

3.4. Growth of algae on mixed wastewaters

3.4.1. Biomass yields of algae

The experiment data show that KILL was the best culture medium among all the wastewaters. Adding KILL to other wastewaters would be expected to improve the nutrient profiles of the other wastewaters. In this experiment, KILL was mixed with MPGP, CUT, REFINERY, and DS by 1:1 (v/v), individually. The nutrient profiles of mixed wastewater were shown in Table 1. Nutrient profiles (TN, TP, NH_3–N, and COD) of mixed meat processing wastewater reached same or similar level with those of TAP medium.

The algal biomass yields in the mixed wastewaters are shown in Fig. 3. Peak values of algal biomass yields in CUT + KILL, REFINERY + KILL, MPGP + KILL, DS + KILL were 1.538 ± 0.018, 1.400 ± 0.035, 1.388 ± 0.018, and 0.675 ± 0.071 g/L, respectively. Biomass yields on CUT + KILL, REFINERY + KILL, and MPGP + KILL were 39.82%, 27.27% and 26.18%, respectively, higher than that in TAP medium. Therefore, in terms of biomass yield, CUT + KILL, REFINERY + KILL, and MPGP + KILL were much better than TAP. The mixed wastewater media produced higher biomass yields than the individual wastewater media (CUT, REFINERY, and MPGP). They even performed better than the TAP medium. Furthermore, the mixture of DS and KILL enabled healthy algae growth while DS alone failed to do so. However, in comparison with other three types of mixed wastewater and TAP medium, DS + KILL was still the worst. In addition, the yield for DS + KILL was lower than the estimated average of the yields for DS (0 g/L) and KILL (1.800 ± 0.126 g/L), suggesting that DS not only diluted KILL but also had certain factors inhibiting algae growth. High concentration of copper in mixture is the most possible factor which prohibited the algae growth in the mixture of DS and KILL.

Algae growth and nutrient removal in the mixed wastewater samples compared to the theoretical average of the mixtures is shown in Table 4. Result of TVSS indicates that except KILL + DS, other three types of mixed wastewater had much higher biomass yield than non-mixed wastewater. In comparison with theoretical average biomass yields of algae, biomass yields of algae in KILL + CUT, KILL + REFINERY, and KILL + MPGP were improved by 25.96%, 15.13%, and 12.21%, respectively. It was hypothesized that the lack of one or more nutrients was the bottleneck to algae growth on meat processing wastewater. Nutrients in mixed wastewater were more balanced and the bottleneck effect was eased. The synergetic effects of different wastewaters in mixed system modified the nutrient profile and contributed to the improvement of biomass yield.

3.4.2. Nutrients removal

Data in Fig. 4 summarized the daily changes in NH_3–N, TN, TP and COD of mixed wastewaters during cultivation. Fig. 4 (a) indicates that NH_3–N removal efficiencies in DS + KILL, MPGP + KILL, CUT + KILL and REFINERY + KILL were 82.40%, 87.43%, 90.38% and 68.75% on 9th day, respectively. TN removal efficiencies (Fig. 4 (b)) in DS + KILL, MPGP + KILL, CUT + KILL and REFINERY + KILL were 44.46%, 30.06%, 50.94% and 49.48% on 9th day, respectively. Fig. 4(c) indicates that TP removal efficiencies in DS + KILL, MPGP + KILL, CUT + KILL and REFINERY + KILL were 52.11%, 63.51%, 44.95% and 54.45% on 9th day, respectively. COD removal efficiencies (Fig. 4(d)) in DS + KILL, MPGP + KILL, CUT + KILL and REFINERY + KILL were 3.21%, 7.95%, 29.52% and 43.91% on 9th day, respectively.

TN removal efficiencies in KILL + CUT, KILL + REFINERY, and KILL + DS were improved while that in KILL + MPGP was maintained at similar level in comparison with theoretical average nutrient removal efficiency. NH_3–N removal efficiencies in all four types of mixed wastewater were higher in comparison with theo-

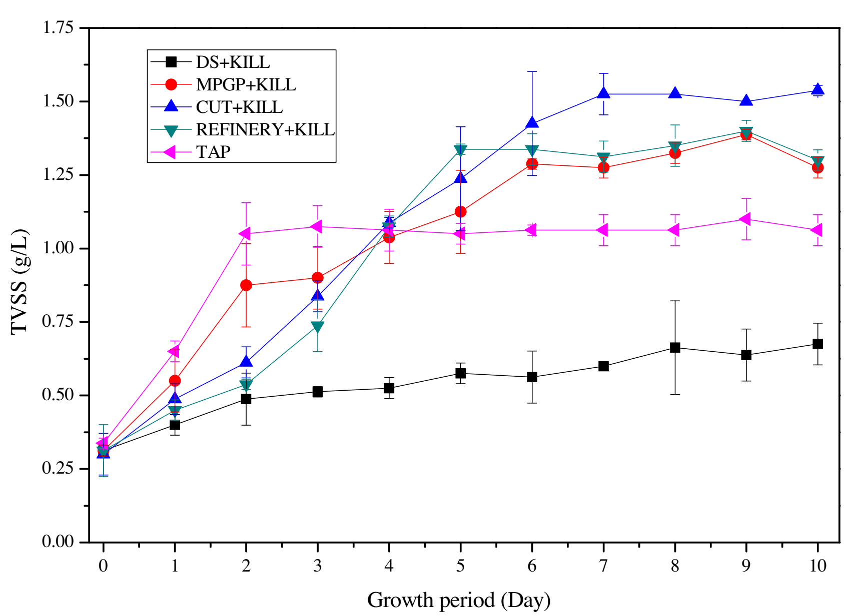

Fig. 3. Growth curve of UM6151cultivated on mixed wastewater.

Table 4
Comparison of algae growth on non-mixed and mixed wastewater.

Wastewater	TVSS (g/L)	TN (%)	TP (%)	NH_3–N (%)	COD (%)
KILL + CUT mixture	1.538	50.94	44.95	90.38	29.52
KILL + CUT theoretical Avg	1.221	27.79	33.06	45.77	40.62
KILL + REFINERY mixture	1.400	49.48	54.45	68.75	43.91
KILL + REFINERY theoretical Avg	1.216	35.39	69.07	50.24	49.45
KILL + MPGP mixture	1.388	30.06	63.51	87.43	7.95
KILL + MPGP theoretical Avg	1.237	33.27	51.26	44.30	38.73
KILL + DS mixture	0.675	44.46	52.11	82.40	3.21
KILL + DS theoretical Avg	0.900	26.99	53.67	41.88	4.32

retical average nutrient removal efficiencies. For example, NH_3–N removal efficiency in KILL + CUT reached 87.43% while the theoretical value was just 44.30%. The main reason for the low theoretical average removal efficiencies of TN and NH_3–N wastewater is that content of nitrogen, in MPGP, CUT, DS, and REFINERY which could be absorbed by algae was low. Previous studies indicate that sometimes in wastewater some necessary nutrients, including nitrogen, were combined with macromolecular materials which could not be utilized by algae (Stehfest et al., 2005). Therefore, algae grown on CUT, REFINERY, DS and MPGP were under the low nitrogen conditions. On the contrary, algae in KILL with high TN concentration could absorb enough nitrogen for growth. After being mixed with KILL, nitrogen concentration in the wastewaters was improved significantly and algae were not impacted by low nitrogen stress. As a result, TN and NH_3–N removal efficiencies in mixed wastewater were much higher than that in non-mixed wastewater.

On the contrary, COD removal efficiency in mixed wastewater was lower than theoretical value. For instance, COD removal efficiency in KILL + CUT was 29.52% while the theoretical value was 40.62%. The difference between COD removal efficiency and NH_3–N and TN removal efficiencies indicates that algae in non-mixed wastewater were prone to utilize organic carbon while those in mixed wastewater were prone to utilize nitrogen. It was reported that algae under low nitrogen pressure are exposed to unfavorable conditions and are more likely to accumulate lipid in cells to survive. For example, content of triacylglycerols in green alga, *Chlamydomonas reinhardtii*, under low nitrogen condition reached 9.5 μg 10^{-6} cell while that in alga under nitrogen sufficiency was less than 0.5 μg 10^{-6} cell (Siaut et al., 2011). In this study, in non-mixed wastewater, particularly MPGP, CUT, DS, and REFINERY, without sufficient nitrogen, lipid synthesis in algae was enhanced (Rodolfi et al., 2009). Therefore, algae were prone to utilize organic carbon to synthesize lipid in non-mixed wastew-

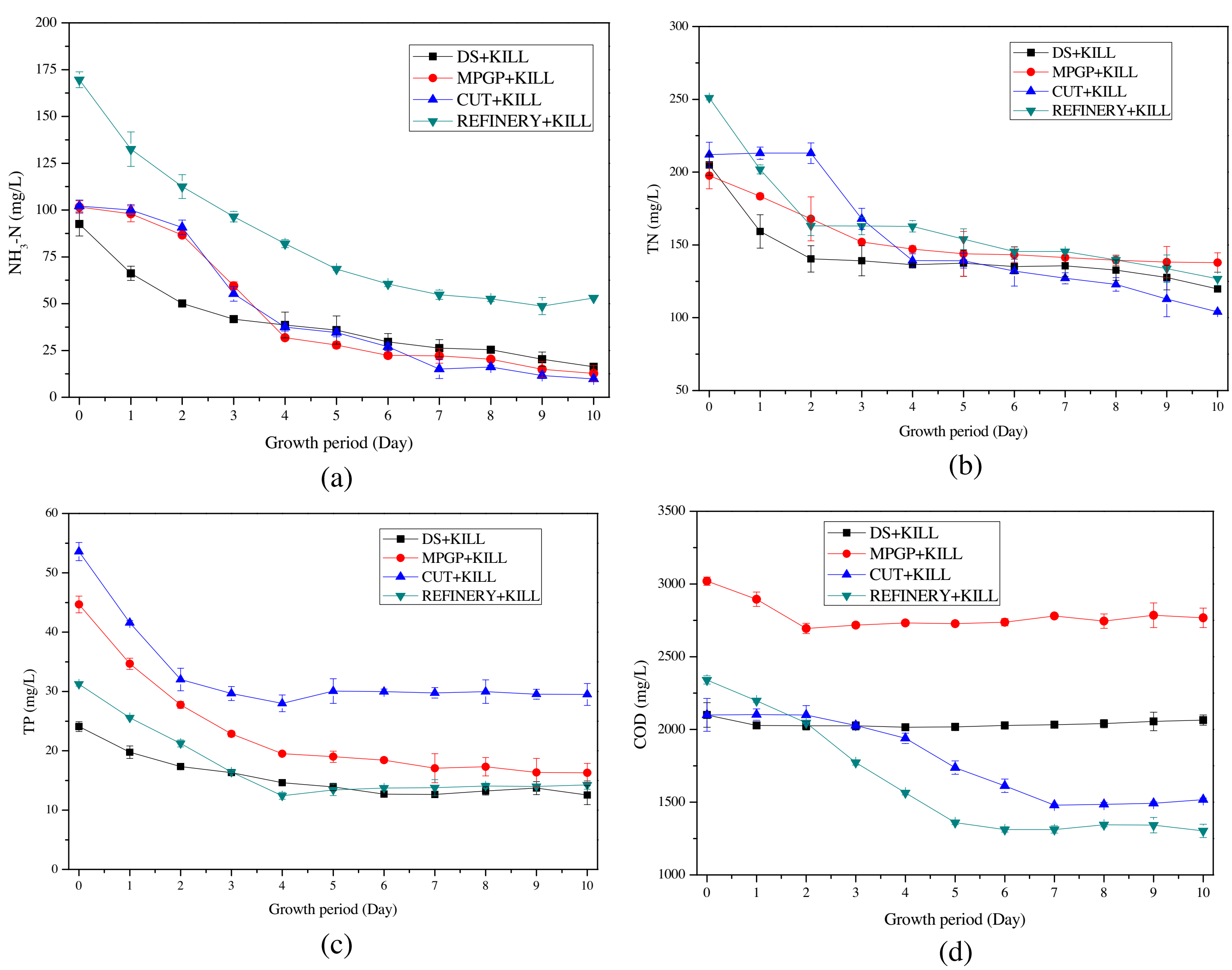

Fig. 4. Removal of nutrients in mixed wastewater (a) removal of NH_3–N in mixed wastewater; (b) removal of TN in mixed wastewater; (c) removal of TP in mixed wastewater; (d) removal of COD in mixed wastewater.

ater. In the mixed wastewater, nitrogen for algae growth was improved due to the addition of KILL with high nitrogen concentration. Under this condition, algae removed nitrogen efficiently for protein synthesis while did not utilize organic carbon to synthesize lipid. Therefore, COD removal efficiency in non-mixed wastewater was higher than that in mixed wastewater.

Data in Table 4 showed that difference between TP removal efficiency in non-mixed wastewater and that in mixed wastewater was minor. For example, TP removal efficiency in KILL + DS was 52.11% while that in non-mixed wastewater was 53.63%. Mixing meat processing wastewater would not change the removal of TP. Table 1 indicates that meat processing wastewater, except DS, contained high concentrations of phosphorous in comparison with TAP medium. Phosphorous in both non-mixed wastewater and mix wastewater was sufficient for algae growth. Therefore, TP removal efficiency was not changed after the mixing process.

3.5. Chemical composition of algal biomass grown on wastewater

Contents of protein, lipid and other components in the harvested algae were shown in Table 5. Protein content of the algae grown on CUT, REFINERY and MPGP ranges from 51.58% to 60.33%, which falls in the range of protein contents in *Chlorella* sp. reported in the literature (Hymowitz et al., 1972; Wolf et al., 1982). However, algae grown on the mixed wastewaters had much higher protein content (60.87–68.65%). The lipid content of algae grown on non-mixed wastewater ranged from 15.87% to 25.60% while lipid content of algae growing on mixed wastewater ranged from 14.50% to 20.57%. The difference in protein and lipid contents between the individual and mixed wastewater algae may be attributed to the variation in nitrogen contents in the wastewaters. In theory, low nitrogen content limited the protein synthesis (Wang et al., 2009) but created stress conditions that triggered enhanced lipid synthesis (Scott et al., 2010). Therefore, protein content of algae grow on mixed wastewater was higher than that of algae grown on individual wastewater, CUT, MPGP, and REFIERY. However, protein content of algae grown on KILL which has the highest nitrogen content was only 63.31% which was lower than protein content of algae grown on some mixed wastewaters, KILL + CUT and KILL + REFINERY. The possible reason is that some nitrogen in large particles could not be utilized by algae and the absorbable nitrogen content in KILL was not as high as the analysis result. After being mixed, the chemical reaction during thermal sterilization might release some nitrogen and improve the absorbable nitrogen content in mixed wastewater.

Table 5
Composition of algae grown on various meat processing waste streams.

Wastewater	Lipid (%)	Protein (%)	Other* (%)
KILL + CUT	17.54	68.65	13.81
KILL + REFINERY	20.57	64.76	14.67
KILL + MPGP	18.89	61.20	19.91
KILL + DS	14.50	60.87	24.63
CUT	21.01	51.58	27.41
REFINERY	23.95	60.33	15.72
MPGP	25.60	55.08	19.32
KILL	15.87	63.31	20.82

* Other components include carbohydrates, nucleic acids, etc.

In comparison with soybean proteins, which have commonly used in our diets, algae have advantages in three aspects. Firstly, protein content in alga UM6151 grown on wastewater is much higher than that in soybean (33.1–49.2%) (Hymowitz et al., 1972; Wolf et al., 1982). Secondly, although oil is not the major product of algae grown on mixed wastewaters, average oil content (17.88%) of algae is on the same level with the oil content (18%) in soybean (Mata et al., 2010). Thirdly, oil yield of algae is 136,900 L/ha year while that of soybean is only 446 L/ha year (Chisti, 2007). Accordingly, in terms of biomass and protein productivities, algae cultivation is much better than soybean cultivation. Finally, the cost of producing protein and oil by growing algae in wastewater is lower than that of growing soybean crop. Therefore, algae grown on mixed wastewater without toxic contaminants could be a potential source of protein in human diets.

4. Discussion

In previous studies, different types of wastewater, such as municipal wastewater and dairy wastewater, have been used to produce algae for feedstock or biofuel (Pittman et al., 2011). The data of some typical studies are presented in Table 6. Generally, biomass yield of algae grown on wastewater is low due to the lack of nutrients or the high content of toxic ingredients. For example, algae biomasses in dairy wastewater and carpet industry wastewater were only 0.5 and 0.34 g/L, respectively, after 9 days. In comparison with algae biomass (above 1.0 g/L) in artificial medium (Farooq et al., 2013; Kumar and Das, 2012), that in most wastewater was much lower. Low biomass yield of algae on wastewater would reduce the economic profit and prevent the application of algae in wastewater treatment. To improve biomass yield, some methods, such as adding CO_2, continuous feeding and so on, were used. For instance, algae biomass in municipal wastewater was improved to 1.5 g/L by continuous feeding (Zhou et al., 2012). However, adding CO_2 and continuous feeding would increase the cost of infrastructure for algae production. Current research improved the biomass yield of algae by mixing different types of wastewater. In comparison with continuous feeding and adding CO_2, mixing wastewater is low cost and the operation is simple.

Table 6
Characteristics of wastewater and biomass of microalgae grown in wastewater.

Wastewater	Algae strain	Nutrient concentration (mg/L)			Yield of algal biomass (g/L)	Period (days)	References
		TN	TP	COD			
Carpet industry wastewater	*Chlorella saccharophila*	NA	3.47–7.89	106–183	0.34	9	Chinnasamy et al. (2010)
Chicken manure	*Chlorella pyrenoidosa*	NA	NA	NA	0.60	NA	Cheung and Wong (1981)
Concentrated municipal wastewater	*Chlorella* sp.	134	212	2324	0.9	9	Zhou et al. (2011)
Dairy wastewater	*Scenedesmus* sp.	36.6	1.8	NA	0.5	9	Woertz et al. (2009)
Industrial and municipal wastewater	*Chlorella vulgaris*	NA	NA	NA	0.21	NA; 6% CO_2 supplied	Chinnasamy et al. (2009)
Municipal wastewater	*Auxenochlorella protothecoides*	134	211	2344	1.5	Continuous feeding	Zhou et al. (2012)
Municipal wastewater	*Chlorella* sp.	51	2.1	NA	0.84	4; (CO_2 supplied)	Woertz et al. (2009)
CUT + KILL	*Chlorella* sp.	212.0	53.6	2100	1.538	9	Current work
DS + KILL		204.9	24.1	2100	0.675		
MPGP + KILL		197.6	44.7	3020	1.388		
REFINERY + KILL		251.0	31.3	2340	1.400		

In comparison with previous studies, the new algae cultivation model based on wastewater mixing developed in this study has advantages in two aspects. First, algae growing on food processing wastewater which contains few toxic ingredients could be used for feed or food while algae growing on municipal wastewater could not reach feed or food grade. Therefore, the application range of algae growing on food processing wastewater is much wider. Second, improving yield of algae biomass by mixing different types of wastewater has low maintenance cost and does not need to update the algae cultivation facilities. In comparison with adding CO_2 and continuous feeding, mixing wastewater to improve biomass yield has much lower cost.

5. Conclusions

It was concluded that (1) *Chlorella* sp. (UM6151) is the most robust algal strain for the treatment of meat processing wastewater; (2) the lack of one or more nutrients is considered as the bottleneck to algae growth on individual wastewater, except KILL; (3) mixed wastewaters had positive synergetic effects on algae growth; (4) mixing wastewater is an economic and efficient way to balance the nutrient profile and improve algae growth and nutrient removal efficiency in wastewater.

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