Mitigating ammonia nitrogen deficiency in dairy wastewaters for algae cultivation

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HIGHLIGHTS

- Limiting factor to algae growth is the ammonia nitrogen deficiency in dairy wastewaters.
- Mixing dairy wastewater with slaughterhouse wastewater balanced the nutrient profile.
- Algae biomass yield on mixed wastewater had been improved to 1.32–2.66 g/L.
- Algal grown on mixed wastewater had high protein content (55.98–66.91%).

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ABSTRACT

This study demonstrated that the limiting factor to algae growth on dairy wastewater was the ammonia nitrogen deficiency. Dairy wastewaters were mixed with a slaughterhouse wastewater that has much higher ammonia nitrogen content. The results showed the mixing wastewaters improved the nutrient profiles and biomass yield at low cost. Algae grown on mixed wastewaters contained high protein (55.98–66.91%) and oil content (19.10–20.81%) and can be exploited to produce animal feed and biofuel. Furthermore, algae grown on mixed wastewater significantly reduced nutrient contents remained in the wastewater after treatment. By mitigating limiting factor to algae growth on dairy wastewaters, the key issue of low biomass yield of algae grown on dairy wastewaters was resolved and the wastewater nutrient removal efficiency was significantly improved by this study.

1. Introduction

Algal biomass has become a potential resource for animal feed and biofuel production. To reduce the production cost of biomass, algae cultivation is often combined with wastewater treatment. Algae have been successfully cultivated on brewery wastewater (Farooq et al., 2013), municipal wastewater (Li et al., 2011a), animal manure (Mulbry et al., 2008), meat processing wastewater (Lu et al., 2015), etc. for biomass production. Different technologies, such as aeration (Mata et al., 2012), acid digestion (Hu et al., 2013), fermentation (Hu et al., 2012), have been developed to improve the biomass yield of wastewater-grown algae. Wastewaters, which are available at no or low cost, provided a cost-effective and sustainable means for algae cultivation and biomass production (Pittman et al., 2011).

It was reported that in US the milk yield increased from 53.1 billion kg during 1944–84.2 billion kg in 2007 (Capper et al., 2009). Such fast development of dairy processing industry is accompanied by increasing amounts of dairy wastewater, which is a source of surface and ground water pollution. Previous studies showed that dairy wastewater with high contents of organics, which could be utilized by fungi, bacteria and some microalgae, was the chief cause of water pollution in some areas (Yu et al., 2014). The conventional treatments of dairy wastewater included direct reutilization of waste components and anaerobic digestion. In the past several years there has been an increasing interest in cultivating

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algae on dairy wastewater for both biomass production and wastewater treatment (El-Sikaily et al., 2007). The treatment based on algae cultivation had low demand on the infrastructure and could produce some valuable compositions. Furthermore, compared with municipal wastewater and animal manure, dairy wastewater contained more nutrients which are essential for algae growth.

Many studies have used dairy wastewater for algae cultivation. However, one problem is that biomass yield of algae grown on dairy wastewater was low. Literature search showed that the highest biomass yield of algae grown on dairy wastewater was less than 0.7 g/L (Blier et al., 1995; El-Sikaily et al., 2007). Dairy wastewater containing complex organics had high contents of COD (950–7500 mg/L) and BOD (500–4500 mg/L) (Christenson and Sims, 2011). The ranges of COD and BOD in dairy whey even reached 18,400–69,500 mg/L and 40,000–83,000 mg/L, respectively (Öztürk et al., 1993). To prevent the inhibition of excessively high content of organics on algae growth, Woertz et al. (2009) diluted dairy wastewaters by 10% before the algae inoculation. However, the harvested algal biomass was less than 0.6 g/L (Woertz et al., 2009). Dairy wastewater also contained various metal elements (Markou and Georgakakis, 2011). Previous studies indicated that the average ammonia nitrogen (NH_3-N) content in dairy wastewater was only 48 mg/L and that NH_3-N contents in some dairy wastewaters were even less than 5 mg/L (Longhurst et al., 2000). The research of Lincoln et al. (1996) showed that NH_3-N in dairy wastewater was consumed totally by algae in 72 h (Lincoln et al., 1996) while the removal efficiencies of other nutrients were not high. Therefore, we hypothesized that it is the deficiency of NH_3-N that leads to the low biomass yield of algae grown on dairy wastewater. By mixing dairy final effluent with pulp and paper influent, Gentili (2014) improved the nutrient profile of dairy wastewater and obtained higher biomass yield (1.12 g/L) (Gentili, 2014). This study revealed that mixing is a possible pathway to improve the nutrient profiles of wastewaters and the biomass yield of algae. However, due to the toxicity of pulp and paper influent, harvested algal biomass could not be used for food or animal feed.

The main aim of this study was to confirm the hypothesis and develop a cheap and efficient method to improve the biomass yield of algae grown on dairy wastewaters. The specific objectives included: (1) Analyzing the nutrient profile and metal element profile of dairy wastewaters; (2) Measuring nutrient removal efficiencies in individual dairy wastewaters and algal biomass yields; (3) Determining the limiting factor to algae growth on dairy wastewaters; (4) Adding certain chemicals to mitigate the limiting factor and measuring the biomass yield; (5) Mixing dairy wastewaters with other wastewater to improve the nutrient profile at low cost; and (6) Comparing the nutrient compositions of algae grown on individual dairy wastewaters and mixed wastewaters.

2. Methods

2.1. Materials and chemicals

Analysis kits for chemical oxygen demand (COD), total nitrogen (TN), ammonia nitrogen (NH_3-N), and total phosphorous (TP) were purchased from Hach (USA). Chloroform, ammonium chloride, methanol and other chemicals were purchased from Sigma–Aldrich (USA). Three dairy wastewaters, mother liquor, salt whey and liquid whey, were obtained from different processing steps in a dairy processing plant in Minnesota, USA. Prior to use for algae cultivation, all wastewaters were centrifuged at 8000 RPM for 10 min to remove the large particles and sterilized at 121 °C for 30 min.

2.2. Algal strains screening

To select the most robust algal strain for dairy wastewater treatment, ten different algal species, collected from lakes and rivers of Minnesota (Zhou et al., 2011) or obtained from UTEX, were cultivated on the agar plates containing one of the three dairy wastewaters. Before the screening process, algae were preserved on agar plate based on autotrophic (AC) medium at 25 °C under continuous fluorescent light (120 μmol photons m^{-2} s^{-1}). Nutrient profile of AC medium was listed as follows: Glycine (5 g/L), H_3BO_3 (14.26 mg/L), $MgSO_4{\cdot}7H_2O$ (0.15 g/L), $ZnSO_4{\cdot}7H_2O$ (22.22 mg/L), K_2HPO_4 (0.3 g/L), $(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$ (1.10 mg/L), $Na_2MoO_4{\cdot}2H_2O$ (0.04 mg/L), $MnCl_2{\cdot}4H_2O$ (5.87 mg/L), KH_2PO_4 (0.7 g/L), $CoCl_2{\cdot}6\ H_2O$ (1.61 mg/L), EDTA disodium salt (50 mg/L), $CuSO_4{\cdot}5\ H_2O$ (1.57 mg/L), $CuSO_4{\cdot}5H_2O$ (0.07 mg/L), and $FeSO_4{\cdot}7\ H_2O$ (4.99 mg/L). The agar plates were prepared according to method described by Zhou et al. (2012).

Dairy wastewaters were divided into four groups: (A) No dilution and no pH adjustment; (B) No dilution but adjusted pH to 6.5; (C) 10 times dilution and no pH adjustment; and (D) 10 times dilution but adjusted pH to 6.5. Algae were grown on the agar plate of four groups of dairy wastewaters. Algal strains having good growth on agar plates of all three dairy wastewater were considered as robust strains and used in the rest of experiments. To examine the growth characteristics of the selected strains on wastewaters, each strain was inoculated onto an agar plate containing one type of dairy wastewater (15 g agar in 1 L wastewater). The algae strains were divided into three categories based on their growth characteristics: (1) "no growth": color of algae colony turns yellow and algae could not survive; (2) "growth": color algae colony is light green but the colony size does not increase; and (3) "good growth": color of algae colony turns dark green and colony size increases obviously.

2.3. Experimental design

The experiments of this work were carried out in five steps. The first step was intended to analyze the nutrient and metal profiles of the dairy wastewaters and select robust algal strains for wastewater treatment. The second step was to study the growth and nutrient removal characteristics of the selected algae strains, from which the limiting factor to algae growth would be determined. The third step was to examine the feasibility of eliminating or mitigating the limiting factor by compensating the deficient nutrients using chemicals. The fourth step was to mitigate the limiting factor by mixing wastewaters of complementary nutrient profiles. The final step was to compare the chemical compositions, including lipid and protein, of the harvested algae grown on individual and mixed wastewaters.

2.4. Growth and chemical analysis

2.4.1. Algal growth and nutrient removal

Algae were cultivated in 250-mL flasks containing 100 mL medium or wastewaters at 26 °C placed on a rotary shaker. Illumination was provided by cool white fluorescent lamps, giving a light intensity of 120 μmol photons m^{-2} s^{-1}. Densities of algae inoculated in wastewaters or artificial medium were around 0.25 g/L.

The biomass yield of algae was expressed as TVSSs (Total volatile suspend solids) which were measured daily according to previously published method (Zhou et al., 2012). Average growth rate of algae was calculated according to Eq. (1)

$$R = (W_t - W_0)/t \tag{1}$$

where R is the growth rate of microalgae based on TVSS; t is the time interval (days); W_t and W_0 are the TVSS at day t and day 0, respectively.

A linear model (Eq. (2)) was used to describe the relationship between algae growth and biomass density (Yang et al., 2011)

$$N = K/(1 + e^{a-rt}) \quad (2)$$

where N (mg/L) is the dry weight of algal biomass at time t (day); K (mg/L) is the maximum biomass accumulated in the culture; a is a constant; and r (day^{-1}) is the specific growth rate.

Eq. (2) could be transformed into a linear form

$$\mathrm{Ln}\left(\frac{K}{N} - 1\right) = a - rt \quad (3)$$

2.4.2. Nutrient profile analysis

Nutrient profiles of dairy wastewater, including COD, NH_3-N, TN, and TP were analyzed by using a Hach DR 5000 Spectrophotometer according to the previously published method (Li et al., 2011b). The concentrations of nutrients in wastewater were expressed as mg/L.

2.4.3. Protein content analysis

The protein content in the harvested algal biomass was analyzed using a CE-440 elemental analyzer (Exeter Analytical Inc., Chelmsford, MA) according to previously published method (Hu et al., 2013). In this work, nitrogen-to-protein conversion factor (NTP) of 6.25 was used for the calculation of protein content (Dominguez, 2013).

2.4.4. Total lipid content analysis

Before subjected to oil extraction, algae were harvested through centrifuge and dried in a vacuum dryer. The total lipid in algae was measured according to the previously published one-step extraction method with minor modification (Sirés and Brillas, 2012): (1) Dry algal powder (around 40 mg) was added into glass tube and mixed with 2 mL of 2:1 chloroform/methanol (v/v) mixture; (2) Oil extraction was carried out in water bath with ultrasound assistance for 15 min; (3) After completion of oil extraction, organic solution and residual algal powder were separated through centrifuge; (4) The extraction process was repeated three times; (5) NEVAP Analytical Nitrogen Evaporator (Organomation Associates, Inc., USA) was used to remove the organic solution; (6) The lipid left in the bottom was weighed.

2.5. Statistical analysis

All experiments and tests were conducted in triplicate. The results were expressed as means ± standard deviation values. Analysis of variation (ANOVA) was employed to analyze the variance of results.

3. Results and discussion

3.1. Dairy wastewater characteristics and algae screening

3.1.1. Nutrient and metal profile

Four parameters, COD, NH_3-N, TN, and TP, in wastewaters were measured and compared with those in TAP medium, an artificial medium commonly used for algae culture. The results (Table 1) indicate that the dairy wastewaters had extremely high concentration of COD and TP. For example, COD contents in mother liquor, salt whey and liquid whey were 48 times, 7 times and 19 times more than that in TAP medium. The main reason for the high COD content is that dairy wastewaters contain high concentration of organic carbon. It was reported that a too high nutrient concentration can seriously inhibit algae growth (Wang et al., 2010). To prevent the negative effect of high concentrations of organic carbon on algae growth, dairy wastewater must be diluted before being used for algae cultivation.

Table 1
Nutrient profile of dairy wastewater.

	NH_3-N (mg/L)	TN (mg/L)	TP (mg/L)	COD (mg/L)
Mother liquor	429	3570	22,350	191,000
Salt whey	57.8	935	735	30,700
Liquid whey	24.8	2164	1012.5	76,350
TAP medium	132.0	364.4	28.6	3870
Mother liquor (mixed)	151.3	281.3	565.3	6000
Salt whey (mixed)	151.7	322.9	59.47	3130
Liquid whey (mixed)	172.3	351.6	157.0	4693

Metal profile analysis showed that the dairy wastewaters contained high concentrations of macro metal elements, such as calcium (Ca), potassium (K), manganese (Mg), and sodium (Na), which are essential to algae growth (Table 2). However, when the contents of these elements exceeded certain thresholds, algal biomass yield would not increase and the metal utilization efficiency would not be improved. Sometimes, high contents of essential elements may prohibit algae growth. To improve the utilization efficiency of metal elements and prevent the negative effects of high contents of elements on algae growth, it is necessary to dilute the wastewater before use. The wastewaters also contained a lot of micro metal elements, such as copper (Cu), iron (Fe), manganese (Mn), and so on, although concentrations of these micro metal elements were lower than the concentrations in TAP medium. It was reported that some metal elements, such as aluminum (Al) and plumbum (Pb), are toxic to algae and lead to low biomass yields (Davis et al., 2003). Furthermore, toxic metal elements in wastewaters might be absorbed by algae. As a result, harvested algal biomass containing toxic metal elements is unsuitable for feed or food use. Table 2 indicated that the concentrations of Al and Pb in dairy wastewater were extremely low. The dilution of wastewater will further reduce the concentrations of toxic elements. Based on the metal profile analysis, dairy wastewaters containing various essential metal elements could be used as medium alternatives for algae cultivation. Dilution of wastewater may minimize the negative effects of toxic metal elements and high concentrations of essential elements on algae growth.

3.1.2. Algae screening

Ten algal strains were cultivated on agar plates containing only one type of dairy wastewater with or without pH adjustment. The pH values of the raw mother liquor, salt whey, and liquid whey

Table 2
Metal profile of dairy wastewater.

mg/L	Mother liquor	Salt whey	Liquid whey	TAP medium
B	1.77	0.22	0.11	2.02
Ca	1726.00	983.90	329.20	13.60
Co	<0.01	<0.01	<0.01	0.40
Cu	<0.02	<0.02	<0.02	0.40
Fe	0.33	0.17	0.25	1.00
K	1407.00	69.46	94.85	63.90
Mg	615.20	99.65	57.01	9.76
Mn	<0.01	<0.01	<0.01	1.41
Mo	0.22	<0.01	<0.01	0.60
Na	3400.00	2462.00	112.60	6.18
Zn	1.08	0.34	0.17	4.93
Al	<0.08	<0.08	<0.08	–
Pb	0.24	0.26	<0.18	–

were 5.10, 5.15, and 5.11, respectively. Effects of dilution and pH on the growth of the ten strains were evaluated. The result indicates that most of strains did not grow without dilution. Only a few algal strains, such as UM 665, UM 4255, and UTEX 2714, exhibited growth on liquid whey without dilution while all others did not survive on mother liquor and salt whey without dilution. Dilution greatly improved algae growth. The optimization of dilution rate was conducted in further experiment. In addition, pH adjustment slightly improved algae growth. Some algal strains, such as UM 667 and UTEX 2229, which could not survive on dairy wastewaters without pH adjustment, had growth in wastewaters with pH adjustment. Therefore, in the rest of this study, dairy wastewaters were diluted and subjected to pH adjustment before use.

Result of algae screening also indicated that UTEX 2714 (*Chlorella vulgaris*) was the most robust algal strain. Previous studies showed that *Chlorella* sp. which contains many valuable compositions, such as chlorella growth factor (CGF), vitamins, and dietary fiber, is regarded as a potential feed and food resource (Safi et al., 2014). In this work, the robust algal strain, *C. vulgaris* (UTEX 2714), was used to treat dairy wastewaters for nutrient removal and biomass production.

3.2. Algae growth and nutrient removal

3.2.1. Optimization of dilution rate

Based on the analysis of nutrient profile and metal profile we decided that dairy wastewaters should be diluted before use to prevent the negative effects of toxic metal elements and high concentrations of some nutrients. The result of algae screening which showed that algae did not grow on liquid whey, salt whey and mother liquor without dilution confirmed this hypothesis. Therefore, according to the concentrations of nutrients, dilution rates of salt whey and liquid whey were set as 5, 10, and 20 times, and dilution rates of mother liquor were 5, 10, 20 and 40 times. The dilution led to very low concentrations of NH_3-N in wastewaters. The highest biomass yields of algae grown on wastewaters at different dilutions rate were presented in Fig. 1.

Algae grown on liquid whey had highest biomass yield (0.95 ± 0.13 g/L) on the 5th day at 5 times dilution while the biomass yields at 10 times dilution and 20 times dilution were much lower. Algae grown on salt whey had the highest biomass yield on the 4th day (0.97 ± 0.14 g/L) at 5 times dilution while the biomass yield at 10 times dilution and 20 times dilution were only 0.56 ± 0.02 g/L and 0.34 ± 0.10 g/L, respectively. The optimum dilution rate of mother liquor was 20 times at which the biomass yield of algae on the 5th day was 1.16 ± 0.21 g/L. When dilution rates of mother liquor were 5 time, 10 times, and 40 times, biomass yields of algae were only 0.68 ± 0.09, 0.82 ± 0.13, and 0.59 ± 0.16 g/L respectively. Therefore, optimum dilution rates of mother liquor, salt whey and liquid whey were 20 times, 5 times and 5 times, respectively, which were used in the rest of this study.

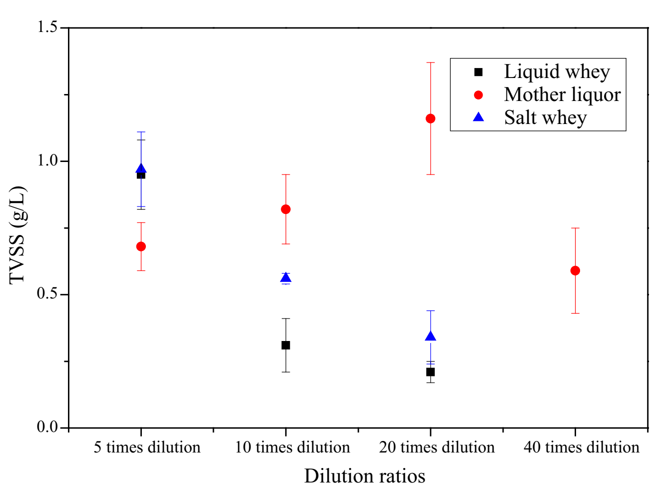

Fig. 1. Growth of algae in wastewaters with different dilution rates.

Fig. 1 showed that both low dilution rate and high dilution rate had negative effects on biomass yield of algae. The main reason for low biomass yield at high dilution rate is that low concentrations of nutrients in wastewater were not enough to support algae growth. At low dilution rate, high concentrations of some nutrients and metal elements were unfavorable to algae growth on wastewaters. The mechanism for this phenomenon is that high concentrations of nutrients, particularly the organic materials, make the environment unfavorable to algae growth. Under this condition, self-protection mechanism of algae was activated and the growth of algae was prohibited (Stehfest et al., 2005). To achieve high biomass yield, optimum dilution rates of three types of dairy wastewater were applied in the rest of experiments.

Wastewaters which have been used in previous studies include concentrated municipal wastewater, meat processing wastewater, carpet industry wastewater and so on (Chinnasamy et al., 2010; Lu et al., 2015; Zhou et al., 2011). Compared with dairy wastewaters, these wastewaters had lower nutrient contents but higher algal biomass yields. For example, Zhou et al. (2011) cultivated Chlorella *sp.* on concentrated municipal wastewater, in which the concentrations of TN, TP and COD were only 134, 212 and 2324 mg/L, respectively, and obtained 0.9 g/L dry algal biomass. Table 1 indicated that nutrient profile of dairy wastewater was not balanced compared with TAP medium. Concentrations of some nutrients, particularly NH_3-N, were extremely low in diluted dairy wastewater. Liebig's law of the minimum demonstrated that the exhaustion of one or several nutrients in medium could prohibit the algae growth even if other nutrients were still sufficient. Therefore, we supposed that the dilution of dairy wastewater led to low concentrations of some nutrients which became the limiting factor or bottleneck for algae growth. As a result, although other nutrients in diluted wastewater were sufficient, this limiting factor or bottleneck inhibited synthesis metabolism in algal cells and led to low biomass yield.

3.2.2. Nutrients removal in wastewaters

To determine the limiting factors, algae were cultivated on dairy wastewater at optimum dilution rates. Changes in NH_3-N, TN, TP, and COD during culture were shown in Fig. 2. The result showed that NH_3-N removal efficiencies in all three types of wastewater were 100% during the growth period. Algae grown on mother liquor, salt whey and liquid whey removed all NH_3-N on the 2nd day. The main reason for the fast exhaustion of NH_3-N is that the initial concentrations of NH_3-N (4.96–21.45 mg/L) in diluted dairy wastewaters were extremely low. TN removal efficiencies were 42.58%, 64.98% and 54.37%, TP removal efficiencies 21.79%, 40.82% and 64.15%, and COD removal efficiencies 16.44%, 46.51% and 41.39%, for mother liquor, salt whey, and liquid whey, respectively.

The above results showed that except for NH_3-N, other nutrients remained at relatively high levels. It is therefore hypothesized that deficiency of NH_3-N was the limiting factor to algae growth and nutrient removal in these dairy wastewaters. Moreover, these wastewaters, which contained high levels of nutrients, if discharged, will not only waste a lot of nutrients, but also cause pollution to the surface and ground water.

3.3. Biomass improvement by adding chemicals

To assess the hypothesis discussed above, ammonium chloride (NH_4Cl) was added into dairy wastewaters to increase the NH_3-N

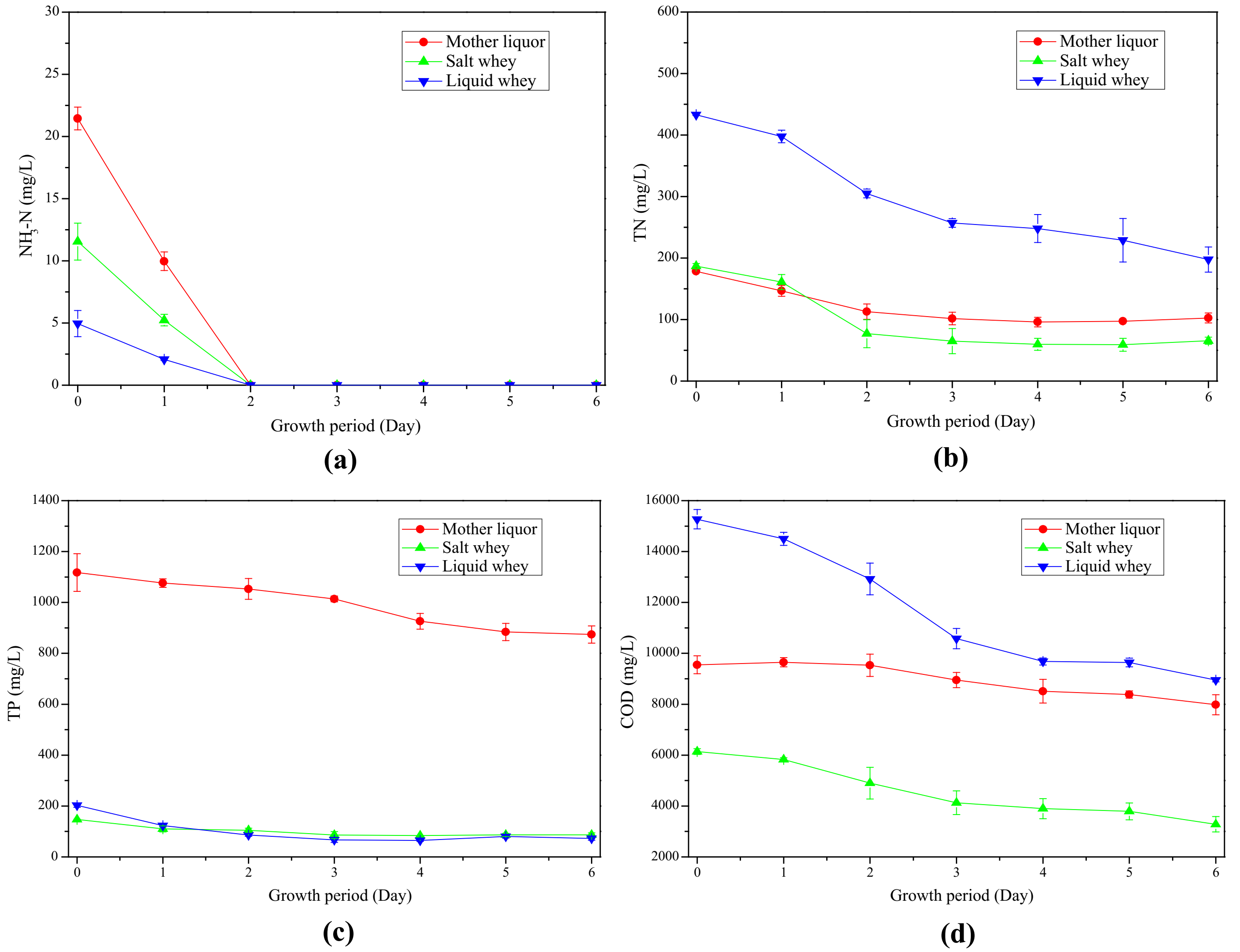

Fig. 2. Nutrients removal in individual wastewaters (a) removal of NH_3-N in individual wastewaters; (b) removal of TN in individual wastewaters; (c) removal of TP in individual wastewaters; (d) removal of COD in individual wastewaters.

concentration in wastewaters to the same level of that in TAP medium. The growth curves of algae on NH_3-N enriched dairy wastewater (Fig. 3) showed that the maximum algal biomass yields on NH_3-N enriched mother liquor, salt whey, and liquid whey were 3.24, 1.65, 2.34 g/L, respectively, representing 179.31%, 70.10% and 146.32% improvement over the raw wastewaters, respectively. Algal biomass yields on NH_3-N enriched dairy wastewater were much higher than those reported in previous studies. Therefore, adjusting NH_3-N concentration in dairy wastewater is an effective way to eliminate or mitigate the limiting factor to algae growth. This result validated the hypothesis that deficiency of NH_3-N was indeed the limiting factor to algae growth on these dairy wastewaters.

This result is in agreement with some previous studies. It was reported that population densities of some algal species were doubled with NH_3-N addition (Muller-Parker et al., 1994). Mechanism for the relationship between NH_3-N addition and biomass yield improvement is that adding NH_3-N in medium provides sufficient nutrient for the synthesis of proteins which are essential to algae replication. On the NH_3-N enriched dairy wastewaters, due to the protein synthesis, algae performed active metabolism which contributed to the improvement of biomass yield.

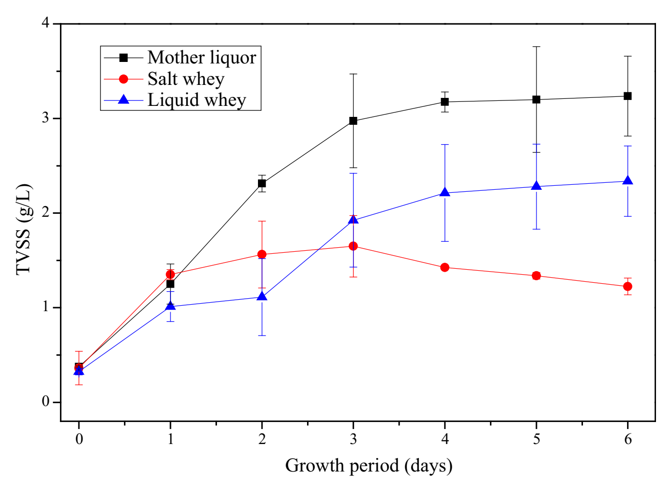

Fig. 3. Growth of algae on dairy wastewater with NH_3-N addition.

3.4. Cultivation of algae on mixed wastewater

Although adding chemicals into dairy wastewaters improved the biomass yield of algae, the high cost of artificial chemicals prevents the wide application of this method in large scale production. Some wastewaters, for example those from meat processing plants, contain high concentration of NH_3-N. In this study, we mixed a meat processing wastewater (MPW) obtained from a local slaughterhouse with the dairy wastewaters by 1:1 (v/v). Nutrient profile of MPW was listed as follows: TN, 416.0 mg/L; NH_3-N, 307.5 mg/L; TP, 97.1 mg/L; and COD, 7940 mg/L. Adding MPW into dairy wastewaters would be expected to improve the NH_3N level and mitigate the limiting factor to algae growth. Nutrient profile of mixed wastewater presented in Table 1 showed that mixed wastewater had almost same NH_3-N content with artificial medium.

3.4.1. Biomass yields of algae

Growth curves of algae grown on mixed wastewater (Fig. 4) indicate that the biomass yields reached peak value on the 5th day and the maximum biomass yields on mother liquor (mixed), salt whey (mixed), and liquid whey (mixed) were 2.66, 1.32, and 2.00 g/L, respectively, which were 129.31%, 26.52%, and 110.53% higher than the biomass yields on individual dairy wastewaters. The possible reason for the declination of algal biomass after the 5th day is that wastewater mixing introduced some new limiting factors. For example, some sedimentation in mixed wastewaters made the environment unfavorable to algae growth after the 5th day. Assessment of model fitness demonstrated that the growth of *C. vulgaris* on dairy wastewaters follows the linear model (Table 3). The specific growth rates of algae on mother liquor (mixed), salt whey (mixed), and liquid whey (mixed) reached 1.15, 1.59, and 2.93 day^{-1} (Table 3), respectively, which were 88.52%, 50.00% and 285.53% higher than the specific growth rates of algae on individual dairy wastewaters. Therefore, although mixing dairy wastewater with NH_3-N-rich wastewater may cause some limiting factors, it is still an effective way to mitigate NH_3-N deficiency and improve the biomass yield of algae.

Among three types of dairy wastewaters, salt whey had the lowest improvement rate upon addition of NH_3-N and mixture of MPW. The most possible reason is that in salt whey there are some other factors limiting the growth of algae. The improvement of NH_3-N concentration is not enough to mitigate or remove all the limiting factors. Although the improvement rate of biomass in salt whey was not very high, wastewater mixing still promoted algae growth in salt whey.

3.4.2. Nutrients removal

Nutrients removal in mixed dairy wastewater (Fig. 5) showed that removal efficiencies of NH_3-N in mother liquor (mixed), salt whey (mixed), and liquid whey (mixed) were 100%, 92.14%, and 98.08%, respectively. TN removal efficiencies in mother liquor (mixed), salt whey (mixed), and liquid whey (mixed) were improved to 60.54%, 71.10% and 57.81%, respectively, while the removal efficiencies in individual dairy wastewaters were 42.58%, 64.98%, and 54.37%. It is believed that high concentrations of NH_3-N in mixed wastewaters mitigate the limiting factor and promoted the greater growth of algae.

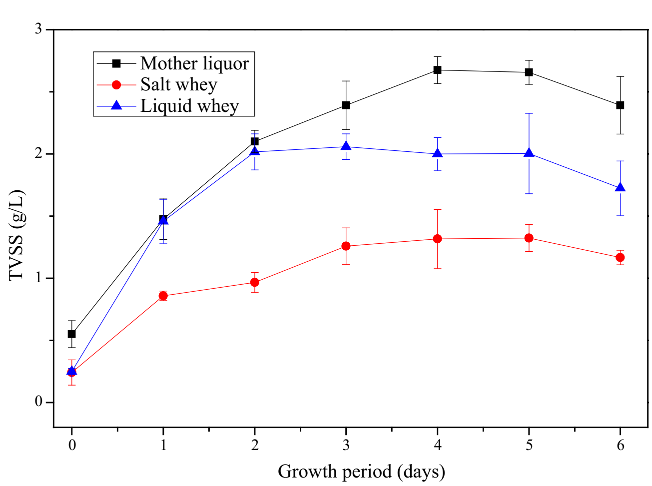

Fig. 4. Growth of algae on mixed wastewaters.

TP in mother liquor (mixed), salt whey (mixed), and liquid whey (mixed) after algae cultivation were 450.7 ± 10.7, 27.9 ± 0.9, and 63.8 ± 2.1 mg/L (Table 3), respectively, which were 48.44%, 67.85% and 12.12% lower than contents of TP left in individual dairy wastewaters (Table 3). After algae treatment, COD left in mother liquor (mixed), salt whey (mixed), and liquid whey (mixed) were 1377 ± 45, 947 ± 22, and 1619 ± 152 mg/L (Table 3), respectively, which were 82.74%, 71.16% and 81.91% lower than the COD left in individual dairy wastewater (Table 3). The comparison showed that the TP and COD left in mixed dairy wastewaters were reduced to a much lower level.

Based on the discussion above, dairy wastewater mixed with slaughterhouse wastewater with higher NH_3-N content improved both biomass yields of algae and nutrients removal efficiencies. As a result, the economic benefits of algae cultivation are improved and the risks of potential pollution caused by high concentrations of nutrients in wastewaters are controlled. This study certified that mixing dairy wastewaters with other wastewaters containing high NH_3-N content is an effective way to both treat wastewaters and obtained algae biomass. To achieve the industrial practice of this strategy, food companies could try to combine the discharge of dairy wastewaters with the discharge of wastewaters with high NH_3-N content and grow algae on mixed wastewaters. In practice, some pathways should be explored to reduce the production cost and improve the economic benefits.

3.5. Composition of algae grown on dairy wastewater

Compositions of algae grown on non-mixed wastewater and mixed wastewater are shown in Table 4. Protein content of algae grown on individual dairy wastewaters ranged from 43.16% to 49.14% while that of algae grown on mixed wastewaters ranged from 55.98% to 66.91%. Lipid content of algae grown on individual dairy wastewaters ranged from 23.95% to 34.04% while that of algae grown on mixed wastewaters was much less (19.10–20.81%). The main reason for the improvement of protein content is that mixed wastewater contained more NH_3-N which was essential to protein synthesis in algal cells. Under the environment with enough NH_3-N, algae were prone to synthesize more protein. Algal biomass with high oil content can be used for biofuel production while that with high protein content can be used for animal feed (Gouveia and Oliveira, 2009). Therefore, algae grown on mixed wastewater are more likely to be used for animal feed. In practice, mixture ratios and dilution rates of wastewater can be changed to improve certain composition in algal biomass.

Protein content of soybean which is the main plant protein source is 33.1–49.2% which is much lower than that (55.98–66.91%) of algae grown on mixed dairy wastewater (Hymowitz et al., 1972; Wolf et al., 1982). Furthermore, productivity of algae was higher than that of traditional crop (Chisti, 2007). Therefore, algae cultivation on mixed dairy wastewater can be a new and cheap protein source for animal feed. Although oil content in algae grown on mixed wastewater was lower than that in algae grown on non-mixed wastewater, it still reached 19.10–20.81%. Based on the compositions of algae in this study, the algal biomass can be exploited to make both protein-based products and oil-based products. Wastewater mixing strategy developed in this work will not only improve the biomass yield, but also change the nutrient

Table 3
Numerical values for the parameters of wastewater treatment.

	K (g/L)	a	r (day^{-1})	R^2	Residual TN (mg/L)	Residual NH_3-N (mg/L)	Residual TP (mg/L)	Residual COD (mg/L)
Mother liquor	1.16	0.87	0.61	0.9633	102.5 ± 8.0	0	874.0 ± 33.9	7980 ± 396
Salt whey	0.97	0.82	1.06	0.9875	65.5 ± 5.8	0	87.0 ± 7.8	3284 ± 305
Liquid whey	0.95	0.85	0.76	0.9678	197.5 ± 20.5	0	72.6 ± 7.1	8950 ± 71
Mother liquor (mixed)	2.68	1.16	1.15	0.9806	111.0 ± 5.3	0	450.7 ± 10.7	1377 ± 45
Salt whey (mixed)	1.32	1.51	1.59	0.9615	93.3 ± 3.8	11.9 ± 2.5	27.9 ± 0.9	947 ± 22
Liquid whey (mixed)	2.06	2.00	2.93	0.9998	148.3 ± 13.5	3.3 ± 1.7	63.8 ± 2.1	1619 ± 152

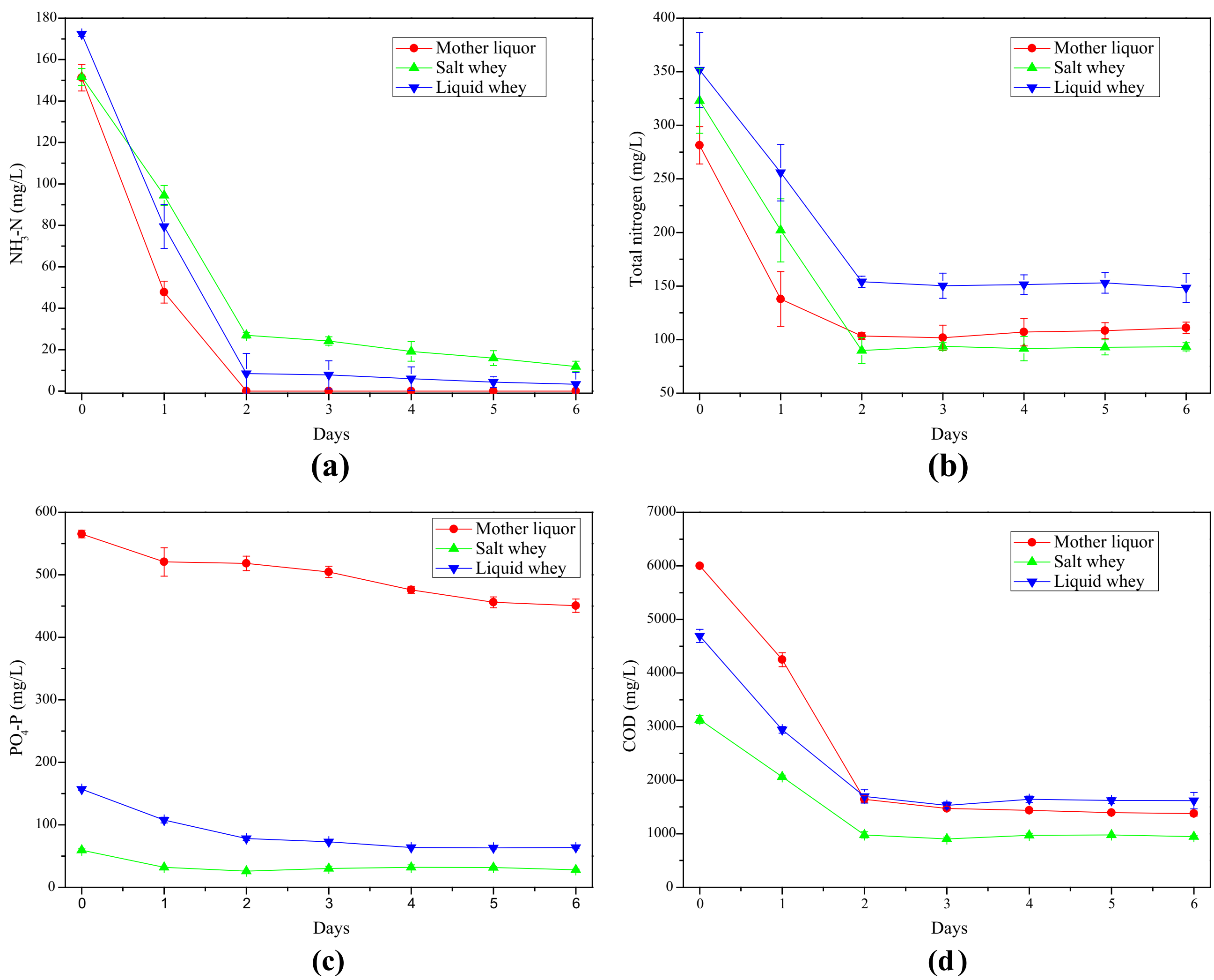

Fig. 5. Nutrient removal efficiencies of mixed wastewaters (a) removal of NH_3-N in mixed wastewaters; (b) removal of TN in mixed wastewaters; (c) removal of TP in mixed wastewaters; (d) removal of COD in mixed wastewaters.

Table 4
Composition of *Chlorella vulgaris* growing on dairy wastewater.

	Protein (%)	Lipid (%)	Other* (%)
Mother liquor	49.14	28.74	22.12
Salt whey	43.89	34.04	22.07
Liquid whey	43.16	23.95	32.89
Mother liquor (mixed)	57.20	20.25	22.55
Salt whey (mixed)	55.98	20.81	23.21
Liquid whey (mixed)	66.91	19.10	13.99

* Other components include carbohydrates, nucleic acids, etc.

composition in algae. In large scale production, to produce algae enriched with certain composition, dilution rates and mixing ratio can be modified according to actual needs.

4. Conclusions

It was concluded that (1) Limiting factor to algae growth on dairy wastewater was NH_3-N deficiency; (2) Wastewater mixing strategy mitigated the limiting factor to algae growth on wastewaters and significantly improved yields of algal biomass; (3) Wastewater mixing strategy led to low concentrations of TP and COD in the treated wastewaters after algae cultivation; (4) Algal grown on mixed wastewater with high protein content (55.98–66.91%) and oil content (19.10–20.81%) can be exploited to make both protein-based products and oil-based products; and (5) In the future, some pathways could be explored to achieve the industrial practice of this strategy.

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