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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), $MgSO_4·7H_2O$ (0.15 g/L), $ZnSO_4·7H_2O$ (22.22 mg/L), K_2HPO_4 (0.3 g/L), $(NH_4)_6Mo_7O_{24}·4\ H_2O$ (1.10 mg/L), $Na_2MoO_4·2H_2O$ (0.04 mg/L), $MnCl_2·4H_2O$ (5.87 mg/L), KH_2PO_4 (0.7 g/L), $CoCl_2·6\ H_2O$ (1.61 mg/L), EDTA disodium salt (50 mg/L), $CuSO_4·5\ H_2O$ (1.57 mg/L), $CuSO_4·5H_2O$ (0.07 mg/L), and $FeSO_4·7\ H_2O$ (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)

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