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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 world-wide 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.