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Cultivation of *Chlorella vulgaris* on anaerobically digested swine manure with daily recycling of the post-harvest culture broth

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ABSTRACT

In this work, a cultivation system with daily recycling of the post-harvest culture broth was set up and performed in order to reuse the water and nutrients in pretreated anaerobically digested swine manure, which was used as media to cultivate *Chlorella vulgaris* (UTEX 2714) at different recycling ratios. Results showed that the alga grew well in the system with an accumulative algal biomass and productivity of 1.68–3.47 g/L and 234.1–532.2 mg/L/d, respectively, at the end of the cultivation. Additionally, chemical compositions in this alga varied with the change of recycling ratios, and the highest productivities of carbohydrate, protein and lipids (76.4, 257.2 and 183.7 mg/L/d, respectively) were obtained in the system with a recycling ratio of 1/4 or 1/6. Fatty acid profiles indicated that this alga could be used as a good-quality biodiesel feedstock with a biodiesel productivity of 9.65–40.1 mg/L/d.

1. Introduction

Microalgae have attracted great attention nowadays because of their capability of fixing carbon dioxide (CO_2), removing nutrients from wastewater, and producing biomass (Zhou et al., 2014). The algal biomass can then be used as high impact feedstock for a variety of high-value products, such as biofuel, pigments, pharmaceuticals, and nutraceuticals (Lowrey et al., 2015). Thus, the microalgae technology is showing promising prospects with environmental, energy and economic benefits. However, a large quantity of water and nutrients are needed for microalgae cultivation in industrial scale. For example, 3726 kg water, 0.33 kg nitrogen and 0.71 kg phosphate were required to generate 1 kg algal biodiesel according to a life-cycle analysis (Yang et al., 2011). Recently, researchers have proved the feasibility of growing microalgae on animal manures, which have similar nutrient compositions with classic algal culture media and could support the growth of some algal strains well (Zhou et al., 2014). Furthermore, manure-based algae cultivation is a cost-effective tool for manure waste management in addition to cost saving from nutrients and water provided by the manures.

In previous studies, the nutrient profiles of raw and digested swine manure (SM) were analyzed (Hu et al., 2012), which showed that the major substances in SM were sugar, acetic acid, propionic acid, and butyric acid. The ammonium (NH_4^+-N) concentration and salinity of SM were in the range of 3.0–4.0 g/L and 30.0–50.0 ppt, respectively, which exceeded the tolerance levels of microalgae on NH_4^+-N and salinity, and would inhibit the algal growth (Yao et al., 2007; Niu et al., 2013). For addressing these issues, the SM was diluted with freshwater and used as media to cultivate microalgae from laboratory to pilot scale (Min et al., 2014). Moreover, an innovative thermal-vacuum stripping process was developed and performed in a previous study (Deng et al., 2017), where about 90% of NH_4^+-N would be removed from the pretreated anaerobically digested swine manure (PADSM) within 2 h, and *Chlorella vulgaris* (UTEX 2714) could grow well on the minimally diluted PADSM media. Based on the results of previous studies, a cultivation system with daily recycling of the post-harvest culture broth was firstly set up and performed in this study for reuse of water and nutrients in the PADSM media.

Recently, researchers have attempted to recycle the culture broth after biomass harvest for the reuse of water and nutrients in culture media. Bilad et al. (2014) recycled the culture broth (permeate) left after algal biomass was removed through membrane filtration for

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cultivation of *C. vulgaris* (SAG, Germany, 211-11B) and obtained a 77% reduction of water footprint. By recycling the culture broth, the inputs of water and nutrients decreased 60% during the cultivation of *C. vulgaris* (FACHB-31) for 7 days (Huang et al., 2016). Additionally, it was demonstrated that the recycled culture broth obtained from centrifugation had a positive effect on biomass and lipid productivity of *C. vulgaris* (UTEX 265) (Farooq et al., 2015), and daily recycling was the best frequency for the growth of *C. vulgaris* (FACHB-31) (Huang et al., 2016). Until now, the feasibility of recycling post-harvest animal manures for microalgae cultivation has not been reported previously; and researchers still know little about the algal growth and nutrients removal in animal manures when the cultivation mode with recycling of the post-harvest culture broth is used.

In the light of the above discussion, minimally diluted (3×) PADSM was used as media to cultivate *C. vulgaris* (UTEX 2714) in the system with daily recycling of the post-harvest culture broth at different recycling ratios. The aim of this work was to investigate the feasibility of recycling PADSM media for cultivation of *C. vulgaris* in this system to reuse the water and nutrients. The specific objectives were (1) to determine whether this system was feasible and efficient in cultivating *C. vulgaris*; (2) to investigate the algal growth characteristics in this system; and (3) to study the nutrient removal, biomass production, chemical compositions, and fatty acid profiles of *C. vulgaris* grown in this system. It was hoped that the results of this study would provide a scientific basis and support for low-cost microalgae cultivation using this system in large scale.

2. Materials and methods

2.1. Swine manure collection, pretreatment, and analysis

Swine manure (SM) used in this study was collected from the University of Minnesota Southern Research and Outreach Center (Waseca, Minnesota). Prior to use, the SM was pretreated according to the method described in a previous study (Deng et al., 2017). In brief, the SM was anaerobically digested at 55 °C for 16 days with activated sludge. After anaerobic digestion, the SM was divided into two portions. One portion was precipitated naturally and centrifuged at 10,000×*g* and 25 °C for 10 min to remove solid particles from the SM in order to decrease the turbidity, resulting in anaerobically digested swine manure (ADSM), which was used to regulate the concentration of NH_4^+-N during the subsequent test cycles. For another portion, ammonia was stripped from the SM at 55 °C for 2 h under a vacuum of 63.5 cm Hg; solid particles were removed using the above-stated methods; salinity was decreased by diluted with distilled water (*v/v*) to a dilution multiple of 3; and pH value was adjusted to about 7.05 with 5 mol/L HCl, resulting in pretreated anaerobically digested swine manure (PADSM), which was used as the main culture media at the beginning of subsequent experiments. Physicochemical characteristics of the ADSM and PADSM were determined and shown in Table 1.

2.2. Algal strain and culture conditions

Chlorella vulgaris (UTEX 2714) was selected for this study because it grew well on the PADSM media and could remove nutrients from the media effectively (Deng et al., 2017). This alga was preserved in Tris-Acetate-Phosphorus (TAP) media (Harris, 1989; Ma et al., 2016), and cultivated in 1000 mL Erlenmeyer flasks with 300 mL TAP media under the conditions described previously (Ma et al., 2014; Deng et al., 2017).

2.3. Experimental setups and system operation

In this work, a cultivation system with daily recycling of the post-harvest culture broth was set up, which consisted of microalgae cultivation unit, feeding unit and harvest unit (Fig. 1). For the microalgae cultivation unit, 500 mL Erlenmeyer flasks were employed as photobioreactors, which were filled with 240 mL PADSM media and kept on a shaker at 100 rpm rotation speed under 25 ± 2 °C and continuous cool-white fluorescent light illumination at 50 μmol/m^2/s. For feeding unit, ADSM and distilled water were added to the cultivation unit periodically to maintain the NH_4^+-N concentration at the initial level and the culture volume constant before the next cycle repeat. Additionally, centrifugation was used for harvest because it was a very suitable method for microalgae recovery when the culture broth was used for microalgae cultivation again (Farooq et al., 2015).

Table 1
Physicochemical characteristics of anaerobically digested swine manure (ADSM) and pretreated ADSM (PADSM). All measurements were performed in triplicate, and results are expressed as mean value ± standard deviation (SD).

Parameters	ADSM	PADSM
Total solids (TS) (g/L)	30.8 ± 0.26	9.93 ± 0.03
Total volatile solids (TVS) (g/L)	8.35 ± 0.07	2.76 ± 0.00
Chemical oxygen demand (COD) (g/L)	20.3 ± 0.88	6.47 ± 0.14
Ammonium(NH_4^+-N) (mg/L)	1105.0 ± 18.0	101.2 ± 5.21
Total nitrogen (TN) (mg/L)	1985.7 ± 25.8	172.7 ± 7.11
Total phosphorus (TP) (mg/L)	348.4 ± 5.26	113.8 ± 2.12
pH	7.08 ± 0.03	7.05 ± 0.02
Ion conductivity (ms/cm)	28.6 ± 0.09	9.78 ± 0.03
Copper (mg/L)	7.53 ± 0.04	2.53 ± 0.02
Iron (mg/L)	13.8 ± 0.11	4.58 ± 0.03
Lead (mg/L)	3.05 ± 0.09	1.00 ± 0.06
Cadmium (mg/L)	Not detected	Not detected

After pre-cultivation, *C. vulgaris* (UTEX 2714) grew into the exponential growth phase and was inoculated into the above-mentioned system. The details of inoculation were described in a previous study (Deng et al., 2017). During microalgae cultivation, a portion of the culture volume (1/2, 1/3, 1/4, 1/6, 1/12 and 0) was processed everyday using centrifugation (10,000×*g*, 25 °C, 10 min) to separate the algal biomass and culture broth, and the resultant culture broth was returned to the cultivation unit for the next cycle repeat, which allowed the reuse of remaining water and nutrients but at the same time diluted the algal biomass density. These experiments were labeled as having different recycling ratios ranging from 0 to 1/2. According to the removal rate of NH_4^+-N, small amount of ADSM (1–12 mL) and distilled water (0–6 mL) were added to the cultivation unit after the daily harvest in order to regulate the NH_4^+-N concentrations to the initial level (about 100 mg/L) and maintain the culture volume constant. All the experiments were performed in a batch mode with daily recycling of the post-harvest culture broth from Day 1, and the initial biomass was about 0.25g/L in all runs, which were carried out in three replicates. Samples were taken at the designated time for evaluation of algal growth, nutrients consumption, and chemical compositions as described below.

2.4. Growth and biomass analysis

Algal biomass (*X*, g/L) was determined as the total biomass minus the biomass in control experiments, where *C. vulgaris* was not inoculated into the cultivation system. The details of biomass measurements were described in a previous study (Deng et al., 2017).

The accumulative algal biomass ($X_{a,t}$, g/L) was defined as the algal biomass in photobioreactors at time *t* by adding all the harvested biomass during the cultivation period from the beginning to time $t-1$, which was calculated by the following equations:

$$X_{a,t}(\mathrm{g/L}) = X_t (t \leqslant 1) \tag{1}$$

$$X_{a,t}(\mathrm{g/L}) = \frac{X_t \times V_R + \sum_{n=1}^{t-1}(X_n \times V_h)}{V_R} (t \geqslant 2) \tag{2}$$

where $X_{a,t}$ (g/L) and X_t (g/L) are the accumulative algal biomass and the biomass at time *t*, respectively. V_R (L) and V_h (L) are the culture