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Growing *Chlorella vulgaris* on thermophilic anaerobic digestion swine manure for nutrient removal and biomass production

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HIGHLIGHTS

- *Chlorella vulgaris* grew well on minimally diluted pretreated swine manure.
- Nutrients could be rapidly removed by *C. vulgaris*, particularly with bacteria.
- Chemical compositions in *C. vulgaris* changed with the culture conditions.
- The alga could be used as biodiesel feedstock if cultivated in PADSM.
- The algal biomass produced could be used as animal feed.

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ABSTRACT

Liquid swine manure was subjected to thermophilic anaerobic digestion, ammonia stripping and centrifugation in order to increase the available carbon sources and decrease the ammonia concentration and turbidity. *Chlorella vulgaris* (UTEX 2714) was grown on minimally diluted (2×, 3× and 4×) autoclaved and non-autoclaved pretreated anaerobic digestion swine manure (PADSM) in a batch-culture system for 7 days. Results showed that *C. vulgaris* (UTEX 2714) grew best on 3× PADSM media, and effectively removed NH_4^+-N, TN, TP and COD by 98.5–99.8%, 49.2–55.4%, 20.0–29.7%, 31.2–34.0% and 99.8–99.9%, 67.4–70.8%, 49.3–54.4%, 73.6–78.7% in differently diluted autoclaved and non-autoclaved PADSM, respectively. Results of chemical compositions indicated that contents of pigment, carbohydrate, protein and lipid in *C. vulgaris* (UTEX 2714) changed with the culture conditions. Moreover, its fatty acid profiles suggested that this alga could be used as animal feed if cultivated in autoclaved PADSM or as good-quality biodiesel feedstock if cultivated in non-autoclaved PADSM.

1. Introduction

Microalgae technology has attracted considerable attention nowadays because of its potential as high impact feedstock for production of biofuel, high value pigments, nutraceuticals and therapeutic compounds (Lowrey et al., 2015). However, the high cost of microalgae cultivation limits its commercial applications. Nutrients and water are important cost factors, accounting for 10–20% of the total cultivation cost (Singh et al., 2011). Many animal manures have nutrient compositions similar to classic microalgae culture media and were found to support the growth of some microalgal strains well (Zhou et al., 2014). In addition to large amounts of nitrogen and phosphorous, volatile fatty acids (VFAs) such as acetic acid, propionic acid and butyric acid were also found in animal manures (Hu et al., 2013), and considered as potential soluble organic carbon substrates for microalgae cultivation (Hu et al., 2012). There is an increasing interest in using animal manures to grow microalgae because manures provide low cost nutrients and water, and at the same time manure based algae production is a cost effective tool for manure waste management.

Recently, researchers have demonstrated the feasibility of growing microalgae on swine manure (Ji et al., 2014; Luo et al., 2016; Nam et al., 2017). However, there are some major issues with the use of animal manures for microalgae cultivation, includ-

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ing (1) high turbidity due to the presence of solid particles; (2) high ammonia concentration; (3) low available carbon sources, most of which are locked in the insoluble organic compounds; and (4) lack of high performance microalgal strains capable of adapting to the environment of animal manures (Zhou et al., 2014). Some of these issues could be addressed through diluting the manures 20–100 times with water (Zhou et al., 2014); however, this requires a large quantity of freshwater. In addition, researchers have attempted to convert organic materials to usable carbon sources through anaerobic digestion (Hu et al., 2012), remove NH_4^+-N through aeration and air stripping (Liao et al., 1995; Min et al., 2014), and separate solid particles using centrifugation to reduce the turbidity (Hjorth et al., 2008).

In the light of above discussion, the aim of this work was to investigate processes enabling fast growth of a selected microalgal strain on swine manure with minimal dilution. The specific objectives were (1) to determine whether the pretreatment methods of liquid swine manure (LSM) were feasible and efficient; (2) to investigate if *C. vulgaris* could grow well in minimally diluted pretreated anaerobic digestion swine manure (PADSM); (3) to understand how the growth of *C. vulgaris* could be affected by the bacteria in PADSM; and (4) to study the nutrient removal efficiency, biomass production, chemical composition, and fatty acid profiles of *C. vulgaris* grown on PADSM. It is hoped that the results of this study could provide a scientific basis and support for microalgae cultivation using minimally diluted animal manures in large scale.

2. Materials and methods

2.1. Swine manure collection, pretreatment and analysis

Liquid swine manure (LSM) was collected from the University of Minnesota Southern Research and Outreach Center (Waseca, Minnesota), and anaerobically digested at 55 °C for 16 days with activated sludge for biogas production, resulting in anaerobic digestion swine manure (ADSM). Then, ammonia was stripped from the ADSM at 55 °C for 2 h under a vacuum of 25 inch Hg (84.7 kPa), the solid particles were removed using the methods of natural precipitation and centrifugation (10,000*g*, 10 min) to decrease the turbidity of the ADSM, and pH value of the ADSM was adjusted to about 7.0 with 5 mol/L HCl, resulting in the pretreated anaerobic digestion swine manure (PADSM). Physicochemical characteristics of the PADSM before and after autoclave were determined, and are presented in Table 1.

2.2. Microalgal strain and culture conditions

Chlorella vulgaris (UTEX 2714) was chosen for this study because it could grow in a mixotrophic mode to achieve high biomass production and high lipid content simultaneously (Hu et al., 2013; Ma et al., 2014; Ma et al., 2016). For the experiments, this alga was cultivated in 300 mL autoclaved Tris-Acetate-Phosphorus (TAP) media (Harris, 1989; Ma et al., 2016) within 1000 mL Erlenmeyer flasks, which were kept at 25 ± 2 °C on a shaker at 100 rpm rotation speed under a continuous cool-white fluorescent light illumination at 50 μmol/m^2/s.

Table 1
Physicochemical characteristics of pretreated anaerobic digestion swine manure (PADSM) before and after autoclave. All measurements were performed in triplicate, and results are expressed as mean value ± standard deviation (SD).

Parameter	PADSM	Autoclaved PADSM
TVSS (g/L)	0.3 ± 0.03	0.4 ± 0.06
Chemical oxygen demand (COD) (g/L)	18.6 ± 0.2	19.7 ± 0.4
Ammonium (NH_4^+-N) (mg/L)	255.4 ± 4.9	169.4 ± 2.8
Total nitrogen (TN) (mg/L)	463.0 ± 6.7	400.8 ± 13.0
Total phosphorus (TP) (mg/L)	113.3 ± 7.0	116.6 ± 2.5
Total volatile fatty acids (g/L)	7.9 ± 0.3	7.5 ± 0.3
Acetic acid (g/L)	3.8 ± 0.1	3.9 ± 0.1
Propionic acid (g/L)	1.4 ± 0.07	1.3 ± 0.02
Butyric acid (g/L)	0.3 ± 0.08	0.3 ± 0.06
Salinity (%)	2.3 ± 0.03	2.4 ± 0.02
pH	7.0 ± 0.03	7.0 ± 0.01

2.3. Experimental procedures

The PADSM was diluted with distilled water (*v*/*v*) to three different dilution multiples of 2, 3 and 4 before inoculation, which were labeled as 2×, 3× and 4×, respectively. Then, the 2×, 3× and 4× PADSM were divided into two equal aliquots. One aliquot was autoclaved at 121 °C for 20 min in order to prevent interference from other microorganisms (mainly bacteria), and the other non-autoclaved aliquot was directly used for experiments where bacterial growth was expected.

When *C. vulgaris* (UTEX 2714) grew to the exponential growth phase with a biomass of 0.8 g/L during pre-cultivation on TAP media, a total of 25 mL of the pre-culture was centrifuged (6000*g*, 4 °C, 10 min) to collect the microalgal cells. The collected cells were washed twice with sterile distilled water, and then inoculated into the 2×, 3× and 4× autoclaved and non-autoclaved PADSM with an initial biomass of about 0.1 g/L, which were labeled as 2 × A, 3 × A, 4 × A and 2 × (A + B), 3 × (A + B), 4 × (A + B), respectively. The other flasks filled with 2×, 3× and 4× autoclaved and non-autoclaved PADSM without algae inoculation were labeled as 2 × C, 3 × C, 4 × C and 2 × B, 3 × B, 4 × B. In this work, batch experiments were performed in 500 mL Erlenmeyer flasks, and the culture conditions were stated in Section 2.2. All the experiments were carried out in three replicates, and the 2 × C, 3 × C and 4 × C flasks were set up as the controls. Samples were taken at the designated times for evaluation of growth, nutrients consumption, pH value, and chemical composition as described below.

2.4. Growth analysis

Based on the method of Zhou et al. (2012), 5 mL of well-mixed culture broth was filtered with a 0.45 μm glass microfiber filter (GF/C; Whatman, UK) pre-ignited at 550 °C for 20 min. The filtered sample was dried at 105 °C for 10 h and then ignited at 550 °C for 20 min to remove organic matters again. The weight difference between the dried and ignited sample was defined as biomass (g/L). The algal biomass was determined as the total biomass minus the biomass in control experiments, where non-autoclaved or autoclaved PADSM was not inoculated with algae. The growth rate of *C. vulgaris* (UTEX 2714) was calculated according to the method described by Lu et al. (2015). All measurements were performed in triplicate.

2.5. Physicochemical analysis

For physicochemical analysis, the value of pH was measured with a pH meter (P100, Cole-Parmer, USA). Concentrations of acetic acid, propionic acid, butyric acid and VFAs were determined using a gas chromatography with flame ionization detector (GC-FID) (7820A, Agilent, USA) according to the method of Zhou et al. (2012). In addition, a volume of 2 mL sample was collected daily from each flask starting from inoculation, and centrifuged at 12,000*g* for 5 min. The supernatant was appropriately diluted for analyses of ammonium (NH_4^+-N), total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD) according to the Hach DR 5000 Spectrophotometer Manual (Hach, 2008). Nutrient removal efficiency (%) was calculated according to the method of Ji et al. (2014) with the following equation: