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Development of an effective acidogenically digested swine manure-based algal system for improved wastewater treatment and biofuel and feed production

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HIGHLIGHTS

- ▸ Coupling low cost and eco-friendly algae-based biofuel with animal feed production.
- ▸ Developing an effective algal system on acidogenically digested manure by a 2^2 CCD way.
- ▸ High algal growth rate and nutrient removal rates are obtained from the algal system.
- ▸ Having high algal lipid productivity (3.63 g m^2 d^{-1}) for low-cost biofuel production.

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ABSTRACT

An effective semi-continuous process was developed to grow a locally isolated green microalga *Chlorella* sp. on acidogenically digested swine wastewater in bench scale for improved algal biomass production and waste nutrient removal using central composite design (CCD). The influences of two key parameters, namely wastewater dilution rate (DR) and hydraulic retention time (HRT), on algal biomass productivity and nutrient removal rates were investigated. The optimal parameters estimated from the significant second-order quadratic models ($p < 0.05$) were 8-fold DR and 2.26-d HRT. The cultivating experiment in a bench-scale multi-layer photobioreactor with the optimized conditions achieved stable algal productivity and nutrient removal rates, which fitted the predictive models well. Moreover, relatively high and stable protein and lipid contents (58.78% and 26.09% of the dry weight, respectively) were observed for the collected algae sample, indicating the suitability of the algal biomass as ideal feedstock for both biofuel and feed production.

1. Introduction

Biomass energy made from traditional crops (oil seeds, sugar crops, wheat, etc.) is considered as a viable alternative to fossil fuels. However, advances in first generation biofuel technologies have encountered economic, ecological, and policy concerns, including competition for arable land with food and feed production, consequential significant food price hike, etc. Microalgae have great potential to replace current feedstock crops, because their productivity is much higher than terrestrial energy crops, and is not constrained by season and land availability and quality. Algal cells provide lipids for biodiesel or crude oil production, carbohydrates for bioethanol and biobutanol production, and/or nutritional compounds for animal feed production [1]. Cultivation of algae on swine manure is considered to be a potentially practical and economical strategy for algal feedstock production and wastewater treatment. The combination could help relieve the livestock producers from the significant financial burden associated with the treatment of the unmanageable growing manure prior to discharge. However, the process has not been commercialized yet, since the strategy is still faced with the lack of suitable algae strains and the dearth of carbon in the wastewater [2,3].

Our previous report showed that locally isolated facultative heterotrophic microalga strain *Chlorella* sp. (UMN271) was capable of

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utilizing volatile fatty acids (VFAs) including acetic, propionic and butyric acids, which are the major soluble organic carbon substrates in swine manure [4]. This *Chlorella* strain grew well on the diluted VFA-enriched swine wastewater effluent from the acidogenic fermentation, with the algal growth rate as high as 0.90 d^{-1}, the lipid content of approx 30%, and the nutrient removal of 458.4 mg COD L^{-1}, 69.5 mg N L^{-1} and 12.03 mg PO_4–P L^{-1} after the 5-day lab-scale batch cultivation.

The next step is to build a system to continuously produce algal feedstock, in which the practical culture conditions for high biomass production and nutrient assimilation can be established. In semi-continuous mode, a proportion of the culture is replaced with fresh media when the majority of microalgae reach late logarithmic growth phase, and then the culture is maintained for days to increase cell density before a next replacement. The repeated harvest-regrowing process can be maintained for a week or for several months without apparent growth decline in the system [5]. Algae growth and their cellular biochemical composition are able to be affected by environmental and culture conditions, such as irradiance, temperature, salinity, and nutrient balance [6,7]. However, the factors that could be adjusted in a pilot-scale semi-continuous culture are limited, among which hydraulic retention time (HRT) and medium dilution rate (DR) are important for algal growth and wastewater treatment.

HRT is the length of time that a soluble compound remains in a constructed bioreactor. In a semi-continuous process, the ratio of the culture volume in the bioreactor to the daily replaced volume is the HRT, which is a key factor influencing algae growth and nutrient uptake. Olguín [8] reported that a high-rate algal pond could be operated at short HRTs in the range of 4–10 days. Uwimana et al. [9] found that HRT was an influential factor that determined the removal efficiency of pathogens from the algal ponds fed with municipal wastewater. Sreesai and Pakpain [10] concluded that HRT influenced both nutrient uptake and growth development of *Chlorella vulgaris* in treated septage wastewater.

Wastewater DR affects the turbidity and nutrient concentrations in the algae cultures, which is also an important factor for algae growth. Cheunbarn and Peerapornpisal [11] reported that *Spirulina platensis* grown on highly diluted anaerobically treated swine manure (5-fold and 10-fold dilutions) resulted in higher cell densities than those on more concentrated manure (1-fold–3.33-fold dilutions) during the 2-week batch study because the darkness and turbidity in concentrated swine manure significantly affected algal photosynthesis. Wang et al. [12] reported that the growth rates and nutrient removal rates by *Chlorella* sp. on 10-fold and 15-fold diluted manure were initially slower than those on 20-fold and 25-fold diluted manure, but caught up in the latter part of the 21-day batch cultivation probably due to the continued algal growth sustained by the higher nutrient concentrations in the more concentrated manure.

Although several literatures have shown the appropriate dilution of swine manure [13] or the preferable HRT [14] for algae growth, it is difficult to directly apply the DR and HRT values in the microalgae-acidogenically digested swine manure system, because there are significant differences in composition between acidogenically digested swine manure and manure media used in the previous reports. Therefore, it is necessary to investigate the effects of DR and HRT on algae growth and waste nutrient removal, and then determine the optimal conditions specifically for the system by using some optimization method.

The Box–Wilson central composite design (CCD) is a useful mathematical approach widely used in the optimization of cultivation processes, in which treatment time and process variability could be reduced, the predictive responses could be closer to the target achievement, and interactions of two or more variables could be studied simultaneously. Kim et al. [15] used CCD to optimize the culture conditions (initial pH, nitrogen and phosphate concentrations) for the mass production of three green algae *Chlorella* sp., *Dunaliella salina* DCCBC2 and *Dunaliella* sp. Khataee et al. [16] used CCD to optimize the biological decolorization of textile wastewater by macroalgae *Chara* sp. However, there is still little research using CCD for the optimization of culture conditions for both algal mass production and wastewater treatment.

In the light of the above discussion, CCD was used in the study to develop a quadratic mathematical model for the prediction of the optimum HRT and DR for the *Chlorella* sp. mass production and the removal of swine wastewater nutrients. Another objective of this study was to develop an effective algae production process using acidogenically digested swine manure with a novel bench-scale photobioreactor.

2. Materials and methods

2.1. Algae strain and seed culture preparation

Alga strain *Chlorella* sp. UMN271, which was isolated from Loon Lake, Waseca MN, was used in the study. The preparation and maintenance of the inoculums was accomplished using 250 mL Erlenmeyer flasks containing 100 mL BG-11 medium with 2 g L^{-1} glucose according to Hu et al. [4]. After 1–2-week cultivation at 25 ± 2 °C under a continuous cool white fluorescent light illumination of 100 μmol m^{-2} s^{-1}, the algal cells were separated from the culture broth using centrifuge at 2000 rpm for 5 min, followed by washing with deionized water and another centrifugation–suspension process.

2.2. Characteristics of swine wastewater

The fresh swine manure and the inoculum sludge were collected from the University of Minnesota Southern Research and Outreach Center, Waseca MN. The fresh swine manure was used as the substrate during the acidogenic digestion in the study, and its characteristics are shown in Table 1. The sludge was anaerobically cultivated for 5 days with 5 g L^{-1} glucose at temperature 38 ± 1 °C to get the activated and concentrated inoculum, and then was heat-treated at 80 °C with a water bath (Thermo Fisher Scientific Inc., Waltham, MA) for 30 min to kill methanogenic bacteria from the community according to Wang et al. [17].

2.3. Acidogenic digester setup and operation

A glass bioreactor with the working volume of 4 L was used as the anaerobic digester. The reactor was operated in semi-continuous mode. At the beginning, 1.0 L concentrated inoculum was added into the reactor containing 3.0 L fresh manure substrate. The mixture was adjusted to approx pH 5.3 with sulfuric acid (4 mol H_2SO_4 L^{-1}) solution, and was maintained at pH 5.3–5.6 and 38 ± 1 °C for the acidogenic fermentation for 48 h as described by

Table 1
Characteristics of fresh swine manure.

Parameter	Value
pH	7.58 ± 0.31
TVSS (mg L^{-1})	2580.01 ± 300.01
Total nitrogen (mg TNL^{-1})	2031.43 ± 66.19
Ammonia–nitrogen (mg NH_3–N L^{-1})	1602.86 ± 84.72
Phosphate–phosphorus (mg PO_4–P L^{-1})	407.43 ± 99.58
COD (mg L^{-1})	17,240 ± 816.66
Total VFAs (mg L^{-1})	7676.26 ± 576.37
Acetic acid (mg L^{-1})	4957.08 ± 357.48
Propionic acid (mg L^{-1})	1612.03 ± 116.13
Butyric acid (mg L^{-1})	1107.16 ± 105.48