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Sustainable microalgae cultivation by using anaerobic centrate and biogas from anaerobic digestion

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ABSTRACT

This research was conducted to determine the effectiveness of using biogas generated from the anaerobic digestion of septic tank sludge and its anaerobic centrate on microalgal cultivation. Three types of sparging gas (biogas, air, and nitrogen gas) and two culture media (anaerobic centrate and Bold Basal Medium) were used to generate six different experimental settings. The most microalgal growth of 1074 mg VSS/L was achieved when both biogas and anaerobic centrate were used after 10 days of cultivation at 30 °C. In addition, the anaerobic centrate used had a reduction of 89% in its soluble chemical oxygen demand, a 97.3% reduction in the phosphorus concentration, and a 71.5% reduction in the nitrogen concentration. The results demonstrated tremendous benefits of biogas reuse and anaerobic centrate reclamation via microalgae cultivation.

1. Introduction

The main components of biogas include methane (CH_4) (40–70%), carbon dioxide (CO_2) (30–60%) and some trace gas compounds, such as hydrogen sulfide (H_2S) and water vapor [1]. Considering that high CO_2 content may downgrade engine efficiency, it is necessary to scrub the biogas before using it for power generation [2]. Different technologies were developed to remove the impurities from biogas, for example physical absorption, chemical solvents, membrane separation, and cryogenic separation [1]. Comparing to those physiochemical methods, biological purification using microalgae is able to offer many economic and environmental benefits [3]. The first benefit is that CO_2 in the biogas can be removed cost-effectively by microalgae and leave the biogas refined with mostly methane that is readily to be used for power generation [4]. Second, the microalgal productivity and nutrient assimilation can be increased due to the buffering capacity offered by CO_2. Third, the culture solution's pH can be controlled by the addition of CO_2 to prevent ammonia volatilization [5] and phosphorus sedimentation [2,6], reactions that are not conducive to microalgae growth. Furthermore, the removal of CO_2 from biogas can reduce the total greenhouse gas emission from these integrated processes.

Cultivation of microalgae in different types of wastewater to reduce nitrogen, phosphorus, and chemical oxygen demand (COD) has been intensively studied. Nitrogen can be removed in algae-based treatment by biological assimilation and ammonia stripping due to the increase of pH during algal growth [7]. Another important nutrient for microalgae, phosphorus, also can be removed by algal bioassimilation and adsorption. But unlike nitrogen removal, phosphorus reduction may be achieved by chemical precipitation once pH rises above 8 [8]. Wang et al. [9] analyzed the kinetics of nutrient removal for different types of wastewater by microalgae, and found that *Chlorella* sp. and *Micractinium* sp. could effectively grow and remove nutrients from both primary effluent and high-strength wastewater under the conditions of high nitrogen concentration (197 mg/L). Similarly, Wang et al. [10] evaluated the growth of *Chlorella* sp. on wastewaters sampled from four different points of a conventional wastewater treatment process, and found that the centrate from the activated sludge centrifuge was the best medium for algal growth. This centrate had the highest nutrient loading with a high total nitrogen concentration of 131.5 mg/L, total phosphorus concentration of 201.5 mg/L, plus excessive COD concentration of 2250 mg/L.

One potential source of high nitrogen and phosphorus in a wastewater treatment facility, is the liquid digestate obtained after anaerobic digestion. This liquid digestate needs to be further separated into a high solids content stream called “cake” and a liquid stream, the anaerobic centrate, by various dewatering methods, such as solid bowl centrifuge,

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belt filter press, rotary press, and sludge drying bed [11]. The anaerobic centrate usually contains fine, low-density solids and a high concentration of nutrients and is typically recirculated back to the main wastewater treatment. However, recirculation of this anaerobic centrate can add a significant load to the treatment facility and cause decreasing treatment efficiency. For example, Wett and Alex [12] investigated the performance of a separate rejection water treatment after an anaerobic sludge treatment process. It was found that approximately 16.3% of total nitrogen load was returned to the mainstream treatment, without the separate treatment, and this additional load can cause decreasing rates of denitrification and result in a lower overall process stability.

As a consequence, using microalgae to biologically treat the anaerobic centrate has been explored. For example, Uggetti et al. [13] used a mixed culture, dominated by *Scenedesmus* sp., to determine the feasibility of cultivating microalgae in an anaerobic centrate, and found the biomass production, up to 2.6 gTSS/L, was positively correlated with the proportion of the anaerobic centrate added. del Mar Morales-Amaral et al. [14] cultivated *Muriellopsis* sp. and *Pseudokirchneriella* sp. within 40–50% of an anaerobic centrate and achieved the microalgal productivities of 1.13 and 1.02 $g/L * d^{-1}$, respectively. Meantime, the removals of nitrogen and phosphorous were over 90%.

The CO_2 from biogas is able to assist microalgae to overcome inorganic carbon limitation and increase the capacity of algal photoautotrophic growth rate and nutrient assimilation [15]. Besides the photoautotrophic growth, microalgae are able to perform heterotrophic growth simultaneously once the organic carbon was available [16]. Mixotrophy is a unique algal metabolism model given by its capability for fixation of inorganic carbon and assimilation of organic carbon [17]. If both inorganic carbon and organic carbon are available in water, the specific growth rate of microalgae in the mixotrophic metabolism is approximately the summation of photoautotrophic and heterotrophic growth rate which may result in the maximal growth rate of microalgae [17,18]. Martínez and Orús [19] studied the interactions between the inorganic carbon and organic carbon metabolism in *Chlorella* sp. and found the presence of organic carbon could stimulate algal growth even at the elevated CO_2 concentrations (2%).

Lipid content exists in microalgal cells in the form of membrane and other energy storage bodies. But the quantity of lipids within the cell can vary due to changes in growth conditions (e.g., temperature, salinity and light) or the characteristics of nutrients (e.g., nitrogen, phosphorus, and iron) [20–22]. The lipids from microalgae could be used to generate many different products, such as biodiesel, biogas, foods, pharmaceutical products, or food for aquaculture [23], therefore, it is important to evaluate the direct or indirect impact of the external carbon (inorganic and organic) source on lipid accumulation of microalgae.

Anaerobic digestion is being widely used in the treatment of organic-enriched sludge in many medium and large-scale wastewater treatment plants. The produced biogas is a valuable energy source [24]. In the United State 20% of the population relies on onsite small septic systems to treat their wastewater [25]. Septic sludge is the final product pumped out from the reaction tank and usually has high concentrations of organic carbon (~10,000 mg/L), ammonia nitrogen (~250 mg/L), and phosphate (~55 mg/L) [25]. With high concentrations of organic carbon and other key nutrients, anaerobic sludge from the septic tank has the potential to serve as a culturing medium for microalgae growth. If the processes of microalgae-based biogas purification and anaerobic centrate reclamation can be integrated and cooperated with anaerobic co-digestion of microalgae, the benefits to algal biomass assimilation, biogas production, nutrients reduction, and the reduction of greenhouse gas can be significantly increased. However, little information is available on this integrated approach.

The objective of this study was to evaluate the effects of using both biogas and anaerobic centrate as the major carbon sources and growth medium for microalgal cultivation. To accomplish this objective, two major tasks were performed:

1. Cultivate microalgae by using three different types of gas (biogas, air and nitrogen) and two different types of nutrients to determine the effects of biogas and anaerobic centrate on the microalgae growth.
2. Determine the extent and kinetics of nutrients removal from the anaerobic centrate during algae cultivation.

2. Materials and methods

2.1. Sources of gas

Septic tank sludge, collected from residential septic tanks in the vicinity of Lowell, MA, was used throughout this research. It was stabilized in a BioFlo 110 Modular Bioreactor (New Brunswick Scientific Co., Inc.) at 35 °C under 50 rpm mixing for 20 days. Its pH was adjusted to 6.8–7.0 manually by injecting 5 N sodium hydroxide solution. Biogas was collected towards the end of the anaerobic digestion when the reactor had reached stabilization by using several 1 L Tedlar Sampling Bags (CEL Scientific Co.) and then stored at 4 °C for the following microalgae cultivation. The gas chromatography (GC) analysis (see Analytical methods section) revealed the biogas consisted of 31% CO_2 and 60% CH_4.

Air was directly collected in the environmental research laboratory at the University of Massachusetts Lowell (UML) at a room temperature of 25 °C. The real-time CO_2 content in the air was analyzed using the same GC method for the biogas analysis and the result showed 0.04% of CO_2 in the air. The nitrogen gas used in this experiment was commercial grade.

2.2. Microalgae strain and cultivation media

The microalgae strain was isolated from the primary clarifiers at the Lowell Regional Wastewater Utility (Lowell, MA). The Chlorella-like single cell algal strain was then cultivated by using the modified Bold Basal Medium (BBM). Microalgae cultivation and enrichment were previously described by [26]. The microalgal stock was prepared by centrifugation at 3000 rpm for 10 min followed by washing with distilled water. The centrifugation-washing was repeated three times to ensure the microalgae culture was free from the chemical residues of BBM. The prepared microalgal stock cultures were kept suspended in tap water and stored at 4 °C. The characteristics of the modified BBM are listed in Table 1. The BBM contains neither organic nor inorganic carbon.

After 20 days of anaerobic digestion, the stabilized septic sludge in the BioFlo 110 reactor could settle. After 1 h of settling, the upper grayish-color supernatant of the septic sludge was collected and filtered by using A/E 47 mm glass fiber filters (Gelman Sciences Inc.) to remove high turbidity, and then autoclaved at 120 °C for 30 min to sterilize the

Table 1
Characteristics of the diluted anaerobic centrate (DAC) and the modified Bold Basal Medium (BBM).

Parameters	DAC	BBM
pH	6.90	6.85
NH_3-N (mg/L)	103.1 ± 2.0	ND
NO_2-N + NO_3-N (mg/L)	ND	55.2 ± 0.6
PO_4-P (mg/L)	8.3 ± 0.7	47.1 ± 3.4
Total nitrogen (TN) (mg/L)	106.0 ± 3.2	56.1 ± 1.6
Total Phosphorus (TP) (mg/L)	8.7 ± 0.4	48.0 ± 1.7
N/P ratio (mg/mg)	12.2:1	1.2:1
Soluble chemical oxygen demand (sCOD) (mg/L)	656.0 ± 70.0	ND

ND: not detectable. The data are expressed as mean ± SD of triplicate measurements.