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Full Length Article

Recycled wastewater from anaerobic digestion of lipid extracted algae as a source of nutrients

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ABSTRACT

Nutrient supply, especially nitrogen and phosphorus, is one of the key obstacles limiting industrialization of algal biofuel. To help enhance nutrient utilization efficiency, our research focused on recycling of nutrients from lipid extracted algae biomass by the method of anaerobic digestion. Two methods of lipid extraction were performed, nutrients were released during anaerobic digestion of the lipid extracted biomass, the recycled nutrients were collected for algae cultivation, and the cycle was repeated: cultivation-extraction-digestion-cultivation. The results show that anaerobic digestion released nitrogen and phosphorus, and methane as a gaseous by-product. The algae grew well on the recycled nutrients. Ammonia is the limiting macro element, and extra trace elements enhanced algae production as well. Ash free dry weight, lipid content and lipid components were monitored and did not vary when the cycle was repeated. This system for recycling nutrients with supplementary nutrients holds potential for producing biofuel from algae.

1. Introduction

Micro algae have the potential to serve as a food source for fish and mammals and as a fuel source. Compared to biofuels from crop residues [1], algal biofuel has some advantages including a higher efficiency for converting sunlight into stored energy (3% for algae compared to 0.2–2% for crops) [2], a high oil content (the yield of oil from algae is expected to be 20,000 to 80,000 per acre, that is 7–31 times better than terrestrial crops) [3], and the ability to grow in saline and impaired waters. However, there are still some challenges associated with commercialization of algal biofuel [4]. The estimated cost per GGE varies greatly depending on the methods used for conversion and extraction, for example Dutta et al. [5] found that the minimum fuel selling price varied from $4.35/GGE to $10.55/GGE. Yet progress continues to be made and comparative studies on different technologies and modelling approaches show further improvements in the required estimated fuel selling price [5,6]. Nutrient supply is one critical obstacle for efficient production of algal biofuel. To produce an estimated 82 million tons of algal biomass per year, approximately 5.4 million tons of N are needed, which accounts for nearly 44% of the total yearly N consumption in the US in 2010. Similarly, 27.5% of the US phosphorus will be consumed [7]. Directing so much of the nitrogen and phosphorous supply to fuel production would negatively affect the agriculture sector. Thus, nutrient recycle is necessary for algae production prior to large scale commercialization.

One method that has been studied for nutrient recycle is anaerobic digestion of whole algae cells. This method has the added benefit of producing energy in the form of methane [8–11]. Some of the previous research related to nutrient release (typically ammonia) by anaerobic digestion of algae is summarized in Table 1. Frigon et al. [12] demonstrated experimentally that digestate (referred as the nutrient effluent from anaerobic digestion) from whole cell algae was rich in ammonia, and Verstraete demonstrated the availability of N and P for nutrient recycle in an open pond [13]. Although these researchers mainly focused on the application of anaerobic digestion of the algae without simultaneous production of biodiesel by oil extraction, the potential of recycling nutrients by anaerobic digestion to decrease fertilizer consumption was proven to be feasible.

If the ultimate goal is to produce biodiesel or biofuel from algal lipids, then N and P should be recycled from lipid extracted algae (LEA)[1] or the residual after lipid extraction [14]. Neves et al. showed that anaerobically digested LEA usually produced more methane compared to whole cell algae [15]. Hemandez et al. demonstrated that digestate from LEA was rich in ammonia and the concentration could be higher than the amount obtained from digestate from whole cell algae [16]. The extraction processes destroys the algal cell wall and therefore facilitates the biodegradation of algae cells by anaerobic digestion. Morken calculated the efficiency of energy production and nutrient

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[1] LEA – lipid extracted algae; Digestate – the nutritious effluent from anaerobic digestion.

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Table 1
Literature reviews of anaerobic digestion of algae.

Reference	Algae Strain	Oil extracted	Descriptions of Experiments and Conclusions
B. Sialve et al. [9]	C. vulgaris	No	Based on the gross composition the theoretical calculation of N-NH_3 yield as 47.5–54.0 mg g VS^{-1}
J. Frigon et al. [12]	C. sorokiniana	No	Different strains were tested and the ammonia yield of *C. sorokiniana* was calculated as 39.4 mg g VS^{-1}
Schamphelaire, Verstraete [13]	C. reinhardtii/P. subcapitata	No	The use of algae for energy generation by anaerobic digestion in a closed-loop system was tested and nitrogen was proven to accumulate as a result of recycle from anaerobic digestion
D.Hernandez et al.[16]	Four algae species	Yes*/No	N-NH_4^+ concentrations after anaerobic digestion were measure for 4 kinds of algae, both with and without lipid extraction. Higher nitrogen release from LEA than whole cell algae was shown
J. Morken et al. [17]	Model calculation	Yes	A model was established calculating the theoretical nutrient recycle efficiency and composition needed to make a closed-loop

* Supercritical fluid extraction was used in this research.

recycle by anaerobic digestion [17], but stated that many inhibitory factors could lower the effectiveness of such a system, such as ammonia toxicity, algal cell resistance, and nutrient consumption by bacteria [7,9]. Therefore, the practicality of the system needs to be further verified experimentally.

In summary, previous work demonstrated the feasibility of anaerobic digestion of whole cell algae and of LEA to produce methane and recycle nutrients. However, the algal process of cultivation-extraction-digestion-cultivation has not been experimentally tested and is the focus of this study. The amount of methane produced, nutrients recycled, lipids extracted and the rate of algal growth were monitored to assess the feasibility of incorporating anaerobic digestion for nutrient recycle into an algal cultivation scheme. As trace nutrients also improve algae production [18], the impact of trace nutrients was also determined.

2. Methods

2.1. Algae strain and medium

The algae species used was *Chlorella sorokiniana* (DOE 1412), a species of algae that has high productivity in indoor and outdoor cultivation systems [19,20]. The cultivation medium is shown in Tables S1–S2 and was developed by colleagues from Texas A & M Agrilife in Pecos, Texas [21] and is referred to as Pecos medium. Anaerobic sludge and secondary wastewater were received from the Pima County Ina Road Wastewater Treatment Plant in Tucson, Arizona. All solvents used in this study were analytical grade reagents.

2.2. Initial algae cultivation

C. sorokiniana was first cultivated in Pecos medium (Tables S1, S2) in a 4 L flask, and then transferred into a 40 L flat plate photobioreactor indoors with Pecos medium. Small bubbles of air and CO_2 were uniformly generated, and pH was maintained at 8 through CO_2 addition. A 12 h on/12 h off light/dark cycle was used. The light intensity was 80 μmol photos m^{-2} s^{-1} on the flask surface and was measured by a quantum meter (MQ-200, Apogee Instruments, Logan, UT). When the density reached a maximum of 0.85 g/L, the algae were harvested by centrifugation and stored in the refrigerator prior to lipid extraction.

2.3. Lipid extraction

To experimentally test the effectiveness and impact of lipid extraction, two methods were used: Soxhlet extraction and microwave extraction. Soxhlet extraction is a typical chemical method for oil extraction which is applied in some industries [22,23], and microwave extraction is a typical mechanical assisted extraction which is often identified as the most easy and effective method of lipid extraction from microalgae [24].

Algae were dried in a horizontal air flow oven (VWR International West Chester, PA) at 70 °C for 24 h and an aliquot of 8 g was weighed for extraction. Hexane was used as the extracting agent in the Soxhlet apparatus. A total volume of 300 mL of hexane were heated to 55 °C and recycled to extract lipid from the algae [25]. The extraction lasted 8 h. For the microwave extraction, methanol-chloroform (1:2) was used as extracting agent. Algae were dissolved in methanol-chloroform overnight, and then heated in the microwave (CEM MDS 2100 Microwave oven with solvent sensor) at 70 °C for 75 min. After lipid extraction, the lipids were dried and weighted to determine the lipid content [26,27]. The biomass was washed with hexane twice after microwave extraction to remove any residual chloroform and stored for anaerobic digestion.

2.4. Anaerobic digestion

Four sets of algae were anaerobically digested: lipid extracted algae using either the Soxhlet or microwave methods, and two controls – whole algal cells and endogenous digestion with no algae. Twelve liters of anaerobic digestion medium (Tables S3, S4) were prepared and divided uniformly into four 4 L flasks. Then 35 g of anaerobic sludge were added and mixed into the medium. The 8 g LEA or whole cell algae were also added. Basal medium and operating conditions were the same as those used by Ayala-Parra et al. [28]

The anaerobic digestion system is shown in Fig. 1. The 4 L flasks were sealed and the air inside was cleared by a mixture of 80% N_2 and 20% CO_2. A stir bar was used to keep the sludge and algae mixing. A safe bottle was connected to the outlet of the 4 L flask, followed by a sample-collection flask filled with 5% NaOH. The whole system was

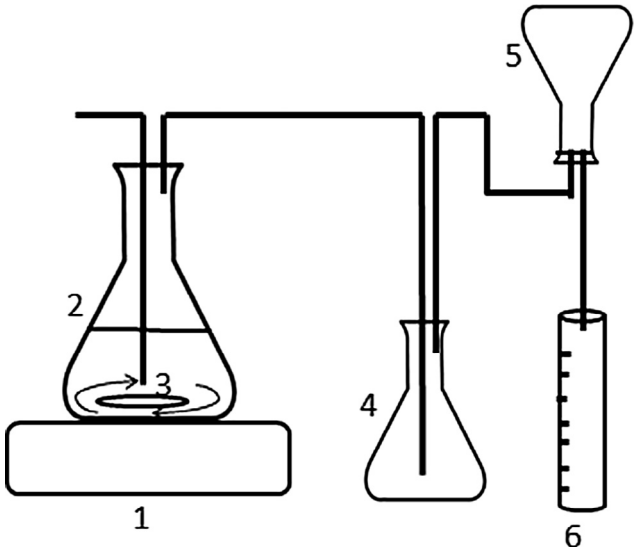

Fig. 1. Scheme for the anaerobic digestion system. In the system, 1 is the magnetic stirrer; 2 is the 4 L flask containing sludge, algae and medium; 3 is the stir bar; 4 is the safe bottle; 5 is the collection flask; 6 is the graduated cylinder. The entire system was sealed and controlled with switches.