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Nutrients recycling and energy evaluation in a closed microalgal biofuel production system

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ABSTRACT

A closed process for microalgal biofuel production involving in lipid extraction, anaerobic digestion, and microalgal cultivation was proposed. Nutrients recycling and energy in the system were evaluated. During the anaerobic digestion process, 69.37% of nitrogen and 60.22% of phosphorus resided in the lipid-extracted microalgae (LEM) were released into the digested liquid (DL). Microalgae (*Chlorella pyrenoidosa*) could grow normally in mixed 10% DL and Selenite Enrichment (SE) medium (without nitrogen and phosphorus additives). The maximum biomass concentration was 1.25 g/L (dry biomass), which was higher than that obtained with the standard SE medium (1.18 g/L dry biomass). This indicates that the abundant nutrients in the DL can be recycled for more sustainable microalgae growth. Economic analysis evaluated that recycling of nutrients in the DL could potentially reduce nutrient cost by 41.77%. Additionally, 28.38% more energy was recovered as methane in LEM during the anaerobic digestion process. This would compensate for the cost of microalgae biofuel production.

1. Introduction

Environmental protection and fossil fuel consumption are two of the most significant topics related to the sustainable development of human society and natural resources [1]. Microalgal biofuel shows great potential to complement fossil fuels to meet our energy needs and carbon capture [2–4]. However, current microalgae cultivation techniques have high nutrients demand and negative energy production. Yang et al. have proved that 0.33 kg of nitrogen and 0.71 kg of phosphate are required to produce 1 L biodiesel without nutrients recycling [5]. In addition, researches have estimated that meeting 5% transportation fuels from microalgae requires 44–107% of total nitrogen use and 20–51% of total phosphorus use in United States [6]. Thus, increasing microalgal biofuel production could cause negative impacts on the global fertilizer market, and even increase the price of agricultural commodities [7]. Moreover, the energy return/investment ratio (0.09–4.3) in microalgal biofuel production is much lower than that of fossil fuel, making it not yet competitive with fossil fuel [8–10]. Therefore, lower nutrients addition and higher energy generation are essential for competitive and sustainable development of microalgal biofuel.

At present, considerable attention has been given to converting the lipid fraction of microalgae biomass into biofuel. However, approximately 45–85% of raw microalgae biomass residues that remain after lipid extraction are disposed of without utilization [11]. Lipid-extracted microalgae (LEM) have a high level of nitrogen and phosphorus, in which about 35–75% of the original energy is stored. Therefore, LEM are viewed as attractive feedstocks for producing energy and recovering nutrients [12]. Chisti pointed out the importance of energy and nutrients recovery from LEM and highlighted the potentiality to defray most of the energy demand for microalgal biofuel production [13]. It is estimated that the energy transferred from LEM to methane could be up to 50–100% of the theoretical energy produced from microalgal biodiesel [14]. Moreover, Bohutskyi et al. found that these nutrients recycling from LEM would reduce the required addition of nitrogen, potassium, phosphorus, sulfur and boron to the culture medium by 30–70% [15].

There has been a great deal of work focused on biogas production from LEM using anaerobic digestion [16, 17]. However, little has been done to create an integrated system for energy recovery from LEM by

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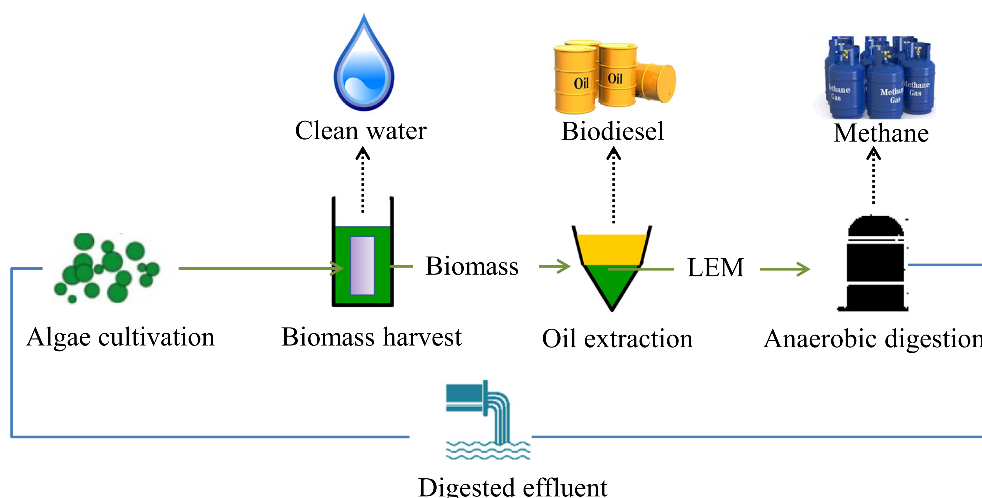

Fig. 1. Schematic of the closed-loop processes used for biofuel production from microalgae.

anaerobic digestion while recycling nutrients by cultivating microalgae. Therefore, a closed loop process, coupled lipid extraction, anaerobically digested LEM, and microalgae cultivation using digested liquid, was proposed for this study. Microalgae cultivation using the digested liquid from LEM is the core issue during this closed process. In this study, the species *Chlorella pyrenoidosa* was chosen as the inoculation candidate, due to its excellent tolerance to the high ammonia concentration in the digested liquid and its mixotrophic ability to utilize organics as carbon source [18, 19]. The results from this study are important for developing sustainable measures for microalgal biofuel production with lower cost and higher energy efficiency.

2. Materials and methods

2.1. Closed microalgae biofuel production system

Fig. 1 illustrates the closed loop process, primarily including an anaerobic digestion of lipid-extracted microalgae (LEM) for biogas production and cultivation of microalgae using the anaerobic digestion effluent. After oil extraction, the LEM was used for anaerobic fermentation. The organic matters remaining in the LEM was degraded to produce biogas (including methane) to recover energy, and nutrients (e.g., nitrogen, phosphorus, and other trace elements) were released into the digested liquid for subsequent microalgae culture.

2.2. Lipid-extracted microalgae (LEM)

A portion (20 g) of the dried *Chlorella pyrenoidosa* (*C. pyrenoidosa*) that was cultivated using anaerobically digested starch processing wastewater was used for lipid extraction according to the methods described by Bligh and Dyer [20]. The algal biomass was placed in a 500 mL conical flask together with 300 mL of methanol-chloroform solution (volume ratio of 2:1), stirred at 400 rpm for 24 h at room temperature. The biomass was filtered out using filter paper and then the liquor was evaporated at 100 °C. The crude lipid in the biomass was collected after all the solvent was evaporated. The filtered biomass was then mixed with the recovered solvent for the second cycle of lipid extraction. A total of 15.28 g LEM was collected.

2.3. Bio-methane potential (BMP) test

The BMP test was used to evaluate biomass production from the LEM. The assays were performed in 120 mL serum bottles. Anaerobic granular sludge (AGS) from a beer factory in Wuxi was used as the inoculum. The total solids (TS) and volatile solids (VS) of AGS were 6.10% and 3.40%, respectively. The fermentation concentration was kept at 20 g TS/kg, and the inoculum to substrate ratio was 1.0 (VS basis). The inoculum was transferred to the bottle, followed by the addition of substrate. Then, distilled water was added to the bottles to make up the volume to 50 mL. Bottles with inoculum but without substrate were used as controls. All the bottles were placed in an incubator shaker at 37 °C and were processed for 30 d. At the end of the experiment, the digested liquid (DL) was collected for microalgae cultivation.

2.4. Microalgae growth experiment

C. pyrenoidosa (FACHB-9) was initially obtained from the Institute of Hydrobiology (the Chinese Academy of Sciences, Wuhan, China). Prior to being used in the experiment, *C. pyrenoidosa* was cultured under sterile conditions in sterilized Selenite Enrichment (SE) medium based on previous research [21]. This medium consisted of $NaNO_3$ (250 mg/L), K_2HPO_4 (75 mg/L), $MgSO_4·7H_2O$ (75 mg/L), $CaCl_2·2H_2O$ (25 mg/L), KH_2PO_4 (175 mg/L), NaCl (25 mg/L), $FeCl_3·6H_2O$ (5 mg/L), H_3BO_3 (2.86 mg/L), $MnCl_2·4H_2O$ (1.86 mg/L), $ZnSO_4·7H_2O$ (0.22 mg/L), $Na_2MoO_4·2H_2O$ (0.39 mg/L), $CuSO_4·5H_2O$ (0.08 mg/L), and $Co(NO_3)_2·6H_2O$ (0.05 mg/L). The pH value of the SE medium was ~7. The cultivation conditions were as follows: light intensity was 127 $\mu mol·m^{-2}·s^{-1}$, light/dark ratio was 12:12, temperature was 25 ± 1 °C, and intermittent artificial shaking was done four times per day for 12 d.

To investigate the efficiency of nitrogen and phosphorus recovery for cultivating microalgae, four experimental groups and one control group (total volume of 100 mL) were evaluated: G1 (90 mL SE without nitrogen (SE-N) + 10 mL DL), G2 (90 mL SE without phosphorus (SE-P) + 10 mL DL), G3 (90 mL SE without nitrogen and phosphorus (SE-N &P + 10 mL DL), G5 (90 mL water + 10 mL DL). In addition, the standard SE (G4) was used as the control group.

All of the experiments were performed in triplicate. The concentration of the initial inoculation of *C. pyrenoidosa* was maintained at 0.10 g/L (dry weight). The initial pH was controlled at 6–7 by using 1 mol/L NaOH and 1 mol/L HCl. *C. pyrenoidosa* was cultivated in 150 mL glass conical flasks within an illumination incubator (GZX-300BS-III, CIMO Medical Instrument, Shanghai, China).

2.5. Analytical methods

2.5.1. Biogas determination

Biogas production was analyzed by calculating the volume and pressure in the headspace of the serum bottle. The pressure was

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