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Enhancement of biodiesel production by cultivating *Dipodascaceae* moderated-filamentous granular sludge with sugar-containing wastewater

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ABSTRACT

Utilizing excess sludge from a wastewater treatment plant to produce biodiesel has become an increasingly popular topic. However, the low lipid content in activated sludge has hindered the application of biodiesel production from excess sludge. This study was designed to enhance biodiesel production from granular sludge fed with synthetic sugar-containing wastewater. Granules cultivated at different initial sludge loading rates had different morphologies and microbial structure. Low seed biomass and high initial sludge loading resulted in the dominance of the white filamentous fungi *Dipodascaceae* in granular sludge, thereby enhancing the lipid accumulation of granular sludge and changing the biodiesel constituent produced from the sludge. However, overgrowth of filamentous fungi deteriorated the settling and compression ability of granular sludge. Thus, controlling the filamentous fungi at a moderate level through an effective operating strategy is of great importance to biodiesel production from a granular sludge system fed with sugar-containing wastewater.

1. Introduction

Energy and environment are two hot topics since they are both closely related to the sustainable development of our society. The combination of shrinking reserves of fossil fuels due to the rising demand for primary energy, fuel price spikes, climate change concerns, public awareness and advancements in renewable energy technologies has generated great interest in renewable energy (Tyagi and Lo, 2013). Although people paid much attention to wastewater treatment in the past, issues with the sludge disposal is a problem that must be addressed with the increasingly stringent environmental standards. Wastewater sludge is naturally produced in large quantities all over the world (Zhang et al., 2014). Meanwhile, the wastewater sludge could fulfill the requirements of lipid feedstock for biodiesel production due to its low cost and continuous supply. Recently, the studies involving the utilization of sewage sludge as a raw material to produce biodiesel has been increasing (Dufreche et al., 2007; Pastore et al., 2013; Liu et al., 2014; Olkiewicz et al., 2015).

The low biodiesel yield resulting from low lipid content of wastewater sludge has indirectly increased the cost of biodiesel production. Therefore, researchers have tried to enhance lipid accumulation of activated sludgeby increasing the organic loading and the ratio of carbon and nitrogen, utilizing different carbon substances, and so on (Mondala et al., 2012, 2013, 2015; Sun et al., 2015). A lot of research showed that increasing the organic loading rate could promote the production of microbial lipid. Meanwhile, the sugar-containing wastewater with high concentration of organic matter is another difficult issue to address. Compared with the conventional activated sludge, the dense structure and good settling properties of biogranules could enable high biomass retention and the tolerate high-strength wastewater and shock loadings (Liu and Tay, 2004; Su and Yu, 2005; Li et al., 2008; Yilmaz et al., 2008; Corsino et al., 2015). Thus, aerobic granulation may be an attractively new technology, both for sugar-containing wastewater treatment and lipid accumulation.

The purpose of this study was to investigate the feasibility of using granular sludge to degrade synthetic sugar-containing wastewater and to produce biodiesel simultaneously. The effect of SBR operating parameters, including seed sludge concentration,

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hydraulic retention time (HRT) and sludge loading rate, on glucose-containing wastewater treatment and lipid accumulation of granular sludge were examined. In addition, the relationships among operation conditions, characteristics of granular sludge and biodiesel production were also investigated. This work represents the first attempt to optimize the biodiesel yield and fatty acid methyl esters (FAMEs) distribution from aerobic granular sludge. What's more, to the best of our knowledge, this is also the first time to propose the controlling strategy of microbial structure for increasing the biodiesel yield from a wastewater biological treatment system.

2. Materials and methods

2.1. Experimental set-up and SBR operation

Three plexiglass columns (5 cm in diameter and 80 cm in height) with a working volume of 1.2 L were used as the SBR reactors. The seed granular sludge was cultivated with activated sludge that was obtained from a full-scale sewage treatment plant (Xiaojia River, Beijing, China) in glucose-based synthetic wastewater. Three reactors were operated in a sequential mode with 5 min of feeding, 3 min of sludge settling and 8 min of effluent withdrawal from the middle ports of the columns. Aeration was conducted at an air flow rate of 1.0 L/min during the aeration phase. Glucose and ammonium chloride were added as carbon and nitrogen sources, respectively, in amounts corresponding to the influent COD concentration of 2000 mg/L and the COD:N ratio of 50:1. The other nutrition contained the following components: 20.1 mg/L of Na_2HPO_4, 6.6 mg/L of KH_2PO_4 and 5 mL of trace mineral solution. $NaHCO_3$ was added into the feeding solution to maintain the pH of the reactors in the range between 7.0 and 8.0. The bioreactors were operated at room temperature, and the water temperature was 20–22 °C.

The whole experiment could be divided into three phases. Phase I: Set the cycle time, HRT and volumetric loading rate to 6 h, 12 h and 4 kg COD/(m^3·d), respectively. Three different biomass concentrations – 8, 5.3 and 4 g MLSS/L – were fed into three SBRs, R1, R2 and R3, resulting in the initial sludge loading rates of 0.5 (R1), 0.75 (R2) and 1.0 (R3) kg COD/(kg MLSS·d), respectively. Phase II: Shorten the cycle time, hydraulic retention time (HRT) and volumetric loading rate to 4 h, 8 h and 6 kg COD/(m^3·d), respectively. Phase III: Discharge 100 and 150 mL sludge from R2 and R3, respectively, in the stage of aeration manually every day, while keeping R1 the same as in phase II. Thus, the sludge loading rate among the three reactors could be maintained at three different levels.

2.2. In situ transesterification

The granular sludge (GS) was treated for in situ transesterification using a modified method based on a previous research report (Mondala et al., 2009). The GS samples were centrifuged at 5000 r/min for 5 min to be dewatered (TSS: 10–15 wt %). Two grams of dewatered sludge were weighed into 100-mL flasks. 30 mL of sulfuric acid-methanol (5%, v:v) and 10 mL of hexane were added to the flasks, and then the mixture was heated 7 h at 75 °C. A condenser was used to minimize the loss of methanol and hexane due to evaporation with water at room temperature. After the reaction was stopped, the mixture was cooled and transferred into a 50-mL tube. Next, 5 mL of saturated NaCl solution was added, and then the mixture was shaken vigorously for 3 min and centrifuged at 3000 r/min for 3 min. Afterwards, the hexane phase was withdrawn and transferred into another 50-mL tube. The extraction procedure was repeated three times. Subsequently, the total hexane phase was washed with 10 mL of a 2% (w/v) sodium bicarbonate and then dried by 3 g of sodium sulfate. The hexane layer was taken and evaporated completely at the temperature above its boiling point to obtain the product of esterification. All of the samples were stored at −20 °C. The experiment was performed in duplicate.

2.3. Analytical methods

Culture samples were collected every week to measure the amount of effluent and to determine the characteristics of the granular sludge. The concentration of effluent glucose was determined via the phenol-sulfuric acid method (Gerhardt et al., 1994) using glucose as the standard. The concentration of ammonium-nitrogen (NH_4^+–N) in the effluent was measured by Nessler's reagent colorimetry. The sludge MLSS concentration and sludge volume index (SVI) were measured according to the Standard Methods (APHA, 2005). The measurements of these parameters were performed in duplicate.

All samples of biodiesel were re-dissolved with hexane and then analyzed using an Agilent 7890A gas chromatograph equipped with a flame ionization detector after being filtered through a 0.45-μm PTFE membrane. The column used was a 30 m × 0.320 mm × 0.25 μm HP-5 capillary column (19091J-413, Agilent) with 5% phenylmethylsiloxane as the stationary phase. The column temperature was programmed to start at 50 °C, maintained for 2 min, increased from 50 to 130 °C t 10 °C/min, ramped to 200 °C at 4 °C/min, and then raised at 3 °C/min to 220 °C, before finally reaching 270 °C at 5 °C/min. The flow of H_2, air and N_2 were 30, 300 and 25 mL/min, respectively. In addition, the sample injection volume was 1 μL, running in splitless mode. The temperature of the injector and detector were set at 200 and 270 °C, respectively. Instrument calibration was conducted using a standard FAME mixture containing C8–C24 methyl esters (Sigma-Aldrich, USA) and four single standards with 100 mg/L 1, 3-dichlorobenzene as the internal standard, including methyl pentadecanoate, methyl heptadecanoate (AccuStandard, Inc., New Haven), methyl 12-methyltetradecanoate and methyl 14-methylpentadecanoate (Larodan Fine Chemicals AB, Sweden). The yield of FAMEs was calculated based on the dry weight of the sludge and the volume of wet sludge (Eqs. (1) and (2)), and the latter could be an important indicator to evaluate the volumetric productivity of production equipment for biodiesel:

$$\text{FAMEs}(mg/g\ SS) = \frac{\sum FAME_i}{The\ weight\ of\ dry\ sludge} \quad (1)$$

$$\text{FAMEs}(g/L\ wet\ sludge) = \frac{FAMEs(mg/g\ SS)}{SVI(mL/g\ SS)} \quad (2)$$

2.4. Microbial analysis

In this study, high-throughput sequencing and qPCR were used to analyze the microbial population structure and the distribution of bacteria and fungi, respectively. GS culture samples (2 mL) were collected and concentrated by centrifugation (13,400g for 5 min). Next, the supernatant was discarded, and then the cell pellets were stored at −80 °C until further processing. Microbial DNA was extracted from the sludge samples using the E.Z.N.A.® Bacteria DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.) according to the manufacturer's protocols. The V3–V4 region of the bacteria 16S and fungi 18S ribosomal RNA gene were amplified by PCR (95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for