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Biodiesel production from granular sludge fed with sugar-containing wastewater

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ABSTRACT

Utilizing excess sludge from wastewater treatment plant to produce biodiesel is becoming increasingly popular, but it has been limited by the low lipid content in activated sludge. This study was designed to enhance biodiesel production from granular sludge fed with synthetic sugar-containing wastewater. The experiments were conducted in two 1.2-L sequencing batch reactors under different sludge settling times to cultivate granular sludge and activated sludge. The biodiesel yield of the granular sludge (30.14 ± 0.73 mg/g suspended solids [SS]) was higher than that of the activated sludge (21.56 ± 0.95 mg/g SS) with the same feedings. The distributions of fatty acid methyl esters varied among the seed sludge, the cultured sludge flocs and the granular sludge. Methyl 14-methylpentadecanoate (14MeC15:0), methyl oleate (C18:1) and methyl linoleate (C18:2) were the three components of the biodiesel produced from the granular sludge that increased the fastest, which might promote the heating value and the low temperature fluidity of biodiesel. The remarkable change of microbial community and the dominance of filamentous fungi *Phialophora* after sludge granulation might contribute to the intrinsic change in the fatty acids, which resulted in a higher yield and a better quality of biodiesel.

Keywords: Biodiesel; Fatty acid methyl esters; Granular sludge; Sugar-containing wastewater; Microbial population

1. Introduction

The massive amount of excess sludge from wastewater treatment plants could be a potential feedstock for nutrient recycling and energy recovery. The resources recycled from excess sludge include nutrients (nitrogen and phosphorus), electricity, hydrogen, syngas, bio-oil and biodiesel [1]. Biodiesel, or fatty acid methyl esters (FAMEs), is a renewable and environment-friendly energy source that could be directly used in traditional engines. Thus, the study of utilizing the microbial lipids in excess sludge to produce biodiesel has received a great amount of attention in recent years [2–4]. However, the low biodiesel yield resulting from the low lipid content of wastewater sludge has indirectly increased the cost of biodiesel production. Therefore, researchers have tried to enhance the lipid accumulation of activated sludge (AS) by increasing the organic loading, the ratio of carbon and nitrogen as well as utilizing various carbon substances [5–8].

Previous research has shown that increasing the organic loading rate could promote the production of microbial lipids. Meanwhile, sugar-containing wastewater with its high concentration of organic matter is another difficult issue to address. Compared with conventional AS, the dense structure and good settling properties of biogranules could enable high biomass retention and the toleration of high-strength wastewater and shock loadings [9–13]. Aerobic granular sludge in an sequencing batch reactor (AGS-SBR) could be a competent small wastewater system for the degradation of industrial wastewaters with high organic loading [14,15]. Granular sludge (GS) could contain special microbial populations responsible for

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the biological treatment of sugar-containing wastewater. This microbial population possibly consists of some heterotrophic bacteria that utilize the organic content of the wastewater to produce microbial lipids for biodiesel production. Therefore, GS for the treatment of sugar-containing wastewater may be a promising raw material for biodiesel production, which could also reduce the environmental contamination from wastewater discharge and convert excess GS into valuable resources.

The purpose of this study was to investigate the feasibility and the mechanisms of biodiesel production from GS during the treatment of sugar-containing wastewater compared with AS with the same feeding conditions. The relationships among sludge type, microbial population and biodiesel production were investigated. These research findings will help to establish a new technical approach to biodiesel production from wastewater sludge so that an alternative means of waste disposal can be achieved. Moreover, a new low-cost feedstock source of lipids for biodiesel production could be obtained.

2. Materials and methods

2.1. Cultivation of activated sludge and granular sludge

Two 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 sludge was obtained from a full-scale sewage treatment plant (Xiaojia River, Beijing, China), and the initial biomass concentrations were 2.8 g mixed liquid suspended solids (MLSS)/L in the two reactors. They were operated in a fixed regular mode for a 4-h cycle with 5 min of feeding and 8 min of effluent withdrawal from the middle port of the column. For the cultivation of AS, the settling time was kept at 30 min in R1, whereas the settlement time was gradually reduced from 30 to 2 min in R2 to promote the granulation process. The aeration rate was 1.0 L/min in the two reactors. The reactors were fed with synthetic sugar-containing wastewater that consisted of glucose, ammonium chloride and other nutrients. The COD concentrations of the influent were both 1,000 mg/L in R1 and R2, and the COD:N ratio was 50:1 in two bioreactors. The other nutrients contained the following components: 25 mg/L of Na_2HPO_4, 20 mg/L of KH_2PO_4 and 5 mL of trace mineral solution (H_3BO_4 0.25 mg, $CuCl_2 \bullet 2H_2O$ 0.32 mg, $MnSO_4 \bullet H_2O$ 0.25 mg, $(NH_4)_6Mo_7 \bullet 4H_2O$ 0.25 mg, $AlCl_3$ 0.25 mg, $CoCl_2 \bullet 6H_2O$ 0.25 mg and $NiCl_2$ 0.25 mg). $NaHCO_3$ was utilized to maintain the pH of the reactors in the range between 7.0 and 8.0.

2.2. In situ transesterification

Both the AS and the GS were treated for in situ transesterification using a modified method based on a previous research report [16]. The sludge samples were dewatered by centrifugation at 5,000 rpm for 5 min (total suspended solids: 6%). One gram of dewatered sludge, 7.5 mL of sulfuric acid–methanol (5%, v:v) and 10 mL of hexane were added to a 100-mL flask, and then the mixture was heated for 7 h at 75°C for in situ transesterification. A condenser was used to minimize the loss of methanol and hexane due to evaporation with water at room temperature. After the transesterification was completed, the FAMEs were extracted, pooled, dried, re-dissolved and analyzed by gas chromatograph–flame ionization detector based on the procedures presented in a previous study [17].

2.3. Analytical methods

To determine the growth characteristics and the degradation ability of the AS and the GS, the sludge MLSS concentration, the sludge volume index (SVI), the particle size distribution and the glucose and ammonium–nitrogen concentrations in the effluent were regularly examined. The sludge MLSS concentration and the SVI were measured according to the standard methods [18]. The particle size distribution was measured using a laser particle size analyser (S3500, Microtrac, USA). The effluent glucose and ammonium–nitrogen concentrations were determined using the phenol–sulfuric acid method [19] and Nessler's reagent colorimetry, respectively. The microbial population structure was analyzed by high-throughput sequencing on an Illumina MiSieq PE300 platform [17].

3. Results and discussion

3.1. Characteristics of activated sludge and granular sludge

The distinctions between AS and GS became increasingly apparent after cultivation under different settling times. Following granulation, the morphology of sludge from R2 completely changed, with granules becoming larger and settling faster than those of the sludge flocs in R1. The mean size of the sludge increased to 300 and 640 μm for R1 and R2, respectively, after running for 84 d, compared with 60 μm for the seed sludge. Moreover, the value of SVI_{30} decreased from 111 mL/g to 47 and 40 mL/g for the sludge cultured from R1 and R2, respectively. In R2, the small and slow-settling sludge was gradually discharged by decreasing the settling time so that the GS with a larger size and lower SVI value was achieved. The sludge from R1 showed a similar tendency in terms of size and settling ability, but it was still suspended and flocculent sludge (Fig. 1). The sludge flocs in R1 were dominated by light-yellow bacteria with a little white filamentous fungus. However, the granules in R2 were formed by both light-yellow bacteria and brown filamentous fungus that tangled together and extended out of the surface. Although the AS and the GS had different morphologies, they both performed well in the degradation of glucose and ammonium–nitrogen, with removal rates higher than 90%. The effluent of R1 and R2 were similar and remained around 15 mg COD/L and 3 mg NH_4^+-N/L after running 2 weeks.

3.2. Production of FAMEs from activated sludge and granular sludge

The yield and distribution of FAMEs were significantly different among the raw activate sludge (RAS), the cultured activated sludge (CAS) and the cultured granular sludge (CGS) (Figs. 2 and 3). Feeding with sugar-containing wastewater and the sludge granulation process could both increase the accumulation of microbial lipids. The total biodiesel yields from the CGS and the CAS were 30.14 ± 0.73 and 21.56 ± 0.95 mg FAMEs/g suspended solids (SS), respectively, compared with 15.53 ± 0.87 mg/g SS for the RAS (Fig. 2). According to FAMEs constituent of the sludge, the biodiesel yields of RAS, CAS and CGS were calculated as 31.55, 43.89 and 61.55 mg COD/g SS, respectively. Using GS to treat sugar-containing wastewater could apparently accumulate more lipids for biodiesel production.

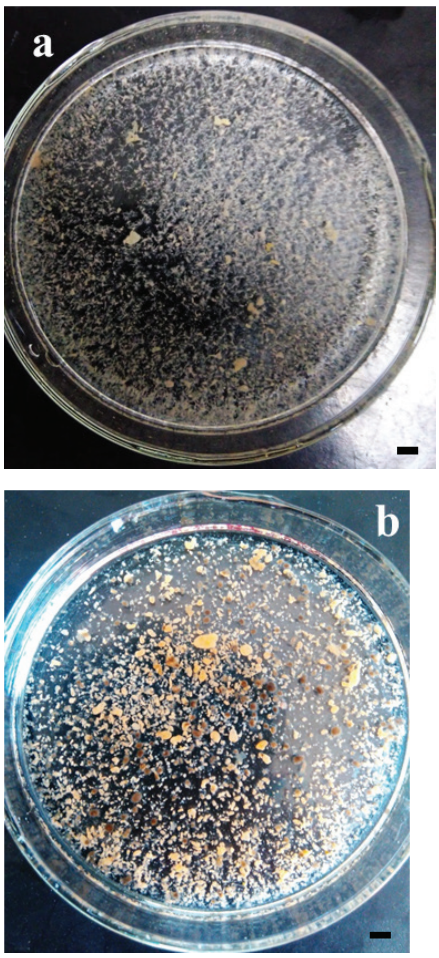

Fig. 1. Morphologies of (a) activated sludge from R1 and (b) granular sludge from R2 after running for 84 d (bar = 5 mm).

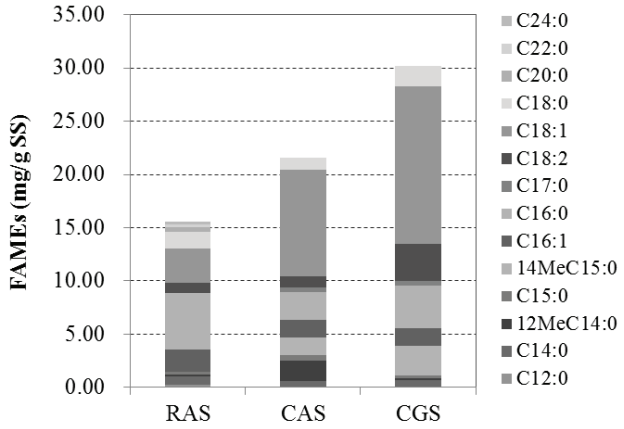

Fig. 2. Yield of each FAME based on the weight of dry sludge. RAS, raw activated sludge, CAS, cultured activated sludge, CGS, cultured granular sludge.

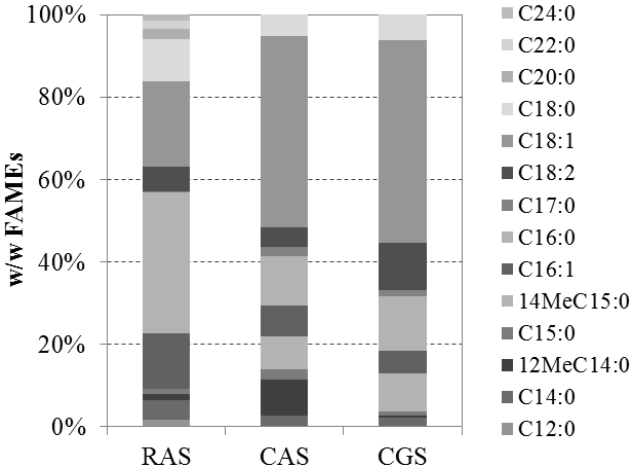

Fig. 3. Distributions of FAMEs in the biodiesel produced from the sludge. RAS, raw activated sludge, CAS, cultured activated sludge, CGS, cultured granular sludge.

Methyl palmitoleate (C16:1), methyl palmitate (C16:0), methyl oleate (C18:1) and methyl stearate (C18:0) were the dominant components in the biodiesel produced from seed sludge, and their yields were 2.08, 5.31, 3.23 and 1.58 mg/g SS, representing 13.4%, 34.2%, 20.8% and 10.2%, respectively (Figs. 2 and 3). However, biodiesel produced from cultured sludge from both of the bioreactors contained less methyl palmitoleate (C16:1) and methyl palmitate (C16:0), but more methyl oleate (C18:1) compared with the seed sludge. The methyl palmitoleate (C16:1) from the CAS and CGS was both reduced to approximately 1.6 mg/g SS. Compared with 2.61 mg/g SS methyl palmitate (C16:0) from the CAS, the CGS led to a higher yield of 3.99 mg/g SS. In addition, the yield of methyl oleate (C18:1) reached 9.98 and 14.79 mg/g SS for the CAS and the CGS, which accounted for 46.3% and 49.1% in the biodiesel. The changes in methyl palmitoleate (C16:1) and methyl oleate (C18:1) were consistent with the study of Mondala et al. [5]. Although more methyl oleate (C18:1) was found both in the CAS and the CGS, only the CGS led to more methyl linoleate (C18:2), which was as much as 3.5 times that of the RAS and the CAS. The percentages of FAMEs with 18 carbons in the biodiesel from the three types of sludge were 37.0% (RAS), 56.4% (CAS) and 66.8% (CGS), whereas the proportions of 16-carbon FAMEs were 47.6% (RAS), 19.6% (CAS) and 18.8% (CGS), respectively (Fig. 3). The heating value would rise with the increase of the chain length [20]; thus, the biodiesel produced from aerobic granular sludge may have a higher heating value. Apart from the increase of fatty acids with 18 carbons, more branched FAMEs were also found in the CAS and the CGS fed with synthetic sugar-containing wastewater. Branched FAMEs accounted for 16.6% and 9.6% of the biodiesel produced from the CAS and the CGS compared with 1.6% for the RAS. The CAS accumulated more methyl 12-methyltetradecanoate (12MeC14:0) and methyl 14-methylpentadecanoate (14MeC15:0), whereas only the CGS produced more methyl 14-methylpentadecanoate (14MeC15:0). Methyl branched FAMEs may improve the low temperature fluidity of biodiesel, which is one of the major problems associated with the use of biodiesel [21].

3.3. Effect of the microbial community on the production of FAMEs

The microbial lipid in sludge was the only lipid feedstock for biodiesel production in this study. Thus, the

variation in the microbial community may account for the difference of biodiesel yield and the distribution of FAMEs. Figs. 4 and 5 illustrate the differences in the microbial communities between the CAS and the CGS, including fungi and bacteria at the genus level. The results showed that *Phialophora* was the dominant fungi in GS, representing 73.1%, followed by *Hypocreales_unclassified* (23.2%). Liu et al. [22] reported a new species of *Phialophora*, which was characterized by a brown colony. This is consistent with the previously mentioned existence of brown filamentous cells in the GS (Fig. 1). The concentration of oleic acid (C18:1) was very high (69.9%–73.6% of the total fatty acids) in *Phialophora dermatitidis*, and the yield of oleic acid (C18:1) could reach 236 mg/g cells [23]. The growth of *Phialophora* may be one of the reasons for the rapid increase in methyl oleate (C18:1) in biodiesel produced from the GS of R2. Unfortunately, being limited to the current information, 72.6% of the fungi in the cultured AS were still *Incertae sedis*, whereas *Hypocreales_unclassified* and *Dipodascus* were identified as the other two main fungi from the CAS, representing 11.5% and 10.1%, respectively. *Dipodascus* were members of the family of yeasts in the order *Saccharomycetales*, and the main fatty acids were oleic acid (C18:1) and linoleic acid (C18:2) from many species of *Dipodascus* [24]. Therefore, the existence of fungi (such as *Dipodascus* and *Phialophora*) may cause the increase of unsaturated FAMEs with 18 carbons in the biodiesel from the cultured sludge in the two bioreactors.

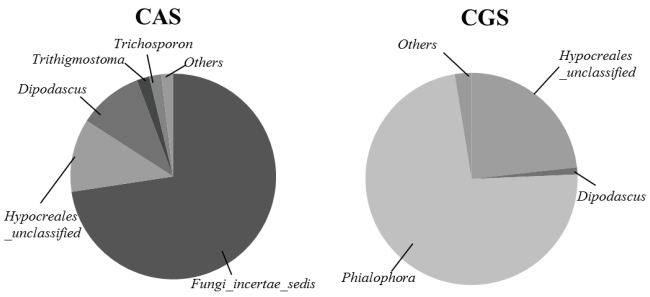

Fig. 4. Distribution of fungi in cultured activated sludge (CAS) and granular sludge (CGS) at the genus level.

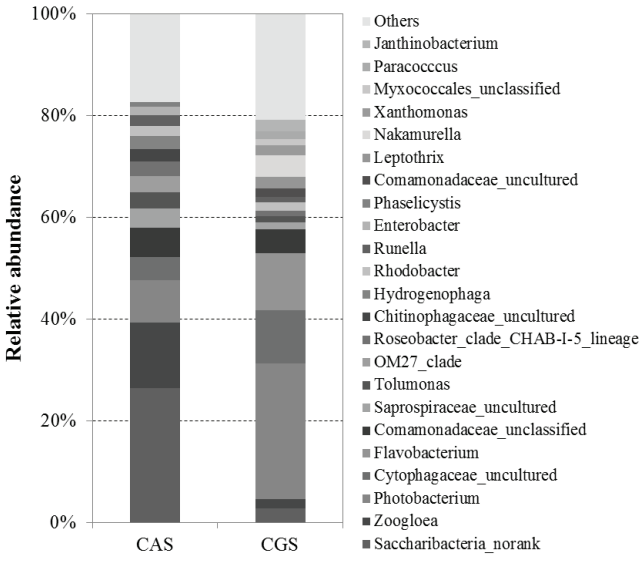

Fig. 5. Distribution of bacteria in the cultured activated sludge (CAS) and the cultured granular sludge (CGS) at the genus level.

As for the bacterial population, the CAS and the CGS were quite different. *Saccharibacteria_norank*, *Zoogloea* and *Photobacterium* were the main members in the CAS at 26.4%, 13.0% and 8.3%, respectively (Fig. 5). *Saccharibacteria*, also known as *TM7*, is a highly ubiquitous phylum in soils, sediments, wastewater and animals [25]. It was reported to be one of the glucose-utilizing species in a full-scale anaerobic sludge digester [26]. *Zoogloea* was found to be one of the genera that showed a high positive correlation with branched fatty acids [27]. The growth of *Zoogloea* in the CAS could result in the increase of branched FAMEs in the biodiesel product (Fig. 2). Additionally, bacteria from the CAS also contained *Comamonadaceae_unclassified* (5.8%), *Cytophagaceae_uncultured* (4.5%), *Saprospiraceae_uncultured* (3.8%), *Tolumonas* (3.2%), *OM27_clade* (3.0%) and *Roseobacter_clade_CHAB-I-5_lineage* (3.0%). However, *Photobacterium*, *Cytophagaceae_uncultured* and *Flavobacterium* became the most dominant species in the CGS, and their proportions were 26.6%, 10.4% and 11.4%, respectively. Palmitic acid (C16:0) was reported to be a major fatty acid from *Photobacterium* [28–30], and the yield of methyl palmitate (C16:0) was higher in the CGS than in the CAS (Fig. 2). It was reported that the *Cytophaga-Flexibacter* group preferentially accumulated iso-branched fatty acid, such as *Cytophaga johnsonae* and *Cytophaga* sp. strain samoa [31]. The occurrence of *Cytophagaceae_uncultured* may also be one of the donors of branched fatty acids in the CAS and the CGS. There were also some other species in the CGS, including *Comamonadaceae_unclassified* (4.6%), *Nakamurella* (4.3%), *Saccharibacteria_norank* (2.7%), *Janthinobacterium* (2.3%), *Leptothrix* (2.2%), *Xanthomonas* (2.0%) and *Zoogloea* (1.9%). Obviously, the variations of the microbial community resulted in corresponding distinctions of biodiesel yield and the distribution of FAMEs between the cultured sludge in the two bioreactors.

4. Conclusions

Feeding with sugar-containing wastewater could promote the lipid accumulation in sludge. By using the same feeding strategy, the GS showed an advantage in biodiesel production both in yield and in quality. Compared with the seed sludge (biodiesel yield of 15.53 ± 0.87 mg/g SS), the GS achieved a higher biodiesel yield (30.14 ± 0.73 mg/g SS) than the CAS (21.56 ± 0.95 mg/g SS). Biodiesel produced from the GS contained more methyl oleate (C18:1) and methyl linoleate (C18:2). FAMEs with longer carbon chains could release more heat energy so that the heating value of biodiesel from the GS would increase. Unsaturated fatty acids also increased in the GS, which may improve the low temperature fluidity of biodiesel. The above variations may be attributed to the change in the microbial community, including the growth of fungi (*Phialophora*) and the change of bacterial population.

Acknowledgments

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