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Influent characteristics affect biodiesel production from waste sludge in biological wastewater treatment systems

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ABSTRACT

Biodiesel is a renewable and environmentally friendly energy source. The use of biological wastewater treatment systems for excess sludge in the biodiesel production can achieve both sludge reduction and resource recovery. In this study, an *in situ* one-step method for producing biodiesel from excess secondary sludge was investigated; then the differences in both biodiesel yield and composition produced from the five industrial wastewater sludges and two municipal wastewater sludges were studied. The results showed that ultrasonic pretreatment of sludge was not necessary for biodiesel production by *in situ* acid-catalyzed transesterification. The addition of *n*-hexane during transesterification was significant to determine the performance of biodiesel production. Significant differences were found in yield and composition of the sludge lipid from seven different types of wastewater. The sludge oil of starch wastewater and restaurant wastewater was significantly higher than those of tannery wastewater, landfill leachate, and livestock wastewater, which was mainly due to the significant differences on wastewater quality and sludge microbial structure. The higher organic content (especially sugar), COD/TN and COD/TP ratios and better biodegradability in wastewater led to the dominance of *Saprospiraceae* and *Anaerolineaceae*, finally benefited the lipid accumulation in excess secondary sludge for biodiesel production.

1. Introduction

Energy and environmental protection are currently hot topics for a sustainable development of society. With the continuing advancement of urbanization and industrialization, energy demand is increasing steadily, but oil, coal, natural gas, and other non-renewable energy are becoming increasingly scarce. In recent years, biodiesel has aroused great interest as renewable and environmentally friendly biomass energy. Biodiesel can be used directly in conventional engines. Compared to fossil fuels, it has advantages of being free of sulfur featuring an inherent lubricity with higher cetane and flash point values (Hajjari et al., 2014; Aghbashlo et al., 2016; Sumprasit et al., 2017). In addition, biodiesel burns less than 20% of unburned hydrocarbons, 30% of CO, and 50% of particulate emissions compared to diesel fuels (Hajjari et al., 2017). Besides, with the urbanization and economic development, a large amount of excess sludge is produced as the most important byproduct of biological wastewater treatment (Folgueras et al., 2013); therefore, its harmless treatment and disposal has become problem that requires to be solved swiftly. The current treatments are mainly incineration, composting or deposition in landfill, and treatment costs account for about 65% of the total cost of sewage treatment, which not only consumes significant energy, but also poses security risks (Liu, 2003; Xiong et al., 2017). Some studies have shown that the main components of residual activated sludge are fat, protein, and carbohydrates (Yang et al., 2015; Xiao et al., 2017); therefore, the excess sludge for secondary development can fully utilize the nutrients to obtain higher value-added products.

At present, the United States, Europe, South Korea, China have conducted preliminary studies about the usage of sewage sludge by extracting microbial oil to produce biodiesel. Dufreche et al. (2007) studied the secondary sludge from the Tuscalusa municipal sewage treatment plant, and used n-hexane, methanol, n-hexane-methanol-acetone mixed solvent and supercritical CO_2 extraction of oil in the sludge, achieving a final yield of fatty acid methyl ester of 4.41 ± 0.63 wt%. At the same time, Mondala et al. (2009) studied waste sludge from the primary and secondary sedimentation, with biodiesel yields of 14.5 wt% and 2.5 wt%, respectively. Zhu et al. (2012) also studied the biodiesel production from activated sludge, and

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extracted oil via acid hydrolysis, soxhlet extraction, and water bath oscillation. The achieved biodiesel yields were 1.33%, 6.73%, and 4.92%, respectively. However, previous studies have focused on the production of biodiesel from municipal sludge, but have not reported the biodiesel production from excess sludge of industrial wastewater, and whether biodiesel production would be different to municipal sludge. Using biological technology to treat different type wastewater, microbial populations with different structures and quantities will form during biological wastewater treatment process. The enriched specific microbial population will finally form the excess secondary sludge via precipitation, and the difference in the sludge microbial community will likely affect the extracted fat and oil composition. Furthermore, the method of biodiesel production from excess sludge also should be considered. Some researchers indicated that transesterification process affected the biodiesel yield from excess sludge (Mondala et al., 2009; Li et al., 2016a). Ultrasonic pretreatment could improve treatment efficiency of sludge anaerobic degradation (Li et al., 2016b). The addition of n-hexane could improve fat solubility from microorganisms (Wu et al., 2017).

Therefore, after optimizing the method of biodiesel production from excess secondary sludge, this study focused on the biodiesel yield and composition of excess secondary sludge from different types of industrial wastewater and municipal wastewater, including starch wastewater, restaurant wastewater, livestock wastewater, landfill leachate, tannery waste, and sewage secondary sludge. These seven wastewater treatment processes used the same A^2/O biological treatment process. This study mainly investigated the effects of different wastewater qualities and the microbial structure of activated sludge on the biodiesel yield and composition produced from secondary sludge, thus providing an alternative and theoretical basis to solve the problem of high cost of biodiesel raw materials, while realizing the energy recovery of excess sludge.

2. Materials and methods

2.1. Optimization of biodiesel production from excess sludge

In this experiment, excess secondary sludge from a municipal wastewater treatment plant in Beijing was used as the raw material. Based on previously reported methods (Revellame et al., 2010; Maeng, 2016), the microbial oil of excess sludge was extracted and methyl esterified via the *in situ* one-step method. The effects of ultrasonic pretreatment (1000 W, running 4 s, suspended 4 s, a total of 5 min), oil extraction reagent n-hexane (10 mL), excess sludge mass and amount of sulfuric acid-methanol on the biodiesel production from excess sludge were investigated in this study. The specific steps are shown in Fig. 1: Weighing of a certain amount of excess sludge after belt filter press dewatering (containing 13%–16% solids), then adding the required reagents for oil extract and transesterification, and running the *in situ* methyl esterification reaction in a water bath at 75 °C for 7 h. During the reaction, the condensate was connected to prevent evaporation of n-hexane and methanol to avoid product loss. After reaction completion, the mixture was allowed to cool to room temperature, mixed with 5 mL of saturated sodium chloride solution, and extracted thrice with n-hexane. The solution was then centrifuged at 3000 r/min for 3 min, and then n-hexane was collected. After 10 mL of 2% (w/v) sodium bicarbonate solution and 3 g of anhydrous sodium sulfate were added and centrifuged, the upper n-hexane layer was extracted and the solvent was evaporated at 80 °C to obtain biodiesel.

2.2. Fatty acid methyl ester (FAMEs) analysis

By using the optimized *in situ* one-step method, the excess secondary sludge from seven wastewater treatment processes were obtained to produce biodiesels which were stored at −20 °C prior to the analysis of gas chromatography. These seven wastewater treatment processes used the same A^2/O biological treatment process to treat different types of industrial wastewater and municipal wastewater, including starch wastewater, restaurant wastewater, livestock wastewater, landfill leachate, tannery waste, and sewages from two different districts. Then, a sufficient amount of n-hexane was added to the extracted biodiesel sample to be dissolved, 1,3-xylene was used as internal standard, and filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter to remove impurities in the solution. Then, it was analyzed with an Agilent 7890A Gas Chromatograph (GC). The column was a 30 m × 0.320 mm x 0.25 μm HP-5 capillary column (19091J-413, Agilent) with a stationary phase of 5% phenylmethylpolysiloxane. The oven temperature started at 50 °C for 2 min, was then increased to 130 °C at 10 °C/min, then further increased to 200 °C at 4 °C/min, then further increased to 220 °C at 3 °C/min, and finally increased to 270 °C at 5 °C/min. The flow rates of Hydrogen, air, and Nitrogen were 30, 300, and 25 mL/min, respectively. The injection volume was 1 μL without the split mode. The inlet and FID detector temperatures were set to 200 °C and 270 °C, respectively. The criteria for qualitative and quantitative analysis of fatty acid methyl esters included FAME mixed with C8 to C24 (18918-1AMP, Sigma-Aldrich, USA), two branched fatty acid methyl esters (12-methyltetradecanoic acid methyl ester, 14-methyl pentadecanoic acid methyl ester, Larodan Fine Chemicals AB, Sweden), and two odd carbonic acid methyl esters (methyl pentadecanoate and methyl heptadecanoate, AccuStandard, Inc., New Haven, USA).

2.3. Microbial structure analysis

In this study, seven excess sludge samples (2 mL) were collected and concentrated via centrifugation (13,400 g for 5 min). Then, the supernatant was discarded, and the cell pellets were stored at −80 °C. High-throughput sequencing and qPCR were used to analyze the microbial population structure of excess sludge, following the protocol described by Li et al. (2016a).

2.4. Wastewater quality analysis

The types of wastewater used for this experiment included starch wastewater, restaurant wastewater, livestock wastewater, landfill leachate, tannery wastewater, and municipal wastewater. The pH values of different types of raw wastewater were adjusted to strong acidity and then stored in a refrigerator at −20 °C until the analysis of raw water quality. The water quality test parameters included COD, ammonia nitrogen, TN, TP, and sugar concentration. The concentration of COD, ammonia nitrogen, TN and TP were measured according to the Standard Methods (APHA, 2005). Sugar concentration was determined via the phenol-sulfuric acid method (Gerhardt et al., 1994).

3. Results and discussion

3.1. Optimization of the in situ one-step method for biodiesel production from excess sludge

3.1.1. Effects of ultrasonic pretreatment

The ultrasonic crushing method is a commonly used method for cell breakage. Breaking of the cell is theoretically beneficial for the release of intracellular matter. However, according to the results of Fig. 2a, ultrasonic treatment did not promote the production of biodiesel for excess secondary sludge. Under both conditions of ultrasonic pretreatment and without ultrasonic pretreatment, the yields of biodiesel were 15.44 ± 3.74 mg/g SS and 15.54 ± 0.58 mg/g SS, respectively, using secondary excess sludge as raw material. Ultrasound pretreatment did not cause a significant increase in biodiesel production, which could be due to the following two aspects: 1) The heat and free radicals released during the ultrasound process may have damaged the oil contained in the microorganism (Sheng et al., 2012); 2) The concentration of added sulfuric acid in the methyl esterification was at a high level in the same