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Enhanced volatile fatty acid production by a modified biological pretreatment in anaerobic fermentation of waste activated sludge

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HIGHLIGHTS

- Enhanced VFAs production was obtained by a novel PEH pretreatment in WAS anaerobic fermentation.
- PEH obtained higher carbon release and utilization rate simultaneously.
- EDTA-2Na solubilized the EPS and then protease lysed sludge cell in PEH process.
- Quality of carbon released by biological pretreatment greatly exceeded physicochemical pretreatment.

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ABSTRACT

A novel protease/EDTA-2Na hydrolysis (PEH) pretreatment method was developed with the combination of EDTA-2Na hydrolysis (EH) and protease hydrolysis (PH) to improve volatile fatty acids (VFAs) production by anaerobic fermentation of waste activated sludge (WAS). Efficiencies of PEH, PH, EH and thermal alkaline hydrolysis (TAH) were compared in releasing SCOD from WAS. Results indicated that physicochemical pretreatment, TAH, had higher SCOD release but less utilization rate of the released SCOD in anaerobic fermentation, biological pretreatments, PH, showed less SCOD release but higher SCOD utilization rate, and PEH could simultaneously obtain higher SCOD release and utilization rate. By TAH, released SCOD could reach 19,273.21 mg/l, but VFAs was only 11,820.36 mg COD/L. By PEH, SCOD and VFAs were 13,628.98 and 12,704.44 mg COD/L, respectively. SCOD released by PEH showed good biodegradability with BOD_5/COD of 0.64%, 25.49% higher than that of TAH. By anaerobic fermentation, volatile suspended solids (VSS) of WAS pretreated by PEH could be reduced by 18.50%, but only 6.16% for TAH treated WAS. In PEH process, synergistic effects between protease and EDTA-2Na were observed, in which EDTA-2Na prevented protease from being trapped on the surfaces of sludge floccules and finally greatly improved protease efficiency. Therefore, it is feasible to apply PEH on enhancing VFAs production in anaerobic fermentation of WAS due to its higher and better bio-available carbon release than other pretreatments.

1. Introduction

In recent years, the production of waste activated sludge (WAS) has increased year by year in wastewater treatment plants (WWTPs) of China and even reached 40 million tons with water content of 80% in 2013 [1]. WAS treatment and disposal has became a critical issue and it accounts for 50–70% of the total cost of WWTPs operation [2]. Anaerobic fermentation for volatile fatty acids (VFAs) production is a promising technology in sewage sludge treatment and reutilization, in which carbon resource in sewage sludge could be converted into useful VFAs, instead of carbon dioxide and methane, then simultaneously achieving waste

Abbreviations: WAS, waste activated sludge; WWTPs, wastewater treatment plants; VFAs, volatile fatty acids; PEH, protease/EDTA-2Na hydrolysis; EH, EDTA-2Na hydrolysis; PH, protease hydrolysis; TAH, thermal alkaline hydrolysis; COD, chemical oxygen demand; BOD, biological oxygen demand; TSS, total suspended solids; VSS, volatile suspended solids; DS, dry sludge; BSA, bovine serum albumin; Y_{SCOD}, yielding rates of SCOD; P_{SCOD}, acidification rates of SCOD to VFAs; P_{VSS}, conversion rates of VSS to VFAs; R_{VSS}, Reduce rates of VSS; U_{Carbon}, utilization rates of different carbon sources; P_{Carbon}, conversion rate of different carbon sources to VFAs; FTIR, Fourier transform infrared spectroscopy; TRCs, total residual carbons; EPS, extracellular polymeric substances.

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sludge disposal, valuable biochemical productions and biological carbon fixation [3–6]. Therefore, it has attracted more and more attentions, one focus of which is how to improve sludge conversion rate [7–9].

Pretreatments have been proved to be effective methods for enhancing the yields of VFAs in sludge anaerobic fermentation process [10,11]. Most of the carbon source in WAS is inside of microbial cells and protected by cell wall. The amount of the carbon that could be directly used by fermentative microorganism is very limited, resulting in low VFAs conversion rates in conventional sludge fermentation processes. Cell lysis could be realized by sludge pretreatments, obtaining thoroughly release of organic matters inside of cells and decomposition of polymeric organics into biodegradable molecules.

There are already many kinds of sludge pretreatment methods reported in previous studies, including mechanical, chemical and biological methods, amongst of which, thermal alkaline pretreatment (TAH) was considered as one of the most effective methods [12–14]. However, there are also many shortages in TAH, such as serious consumption of energy, waste of mineral resources, terrible corruption and difficulty in implementation in practical projects [15,16].

Recent researchers found that biological enzymes pretreatments, such as PH, are also an effective method with characteristics of mild reaction condition, less by-products and lower energy consumption [17–19]. Moreover, protease showed the best efficiency in the pretreatment of WAS. For example, Whiteley et al. [20] reported that the surviving temperatures of proteases could be up to 70 °C while that of phosphatases was 60 °C in WAS pretreatment. Wawrzynczyk et al. [21] compared the efficiencies of enzymes, sodium tripolyphosphate and cation exchange resins in WAS pretreatment, and the solubilizations of protein from WAS by enzymes, sodium tripolyphosphate and cation exchange resin were 22.5, 10.5 and 19.5 mg/g TS. However, there are also some problems in the processes of single biological pretreatment, such as high cost of enzymes and low SCOD release.

In this study, a novel pretreatment method, protease/EDTA-2Na hydrolysis (PEH), was developed by modifying the single biological pretreatment, in which the efficiencies of enzymes were improved greatly, the usage amount of enzymes was reduced and finally those problems occurred in single biological pretreatments were solved. Moreover, different pretreatment methods, namely protease hydrolysis (PH), EDTA-2Na hydrolysis (EH), protease/EDTA-2Na hydrolysis (PEH) and thermal alkaline hydrolysis (TAH), were comprehensively compared for their SCOD release and VFAs yields improvement. The aim of this paper is (1) to investigate the quality and quantity of the carbon released by the modified biologic pretreatment; (2) to evaluate its efficiencies in improving the bioavailability of the carbon resource in WAS and (3) to clarify the influencing mechanism of different pretreatments on VFAs production from anaerobic fermentation of WAS.

2. Material and methods

2.1. Substrates

Waste activated sludge (WAS) used as the substrate for pretreatments was obtained from the secondary sedimentation tank of the urban wastewater plant in Wuxi, China. Fresh WAS was firstly concentrated by settling for 4 h, then filtered by the metal sieve with 0.71 mm aperture and finally stored at 4 °C for later use. WAS was characterized with pH of 6.53, TCOD of 55–60 g/l, SCOD of 0.40–0.44 g/l, TSS of 73.0–75.0 g/l, VSS of 42–46 g/l, protein concentration of 22–25 g/l and reduction sugar concentration of 3.5–4.5 g/l.

2.2. Seeding sludge for anaerobic fermentation

Anaerobic sludge from an UASB reactor for brewery wastewater treatment was collected as the seeding sludge. In order to accumulate acetogenic bacteria, the anaerobic sludge was firstly concentrated by setting for 24.0 h at ambient temperature and the precipitated sludge was then treated at 105 °C for 2.0 h to kill methanogens. To reactivate acetogenic bacteria, the heat-treated sludge was added into a 1000 ml shaking flask holding nutrient solution whose compositions, concluding 3.0 g/l glucose, were referred to previous reports [22,23]. When the mixed-liquor suspended solids (MLSS) was 9.0 g/l, pH was about 6.5, stirring speed was 120.0 rpm and temperature was 35.0 °C, the heat-treated sludge was cultivated for 24.0 h in the completely anaerobic flask. Finally, the seeding sludge was obtained by centrifuging the cultivated sludge at 4800 rpm for 10 min. In cultivation process, pH was adjusted by dilute HCl and NaOH, oxygen in headspace of flask was removed by injecting nitrogen for 10.0 min, dissolved oxygen was removed by adding L-cysteine solution and phosphate was used as the buffer.

2.3. Enzymes for pretreatments

Enzymes used in the pretreatment processes were commercial neuter protease with activities of 80,000 U/g (from Amano in Japan).

2.4. WAS pretreatment methods

Protease hydrolysis (PH): seven beaker flasks of 250 ml were filled with 150 ml WAS and then added proteases with dosages of 0, 5, 10, 15, 20, 25 and 30 mg/l dry sludge (DS), respectively. When the stirring speed was 120 rpm and temperature was 25 °C, samples taken from beaker flasks at the interval of about 30 min were analyzed. Results indicated that carbon release could not further increase by increasing protease dosage when it reached 25 mg/g DS. Therefore, the data presented in this paper were obtained under protease dosage of 25 mg/g DS.

EDTA-2Na hydrolysis (EH): seven beaker flasks of 250 ml were filled with 150 ml WAS and then added EDTA-2Na with dosages of 0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 g/g DS, respectively. The following process of EH was the same as that of PH, and the data presented in this paper were obtained under the most optimal EDTA-2Na dosage of 0.2 g/g DS.

Protease/EDTA-2Na hydrolysis (PEH): a beaker flask of 250 ml was filled with WAS of 150 ml and then added proteases with dosage of 25 mg/g DS and EDTA-2Na with dosage of 0.2 g/g DS. With stirring speed of 120 rpm and temperature of 25 °C, samples taken from the beaker flask at certain intervals were analyzed.

Thermal alkaline hydrolysis (TAH): 250 ml WAS was put into a beaker of 500 ml and rapidly heated to 90 °C. Then, pH of the heated WAS was adjusted to about 12 and TAH process started [24]. Samples were taken at interval of about 30 min for analyzing.

With stirring speed of 120 rpm and temperature of 25 °C, experiments without the additions of EDTA-2Na and protease were set as the blank. All tests were conducted in triplicate and presented data were the average of three independent experiments.

2.5. Anaerobic fermentation

Four beaker flasks of 1000 ml were filled with 500 ml WAS pretreated by PH, EH, PEH and TAH, respectively, and adjusted pH of 10.0 by dilute HCl and NaOH. Dissolved oxygen in WAS and gaseous in the headspace of flasks were removed by sparging gaseous nitrogen for about 30 min to maintain strict anaerobic condition. In the whole process of fermentation, flasks were placed in orbital