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Enhancement of sludge reduction and methane production by removing extracellular polymeric substances from waste activated sludge

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HIGHLIGHTS

- The removal of EPS from WAS increased sludge reduction and methane production.
- The enhancement was due to the increased hydrolytic activity during fermentation.
- *Clostridium* species contributes to the enhancement of methane production.

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ABSTRACT

The management of waste activated sludge (WAS) recycling is a concern that affects the development of the future low-carbon society, particularly sludge reduction and biomass utilization. In this study, we investigated the effect of removing extracellular polymeric substances (EPS), which play important roles in the adhesion and flocculation of WAS, on increased sludge disintegration, thereby enhancing sludge reduction and methane production by anaerobic digestion. EPS removal from WAS by ethylenediaminetetraacetic acid (EDTA) significantly enhanced sludge reduction, i.e., 49 ± 5% compared with 27 ± 1% of the control at the end the digestion process. Methane production was also improved in WAS without EPS by 8881 ± 109 CH_4 μmol g^{-1} dry-weight of sludge. Microbial activity was determined by denaturing gradient gel electrophoresis and real-time polymerase chain reaction, which showed that the hydrolysis and acetogenesis stages were enhanced by pretreatment with 2% EDTA, with a larger methanogenic community and better methane production.

1. Introduction

Thus far, most domestic and industrial wastewater treatment plants (WWTP) use the activated sludge process because of its ability to degrade organic contaminants in wastewater and discharge clean water, carbon dioxide, and biomass (Rocher et al., 1999). However, the major disadvantage of this method is the generation of a large amount of waste activated sludge (WAS). In Japan, WAS accounts for approximately 47% of industrial waste (Yoshida et al., 2009) and 25–60% of the operating costs of WWTPs (Yan et al., 2008). By some modifications through operational control in the industrial scale treatment such as extended and improved aeration, equipped membrane bioreactor or added additives, the ability to reduce WAS is about 20–30% (Mahmood and Elliott, 2006). The hazardous materials present in WAS, such as pathogens and toxic chemicals, can harm the environment if it is inappropriately discharged (Li et al., 2009). Landfill and incineration are the conventional methods used to dispose of WAS; however, these solutions create a burden for society because of their land space requirements, operational costs, and the need for strict human health and environmental protection regulations (Wei et al., 2003). Various methods and strategies have been developed to reduce WAS generation, such as chemical treatments using metal cations (Kim et al., 2002) and oxidizing substances (Weemaes et al., 2000; Neyens et al., 2003). Individual or combined thermal processes and ultrasound and alkaline treatments have been widely used for sludge solubilization (Kepp et al., 2000; Vlyssides and Karlis, 2004; Bougrier et al., 2005; Li et al., 2008). Combined thermal, chemical, and mechanical treatments have also been used as a sludge reduction strategy (Nah et al., 2000; Valo et al., 2004).

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The utilization of carbon source in waste activated sludge is a promising approach to produce valuable energy sources such as biohydrogen (Mohd Yasin et al., 2013), and for electricity generation on the basis of microbial fuel cells (Mohd Yusoff et al., 2013). Methane production by anaerobic digestion has gained popularity for reducing the quantity of sludge generated and the recovery of renewable energy (Kanai et al., 2010). Methane is generated by the continuous degradation of organic materials in WAS by various microorganisms (Buczkowska et al., 2010). This fermentation process generally comprises four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Demirel and Scherer, 2008). In general, methane is produced by methanogens from acetic acid, carbon dioxide, and hydrogen generated in the earlier reactions. In WAS, extracellular polymeric substances (EPS) play important roles in sludge flocculation and cohesion (Frølund et al., 1996). EPS are metabolic products secreted by microorganisms during their growth, which cover the cell surface layer to protect against the severe external environment (Liu and Fang, 2002). EPS have a complex biochemical composition, including protein, carbohydrate, lipid, humic substances, uronic acid, and deoxyribonucleic acids (Tsuneda et al., 2003). EPS are considered important for the physicochemical properties of the activated sludge floc and have been implicated in determining the structure, charge, and flocculation of the WAS floc (Frølund et al., 1996). Therefore, the present study aimed to disintegrate WAS by extracting the EPS before anaerobic digestion. We hypothesize that removing EPS from WAS may reduce the sludge flocculation and subsequently more materials and bacteria would be exposed in the liquid phase, thereby enhancing the later stages of anaerobic digestion. In addition, no previous studies have mentioned the effect of EPS extraction on sludge reduction and methane production from WAS. This study provides an overview of the effect of EPS removal on anaerobic fermentation.

2. Materials and methods

2.1. Sludge preparation

Raw sludge was collected from Hiagari WWTP in Kitakyushu City, Japan. The sludge composition was previously reported (Maeda et al., 2009). Total solid (TS) composed of 3.3–4.0%, volatile solid (VS) (2.6–3.1%), suspended solid (SS) (30–41%), volatile suspended solid (VSS) (26–33%) and chemical oxygen demand (COD) of 44–51 g L^{-1}. Protein was the main component of the sludge (40–45% of total solid), followed by carbohydrate (12–14% of total solid) and lipid (11–13% of total solid). Raw sludge was washed three times by centrifugation at 8000 *g* for 10 min at 4 °C, and the pellet was resuspended in distilled water, before adjusting its concentration to 25% (w/w) with distilled water.

2.2. EPS extraction

The prepared sludge was used for EPS extraction according to published methods using ethylenediaminetetraacetic acid (EDTA) as an extractant as well as ultrasonic and autoclave treatments (Liu and Fang, 2002). As reported, formaldehyde plus NaOH is the most efficient method for EPS extraction and follow by EDTA method, however the difference of the amount of extracted EPS is not major (Liu and Fang, 2002). In addition, under treatment of NaOH not only EPS is extracted but also sludge components are breakdown and at a high pH the cellular substance is more susceptible by the effect of alkaline treatment (Kim et al., 2010). This study mostly focused on the effect of EPS removal on methane production by anaerobic digestion therefore EDTA method was selected since its high ability of EPS extraction. A flowchart illustrating each method is shown in Fig. 1. After EPS extraction, the pellet was washed twice to completely remove the extractant that remained in sludge, and it was used for methane fermentation, while the supernatant was processed by centrifugation and membrane dialysis. The supernatant included extracted EPS was transferred to a white porcelain dish and dried in drying-oven at 105 °C for two days. The extracted EPS was measured by the weight subtraction of the dish before and after drying process. According to a reported method (Liu and Fang, 2002), 2% EDTA should be used for the extraction process, but different EDTA concentrations (0.1%, 0.5%, 2%, and 4%) were tested in the present study to determine the effects of different degrees of EPS extraction by EDTA in methane fermentation. To verify that the effects of EDTA on anaerobic digestion with EPS-extracted sludge were not due to the presence of EDTA itself in the fermentation culture, an additional fermentation vial (20 g of 25% raw sludge) with EDTA at a 2% final concentration was incubated at 37 °C in a shaking incubator at 120 rpm for 15 d (Bio-shaker, BV-180LF, Taitec, Japan). To observe any structural changes in the sludge after the EPS extraction process, 5 mL of sludge samples with or without pretreatment were directly analyzed using a scanning electron microscope (SEM) where the surfaces of the samples were compared by SEM visualization (Hitachi S-3500N) (Mohd Yasin et al., 2013).

2.3. Methane production

The fermentation process used 20 g of 25% sludge (16 g of treated sludge as a substrate and 4 g of raw sludge as an inoculum) in 50-mL vials. The inoculum to sludge ratio (0.25) was based on dry solid basis (Raposo et al., 2006). The fermentation vials were sealed with crimped butyl rubber stoppers and sparged with nitrogen for 5 min and subsequently incubated at 37 °C in a shaking incubator at 120 rpm for 15 d (Bio-shaker, BV-180LF, Taitec, Japan). Each day, 50 μL of the headspace gas was analyzed using gas chromatography (GC) using a 6890 N gas chromatograph (Agilent Technologies, Glastonbury, CT) equipped with a 80–100 mesh Porapak Q column (Suppelco, Bellefonte, PA) and a thermal conductivity detector. The injector and detector were maintained at 100 °C and 200 °C respectively. The nitrogen carrier gas flow rate was maintained at 20 mL min^{-1} and the column temperature was 70 °C.

2.4. Vital microbial community analysis

2.4.1. RNA extraction and complementary DNA synthesis

A 4-mL sample of fermented sludge was mixed with 3 mL of RNAlater solution (Formerly Ambion, Applied Biosystems) in a 15-mL falcon tube before centrifugation at 15 000 rpm for 2 min. The cell pellet was chilled using dry ice dissolved with ethanol for 30 s and stored at −70 °C before RNA extraction. The total RNA was extracted using an RNeasy kit (Qiagen, Inc., Valencia, CA) with a bead beater (Wakenyaku Co. Ltd., Kyoto Japan, model 3011b), as previously described (Mohd Yusoff et al., 2012). The complementary DNA (cDNA) was synthesized from the extracted RNA using a PrimeScript RT reagent kit Perfect Real Time with random oligomers (TAKARA Bio Inc.). Agarose gel electrophoresis was conducted to verify the success of RNA isolation and cDNA synthesis. The cDNA were used later as the DNA template for denaturing gradient gel electrophoresis (DGGE) and real-time polymerase chain reaction (RT-PCR) quantification.

2.4.2. DGGE

To analyze the changes in the bacterial community, DGGE was conducted using the cDNA samples. The PCR amplification was performed using a RoboCycler Gradient 40 (Stratagene). The nucleotide sequences of the primers were shown in Table 1. Primer 3 had the same sequence as primer 1 but its 5′-end had an additional