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Inoculation and alkali coeffect in volatile fatty acids production and microbial community shift in the anaerobic fermentation of waste activated sludge

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HIGHLIGHTS

- We use inocula in acidification of waste activated sludge in alkaline condition.
- Inocula were paper mill anaerobic granular sludge and dying mill anaerobic sludge.
- Inoculation increase volatile fatty acid production at pH 9 but not at pH 10.
- Inoculum make the microbial community shift at pH 9 but not at pH 10.
- Inoculation will decrease alkaline dosage in waste activated sludge acidification.

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ABSTRACT

Batch fermentations of waste activated sludge (WAS) at alkaline pH with different inocula were performed. Paper mill anaerobic granular sludge (PAS) and dyeing mill anaerobic sludge (DAS) were used as inocula. At pH 10 the inoculation did not increase the volatile fatty acids (VFAs) production compared to the non-inoculated samples fermented in the same conditions, and the maximal VFAs yield of non-inoculated WAS was higher than inoculated WAS. However, at pH 9 the inoculation with PAS increased the sludge hydrolysis and VFAs production was 1.7-fold higher than that in non-inoculated WAS (yield 52.40 mg/g of volatile solid). Denaturing gradient gel electrophoresis analysis revealed that 3 bacterial species, identified as *Proteocatella*, *Tepidibacter*, and *Clostridium*, disappeared when inoculated with PAS at pH 9 or at pH $\geqslant$ 10. The results showed that the inoculation with PAS can be helpful to achieve a relatively high VFAs production from WAS in a moderate alkaline environment.

1. Introduction

As consequence of the widespread use of activated sludge technology in wastewater treatment, the management of waste activated sludge (WAS) has become a serious issue. Anaerobic digestion of WAS is a common treatment strategy to minimize the sludge quantity and recover energy. However WAS composed mostly by bacterial mass, its hydrolysis is difficult, representing the main rate-limiting step of treatment process (Li and Noike, 1992). In fact, since WAS has a long fermentation time and a low biogas production rate, its digestion without any pretreatment is characterized by a relatively low efficiency.

An alternative strategy for the treatment of WAS is the fermentation of volatile fatty acids (VFAs) which can also be further used as a carbon source for biological nutrients removal (BNR). In fact to achieve a high performance in phosphorus and nitrogen removal from wastewater during BNR process, additional carbon is needed, since the original carbon concentration is not sufficient (Tan et al., 2012). In recent years it was shown that WAS fermentation at pH 10 can accumulate high concentration of VFAs (Liu et al., 2012; Su et al., 2013; Yu et al., 2008; Yuan et al., 2006). The use of fermented WAS in BNR was also investigated, and it was concluded that VFAs by alkaline fermentation of WAS were more favorable carbon source than pure acetic acid (Elefsiniotis et al., 2004; Tong and Chen, 2007).

The impact of alkaline conditions on sludge hydrolysis, VFAs accumulation, VFAs composition and on the quantification of individual VFAs have been subjects of extensive studies in the past recent years (Chen et al., 2007; Liu et al., 2012; Yu et al., 2008; Yuan et al., 2006; Zhang et al., 2010, 2011). New methods involving the use of additives such as carbohydrates and β-cyclodextrin to improve the bioproduction of VFAs have also been recently published (Feng et al., 2009; Jia et al., 2013; Yang et al., 2012). The

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mechanism underlying VFAs accumulation in alkaline condition is yet not fully understood. The accumulation of VFAs in alkaline condition is considered a direct consequence of the methane synthesis pathway inhibition, which consumes VFAs in normal conditions (Yuan et al., 2006), and a consequence of the sludge matrix breakage induced by the high pH, that allow the contact between extracellular organic matter and enzymes (Yu et al., 2008). As VFAs generation through WAS anaerobic fermentation under alkaline condition is a biochemical process, it is necessary to investigate the impact of microbial inoculum and microbial community shift during the fermentation process when different seed sludge are added. To the best of the authors' knowledge the impact of different inocula on WAS bioproducts under alkaline condition has not been investigated yet.

This work aimed to (1) characterize the impact of pH on WAS hydrolysis and acidification during fermentation performed with different inocula; (2) to investigate the bacterial community shifts during fermentation caused by different inocula at different pH conditions. To achieve these goals several parameters indicative of the hydrolysis and acidification status were monitored: soluble chemical oxygen demand (SCOD), soluble proteins, soluble polysaccharides, volatile fatty acids, hydrolytic enzymes activities (acid phosphatase, alkaline phosphatase, alkaline protease, and α-glucosidase), while denaturing gradient gel electrophoresis (DGGE) was conducted to analyze the bacterial communities.

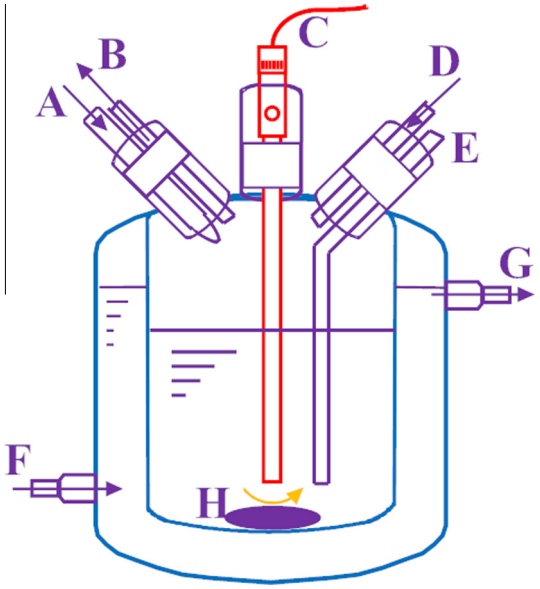

Fig. 1. Diagram of the bioreactor. (A) NaOH inlet; (B) biogas outlet; (C) pH sensor; (D) N_2 displacement inlet for sampling; (E) sampling outlet or N_2 inlet for air purge; (F) water circulation inlet; (G) water circulation outlet; (H) magnetic rod.

2. Methods

2.1. Waste activated sludge and sludge seeds origin

Waste Activated Sludge was obtained from the secondary sedimentation tank of a nucleotide fermentation factory in Zhaoqing (China). WAS was centrifuged immediately by a settling centrifugal machine (2800 rpm), transported to laboratory within 6 h and stored at 4 °C.

Two kinds of sludge were used in this work as seed. Paper mill anaerobic granular sludge (PAS) was obtained from Guangzhou Paper Group LTD (Guangzhou, China). Printing and dyeing mill anaerobic sludge (DAS) was obtained from a lab scale internal circulation reactor used for the treatment of synthetic wastewater. Sludge seeds were centrifuged at 12,000 rpm and the sediment was stored at 4 °C.

2.2. Experiment set-up

Four identical custom-made 3-neck jacketed glass vessels (working volume, 500 mL, Fig. 1) were used as bioreactors. Each reactor was filled with 500 mL of sludge suspension, then the headspace air was purged by inflating nitrogen into the system for 2 min. The pH was controlled by a custom-made system consisting of a pH sensor and a peristaltic pump which automatically added small volumes of an alkaline solution (NaOH 2 mol/L) to keep the pH constant to a set value throughout the fermentation. The graduated cylinders used as alkaline solution reservoirs were monitored every 6 or 12 h to assess the consumption of solution during the fermentation. temperature was controlled by thermostated water circulation. All the reactors were maintained at 35 ± 0.2 °C under continuous stirring (300 rpm) by magnetic rods. The produced biogas was collected by water displacement. The fermentations were run for 10 days and every 2 days 15 mL of fermented sludge were withdrawn from the reactors by nitrogen displacement and analyzed. In each experiment two different pH conditions were run simultaneously, in duplicate.

Sludge samples for non-inoculated experiments were prepared suspending 400 g of WAS in 2 L of distilled water. Sludge samples for inoculated experiments were prepared suspending 80 g of sludge seed and 320 g of WAS in 2 L of distilled water. Fermentations of non-inoculated WAS were run at pH 8, 9, 10, and 11, while fermentations of inoculated WAS were run at pH 9 and pH 10. An additional non-inoculated sample was fermented without change the pH and was used as a blank.

To assess the maximum amount of components soluble in alkaline conditions, an aliquot of freshly prepared sludge sample (10 mL) was added to a strong alkaline solution (NaOH 2 mol/L, 40 mL) and incubated at 30 °C for 24 h 100 rpm in a shaker.

In tables and figures non-inoculated samples were indicated as their pH value or as "WAS" when the pH was not changed. Inoculated samples were indicated as their pH value plus the type of seed employed (e.g. pH 9 + PAS means that the fermentation was performed at pH 9 and the sample was inoculated with PAS) or as "WAS + PAS" and "WAS + DAS" when the samples were inoculated with PAS or DAS, respectively, without change the pH.

2.3. Analysis methods

Total solid (TS), volatile solid (VS), total suspend solid (TSS) and volatile suspend solid (VSS) were determined according to standard methods in triplicate (APHA, 1998). Sodium hydroxide dosing was measured every 6 or 12 h by reading the difference of the graduated cylinders which contained 2 mol/L sodium hydroxide to supply the peristaltic pumps.

Sludge samples were centrifuged at 12,000 rpm for 10 min and the supernatant was used for further analysis. To determine SCOD, soluble proteins and soluble polysaccharides, the sludge supernatants were analyzed in duplicate by potassium dichromate method (APHA, 1998), Bradford method (Bradford, 1976), and anthrone-sulfonic acid method (Herbert and Philips, 1971), respectively. The supernatants of sludge samples pre-incubated in strong alkaline conditions (900 μL) were neutralized by adding sulfuric acid (100 μL, 14.4 mol/L) before soluble protein determination. For VFAs determination the supernatants of sludge samples were treated with phosphoric acid (3 mol/L) to adjust the pH below 5 and then filtered through a 0.22 μm membrane. VFAs concentration was determined in triplicate by a gas chromatograph (GC-2010,