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Characterization of methanogenic activity during high-solids anaerobic digestion of sewage sludge

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ABSTRACT

High-solids anaerobic digestion of sewage sludge has advantages of smaller digesters and a lower energy requirement for heating than conventional digestion, but its efficiency is negatively influenced by high solids concentration. To discover the mechanism causing this deteriorative performance, the specific methanogenic activity (SMA) of high-solids digestate was measured using typical substrates including acetate, propionate, butyrate, glucose, microcrystalline cellulose and hydrogen and carbon dioxide. When the total solids content increased from 4.2% to 14.4%, the SMA of the digestate decreased 40%–50% for most substrates except hydrogen and carbon dioxide, mainly because of blocked mass transfer in the high-solids digestate. On the other hand, some features remained stable: acetotrophic methanogens exhibited more activity than hydrogenotrophic methanogens, butyrate fermentation rather than propionate fermentation was still the main metabolic pathway during the degradation of glucose and cellulose, and hydrolysis was still the rate-limiting step during the anaerobic digestion of cellulose.

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1. Introduction

Anaerobic digestion is a widely-used method of sludge treatment because of its good performance in waste reduction and energy recovery in the form of methane [1,2]. During conventional anaerobic digestion, the total solids (TS) content in feed sludge is usually controlled in the range of 2%–5%. Correspondingly, this process needs large digesters, which are not always feasible in small-scale wastewater treatment plants (WWTPs) or at WWTPs in highly urbanized areas [3]. In such situations, high-solids anaerobic digestion (HSAD) of sewage sludge is often recommended as an alternative because smaller reactor volumes and lower energy requirements for heating are needed and less material handling is required [4].

High-solids anaerobic digestion has been successfully applied to a variety of solid wastes such as the organic fraction of municipal solid waste (MSW), agricultural wastes and green wastes, whereas the knowledge on mono-HSAD of sewage sludge is rare. Unlike uncompacted wastes, high-solids sludge is a sticky semisolid. In fact, sewage sludge with 2%–15% TS behaves as a pseudoplastic fluid and its viscosity increases exponentially as TS increases [5]. Thus, the digestibility of the sludge having a high viscosity cannot be deduced exactly from that of other wastes. A few studies have reported that the HSAD of sewage sludge easily suffers from long digestion time or low biogas production. For example, the time required to complete digestion extended from 16 days to 46 days when sludge TS increased from 1.79% to 15.67% [6].

Three aspects may be involved in the poor performance of HSAD of sewage sludge. The first of these reasons is the low water content in high-solids digestate. Water is essential for biochemical reactions. During anaerobic digestion, water promotes substrate hydrolysis and enables the transfer of hydrolysis products and other intermediates to the sites of bacteria. The lack of free water results in deteriorated molecular diffusive behavior and corresponding poor transfer efficiencies in high-solids digestate. For example, during dry fermentation of MSW, the effective diffusion coefficient decreased drastically when the TS content increased, with numerical values 50 to 185 times smaller than the reference value in water at 8% TS and 25% TS, respectively [7]. A static batch experiment on anaerobic digestion at different TS levels also demonstrated that biogas yield was directly influenced by the decreased water content [8]. The second reason for poor HSAD performance may be related to the accumulation of some intermediate products, which result from blocked mass transfer and high concentration of substrates [6]. These intermediate products, such as free ammonia and volatile fatty acids (VFAs), have a negative impact on microorganisms. Sig-

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Table 1
Main reactions presented in methanogenesis.

Substrate	Microbial population	Reaction
Acetate	Acetoclastic methanogens	$CH_3COOH \rightarrow CH_4 + CO_2$
Propionate	Propionate degradation bacteria	$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$
Butyrate	Butyrate degradation bacteria	$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$
Glucose	Acidogenic bacteria	$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$ $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ $C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$
Cellulose	Hydrolytic bacteria	$(C_6H_{10}O_5)n + nH_2O \rightarrow nC_6H_{12}O_6$
H_2/CO_2	Hydrogenotrophic methanogens	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
H_2/CO_2	Homoacetogenic bacteria	$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$

nificant microbial inhibition was found at free ammonia nitrogen (FAN) concentration range of 600–800 mg/L during the operation of a pilot-scale HSAD reactor [3]. A maximum FAN of 968 mg/L was observed in a batch HSAD test of sewage sludge, resulting in a relatively low specific biogas yield [6]. Finally, due to the difficulty in microorganisms acquiring substrates, as well as the negative effect of accumulated metabolites, microbial activity may decrease and result in the deteriorative performance of HSAD. It was found that the specific methanogenic activity (SMA) decreased linearly with increasing TS content from 18% to 35% during dry fermentation of MSW [9].

Although some previous studies have verified the phenomena of blocked mass transfer and accumulative intermediate products, there is still a lack of direct observation of the microbial activity involved in different reaction steps during mono-HSAD of sewage sludge. Several microbial pathways are related to methanogenesis, as shown in Table 1. Organic wastes are first hydrolyzed into glucose, organic acids and other oligomers, and these intermediate products are then degraded to VFAs. Finally, methane is produced from the decomposition of acetic acids or the synthesis of carbon dioxide and hydrogen. Previous research has reported only the influence of high solids concentration on methanogenesis during dry fermentation of MSW [9]. However, during HSAD of sewage sludge, the efficiency of each step in the complex reaction chain, including hydrolysis, fermentation, acetogenesis and methanogenesis, has been rarely studied so far. Thus, in the present study, the microbial activities in sludge digestate from HSAD and low-solids anaerobic digestion (LSAD) were measured based on different typical substrates. Using these data, the influences of high solids concentration on different reaction steps were analyzed, giving insight into the differences and similarities between HSAD and LSAD.

2. Materials and methods

2.1. Characterization of digestate

The digestate discharged from two laboratory-scale semi-continuous anaerobic digesters was used to assess the microbial activity. One digester (HSAD) was fed with dewatered sludge collected from a full-scale WWTP in Kunming city, China, which had a high TS content of 15.7% and a volatile solids (VS) content of 64.2% based on TS. The other digester (LSAD) was fed with the same dewatered sludge as used in the HSAD digester, except diluted to a TS content of 5.7% and a VS/TS ratio of 63.5%. Both digesters had run steadily for six months at a temperature of 35 °C and a solids retention time of 30 days. The average removal rates of sludge organic matter for the HSAD reactor and the LSAD reactor were 35.7% and 37.2%, respectively, and the specific biogas production per unit VS added for the two digesters averaged 233.0 mL/g and 316.1 mL/g, respectively.

The digestate collected from the two digesters was first stored anaerobically at 35 °C for several days to remove residual biodegradable organic matter and limit endogenous activity [9,10]. Thus, the residual VS was mainly comprised of live microorganisms. The final composition of the digestate was 14.4% TS with 54.5% VS/TS for the HSAD tests, and 4.2% TS with 56.9% VS/TS for the LSAD tests.

2.2. Experimental procedure

The frequently-used indicator of microbial activity during anaerobic digestion is specific methanogenic activity, which is calculated by dividing the maximum methane production rate by the biomass (i.e., the inoculum, based on the VS content) during batch anaerobic digestion experiments using specific substrates [11]. SMA values based on different substrates can reveal the activities of not only methanogens but also other microflora involved [9,12,13], and thus, show the metabolic pathways to some extent.

In this study, the SMA tests were divided into an HSAD series and an LSAD series, and each series included eight groups. Each of the first six groups in each series used one of the following specific substrates: sodium acetate, sodium propionate, sodium butyrate, glucose, microcrystalline cellulose, and hydrogen (H_2) and carbon dioxide (CO_2), respectively. Two "blank" groups without any substrate were also set in each series to eliminate the probable influence of residual biodegradable organic substances in the inoculum and endogenous activity. Each experiment was performed in triplicate in 140-mL glass vials under mesophilic temperature condition (35 ± 2 °C). The digestate (inoculum) and the substrate were mixed well at a 10:1 ratio of inoculum VS to substrate chemical oxygen demand (COD) to ensure sufficient substrate and prevent inhibition [10]. In the LSAD series, 90 g of mixture were added into each vial and the vials were shaken frequently during the subsequent digestion to ensure homogeneity and facilitate the release of biogas. In the HSAD series, 30 g of mixture were smeared on the inner wall of the vials, forming a thin layer to facilitate the release of biogas. All the vials were flushed with nitrogen to remove oxygen, and then immediately sealed with rubber plugs. The biogas produced was transported through a perfusion tube into a fermentation tube that was filled with displacement liquid of a sodium hydroxide solution (3 mol/L) with thymolphthalein pH-indicator, so as to determine the methane production. For the groups using H_2 and CO_2 as the substrates, the volume ratio of H_2 to CO_2 was 4:1, and the gas mixture was injected frequently into the vials. The discharged gas from the vials was measured by displacing saturated $NaHCO_3$ solution in fermentation tubes. In the blank groups, NaOH solution and $NaHCO_3$ solution were used for the measurement of methane and biogas, respectively.

There has been no consensus on whether other nutrients and trace elements have an impact on SMA tests of sludge digestate. Thus, several additional groups of experimental vials were added during preliminary experiments. In these groups, glucose was also used as the substrate, but with supplementary growth factors [10,14] in the form of concentrated solution (Table 2). The methane production from glucose alone and from the mixture of glucose, the

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