



Food waste enhanced anaerobic digestion of biologically pretreated yard waste: Analysis of cellulose crystallinity and microbial communities



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ARTICLE INFO

Article history:

Received 31 March 2018

Revised 12 July 2018

Accepted 17 July 2018

Keywords:

Lignocellulosic biomass

Anaerobic digestion

Biological pretreatment

Simulative chemical pretreatment

Biochemical methane potential

Food waste supplementation

ABSTRACT

Solid waste treatment through anaerobic digestion (AD) technology contributes to energy recycling and reuse of various solid organic wastes. However, yard waste (YW) is generally recalcitrant to AD due to the presence of high cellulose and hemicellulose content, which are difficult to be hydrolyzed. In this study, to enhance hydrolysis efficiency, YW was biologically pretreated with digested sludge and supplemented with food waste (FW) before AD process. Effects of FW supplementation on pH, SCOD, cellulose and hemicellulose content and cellulose crystallinity were examined. The optimal amount of FW supplementation was determined to be 10 wt%. An increase of 6.5–20.3% in cellulose reduction and an increase of 14.8–53.1% in hemicellulose reduction in digesters was achieved within the optimal pretreatment time of 4 days. After hydrolysis, cellulose crystallinity decreased by 23% from 71% in the control digester, which was responsible for improved biodegradability of cellulose in YW. FT-IR analysis of hydrolysis mixture confirmed that partial hydrogen bonds were destroyed in digesters with supplementation of 10 wt% FW, leading to a higher extent of degradation of the feedstock. In the batch AD of FW supplemented YW, results indicated that methane yield was 35% higher than that of the control digester without FW supplementation. Pyrosequencing analysis indicated that the abundance of bacterial genus *Sphaerochaeta* and *Cellulosibacter* in subsequent digestion were enhanced by 10- and 5-folds by 10 wt% FW supplementation, respectively, and were deemed to be responsible for the enhanced anaerobic digestion performance.

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1. Introduction

Ever-increasing solid organic wastes disposal in megacities is a critical global issue (Clarke, 2018). According to the statistics of National Environment Agency (NEA), more than 330,000 tons of horticultural waste or yard waste (YW) are produced annually in Singapore (Waste statistics and overall recycling of Singapore, 2017). Similarly, more than 200 million tons of municipal solid wastes are generated annually in China. Among them, approximately 60% of which by wet weight are YW and food waste (FW), urgently to be treated (Pariatamby et al., 2014). Incineration is currently the main method used to dispose of the non-recycled YW while FW primarily relies on landfill to dispose of. The incineration, however, produces a series of pollutants (e.g., furan and dioxins) that pose potential harm to the human health and cause damages to the environment (Sharma et al., 2017). Also, given the rapid expansion of municipal waste amount, the landfill space

is not able to cater for a long-run requirement. Accordingly, there is an urgent demand to develop more sustainable and environmentally beneficial technologies for waste disposal. Anaerobic digestion (AD) is a promising candidate to satisfy these requirements due to its capability of executing waste treatment and bioenergy recycling simultaneously. AD process of YW and FW generally comprises four steps i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis (Appels et al., 2011; Li et al., 2016a). Among which, hydrolysis was considered as the rate determining step during AD of slowly degradable solid wastes such as YW (Achinah et al., 2017).

However, YW is recalcitrant to AD due to the presence of high cellulose and hemicellulose content, which are difficult to be hydrolyzed into fermentable sugars (Kumari and Singh, 2018; Sun et al., 2015). Very often, pretreatment is necessary to enhance AD of lignocellulosic biomass (Duque et al., 2017; Hassan et al., 2018; Li et al., 2016b; Tian et al., 2018; Yan et al., 2017; Zhang et al., 2016b). Technique proposed comprised physical pretreatments (e.g., torrefaction (Nunes et al., 2017), extrusion (Duque et al., 2017), gamma and electron beam radiation (Kapoor et al., 2017;

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Xiang et al., 2017), ultrasonic pretreatment (Subheddar et al., 2018), microwave pretreatment (Bundhoo, 2018), and steam explosion (Theuretzbacher et al., 2015)), chemical pretreatments using acids or alkali (Al-Mallahi et al., 2016; Kumar et al., 2018; Pellerá and Gidarakos, 2018), biological pretreatments using biological agents or fungal species (Brémond et al., 2018; Ding et al., 2017), and combination of different pretreatment methods (Hassan et al., 2018; Martínez-Patiño et al., 2018). However, these pretreatment methods have individual drawbacks. Physical pretreatments such as smash and mechanical agitation could enhance hydrolysis efficiency, but frequently involve high energy requirement. Chemical pretreatments could exhibit quick and dramatic effect but generally need high cost of chemicals and subsequent chemical disposal. Biological pretreatment methods such as usage of microbial consortium and fungus could make a positive contribution to environmental protection, but frequently need long process time and high cost of bacteria and enzymes. Thus, there is an increased demand for economic and efficient pretreatment methods.

Discarded sludge pretreatment (DSP) method has been proposed and shown to be promising to enhance AD of corn stover (Hu et al., 2015) and algal biomass (Zou et al., 2018). DSP, as a biological pretreatment method, refers to a method in which discarded sludge produced from AD digesters is used to pretreat feedstock for a certain time in a pretreatment digester prior to put all pretreated mixture into an AD digester to start traditional fermentation. This method comprises several advantages such as recycling of bacteria resource, low extra energy input and environmental-friendly feature (Zhang et al., 2017b). DSP was proved to be capable of improving hydrolysis of cellulose and hemicellulose, the rate determining step of YW fermentation (Shrestha et al., 2017). Therefore, there is little doubt that performance of AD of YW will be subsequently improved after DSP process. FW is anticipated to be a good potential feedstock for AD. Mono-digestion of FW could result in decrease in pH to around 3.5 if the digesters are overloaded (Zhang et al., 2017a), which is an advantage for hydrolysis of cellulose and hemicellulose (Hu et al., 2015; Wei, 2016). Specifically, low pH value implies high H^+ concentration and high acidity, which might be efficiently used for pretreatment or pre-acidification of YW. Potentially, the enhanced hydrolysis of cellulose and hemicellulose inside YW would contribute more intermediate metabolites for acidification stage, leading to higher concentration of volatile fatty acids (VFAs). Thus, we hypothesize that FW supplementation might facilitate YW pre-acidification through decreasing the pH of hydrolysis mixture and increasing the VFAs concentration in the hydrolysis and acidification step (acting as simulative chemical pretreatment). But only few previous studies have been done on the use of FW supplementation as a pretreatment method for pre-acidification of YW. Moreover, due to the lack of in-depth understanding of microbial community diversity, the production of methane using YW in a full scale biogas plant remains problematic (Sawatdeenarunat et al., 2015). In addition, a main strength of this integrated approach is that it also allows the valorization of FW, which is known to be complicated due to its low C/N ratio and its high biodegradability and TS content (Capson-Tojo et al., 2016).

Consequently, in this study, in order to realize the potential of using DSP and FW as pretreatment for YW, the effects of supplementation of FW on pH, SCOD, cellulose and hemicellulose hydrolysis efficiency, cellulose crystalline structure and functional groups of hydrolysis mixture, methane production, and microbial community diversity were examined. The information acquired from characterizing the microbiome of dominant microbial communities will help to shed light on enhancing mechanisms of biological pretreatment and food waste supplementation.

2. Materials and methods

2.1. Substrates and inoculum

YW was collected from the National University of Singapore (NUS) campus and comprised mainly tree leaves and twigs. The moisture content of the YW was dried to below 10 wt% at 45 °C for 48 h in a drying oven. Subsequently, this was shredded into particles of 20-mesh (about 0.85 mm diameter), and stored in an airtight container until needed. FW was collected from a NUS canteen and comprised mainly rice, vegetables, noodles, meat and food seasonings. After removing non-biodegradable components such as plastics and bones, the collected FW was homogenized using a blender and then stored at -20 °C until needed. The discarded anaerobic sludge collected from a large-scale anaerobic digester in the Ulu Pandan Water Reclamation Plant (PUB) in Singapore was used as inoculum. The ratio of volatile solids (VS) to total solids (TS) of the discarded sludge was 0.66 with TS of 1.64 ± 0.03 wt%. Before usage, the sludge was starved for 7 days and incubated at 37 °C to activate microbiological activity and to remove any easily degradable VS. Detailed characteristics of the YW, FW and seed sludge are listed in Table 1. Table 1 showed that the relatively high percentages of Ca^{2+} , Mg^{2+} and Al^{3+} in yard waste might have toxic and inhibitory effects to microorganisms, but FW supplementation might reduce the inhibitory effects. Ammonia originated from the degradation of proteins in FW might be an inhibitor to mono-digestion, but synergistic effects could be attained in the co-treatment of YW and FW.

2.2. Determination of optimal FW supplementation dosage

Discarded sludge from PUB was first mixed with YW to a TS of 16 wt%, which was found to be appropriate for complete saturation condition and thorough absorption of liquid sludge by the feedstock. In 1 L digesters, YW mixed with discarded sludge was supplemented with various amounts of FW (at 10, 20, 50 wt% total solids) to a working volume of 400 mL. These were designated R10, R20 and R50 experiments. The substrate to inoculum ratios of the acidification experiments were approximately 5–10 on the basis of TS. The mixing ratios YW to FW on the basis of TS were 9:1 (R10), 4:1 (R20) and 1:1 (R50), respectively. Two control digesters, one with no FW supplementation (R0) and the other acidification of pure FW (R100) were also established. The total solid weight of feedstock in each digester was 64 g. Each of the experiment was conducted in triplicates and results were averaged as representative data for each corresponding digester. During the pretreatment experiments, pH, SCOD, cellulose and hemicellulose content were determined daily. For each digester, a number of same bottles were established and individual bottles were sacrificed at differing sampling time to facilitate characterization of desirable parameters at a different time. The pretreatment experiment was each conducted for 6 days. Subsequently, it was found that 4–5 days was the optimal pretreatment period; during the 6 days of pretreatment experiment, pH decreased gradually and remained relatively stable from the 4th day, indicating that hydrolytic acidification of FW and YW had been completed. From these experiments, it was also concluded that optimal FW supplementation was 10 wt%. In the next set of batch AD tests, YW was supplemented with 10 wt% FW.

2.3. Batch AD tests

Batch AD of YW supplemented with 10 wt% FW (R10) was conducted under mesophilic temperature of 37 °C. A series of 1 L lab-scale anaerobic digesters at a working volume of 800 mL was used

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