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Effect of cycle digestion time and solid-liquid separation on digestate recirculated one-stage dry anaerobic digestion: Use of intact polar lipid analysis for microbes monitoring to enhance process evaluation

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ABSTRACT

One-stage dry anaerobic digestion with solid-liquid-separated digestate recirculation was applied to treat synthetic organic solid waste at Digestion Time (DT) of 15, 25, 35 days for several cycles. The highest methane yield of 0.360 ± 0.045 m^3/kg-VS_{add} with lowest accumulation of hydrolytes was achieved under DT of 15 days. The analysis of intact lipid profiles, including phospholipid fatty acid for bacteria and phospholipid ether lipid for archaea, indicated that the inoculum breakdown occurred, mainly during the start of the process. A significant decline of hydrolytic bacteria was observed during the granular breakdown, which was likely related to the lower methane yield in subsequent cycles. In contrast, the amount of methanogens was still stable even after granular breakdown occurred. The accumulated ammonia in the liquid digestate was partially removed by solid-liquid separation before digestate recirculation, which relieved possible inhibition to some extent with minor microorganism loss. Hence the levels of ammonia, which was highly possible to be the inhibitor causing the decline of methane production, were lower in DT15 than in DT25 and DT35. In this case, it could be implied that the microorganism community reconstruction in DT15 may face less challenges comparing to the other two setups.

1. Introduction

Dry anaerobic digestion, which is considered to be a favorable method for treating organic solid waste, has been gaining increasing attention due to its carbon recovery capability in the form of biogas.

The quantity and type of inoculum—for the purpose of activating the methanogens and alleviating acid inhibition due to high organic loading of a dry process [1–4] — are important parameters of this process. One of the conventional procedures uses an inoculum which is comprised of digestate mixed with the feedstock. Thereafter, the inoculum is transferred back into the digester. Usually, a high-ratio of digestate to substrate (DSR) is preferred for dry anaerobic digestion. Common processes such as DRANCO [5,6], requires 1 ton of feedstock mixed with 6–8 tons of digested residue. Di Maria et al. [7] also reported a 2-fold increase of methane yield when DSR increase from 1:1 to 3:1 in solid state anaerobic digester.

As a result of the hydrolysis, the Total Solid content (TS) of the digestate is usually lower than the feedstock waste. To maintain the TS in the reactor, the digestate is usually subjected to solid-liquid separation by dewatering prior to mixing, using a screw-, filter—press or centrifugation, to remove excess water and possibly some inhibitors in the digestate. The process fluctuation may be caused by filtration of un-degraded substances and suspended microorganisms during dewatering.

Meanwhile, Solid Retention Time (SRT) is another factor which affects the treatment capacity and stability of the process. The effects of SRT on volatile solid (VS) reduction, methane yield, and the stability of methanogens during sludge digestion, have been investigated by a number of research teams [8–11]. In the case of wet digestion, TS level is usually capped around 5%, the optimal SRT

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Nomenclature

DOC	Dissolved Organic Carbon
DSR	Digestate to Substrate Ratio
DT	Digestion Time
DT15, DT25, DT35	The setups with the DT of 15 days, 25 days, and 35 days, respectively
FAN	Free Ammonia Nitrogen
ISR	Inoculum to Substrate Ratio
PLEL	Phospholipid Ether Lipid
PLFA	Phospholipid Fatty Acid
SRT	Solid Retention Time
TAN	Total Solid
TS	Total Ammonia Nitrogen
VFA	Volatile Fatty Acid
VS	Volatile Solid

is usually less than 12 days. For semi-dry and dry digestion, it has been suggested that the optimal SRT should be longer than 15 days, depending on the reactor design, organic loading rate, and waste composition. DNA analysis during sewage sludge digestion [12] indicated that methanogens declined significantly when the SRT was less than 10 days. A similar conclusion was obtained by Fdez-Guelfo et al. [8], who studied the production of methane and the accumulation levels of volatile fatty acids (VFA).

Nevertheless, it is still unknown how SRT influence the characteristics of digestate as an inoculum in the following anaerobic digestion. The activities and relative abundances of microorganisms existing in the digestate may change when subjected to various setups in terms of waste degradation levels and SRTs, which affect the efficiency and stability of the dry anaerobic process. Therefore, it is important to optimize SRT values in terms of harvesting, inoculating materials, maintaining high waste degradation rates, maximizing the capacity and energy potential of the anaerobic digestion.

However, it is very challenging to determine SRT in the overall solid fraction in digestate recirculation process. This is because solid portion of the dewatered digestate will be pre-mixed with the new feedstock when amount of discarded liquid equals the amount of feedstock in the digestate recirculation process. Hence, in digestate recirculation operation, the Digestion Time (DT) of each cycle could be a better parameter for comparison instead of the SRT. In this case, the DT equals to the SRT of the fresh substrate because once one cycle is finished, the fresh substrate will be converted to digestate and serve as the new inocula.

It is necessary to monitor the evolution of microorganism communities in the digester to gain knowledge on the mechanism of digester's performance. Intact polar lipid techniques, such as phospholipid fatty acid (PLFA) analysis for bacteria and phospholipid ether lipid (PLEL) analysis for *Archaea*, are useful culture-independent methods [13] which was chosen in this study over the traditional Real-time PCR and analysis targeting 16S rRNA. Analysis targeting 16S rRNA such as FISH can monitor the active microbial, but the information is limited by the sample size (a few μg) and randomness due to the narrow vision of the microscope and the highly heterogeneous property of solid waste matrix. Real-time PCR can be used for microbial quantification, but DNA-based PCR cannot differentiate the live microbes from the dead ones, while RNA-based PCR demands sophisticated skill of researchers to avoid the rapid degradation of RNA. Furthermore, several sets of primers need to be designed and applied for the amplification of different series of microorganisms, which requires huge amount of time and cost. Additionally, if the microbial community structure of a reactor is not known *a priori*, the use of inappropriate primers may result in bias evaluation. Next-Generation Sequencing (NGS) can also be performed with RNA for active microbial populations without the use of specific primers. At present however, it is still costly to use NGS as a routine measure for frequent monitoring of the reactors' daily performance. Conversely, intact lipid analysis can provide overall and broad information on the living/active microbes at a relatively reasonable throughput and cost. Besides, the sampling volume required for intact lipid analysis is about several grams, which is suitable for the analysis of heterogeneous solid samples from field digesters. The intact lipid analysis has been applied to a wide variety of environmental samples, including soils [14,15] and compost [16,17]. Although the advantages of the intact lipid analysis make it suitable for monitoring anaerobic digestion reactors, the application is still limited. To our best knowledge, only Sundh et al. [18] reported PLFA and PLEL analysis for a glucose-overloaded anaerobic digester, and Schwarzenauer and Illmer [19] used PLFA analysis to monitor the bacterial community in a KOMPOGAS anaerobic digester.

In the present study, a one-stage dry anaerobic digestion of organic solid waste, with digestate recirculation, was operated under different DTs (equivalent to SRTs for the fresh substrate). The aim of this experiment was to evaluate the effects of DT on digestion efficiency and to assess the performance of the digestate as a continuously applied inoculum source. To monitor the evolution of the active microbial populations in the separated solid and liquid digestate, the microbial amount and community structure were determined by PLFA and PLEL analyses. Multidimensional analysis tools, including principal components analysis (PCA) and partial least squares analysis (PLS), was conducted to examine the relationships between the intact lipid profiles, the process efficiency and the process stability.

2. Material and methods

2.1. Waste and initial inoculum

Simulated organic waste—consisting of vegetable (potato, celery, and lettuce), fruit (banana peel, apple peel, orange peel) and green waste—was used as a feedstock to the reactor. The types of fruit were selected because of their high consumption in China. Vegetable, fruit and green waste were mixed together in a ratio of 15:5:1 (wet weight) according to a field survey. The characteristics and proportions of each component in the mixed substrate are shown in Table 1.

Seed sludge was obtained from a commercial anaerobic reactor, with an inner recirculation, for treating paper wastewater. The collected sludge was centrifuged at 2000 × *g* for 15 min and the sediment was used as the initial inoculum. The TS of the inoculum sludge was 14.58 ± 0.07%, with 89.6 ± 0.10% of volatile solid in TS. The proportional content (as dry weight) of carbon, nitrogen and hydrogen content was 42.7 ± 0.03%, 8.3 ± 0.52%, and 6.7 ± 0.16%, respectively.

2.2. One-stage dry anaerobic digestion

As shown in Fig. 1, 4 kg of initial inoculum and 2 kg of substrate (both based on wet weights) were mixed completely prior to feeding into the 25-L reactor. Since the TS of the initial inoculum and substrate were similar, the inoculum-to-substrate ratio (ISR) was also 2:1 based on dry weight. After covering and sealing with flange, nitrogen gas was injected into the system at a flow rate of 10 L/min for 5 min, to maintain the anaerobic environment. Subsequently, the reactor was incubated in semi-batch mode at