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Research article

Correlation between system performance and bacterial composition under varied mixing intensity in thermophilic anaerobic digestion of food waste

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ABSTRACT

This study examines the stability and efficiency of thermophilic anaerobic digesters treating food waste under various mixing velocities (50–160 rpm). The results showed that high velocities (120 and 160 rpm) were harmful to the digestion process with 18–30% reduction in methane generation and 1.8 to 3.8 times increase in volatile fatty acids (VFA) concentrations, compared to mild mixing (50 and 80 rpm). Also, the removal rate of soluble COD dropped from 75 to 85% (at 50–80 rpm) to 20–59% (at 120 –160 rpm). Similarly, interrupted mixing caused adverse impacts and led to near-failure conditions with excessive VFA accumulation (15.6 g l^{-1}), negative removal rate of soluble COD and low methane generation (132 ml gVS^{-1}). The best efficiency and stability were achieved under mild mixing (50 and 80 rpm). In particular, the 50 rpm stirring speed resulted in the highest methane generation (573 ml gVS^{-1}). High-throughput sequencing of 16S rRNA genes revealed that the digesters were dominated by one bacterial genus (*Petrotoga*; phylum *Thermotogae*) at all mixing velocities except at 0 rpm, where the community was dominated by one bacterial genus (*Anaerobaculum*; phylum *Synergistetes*). The *Petrotoga* genus seems to have played a major role in the degradation of organic matter.

1. Introduction

Anaerobic digesters are often designed as continuously stirred reactors with the aim of providing efficient mixing for a homogeneous distribution of substrate and heating energy and to avoid settling of solids and short-circuiting as well as maintaining the design solid retention time (Lindmark et al., 2014b). In addition to physical effects, mixing can have direct impacts on the performance of the microbial system by dispersing inhibitory metabolic by-products, such as volatile fatty acids (VFA) and hydrogen (H_2) (Amani et al., 2010). Accordingly, inadequate mixing in anaerobic digestion (AD) is often associated with various operational challenges, including non-uniform substrate distribution, grit deposition, stratification, formation of dead zones, and most notably, the inhibition of methanogenesis by accumulation of toxic metabolic by-products (Stroot et al., 2001). Thus, adequate mixing was found essential to ensure a homogeneous temperature and an optimum digestion environment, leading to better methane generation, higher effective volume, improved removal of organic matter and enhanced tolerance to higher organic loading rates (OLR) (Lindmark et al., 2014b; Elnekave et al., 2006). However, reported results on mixing impact and optimal intensity remain inconclusive. While vigorous mixing has been suggested to improve the biodegradation of volatile solids by increasing their solubility and area of contact with bacteria (Halalsheh et al., 2011), it has been equally reported to hinder the formation of flocks, where syntrophic microbial interactions can take place, and cause propionate accumulation resulting in lower removal efficiency (Suwannoppadol et al., 2011). In comparison, systems with moderate mixing (80 rpm) seem to have high stability and are able to absorb the disturbance of a shock load, even when stratification occurs (Gomez et al., 2006). Slower mixing at 15 and 25 rpm has also been reported to improve acidogenesis (Yu et al., 2017) and gas generation (Lindmark et al., 2014a), respectively.

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The need for mixing is equally controversial whereby some studies reported that no mixing may cause substrate shortcuts, ultimately decreasing the effective hydraulic retention time (HRT) and the overall efficiency of the digestion process, methane generation and pathogen removal (Elnekave et al., 2006; Ghanimeh et al., 2012). In contrast, the conversion of VFA to methane is inhibited by high concentration of hydrogen and close microbial contact has been found to be a solution for this limitation, which can be achieved by reduced or interrupted mixing. Similarly, the lack of mixing during startup, can reportedly shorten the startup period, stabilize the system, and increase methanogenic abundance (Stroot et al., 2001) – associated with higher methane generation (Traversi et al., 2012). Other work emphasized the importance of mixing during startup for VFA dissipation and stabilization, particularly in the absence of acclimated inocula (Ghanimeh et al., 2012).

Despite the divergence in reported findings, digesters operating at low TS feed or low OLR, appear to be less sensitive to the intensity of mixing or its absence. In low-TS systems, inhibiting byproducts are diluted and adequate mixing can be achieved by the slightest stirring effort, or even by the natural movement of the generated biogas (Wang et al., 2017). In comparison, high-TS can result in high viscosity liquor that requires greater stirring effort to achieve the same level of mixing. The impact of mixing seems also to depend on the type of waste fed into the system as different substrate composition can lead to different microbial setups with varied tolerance, and different abundance of toxins and inhibitors (Lindmark et al., 2014b).

Based on the above, to this date, no optimum mixing pattern can be discerned from the literature (Kuczman et al., 2017). The need for mixing in thermophilic digesters treating food waste, specifically in the absence of acclimated inocula, was previously demonstrated (Ghanimeh et al., 2012), and the current study aims at examining the stability and efficiency of thermophilic anaerobic digesters treating food waste in response to varying the mixing velocity. Furthermore, while microbial communities in anaerobic digesters have been characterized (Li et al., 2015), studies addressing the effect of mixing on the microflora remain divergent and limited (Tian et al., 2014). Accordingly, this paper addresses the correlation between bacterial composition and mixing, on one hand, and system performance, on the other, in thermophilic digesters treating food waste. Four stirring velocities (50, 80, 120, 160 rpm) were tested, for one HRT (30 days) each. The change in stirring speed was initiated after completion of the startup period to avoid the impact of shock loads. The withdrawal of digestate took place after vigorous mixing to ensure constant SRT at all mixing speeds. A semi-continuous feeding mode was adopted because, compared to commonly reported batch systems, it has the advantage of simulating real-life applications. The performance of the reactors was evaluated in terms of methane generation, removal of total and soluble COD and VFA concentration. Also, the bacterial community composition at different mixing velocities was characterized by high-throughput 16S rRNA gene sequencing to correlate with the system's performance.

2. Materials and methods

2.1. Experimental setup and procedures

Two CSTR (9 L working volume, Bioflo 110, New Brunswick Scientific Co.), referred to as digesters A and B, were operated at a HRT of 30 days under stable thermophilic temperature (55 ± 1 °C). The digesters were mechanically stirred by means of an internal impeller. The biogas was collected in gasometers using the water displacement method and was analyzed on a daily basis for CH_4, CO_2 and O_2 content. Prior to feeding, the digesters were vigorously mixed and samples were retrieved by pressure differential and tested on a weekly basis. The experiment lasted for 185 days (26 weeks) split between (a) the **startup** phase (87 days), during which the organic loading rate (OLR) was increased from 0.5 to 2 gVS l^{-1} d^{-1} at a mixing speed of 80 rpm, and (b) the **varied mixing** period where three mixing schemes were applied to each system: 80, 120 and 0 rpm in digester A, and 80, 50 and 160 rpm in digester B. The fluid has a density of 980 kg m^{-3} and a dynamic viscosity of 0.0195 Pa s (Ghanimeh et al., 2017). Accordingly, the highest velocity near the blade edges, at 50, 80, 120 and 160 rpm is around 0.19, 0.30, 0.45 and 0.60 m/s, respectively, resulting in a laminar to transitional flow with Reynolds numbers of 682, 1091, 1636 and 2181, respectively. Also, assuming minimum zero velocity near the cylinder wall, the average velocity gradient (G) at 50, 80, 120 and 160 rpm can be approximated at 2.4, 3.9, 5.8 and 7.7 sec^{-1}, respectively (Equation (1)), requiring a power input of 0.1, 0.3, 0.7 and 1.2 W per cubic meter of digester volume (Equation (2)).

$$G = \frac{(V_{max} - V_{min})}{gap} \quad (1)$$

where G is an approximated value of the average velocity gradient, V_{max} is the maximum velocity (i.e. velocity near the blade edge); V_{min} is the minimum velocity (assumed zero at the cylinder wall); gap is the clearance between the blade edge and the cylinder wall (7.8 cm).

$$G = \sqrt{\frac{P}{\mu V}} \quad (2)$$

where P is the power input (W); μ is the dynamic viscosity (Pa.s); and V is the volume of the compartment (m^3).

At the end of the startup phase, both digesters were run for one HRT under the same mixing speed (80 rpm) for stabilization purposes. Then, the mixer speed in digester A was increased to 120 rpm to achieve high-intensity mixing. The deterioration in the digester's performance necessitated the interruption of mixing (0 rpm). In comparison, the mixing speed in digester B was reduced to 50 rpm, in an attempt to improve the system's performance, prior to applying vigorous mixing (160 rpm). The minimum (50 rpm) mixing speed was experimentally identified, in the current study, as the lowest stirring speed at which stratification does not occur.

2.2. Inoculation and feeding

Both digesters were inoculated with 1.5 kg of fresh cow manure diluted to 3.5 L with de-ionized water. Slow feeding (low OLR) was initiated on the second day. By the second week, inoculation was completed with the addition of 0.5 kg of fresh compost diluted to 4 L with fresh leachate. Upon reaching the full (9 L) digester capacity, daily wasting and feeding was initiated. The feed consisted of a mixture of fruit and vegetable market waste and restaurant leftovers that were ground and mixed to ensure homogeneity, then stored at −20 °C to avoid fluctuation in characteristics. The waste had an initial TS of 33.1% that decreased to 6.5% after dilution with de-ionized water at 1:5 and an HRT of 30 days.

2.3. Analytical methods

Biogas composition (CH_4 and CO_2) was monitored on a daily basis using a dual wavelength infrared cell with reference channels (GEM-5000 monitor, Keison Products, UK). Total, suspended, dissolved and volatile solids were determined using Standard