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Microbial community dynamics linked to enhanced substrate availability and biogas production of electrokinetically pre-treated waste activated sludge

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HIGHLIGHTS

- Electrokinetic pre-treatment enhanced substrate solubility and biogas production.
- Increased substrate availability altered bacterial community composition.
- Abundance of selected OTUs correlated with substrate solubility and biogas yield.
- Methanogenic community composition remained stable during operation.

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ABSTRACT

The restricted hydrolytic degradation rate of complex organic matter presents a considerable challenge in anaerobic digestion of waste activated sludge (WAS). Within this context, application of pre-treatment of digester substrate has potential for improved waste management and enhanced biogas production. Anaerobic degradation of untreated or electrokinetically pre-treated WAS was performed in two pilot-scale digesters for 132 days. WAS electrokinetically pre-treated with energy input 0.066 kJ/kg sludge was used in a first phase of operation and WAS pre-treated with energy input 0.091 kJ/kg sludge was used in a second phase (each phase lasted at least three hydraulic retention times). Substrate characteristics before and after pre-treatment and effects on biogas digester performance were comprehensively analysed. To gain insights into influences of altered substrate characteristics on microbial communities, the dynamics within the bacterial and archaeal communities in the two digesters were investigated using 16S rRNA gene sequencing (pyrosequencing) and quantitative PCR (qPCR). Specific primers targeting dominant operation taxonomic units (OTUs) and members of the candidate phylum Cloacimonetes were designed to further evaluate their abundance and dynamics in the digesters. Electrokinetic pre-treatment significantly improved chemical oxygen demand (COD) and carbohydrate solubility and increased biogas production by 10–11% compared with untreated sludge. Compositional similarity of the bacterial community during initial operation and diversification during later operation indicated gradual adaptation of the community to the higher solubility of organic material in the pre-treated substrate. Further analyses revealed positive correlations between gene abundance of dominant OTUs related to Clostridia and Cloacimonetes and increased substrate availability and biogas production. Among the methanogens, the genus *Methanosaeta* dominated in both digesters. Overall, the results showed that electrokinetic pre-treatment of WAS increases substrate solubility and biogas production. Changes in bacterial community composition and abundances of dominant bacterial OTUs were observed during anaerobic degradation of pre-treated WAS, whereas the relative abundance of methanogenic community members remained stable.

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1. Introduction

Anaerobic degradation of the excess biomass from secondary wastewater treatment systems, often denoted waste activated sludge (WAS), combines generation of energy-rich methane in the form of biogas with efficient sludge stabilisation and reduction of the volume requiring final disposal. Biogas can be used for the generation of heat and electricity or upgraded for use as vehicle fuel. Furthermore, when produced from waste biomass, it plays a vital role within the sustainable renewable energy sector, promoting organic waste as a valuable resource for energy recovery. However, low degradation rates of the organic matter in waste present a considerable challenge for anaerobic digestion at wastewater treatment plants (WWTPs) and need to be addressed to allow future improvement and optimisation of the technology. Management measures that increase energy output, and thereby the profitability of biogas production plants, would increase the potential to meet the economic pressure on energy prices and most likely expand the uptake of this renewable energy technology. In current practice, degradation of organic matter by activated microbial communities commonly requires hydraulic retention times of about 15–20 days and associated bottlenecks are often located in the initial hydrolytic step (Eastman and Ferguson, 1981; Shimizu et al., 1993). The restricted hydrolytic rate influences the downstream degradation steps of acidogenesis, acetogenesis and methanogenesis, since the overall process depends on concerted action by various microorganisms involving interspecies syntrophic interdependencies (Angelidaki et al., 2011). WAS is mainly composed of microbial cells strongly linked together in a floc matrix by extracellular polymeric substances (EPSs) (More et al., 2014). This floc structure constrains the accessibility for extracellular enzymes to the biopolymers, reducing the degree of degradation and hence the biogas production potential. Pre-treating the digester feed prior to anaerobic digestion is a promising strategy for enhancing the rate of the methanisation process, an effect generally ascribed to the associated reduction in particle size and increased solubility of organic matter in WAS (Appels et al., 2008). Previous research on pre-treatment techniques has mainly focused on thermal hydrolysis, ultrasonic treatment, mechanical disintegration, biological and chemical treatments or their combinations, and achievements in increased biogas production are well documented an electrical current through the sludge, commonly referred to as electrokinetic disintegration, is a relatively novel technique relying on the combined effect of motion and electrical effects. This technique has been thoroughly evaluated in the context of sludge dewatering (Mahmoud et al., 2010) and has been shown to increase the solubilisation of organic macromolecules (Chen et al., 2011) and to disrupt flocs networks and microbial cell walls (Gharibi et al., 2013; Yuan et al., 2011; Zhen et al., 2014). An important aspect of electrokinetic disintegration is the need for low amount of energy, much lower than e.g. ultrasound pre-treatment, to yield certain effects on the WAS composition. Commercial biogas plants occasionally operate on the border for economical feasibility and achieving an increase in biogas yield with the use of minimal amounts of energy may then be of crucial importance for continued production. However, little is known about the precise impact of electrokinetic pre-treatment on process performance and microbial community dynamics during long-term anaerobic digestion.

The present study examined application of electrokinetic pre-treatment to enhance biodegradation of WAS with the aim of raising the hydrolytic rate and increasing biogas yield. The specific objective was to investigate the dynamics within the bacterial and archaeal communities and identify significant correlations to operational factors altered by the pre-treatment. Pyrosequencing was applied to reconstruct and comprehensively investigate the microbial community structure throughout 132 days of digester operation. Specific primers targeting selected dominant operation taxonomic units (OTUs) were designed to further evaluate their abundance and dynamics in the digesters. The effects of increased chemical oxygen demand (COD) and carbohydrate solubility and possible links to enhanced biogas production were statistically related to microbial community composition. To the best of our knowledge, this is the first report describing anaerobic degradation of electrokinetically pre-treated WAS in continuous mode.

2. Methods

2.1. Inoculum and sludge origin

Inoculum for the anaerobic digesters was collected from the WWTP of Antwerpen-Zuid, Belgium. During operation, the digesters were fed with thickened WAS from the secondary clarifier of the WWTP of Mechelen-Noord, Belgium.

2.2. Electrokinetic pre-treatment

Electrokinetic disintegration was performed using a BioCrack unit (Vogelsang, Germany), consisting of two modules (each with a power requirement of 35 W) with an internal electrode, through which the WAS was pumped back and forth under constant flow. The pre-treatment was maintained for 6 min or 12 min, resulting in an energy dosage of 0.066 kJ/kg sludge or 0.091 kJ/kg sludge, respectively. Large components within the WAS were removed with a macerator before it entered the disintegration unit in order to protect the equipment.

2.3. Digester operation

The experimental set-up consisted of two analogous pilot-scale digesters, each with a total working volume of 50 L, operated in parallel at 37 °C. The digesters were filled with inoculum, followed by 40 days of no feeding to exhaust the substrate still present in the inoculum sludge. The digesters were then fed three times a day with WAS to obtain an organic loading rate (OLR) of 2 kg COD/m^3 per day and a hydraulic retention time (HRT) of 20 days. Both digesters received untreated WAS during a start-up period of 40 days. Electrokinetically pre-treated sludge was then added to one digester (hereafter referred to as digester D_E), whereas the other digester continued to run on untreated sludge (hereafter the control digester, D_C) throughout the experimental trial (days 0–132). The operating period of D_E was divided into two phases, each of which lasted for at least 3 HRT. In the first phase (days 0–72), the digester was fed WAS pre-treated with an energy input of 0.066 kJ/kg sludge and in the second phase (days 73–132), it was fed WAS pre-treated with an energy input of 0.091 kJ/kg sludge.

2.4. Assessing WAS characteristics

WAS characteristics were determined based on analyses of sludge material from 9 to 11 different feed collection points in each phase. Total solids (TS), volatile solids (VS), total Kjeldahl nitrogen, pH, volatile fatty acid concentration (VFA, including acetic, propionic, butyric, isobutyric, valeric and isovaleric acids) and methane content in the gas were measured as previously described (Westerholm et al., 2016). For analyses of total COD, protein and carbohydrate, and soluble COD and carbohydrate concentrations, 2, 15 and 5 mL sludge were used, respectively, and the analyses were conducted as described previously (Houtmeyers et al., 2014).