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Research paper

Long-term anaerobic digestion of food waste at semi-pilot scale: Relationship between microbial community structure and process performances

Barbara Tonanzi, Agata Gallipoli, Andrea Gianico, Daniele Montecchio, Pamela Pagliaccia, Marco Di Carlo, Simona Rossetti, Camilla M. Braguglia*

Water Research Institute, IRSA-CNR, Via Salaria Km 29.300, 00015, Monterotondo, RM, Italy

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ABSTRACT

Stability and performance of long term semi-continuous Anaerobic Digestion of food waste at semi-pilot scale is here evaluated based on the integration of multiple lines of evidence. In order to elucidate the main microbial components, the core microbiome dynamics were assessed by high-throughput 16 S rRNA gene sequencing over the reactor operation together with the data related to the AD performances. The experimental reactor, after a successful start-up, was operated for more than 200 days at a moderate OLR (Organic Loading Rate) of 1.6 ± 0.4 g VS $L^{-1}d^{-1}$. The availability of readily biodegradable substrate, in particular carbohydrates, favored the fermentative functional redundancy of bacteria promoting the rapid accumulation of acetate first, and propionate afterwards, due to limited methanogenesis. The prolonged operation, despite the moderate OLR, nurtured propionate accumulation, because H_2 concentration exceeded the level capable to render the reaction endergonic, hampering the propionate uptake process. The application of a Pulsed Feeding strategy increased the hydrogenotrophic *Methanomicrobiales* favoring the consumption of propionate most likely through hydrogen utilization.

1. Introduction

In recent years, following worldwide industrial development and population growth, the amount of food waste (FW) is expected to increase. Although FW reduction would be the best option in the FW management hierarchy, subsequent approaches such as recovery in terms of waste-to-energy also require attention and technical development from the research community in order to promote a comprehensive sustainable FW management system [1]. Due to its fundamental characteristics such as wide availability, high biodegradable organic fraction and in particular high carbohydrate and proteins content, FW has been considered an attractive economical source of energy production through Anaerobic Digestion [2,3]. Hydrolysis, considered to be a stage which limits the rate of solid waste digestion [4,5], and acidification phase, where the acidifying bacteria convert soluble chemical substances to short-chain organic acids, CO_2 and hydrogen represent the initial pathways of transformations. Afterwards, acetate, H_2, CO_2 and formate are used to produce methane by methanogenic bacteria. Anaerobic digestion of FW is therefore a complex and delicate process due to the close connection of the degradation and transformation phases, and many parameters can influence the performances and stability of the AD process. Large scale FW anaerobic digesters are usually operated with a low organic loading rate (OLR), from 1 to 4 gVS $L^{-1}d^{-1}$, or long hydraulic retention time (HRT), up to 80 d [6,7] because of the major concern regarding process instability and low biogas productions.

Microorganisms, mainly responsible for the success (or failure) of the process, are the core of the digester. The slow growth methanogenic bacteria are very sensitive to process parameters as pH and temperature. Consequently, it is very important to maintain the correct conditions for the success of the process [8]. Understanding the behaviour and composition of the involved microbial communities may be helpful for improving AD, in particular by long term operation of the digesters. Real-time process monitoring combined with the related microbial management [9–11] may be the key to improve AD stability and efficiency.

Zamanzadeh et al. [12] reported the digestion performances of a single-stage anaerobic mesophilic reactor with HRT of 20 d and OLR of 1.85 gVS $L^{-1}d^{-1}$. No accumulation of VFAs occurred, and the methane yield was 480 mL CH_4 $g^{-1}VS_{added}$. However, it must be pointed out that

* Corresponding author.
E-mail address: braguglia@irsa.cnr.it (C.M. Braguglia).

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the feedstock used in this study was an acidic FW with very high level of VFAs, suggesting the occurrence of a pre-fermentation step just before the AD process. By investigating the microbial community in this digester, the main bacteria phyla were *Firmicutes* and *Chloroflexy* while *Methanosaetae* were the dominant archaea. Li et al. [13] paid attention to the community composition when OLR disturbance is introduced into a mesophilic anaerobic digester treating FW. At the stable phase the common major phyla of bacteria were *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, *Synergistetes*, and *Spirochaetae*. In deteriorative phase, the relative abundance of these phyla decreased while the acidogenic bacteria increased with subsequent metabolic uncoupling among bacteria and methanogens. In fact, acidogenic bacteria showed high functional redundancy and rapid adaptation to OLR increase [9,14]. The microbial community analysis during mesophilic AD of FW at OLR increasing from 1.0 to 2.5 gVS L^{-1} d^{-1} showed high functional redundancy in bacterial community integrated with acetoclastic methanogenesis when OLR increased, and *Methanosaeta* gradually dominated in archaeal community [15].

Most research done on anaerobic digestion of FW was carried out in batch systems, few in continuous reactors, and the correlation between the microbial community structure and the reactor functioning which is still a field full of gaps, especially in terms of long term processing [1,2]. Moreover, the industrial production of energy from organic wastes requires further understanding of the process dynamics at semi-pilot or large scale [16], nevertheless at our best knowledge, just a couple of pilot-scale studies operating in continuous mode that were reported referred however to short operation phases with respect to the applied HRTs [17,18]. Nowadays, as far as we know, there are no multidisciplinary studies at semi-pilot scale regarding the anaerobic digestion of food waste. Moreover, the application of high-throughput 16 S rRNA gene sequencing to determine the composition and gene content of the microbial population in the digesters have become the common platform in environmental microbiology [19].

The aim and the novelty of this study was to verify the process performances of mesophilic AD of food on a semi-pilot scale to obtain robust data throughout a long term performance at fixed moderate OLR of 1.6 g VS $L^{-1}d^{-1}$ monitoring both the microbial community structure (both bacterial and archaeal) and the process parameters. The impact of process duration, HRT and feeding mode on process stability and performances (organics reduction, VFA concentration and type, methane conversion rate) was assessed. Quantitative, predictive relationships between the complex microbial community structure and the digester functional outputs are presented and discussed.

2. Materials and methods

2.1. Inoculum and food waste

For the start-up of the semicontinuous test, an inoculum originated from a full-scale digester treating sewage sludge was used. FW was sampled from the canteen of the research campus “Roma 1” of National Research Council (CNR), producing approximately 400 kg of FW per week. The waste composition, typical for Italy, consisted of mixed raw and cooked food such as pasta and bread (15%), cheese (15%), fruit and vegetable peelings (70%). The mixed material was then shredded with a blender (Kenwood Food Processor) to a final particle size below 3 cm, using a lab-scale knife mill before storage at −20 °C.

2.2. The anaerobic reactor operation

Two digestion tests were performed using 10 L stainless steel anaerobic reactors *(Bioprocess, Sweden)*, completely mixed, operated in semi-continuous mode and maintained at the constant temperature of 37 °C (Fig. 1).

After the acclimation period, one reactor, the experimental one, was operated at HRT 40 d, keeping the OLR of 1.6 ± 0.1 g VS $L^{-1}d^{-1}$, by feeding FW daily (from Monday to Friday). After 130 d, the feeding pattern was switched into a “pulse feeding” mode, by feeding the reactor 3 times a day (keeping constant HRT and OLR). Three recovery phases (R, R1 and R2) were imposed by stopping feeding the substrate, measuring continuously methane production, VFA and COD concentration. Moreover, Na_2CO_3 was added as alkalinity to control pH drops in the reactor. The control reactor was always maintained at the same HRT of 20 d, same OLR of 1.6 ± 0.1 g VS $L^{-1}d^{-1}$, and same feeding procedure (daily feeding, from Monday to Friday, Saturdays and Sundays excluded). The produced biogas was collected and sent through a CO_2 trap (a bottle filled with NaOH 3 M) before a methane detection unit (μFlow, Bioprocess Control, Sweden) equipped with temperature and pressure compensation for the normalization of gas flow rate and volume measurement at 0 °C and 1 atm.

Fig. 1. Semi-pilot stainless steel reactor used for semi-continuous trials.

2.3. Analytical procedure

Total and volatile solids were determined according to standard methods [20]. The pH was detected by a portable pHmeter Eutech Instruments pH 700. To analyse the soluble phase, the particulate sludge matter was removed by centrifugation (10 min at 4000 rpm) and the resulting centrate was filtrated through 0.45 μm pore size membrane filters. Soluble COD (sCOD), measured in duplicates, by means of COD Cell Test by Spectroquant Merck (EPA method 410.4). Ammonium nitrogen (NH_4^+-N) was determined according to APHA [20]. Volatile fatty acids (VFA) were analysed by injecting 1 mL of filtered (0.22 μm porosity) liquid sample into a Perkin Elmer Auto System gas chromatograph equipped with a FID detector (flame ionization detector). To analyse the soluble carbohydrates, samples aliquots were filtrated through 0.45 μm pore size filters and carbohydrates determination on the supernatant was based on a modified Dubois method, reported elsewhere [21]. The biogas composition was measured using a PerkinElmer Auto System Gas Chromatograph equipped with a thermal conductivity detector (TCD).

2.4. In situ detection methods (FISH and CARD-FISH)

Biomass samples were sampled from the anaerobic reactors and immediately fixed in formaldehyde and ethanol (2% and 48% vol/vol final concentration respectively) and stored at −20 °C. Prior to the