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Solid anaerobic digestion batch with liquid digestate recirculation and wet anaerobic digestion of organic waste: Comparison of system performances and identification of microbial guilds

Francesco Di Maria [a,b,*], Martino Barratta [a], Francesco Bianconi [a], Pisana Placidi [a], Daniele Passeri [a]

[a] LAR Laboratory - Dipartimento di Ingegneria, Via G. Duranti 93, 06125 Perugia, Italy
[b] CIMIS, Via G. Duranti 67, 06125 Perugia, Italy

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ABSTRACT

Solid anaerobic digestion batch (SADB) with liquid digestate recirculation and wet anaerobic digestion of organic waste were experimentally investigated. SADB was operated at an organic loading rate (OLR) of 4.55 kgVS/m^3 day, generating about 252 NL CH_4/kgVS, whereas the wet digester was operated at an OLR of 0.9 kgVS/m^3 day, generating about 320 NL CH_4/kgVS. The initial total volatile fatty acids concentrations for SADB and wet digestion were about 12,500 mg/L and 4500 mg/L, respectively. There were higher concentrations of ammonium and COD for the SADB compared to the wet one. The genomic analysis performed by high throughput sequencing returned a number of sequences for each sample ranging from 110,619 to 373,307. More than 93% were assigned to the *Bacteria* domain. Seven and nine major phyla were sequenced for the SADB and wet digestion, respectively, with *Bacteroidetes*, *Firmicutes* and *Proteobacteria* being the dominant phyla in both digesters. Taxonomic profiles suggested a methanogenic pathway characterized by a relevant syntrophic acetate-oxidizing metabolism mainly in the liquid digestate of the SADB. This result also confirms the benefits of liquid digestate recirculation for improving the efficiency of AD performed with high solids (>30%w/w) content.

1. Introduction

Anaerobic Digestion (AD) is a viable method for biodegradable waste treatment, as well as for renewable energy production, contributing to achieving the 2020 EU objective (Beurskens and Hekkenberg, 2014). Concerning renewable energy production, waste materials like manure, crop residues, sewage sludge, the organic fraction of municipal solid waste (OFMSW) and fruit and vegetable waste are of particular importance since they do not compete with food crops as substrate for AD (Apples et al., 2011). Depending on the total solids (TS) concentration of processable substrates, full-scale AD technologies can be grouped into three main classes: wet AD operated with TS < 15%; dry AD operated with TS < 25%; solid state AD operated with TS up to 40%.

Currently mainly wet and dry technologies are used for the AD of OFMSW (De Baere and Mattheuws, 2010). Before being processed in such facilities, the OFMSW requires more or less important pre-treatments, such as mechanical sorting, shredding, metal separation, moisture increase, and pulping, which are costly and complex operations affecting AD viability (Vinot et al., 2010). The main outputs from AD are a biogas mainly rich in CH_4 (*i.e.* about 60%v/v) and CO_2 (*i.e.* about 40%v/v) and a digestate rich in nutrients and organic carbon with a moisture content (MC) usually >80%w/w (Martins das Neves et al., 2009; Di Maria et al., 2013). A digestate with such a high MC is another important technically and economically negative aspect for the AD of waste in many EU areas (Fricke et al., 2005). In this case the digestate must undergo a preliminary solid/liquid separation before successive recovery operations. The liquid fraction is usually processed in wastewater treatment plants before disposal, whereas the solid fraction is composted for the production of a soil amendment. Besides being a further cause of cost increase, the dewatering process can remove up to 80% of the nutrients from the solid fraction of the digestate, consequently reducing their concentration in the final soil amendment (Rico et al., 2011). As already demonstrated in previous studies, Solid Anaerobic Digestion Batch (SADB), which operates with TS up to 40%w/w, can significantly reduce these problems even if the biogas yield is lower (ten Brummeler, 2000; Li et al., 2011; Di Maria et al., 2012a, 2013).

Lower biogas generation is a consequence of complex phenomena occurring at high TS concentrations. Abbassi-Guendouz et al. (2012) reported that TS ⩾ 30% is an obstacle to liquid/gas mass

* Corresponding author at: LAR Laboratory - Dipartimento di Ingegneria, Via G. Duranti 93, 06125 Perugia, Italy.
E-mail address: francesco.dimaria@unipg.it (F. Di Maria).

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transfer, causing a reduction in methanogenic degradation efficiency. In the same study there was also a reduced hydrolytic activity for $10\% \leqslant TS \leqslant 25\%$. The corresponding methane yield was about 170 L/kgVS for TS up to 25% and dropped to less than 50 L/kgVS for TS = 35%. Xu et al. (2014) reported a two-faced effect of TS concentration during AD. An increase in TS up to 10% stimulated microbial growth with a consequent increase in methane generation. On the other hand, higher values >15–20% increased mass diffusion resistance, causing inhibition of hydrolysis and methanogenesis. In this case the maximum methane rate of 1.2 L/day/L of digester was achieved at TS = 18%. At TS = 28% the CH_4 rate was <0.7 L/day/L of digester. Lower water content also caused a rapid accumulation of volatile fatty acids (VFA), generating further inhibition of methanogens microbes (Abbassi-Guendouz et al., 2012; Di Maria et al., 2013; Schievano et al., 2010). Other authors have investigated the correlations between the organic load (*i.e.* gVS/kg) and the biogas yield from AD of OFMSW. For an organic load of 80 gVS/kg, Bouallagui et al. (2005) reported a biogas yield of about 600 NL/kgVS. At higher concentrations of about 200 gVS/kg and 340 gVS/kg, a biogas yield of about 500 NL/kgVS and 400 NL/kgVS was reported by de Laclos et al. (1997) and Di Maria et al. (2013), respectively. Also in these cases a high VS concentration was a consequence of low water content. Even if these results confirm the favorable conditions for microbial communities in those processes operating with $TS \leqslant 15\%$, some authors (Di Maria et al., 2013; Pognani et al., 2015) have proposed recirculating the liquid digestate during SADB in order to enhance process performance. The main results highlighted the central role of the recirculated liquid digestate in methanogenesis, with a high methane yield, also at TS > 30%. Improvement of AD performances is also a consequence of more favorable conditions inside the digester for microbial growth.

Cooperation between the key members of microbial communities is necessary for the optimum performance of AD. This can provide useful information both for the design and management of AD facilities. On the other hand, complexity of the microbial activity is seen as one of the main reasons for the lack of basic knowledge about digestion systems (Apples et al., 2011; Rui et al., 2015).

Current methods used for sizing and optimizing digester performance continue to be based on macro-parameters such as: the organic loading rate (OLR) ($kgVS/m^3$ day); the temperature; the hydraulic retention time (HRT) and sludge retention time (SRT) (day); the amount of inoculum; the co-digestion of different substrates (Curry and Pillay, 2012; Di Maria et al., 2015; Hilkiah Igoni et al., 2008; Poschl et al., 2010). Unfortunately, the effects on microbial guilds are generally disregarded.

In recent studies some efforts have been made to investigate the effects on microbial populations by modifying the internal environment of the digesters. Li et al. (2015) investigated the effect of mesophilic and thermophilic temperatures on the microbial communities of solid state AD of maize. Stolze et al. (2015) investigated the biogas-producing microbial communities for biogas plants processing different ratios of cattle manure and maize silage, operating under wet and dry conditions. Rui et al. (2015) investigated the core populations of prokaryotic communities in different biogas digesters for manure and other bio-waste. Other authors have identified the key role played by the concentration of ammonium in stimulating syntrophic acetate oxidizing *Bacteria* and hydrogenotrophic *Archaea* able to enhance methanogenic activity (Di Maria and Barratta, 2015; Hattori, 2008; Schnurer et al., 1999; Shah et al., 2014). Li et al. (2016) analyzed the correlation between process stability and the microbial community for wet AD of food waste at different OLR. For four parallel wet biogas reactors co-digesting cow manure and protein-rich substrate, Solli et al. (2014) found that the most abundant phyla in the *Bacteria* domain were *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. On the other hand the most abundant genus of the *Archaea* domain were *Methanoculleus*, *Methanobrevibacter*, *Methanosarcina* and *Methanosaeta*. From the pyrosequencing analysis of bacterial and archaeal richness of 21 full-scale wet biogas digesters, Sundberg et al. (2013) reported different results on the basis of the substrate treated. In particular acetoclastic methanogens were detected in sewage sludge digesters, but not in digesters processing various combinations of bio-waste. This suggested that in the latter, methanogenesis takes place mainly by syntrophic acetate oxidation.

All these findings confirmed the need for further investigations in this sector, in particular concerning the microbial guilds operating in digesters with TS > 30% as in SADB with liquid digestate recirculation. Emerging metagenomic approaches based on high-throughput sequencing (HTS) return quite complete DNA sequences, providing useful and easy-to-handle information on microbial populations (Yang et al., 2014), indicating that this could be a suitable approach for such analysis. The aim of the present study was to investigate the performances of digesters and the related microbial populations concerning the anaerobic digestion of OFMSW performed by wet and SADB with liquid digestate recirculation. Microbial profiles were determined using *illumina MiSeq* HTS (Li et al., 2015; Yang et al., 2014).

2. Materials and methods

2.1. Characterization of samples

The OFMSW used in the experimental apparatus was withdrawn from the inlet of an existing composting facility operating in central Italy. After manual removal of bulky and not biodegradable compounds, the amount necessary for the SADB runs was put directly in the pilot apparatus, whereas the amount necessary for feeding the wet process was frozen at −20 °C. Once a week a given amount of OFMSW was thawed, blended and diluted with demineralized water for preparing the mixture for feeding the wet digester (*i.e.* TS = 4%). The mixture was stored at +4 °C. Total solids (TS) (%w/w) and consequently moisture content (MC) (%w/w) were determined by measuring weight loss after heating at 105 °C for 24 h. VS (%TS) were determined by measuring the weight variation of TS after burning at 550 °C for 24 h. Total organic carbon (TOC) (% TS) was determined by the Springler and Klee wet dichromate oxidation method and total nitrogen was determined using the Kjeldahl method (TKN) (%TS). Other chemical parameters were determined using the HACH Lange DR 3900 spectrophotometer. Chemical oxygen demand (COD) (mgO_2/L), and total volatile fatty acids (TVFA), expressed as acetate equivalent (mg/L), were determined using HACH Lange cuvettes LCK 014 and LCK 365, respectively. The same methodology was also used for ammonium ($N\text{-}NH_4$) (g/kgTS, mg/L) determination using cuvette LCK 303. pH was determined with a kp 50 Delta Ohm probe. All analyses were performed at least in triplicate.

2.2. Pilot apparatus and procedure of runs

Two similar pilot apparatuses were used for the present study, one for the SADB and one for the wet process. They consisted of 100-L gas-tight anaerobic reactors (Di Maria et al., 2012b) with removable tops (Fig. 1). The digester temperature (35 ± 2 °C) was controlled by a thermal heating jacket and by a 2 cm thick insulating layer wrapping the digester. The temperature was continuously monitored with a resistance temperature detector inserted inside the processed substrate. Before starting the SADB, 10 L of demineralized water were inserted in the apparatus. During all the runs, a given rate of this liquid was withdrawn from the reactor bottom

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