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Boosting methane generation by co-digestion of sludge with fruit and vegetable waste: Internal environment of digester and methanogenic pathway

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ABSTRACT

The effects of anaerobic co-digestion of waste-mixed sludge with fruit and vegetable waste (FVW) on the methane generation of a mesophilic digester was investigated. Organic loading rates (OLR) were 1.46 kgVS/m^3 day, 2.1 kgVS/m^3 day and 2.8 kgVS/m^3 day. Increase in the OLR due to FVW co-digestion caused modification of the internal environment of the digester, mainly in terms of N-NH_4 (mg/L). Corresponding microbial populations were investigated by metagenomic high-throughput sequencing. Maximum specific bio-methane generation of 435 $NLCH_4$ per kgVS feed was achieved for an OLR of 2.1 kgVS/m^3 day, which corresponded to a biomethane generation per kgVS removed of about 1700 $NLCH_4$. In these conditions the methanogenic pathway was dominated by aceticlastic *Methanosaeta* and hydrogenotrophic/aceticlastic *Methanoscarcinae*. Ammonia concentration in the digester resulted a key parameter for enhancing syntrophic acetate oxidation, enabling a balanced aceticlastic and hydrogenotrophic/aceticlastic methanogenic pathway.

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 (FVW) are of particular importance since they do not compete with food crops as substrate for AD (Apples et al., 2011). Furthermore AD converts biodegradable materials into two main streams: a biogas composed mainly of methane and a quite stabilized organic fertilizer for agricultural use (Martins das Neves et al., 2009; Di Maria et al., 2013). Improving energetic and environmental performances of AD is a key challenge for increasing the viability of this process (Assam et al., 2011). Due to possible optimization of some biochemical parameters, one of the most investigated solutions for achieving this goal is co-digestion (Di Maria et al., 2014; Gomez et al., 2006; Liu et al., 2012; Poschl et al., 2010; Zupancic et al, 2008). Currently AD is widely used for stabilizing the mixture of primary and waste-activated sludge, known as waste-mixed sludge (WMS) generated by large-size wastewater treatment plants (WWTP), *i.e.* >30,000 population equivalent (PE). About 36,000 WWTP operating in the EU use AD to reduce sludge reactivity (Bolzonella et al., 2006) before disposal. About 10 million tonnes on dry basis of sludge are produced in the EU each year, and its disposal amounts to about 50% of the total operating costs of WWTP (Apples et al., 2011). Due to the low organic matter and volatile solids (VS) concentration of WMS originating from WWTP, the digester generally operates at low organic loading rates (OLR) kgVS/m^3 day. This has given rise to the concept of co-digesting WMS with other biodegradable substrates to improve the energetic and environmental performances of the digesters. The co-digestion of OFMSW with sludge in a full-scale facility increased the biogas generated from 600 m^3/day to 950 m^3/day (Bolzonella et al., 2006). Increasing the OLR of a WWTP sludge digester from 2.4 to 6 kgVS/m^3 day by co-digestion with FVW led to a daily increase in biogas production up to 100%, along with a reduction in greenhouse gas (GHG) emissions from 114 to 523 kgCO_2/tonne (Liu et al., 2012) due to energy recovery. Gomez et al. (2006) showed that co-digestion of sludge with FVW increased the specific biogas production from 300 to 800 L/kgVS. Co-digestion has also been shown to be a reliable approach for improving the agronomic quality of the digestate (Di Maria et al., 2014), improving its potential use as a soil improver or fertilizer.

These findings are a consequence of the effects that co-digestion has on the biochemical and microbial processes occurring during

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AD. In particular, the 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). Cooperation between the key members of a microbial community is necessary for the optimum performance of AD and can provide useful information both for the design and management of AD facilities. Several methods have been proposed for specifying the size of digesters and for optimizing their performance. Curry and Pillay (2012) proposed a model for calculating the biogas production from FVW and for sizing the digester based mainly on OLR, hydraulic retention time (HRT) and humidity content. Di Maria et al. (2013, 2012a) investigated the effect of the ratio of inoculum to the fresh OFMSW for batch digesters on biogas generation and investment costs. Hilkiah Igoni et al. (2008) indicated a preliminary design procedure for continuous and batch digesters for OFMSW based on physical and chemical parameters, suggesting that mesophilic temperature was the best. Poschl et al. (2010) evaluated the primary energy input-output factor for anaerobic digestion and co-digestion of several feedstocks. Results showed that co-digestion offers a more stable and energetically and environmentally efficient solution. All these studies confirm that the most widespread design approaches for anaerobic digesters disregard completely the aspect concerning the microbial populations and their relationship with the other operating parameters and with process performances. Nowadays emerging metagenomic approaches based on high-throughput sequencing (HTS) give quite complete DNA sequences able to give useful and easy-to-handle information on microbial populations (Yang et al., 2014). In the present study, with the aid of an experimental apparatus, mesophilic co-digestion of WMS and FVW at different OLRs, aimed to improve AD performances, was investigated. Effects on methane generation, VS removal, digester internal environment and corresponding microbial populations were analyzed. A correlation between the amount of FVW co-digested, OLR, HRT, specific methane generation, VS removal and methanogens *Archaea* was also proposed as support for both designing and managing digesters.

Table 1
Fractions (% by weight) of materials used for FVW generation.

Component	%
Potato	55
Fruit and vegetables	28
Bread	5
Paper	2
Pasta	10

2. Materials and methods

2.1. Characterization of sludge and FVW samples

The WMS used in the run was withdrawn from an existing WWTP of 90,000 PE at the thickener outlet and frozen at −20 °C. The amount of WMS needed to feed the pilot digester for 1 week was thawed and stored at +4 °C. The FVW was generated according to Sosnowski et al. (2003) by blending the components reported in Table 1. Once generated, the FVW was stored in accordance with the WMS procedure.

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). Organic nitrogen (N_{org}) (g/kgTS), ammonium ($N\text{-}NH_4$) (g/kgTS, mg/L) and TVFA (mg/L), expressed as acetate equivalent, were determined according to the HACH Lange methodology. To analyze for heavy metals, samples were digested according to the US EPA 2050B (US EPA, 1996) method. Heavy metals were determined by flame atomic absorption spectrophotometry using a Shimadzu AA-6800 apparatus. All the analyses were performed at least in triplicate.

2.2. Pilot apparatus and procedure of runs

The pilot apparatus used in the analysis consisted of a 100-liter gas-tight anaerobic reactor (Di Maria et al., 2012b) with a removable top (Fig. 1). The digester temperature was controlled by a thermal heating jacket wrapping the digester and by a 2 cm thick insulating layer. The temperature was continuously monitored with a resistance temperature detector inserted inside the processed substrate. Stirring was maintained by a temporized circulating pump. A given rate of liquid was withdrawn from the reactor top and injected at the reactor bottom. Gas produced during the process was continuously withdrawn from the reactor top, then

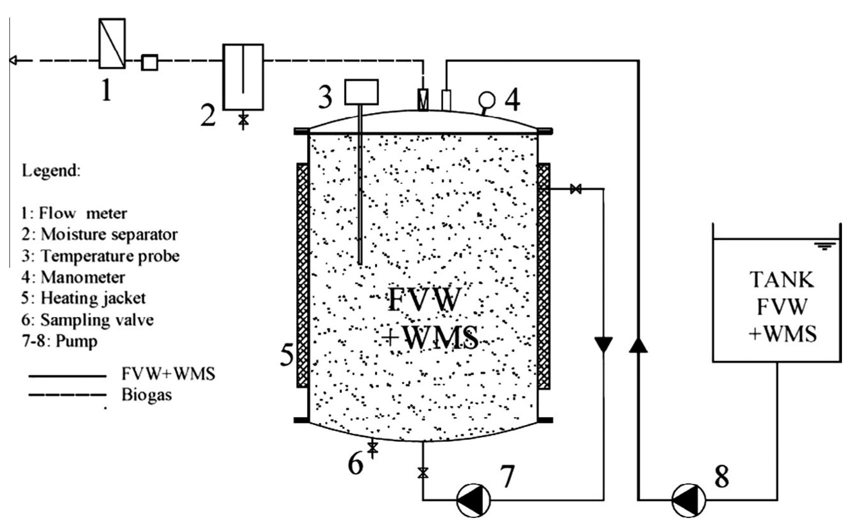

Fig. 1. Scheme of the pilot apparatus.