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Pre-treatment and inoculum affect the microbial community structure and enhance the biogas reactor performance in a pilot-scale biodigestion of municipal solid waste

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During anaerobic digestion of municipal solid waste, organic matter is converted to methane, carbon dioxide, and other organic and inorganic compounds through a complex cooperation among different microbial groups with different metabolic activities. Here, culture-dependent and independent approaches provided evidence for examining the relationship between bacterial and archaeal communities and methane production in a pilot-scale anaerobic digestion. The abundance of aerobic and anaerobic functional groups of C and N cycles, such as cellulolytic, pectinolytic, amylolytic and proteolytic bacteria, was high at the beginning of the experiment and was drastically decreased after anaerobic digestion. In contrast, the ammonifiers increased in the biogas producing reactors in a higher pH environment. The methanogenic archaeal genera recovered were *Methanobrevibacter*, *Methanobacterium*, *Methanoculleus* and *Methanocorpusculum*, thus indicating that methane was formed primarily by the hydrogenotrophic pathway in the reactors.

Moreover, the mechanical pretreatment effects, as well as the effect of pelleted manure as inoculum, were considered. The highest methane production was detected in the biodigesters with minced organic waste, thus indicating that pre-treatment of a heterogeneous starting matrix was essential for improving biogas production and stabilizing the process.

1. Introduction

Presently, anaerobic digestion is the most sustainable, cost-effective and attractive technology for disposing the organic fraction of municipal solid waste (OFMSW), owing to the prospect of producing methane rich gas (Kothari et al., 2014). The digestate can also be treated with an aerobic process to produce a stabilized residue that can be used as a soil additive or for environmental restorations (Castillo et al., 2006). There is growing interest in managing waste by using anaerobic digestion (Banks et al., 2001), and the European Commission has estimated that approximately one-third of the EU's 2020 target for renewable energy in transport could be met by using biogas produced from biowaste (European Commission, 2010).

Anaerobic digestion occurs in four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. During these steps, organic waste is degraded by a consortium of microorganisms and is converted into clean biogas (Kothari et al., 2014; Pagliano et al., 2017). In the first step, organic waste biomolecules are transformed into soluble, biodegradable organics by several microbial groups that can synthesize extracellular enzymes such as cellulase, pectinase, amylase, lipase and proteolytic enzymes (Panico et al., 2014). Subsequently, in the acidogenic phase, acid-forming bacteria metabolize the hydrolysis products towards mainly volatile fatty acids, such as acetic acid, propionic acid, butyric acid and valeric acid (Sans et al., 1995). These acidogenic products are then converted into acetic acid, hydrogen, and carbon dioxide (acetogenic phase), and finally into methane by methane-producing Archaea (methanogenic phase) (Chynoweth et al., 2001). During the methane formation process, principally two different methanogen groups may be present: hydrogenotrophic methanogens, which produce methane by using H_2 and CO_2, and acetoclastic methanogens, which mainly use acetate (Zhang et al., 2014).

Among the factors that affect microbial activities during the phases of anaerobic digestion, the composition, source and structure of the organic waste play fundamental roles, because the waste is extremely heterogeneous and unpredictable (Abdullah et al., 2008). In particular, hydrolysis is reported as the rate limiting step in anaerobic digestion of OFMSW (Fantozzi and Buratti, 2011).

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Therefore, to optimize biological process yields, different technological parameters have been developed. Depending on the substrate type, several pretreatments have been used, such as biological, thermal, chemical, mechanical and a combination of them, to improve the solubility and biodegradability of the organic matter (Lafitte-Trouqué and Forster, 2002) by increasing the organic substrate surface area and/or the microbial enzymatic activity (Cesaro and Belgiorno, 2014) and therefore biogas yield. Among these, the mechanical pretreatment is commonly used to reduce the particle size of organic material and could be successfully applied at full-scale level for its economic feasibility and easy implementation (Ariunbaatar et al., 2014). Besides, the treatment of OFMSW combined with manure in a codigestion process has been established to increase the biogas production and stabilize the process (Hartmann and Ahring, 2005) since the use of inoculum allows to have a fast start up of the process and a right equilibrium of bacterial populations (Fantozzi and Buratti, 2011). However, the use of fresh manure as inoculum involves several disadvantages such as health problems due to the presence of pathogens. To overcome this issue, it could be possible to use stabilized and pathogen-free pelleted manure (Holm-Nielsen et al., 2009).

However, although the anaerobic process is a biological process depending on a complex cooperation of numerous microorganisms with different metabolic capacities, the influence of microbial community structure on digester function as well as on its stability has been poor investigated (Venkiteshwaran et al., 2015). In the recent years, studies have mainly focused on microbial community dynamics using molecular approaches related to chemical parameters (Rui et al., 2015; Solli et al., 2014; Yi et al., 2014). However, the use of a combination of culture-dependent and culture-independent techniques could be a useful approach to investigate the active and growing specific eco-physiological microbial groups.

In the present work, both a culture-dependent and a culture-independent microbial approach were developed to evaluate the relationships between bacterial and archaeal communities, with methane production in a pilot-scale anaerobic digestion using mechanically pretreated OFMSW inoculated with commercial pelleted manure. These results will help us to understand the role of different microorganisms on the performance of anaerobic digestion depending on operating conditions such as temperature, pH, solid content, microbial diversity and abundance, as well as the physical and chemical structure of the organic waste including pretreatment and particle size.

2. Materials and methods

2.1. Biodigestion of organic municipal solid waste and sampling

Small-scale anaerobic digestion of the organic fraction of municipal solid waste (OFMSW) was carried out in ten pilot digesters (NHP s.r.l./ESco, Naples, Italy) at the experimental station of the Department of Agricultural Sciences (Portici, Naples, Italy).

Experiments were carried out in insulated digesters. Each digester was equipped with a plastic collection tank (holding capacity of 100 L, filled to 80 L), a manometer, a pH-meter (Mettler-Toledo S.p.a., Milan, Italy) and thermometer (Mettler-Toledo S.p.a.), a valve for methane detection and an output waste valve. Fig. 1 shows a schematic diagram of the bio-digester set-up.

The OFMSW was composed of 30% cellulose, 21% hemicellulose, 15% starch, 12% pectin and 20% lignin, and inoculated with animal manure pellets (Humoscam, Scam, Modena, Italy) characterized by 0.87 gTSS/g (dry/wet weight ratio) and 0.42 gVSS/g (dry/wet weight ratio) with a pH of 7.8. Four reactors (B1a, B2a, B3a and B4a) were filled with untreated OFMSW and four reactors (B1b, B2b, B3b and B4b) were filled with minced OFMSW (final size 0.5–5 cm) by using a steel hand grinder. Two non-inoculated digesters (Ca and Cb) were used as controls.

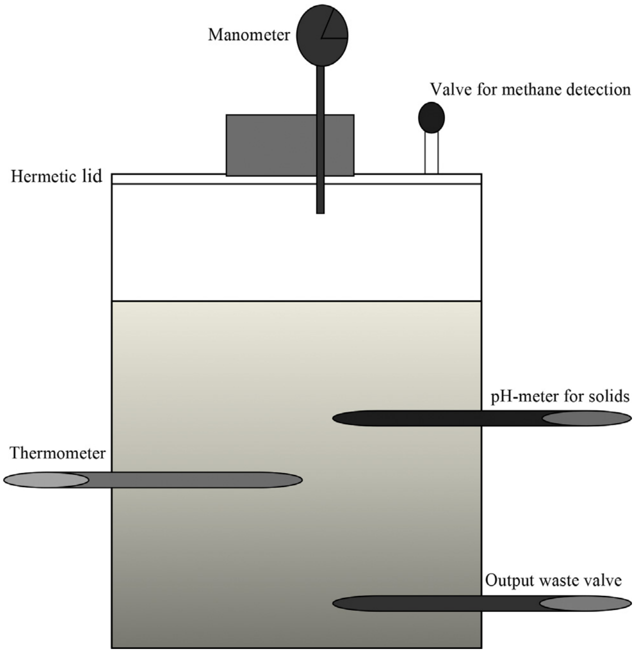

Fig. 1. Schematic representation of 100 L pilot bio-digester (NHP SRL/ESCo, Naples, Italy).

Analysis of the microbial communities in the organic waste samples was conducted on the starting matrix (C0), and then after 21 days of anaerobic digestion, by sampling 1 kg of biomass, as described in Sections 2.3 and 2.4.

2.2. Physico-chemical characteristics

Waste mixture was physically and chemically characterized as follows: during the process, temperature (°C) and pH were measured by using specific temperature sensors (Mettler-Toledo S.p.a.) placed in the core of the biodigester. Total suspended solids (TSS) and volatile suspended solids (VSS) were evaluated as per the standard methods (APHA, 2005); COD value was measured with a thermoreactor (Velp Scientific ECO08, Usmate (MB), Italy) and a photometer (Velp Scientific PF-3 COD-using kit NANOCOLOR®, Usmate (MB), Italy) according to manufacturer's instructions.

The biogas was monitored and collected in 10-L Tedlar bags (Tedlar Gas Sampling Bag, Sigma-Aldrich, Milan, Italy). CH_4, CO_2, and O_2 as a percentage of the sample gas volume (% v/v) and H_2S (ppm) were measured with a Portable Gas Detector (PGD 2 series – Status Scientific Controls, Nottinghamshire, United Kingdom).

2.3. Microbial quantification

Initial OFMSW suspensions were prepared by adding 20 g of sample to 180 mL of quarter strength Ringer's solution (Oxoid, Milan, Italy).

After shaking, suitable dilutions were made in the same solvent and were used to inoculate different solid and liquid media to grow the generic and functional microbial groups. Three plates or tubes of each dilution were analyzed.

Samples were characterized for aerobic and anaerobic bacteria, spore-forming bacteria, and fungi, as well as other microbial groups such as proteolytic, ammonifier, aerobic and anaerobic cellulolytic, pectinolytic, amylolytic, acidifying and methanogen. All

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