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Optimisation of the two-phase dry-thermophilic anaerobic digestion process of sulphate-containing municipal solid waste: Population dynamics

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HIGHLIGHTS

- Biohythane was obtained from sulphate-containing organic industrial solid wastes.
- Different organic loading rates and hydraulic retention times (HRTs) were carried out.
- Maximum of 1.9 l H_2/l/d and 5.4 l CH_4/l/d were obtained in the process.
- The lower HRT the greater acidogenic stage takes place in the second reactor.
- Acetogens and *Archaea* were dominated over sulphate-reducing bacteria in second phase.

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ABSTRACT

Microbial population dynamics and anaerobic digestion (AD) process to eight different hydraulic retention times (HRTs) (from 25 d to 3.5 d) in two-phase dry-thermophilic AD from sulphate-containing solid waste were investigated. Maximum values of gas production (1.9 ± 0.2 l H_2/l/d; 5.4 ± 0.3 l CH_4/l/d and 82 ± 9 ml H_2S/l/d) and microbial activities were obtained at 4.5 d HRT; where basically comprised hydrolysis step in the first phase (HRT = 1.5 d) and acidogenic step finished in the second phase as well as acetogenic–methanogenic steps (HRT = 3 d). In the first phase, hydrolytic–acidogenic bacteria (HABs) was the main group (44–77%) and *Archaea*, acetogens and sulphate-reducing bacteria (SRBs) contents were not significant; in the second phase (except to 2 d HRT), microbial population was able to adapt to change in substrate and HRTs to ensure the proper functioning of the system and both acetogens and *Archaea* were dominated over SRBs. Decreasing HRT resulted in an increase in microbial activities.

1. Introduction

AD is one of the effective technologies used to recover energy resources from organic wastes, in addition to being a simple and effective biotechnological means of reducing and stabilizing organic wastes. It is carried out by the coordinated action of various groups of microorganisms and goes through several intermediate stages. In the first step (hydrolysis), complex organic polymers are hydrolyzed into simpler soluble organic compounds by HABs; in this step large quantities of hydrogen (H_2) are produced. In the second step (acidogenesis), HABs produce volatile fatty acids (VFA), alcohols, H_2 and carbon dioxide (CO_2). In the third steps (acetogenesis), acid acetic, H_2 and CO_2 is produced by obligate H_2-producing acetogens (acetogens), organisms that consume fermentation products, such as propionate, butyrate, lactate, and ethanol. *Syntrophobacter* (propionate-utilizing acetogens (PUAs)) and *Syntrophomonas* (butyrate-utilizing acetogens (BUAs)) are the majority of the acetogens known (Mara and Horan, 2003). Acetogens requires very low H_2 partial pressure to favour the thermodynamics of the reactions (Boone et al., 1989). In the absence of external electron acceptors anaerobic oxidation of butyrate and propionate occurs only in syntrophic association with H_2-utilizing methanogens (HUMs) (Boone et al., 1989; Liu et al., 2011). In the fourth step (methanogenesis), methane (CH_4) is produced by methanogenic population. The methanogens are normally divided into two main groups based on their substrate conversion capabilities. Acetate-utilizing methanogens (AUMs) are capable of converting acetate to CH_4 and CO_2 and HUMs convert H_2 and CO_2 to CH_4.

HABs, acetogens and methanogenic microorganisms differ, not only in terms of their nutrition and pH requirements, but also with respect to their physiology, growth, and nutrient uptake kinetics,

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and in their particular ability to with stand environmental changes, thus different requirements regarding reactor conditions, have led to the development of two-phase AD processes (De la Rubia et al., 2009). The phase-separated anaerobic process including two-stage system normally comprises hydrolysis–acidogenesis in the first phase and acetogenesis–methanogenesis process in the second phase. A step forward of the common AD process, is the separate phase approach finalised to the production of H_2 in the first phase reactor and CH_4 in the second phase reactor. Obtained gases can be used separately or mixed together to obtain biohythane (Cavinato et al., 2011). Methanogenic microorganisms grow more slowly than HABs, at a rate similar to acetogens (3.6 d) and the optimum pH environment for methanogens and acetogens is in the range 6.5–8.5 (Montero et al., 2009; De la Rubia et al., 2009). Consequently, conditions that are non-favourable to the growth of methanogens (short HRTs or/and low pH (5.2–6.5)) should be established in the first phase, while in the second phase, high HRTs and neutral or slightly basic pH should be imposed.

The organic fraction municipal solid waste (OFMSW) used to feed proceeds of the municipal solid waste treatment plant "Las Calandrias" (Jerez de la Frontera, Cádiz-Spain) typically contained sulphate at concentrations higher than 5 g/kg. During AD of sulphate-containing wastes, SRBs can lead to the undesirable production of hydrogen sulphide (H_2S). The nutritional requirements of SRBs are an inorganic electron acceptor, this is usually provided by sulphate ion and an electron donor, and essentially these consist of VFA or H_2, and occasionally sugars and lounge chain fatty acids. Two stages of inhibition exist as a result of sulphate reduction (Chen et al., 2008); primary inhibition is due to competition for common organic and inorganic substrates from SRBs and secondary inhibition results from the toxicity of sulphide to various microbial groups.

Understanding the functioning of anaerobic reactors requires quantitative information on microbial numbers, biomass, and activities of the bacterial groups involved in the process (Montero et al., 2009). The stability of the system depends on the active microbial groups involved in the process (Montero et al., 2009). Molecular tools as fluorescent *in situ* hybridization (FISH), based on sequence comparison of small-subunit (SSU) ribosomal RNA (rRNA) molecules already have been used for the quantification of population abundance in different anaerobic environments (McMahon et al., 2001; Montero et al., 2008, 2009).

This study aims to: (1) establish the optimal conditions (organic loading rate (OLR) or HRT) in order to maximise the gas production (GP); (2) investigate the population dynamics and (3) study the different microbial activities, including SRBs in the two-phase dry-thermophilic AD process of sulphate-containing municipal solid waste. No previous studies had been published about this. For these purposes, the effect of eight different OLRs (from 3 g to 24 g TVS/l/d) or HRTs (from 25 d to 3.5 d) were tested.

The effect of the variations in operating parameters (HRT or OLR) on dissolved chemical oxygen demand (COD_D), VFA, total volatile solids (TVS), hydrogen production (HP), methane production (MP), GP, sulphide production (SP), microbial population and microbial activities to improve the two-phase dry-thermophilic AD process of sulphate-containing municipal solid waste was studied at laboratory scale. FISH was used to determine the main groups involved in the anaerobic process.

2. Methods

2.1. Experimental equipment and operating condition

Two laboratory-scale continuously stirred tank reactors (CSTRs) were employed. The first reactor, dedicated to the HP (first phase), had a 5.5 l working volume, while the second reactor (second phase) dedicated to the MP had a 5 l working volume, both heated by recirculating water through a thermostatic jacket. A PRECISTERM 6000142/6000389 (SELECTA S.A.) baths were used, with a maximum capacity of 7 l of water. The stainless steel reactors lid have a diameter of 200 mm and contain three openings, one for the biogas outlet, a feed inlet and another opening for the stirring system. The bottoms of the reactors have a discharge valve with a 40 mm i.d., used for sampling. The biogas was collected in 40 l capacity Tedlar (a polyvinyl fluoride plastic polymer) bags. The bags are 29.8 cm wide and 45.7 cm long. The stirring systems consist of an IKA EUROSTAR Power Control visc-P4 overhead stirrer coupled to a stainless steel blade with scrapers which allows homogenisation of the waste at a speed of 23 rpm. In CSTRs without recycling of solids, the solid retention time (SRT) and HRT are equal.

The experimental test was divided in eight periods or conditions (runs). A start up phase and a steady state condition protracted for at least 3 consecutive HRTs were clearly defined for any experimental run (except at Run VIII, because the destabilisation was observed). The operational conditions start up and steady state condition periods are shown in Table 1. The whole experiment length was 398 d (Run I, 0–100; Run II, 101–189; Run III, 190–257; Run IV, 258–303; Run V, 304–337; Run VI, 338–368; Run VII, 369–395; Run VIII, 396–398).

2.2. Inoculum, substrate and feeding

The seed used as inoculum for the methanogenic reactor was collected from a single phase dry-thermophilic AD of OFMSW; while inoculum for the acidogenic reactor was collected from an H_2-producing reactor. The total solid (TS) concentration and TVS in the methanogenic inoculum were 67 g/kg and 33 g/kg and in the acidogenic inoculum these were 82 g/kg and 50 g/kg, respectively.

The tested substrate in the first phase was the OFMSW from the 30 mm trommel of the municipal solid waste treatment plant in Cadiz, Spain. The OFMSW was stored in 25 kg drums at −4 °C to avoid anaerobic degradation by the microorganisms found in the solid waste itself. The TS concentration of the feed first reactor was adjusted to 20% (which is characteristic of dry AD) by adding tap water. Characterisation of the substrate used in the assay is showed in Table 2.

The tested substrate in the second phase was the effluent of the first phase.

In the first reactor NaOH 10 M was added to the substrate when the pH of the effluent was below 5.3. In the second reactor, any control of pH was realised.

About the feeding regime, both reactors were fed once a day (semi-continuous).

2.3. Analytical methods

The analytical determinations made in this study can be grouped in two categories: physical–chemical analysis and microbiological analysis.

2.3.1. Physical–chemical analysis

For the control of the reactors the following parameters were determined: Total chemical oxygen demand (COD_T), COD_D, alkalinity, sulphate, TVS, pH, total volatile fatty acids (TVFA), acetic, butyric, propionic, volume and composition of the biogas (H_2, CH_4, CO_2 and H_2S). These determinations were performed according to APHA (1995) and Fdez-Güelfo et al. (2010). The sulphates were analysed from the filtrate supernatant obtained by means effluent sample lixiviation (10 g of digested waste in 100 ml of Milli-Q