Bioresource Technology 136 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Archaeal community dynamics and abiotic characteristics in a mesophilic anaerobic co-digestion process treating fruit and vegetable processing waste sludge with chopped fresh artichoke waste

M. Ros [a,*], I.H. Franke-Whittle [b], A.B. Morales [c], H. Insam [b], M. Ayuso [c], J.A. Pascual [a]

[a] Centro de Edafologia y Biologia Aplicad del Segura CEBAS-CSIC, Campus Universitario de Espinardo, 30100 Murcia, Spain
[b] Leopold-Franzens University, Institute of Microbiology, Technikerstraße 25d, 6020 Innsbruck, Austria
[c] Centro Tecnológico de la Conserva y Alimentación, Avda. de la Concordia s/n, Molina de Segura, 30500 Murcia, Spain

HIGHLIGHTS

- Co-digestion of fruit and vegetable waste sludge with artichoke waste enhanced biogas production.
- *Methanosaeta* and *Methanosarcina* dominanted the methanogenic community.
- *Methanosarcina* numbers increased upon co-digestion with chopped fresh artichoke waste.
- *Methanosaeta* numbers decreased upon co-digestion with chopped fresh artichoke waste.

ARTICLE INFO

Article history:
Received 13 December 2012
Received in revised form 18 February 2013
Accepted 19 February 2013
Available online 26 February 2013

Keywords:
Anaerobic digestion
Sequential batch reactor
Co-digestion
Real time PCR
ANAEROCHIP

ABSTRACT

This study evaluated the feasibility of obtaining methane in anaerobic digestion (AD) from the waste products generated by the processing of fruit and vegetables. During the first phase (0–55 d) of the AD using sludge from fruit and vegetable processing, an average value of 244 ± 88 L kg^{-1} dry matter $d^{-1}$of biogas production was obtained, and methane content reached 65% of the biogas. Co-digestion with chopped fresh artichoke wastes in a second phase (55–71 d) enhanced biogas production, and resulted in an average value of 354 ± 68 L kg^{-1} dry matter d^{-1}, with higher methane content (more than 70%). The archaeal community involved in methane production was studied using the ANAEROCHIP microarray and real-time PCR. Results indicated that species of *Methanosaeta* and *Methanosarcina* were important during the AD process. *Methanosarcina* numbers increased after the addition of chopped fresh artichoke, while *Methanosaeta* numbers decreased.

1. Introduction

The processing of fruit and vegetables to produce finished consumable products (juices, cans, frozen fruits and vegetables) results in the generation of significant amounts of organic wastes (10–65% of the raw material) and sludge from waste water (2–8 tonnes sludge per 100 tonnes of processed raw material). Currently, such organic wastes are used as animal feed, or they are landfilled, although their use in landfill does not meet current legislation, as such organic wastes are highly biodegradable (Misi and Froster, 2001) and a source of nuisance in landfills (odour problems, atmospheric pollution with greenhouse gases) due to their high organic matter content. Fruit and vegetable processing waste and sludges are, however, suitable input materials for anaerobic digesters (AD), allowing the production of biogas, a renewable energy that helps to reduce dependency on fossil fuels and contributes positively to national economies (Mata-Alvarez et al., 2000).

Anaerobic digestion occurs via four main steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Demirel and Scherer, 2008). The anaerobic co-digestion of different wastes has been shown to enhance biogas production and result in a more efficient waste treatment (Mata-Alvarez et al., 2000; El-Mashada and Zhang, 2010), probably due to an improved nutrient ratio with mixed substrates and enhanced pH buffering capacity. Methanogens hold the key position in the anaerobic digestion process because it is in this last step of the process where the valuable methane is produced; for this reason, to maximise methane production and biogas yield, it is necessary to understand the pathway and to enhance the growth and activity of these key taxa.

Molecular techniques based on the comparative analysis of 16S rDNA sequences are currently standard tools in microbial ecology

* Corresponding author. Tel.: +34 968396200.
E-mail address: margaros@cebas.csic.es (M. Ros).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.02.058

studies, and have allowed the discovery and quantification of many novel and non-culturable bacteria. Nucleic acid microarrays represent one of the most recent advances in molecular technologies, and allow a high-throughput format for the parallel detection of 16S rRNA genes from an environmental sample (Bodrossy and Sessitsch, 2004). The ANAEROCHIP microarray offers the possibility to analyse an entire array of methanogens concerning their presence or absence in a particular sludge sample in a single experiment (Franke-Whittle et al., 2009a). Microarray technology has also been applied and used successfully in other habitats and with different target microorganisms e.g. hypersaline cyanobacterial mats (Loy et al., 2002), soils (Small et al., 2001) and composts (Franke-Whittle et al., 2009b).

The aim of this work was to investigate the performance and biogas production of an AD reactor treating sludge from fruit and vegetable processing (artichoke and pepper) and supplemented with chopped fresh artichoke waste. Archaeal communities present in the anaerobic digester sludge at different times of the process were monitored using the ANAEROCHIP microarray, and quantified using real-time PCR.

2. Methods

2.1. Physical and chemical parameter analysis of sludge

Electrical conductivity (EC) and pH of sludge were measured directly with a micro conductivity metre GLP 32 (Crison, Alella, Spain) and micro pH metre GLP 21 (Crison, Alella, Spain), respectively. Total nitrogen was determined by the Kjeldahl method. Total organic carbon (TOC) was determined by the method described by Yeomans and Bremner (1989). Total solids (TS) and volatile solids (VS) were measured according to standard methods for examination of water and wastewater (APHA, 1995). VFA were measured by titration with H_2SO_4. VFA = (volume (ml) of H_2SO_4 from pH 5.0 to pH 4.4 $\times$ 1.66) $\times$500.

2.2. Reactor operation and sampling

2.2.1. Start-up

The experiment was performed using an anaerobic continuously stirred tank reactor (8–10 rpm) with 300 L working volume capacity. The reactor was initially charged with 215 L of mesophilic urban sewage sludge and 20 L sludge from a fruit and vegetable SBR (sequential batch reactor), and heated up to 35 °C. Table 1 shows the physico-chemical properties of the raw materials.

2.2.2. Single digestion process

The reactor was fed daily with 12 kg of sludge (fresh weight) from three fruit and vegetable processing plants (a canning company and two companies producing frozen artichoke and pepper) and an equivalent amount of effluent sludge was removed, corresponding to a hydraulic retention time (HRT) of 22.5 d.

2.2.3. Co-digestion

Once steady state conditions were achieved in the reactor, co-digestion of the fruit and vegetable processing sludge with chopped fresh artichoke waste (60/40) was started (55 d after the beginning of AD). The physico-chemical properties of the mixture are shown in Table 1. A flow rate of 11.75 kg d^{-1} (fresh weight) corresponding to a hydraulic retention time (HRT) of 30 d, was used.

Gas production was quantified daily using a Milligascounter©1 (Ritter, Germany) located on the top of the reactor. Methane production was measured with a Multi Gas Monitor X-am 7000 (Dräger, Spain). Effluent sludge samples were collected at different times after the start of the process (0, 24, 30, 59 and 71 d) from a tap located at the bottom of the reactor.

2.3. DNA extraction

DNA was extracted from 200 mg of pelleted sludge material, obtained after the centrifugation of 1 ml of sludge for 20 min at 13,000 rpm, using the Fast DNA Spin Kit for soil (BIO 101, USA), following the manufacturer's instructions. DNA was subjected to electrophoresis in 1.5% (w/v) agarose gels stained with ethidium bromide and visualised under UV light.

2.4. ANAEROCHIP microarray analysis

The 109F (5′-ACKGCTCAGTAAC ACGT-3′) and 934R (5′-GTGCTCCCCCGCCAATTCCT-3′) primers were used to amplify the 16S rRNA gene of methanogens in the sludge samples by PCR, as described by Franke-Whittle et al. (2009a). Single-stranded Cy5-labelled PCR product was generated using Lambda exonuclease and hybridised on ANAEROCHIP microarrays at 55 °C for 4 h. Arrays were washed after hybridisation, and a ScanArray Gx microarray scanner (Perkin Elmer, MA, USA) was used to scan hybridised microarray slides. The ScanArray Gx software (Perkin Elmer, MA, USA) was used to analyse fluorescent images, as described by Franke-Whittle et al. (2009a). For all spots, the median foreground and background signals were determined. The signal to noise ratio (SNR) for all spots was calculated using the following calculation: $SNR = [I_p - (I_{np} - I_{bnp})]/I_{bp}$ where I_p is median intensity of fluorescence of the probe, I_{np} is the median intensity of fluorescence of the nonbinding control probe, I_{bnp} is the median intensity of fluorescence of the background area around the nonbinding control probe, and I_{bp} is the median intensity of fluorescence of the background area around the probe. Signals were treated as positive if a SNR value of $\geqslant 2$ was obtained (Loy et al., 2002).

2.5. Real-time quantitative PCR

Sludge DNA extracted from samples was subjected to real-time PCR amplification with the genus-specific primers 240F (5′-CCTATCAGGTAGTAGTGGGTGTAAT-3′)/589R (5′-CCCGGAGGACTGACCAAA-3′) for *Methanosarcina*, and MS1b (5′-CCGGCCGGA-

Table 1
Physico-chemical properties of raw materials.

Parameters	Urban sewage sludge	Fruit and vegetable sludge[a]	Fruit and vegetable sludge + chopped fresh artichoke waste[a]
pH	7.8	6.35 (5.93–6.76)	5.85 (5.32–6.41)
EC (μS cm^{-1})	14,000	3492 (2750–4090)	6883 (5080–8810)
Dry matter (%)	1.4	2.94 (1.79–4.09)	8.32 (7.36–9.84)
Total organic carbon (TOC) (%)	31.5	48.87 (45.64–50.97)	50.82 (48.79–51.53)
Hydrosoluble C (mg kg^{-1})	nd	3000	15,000
Total nitrogen (N) (%)	12.86	8.17 (7.87–8.45)	5.05 (4.08–6.6)
C/N ratio	2.45	(5.79–6.03)	10.06 (11.96–7.81)

Values between parentheses mean a range minimum and maximun; nd: not detected.
[a] Average value of a mixture 60/40 (sludge/chopped fresh artichoke waste).