Bioresource Technology 123 (2012) 507–513

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Enhanced methane production from pig manure anaerobic digestion using fish and biodiesel wastes as co-substrates

Leticia Regueiro [a], Marta Carballa [a,*], Juan A. Álvarez [b], Juan M. Lema [a]

[a] Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Rúa Lope Gómez de Marzoa s/n, 15782 Santiago de Compostela, Spain
[b] AIMEN Technological Center, C/Relva, 27 A – Torneiros, 36410 Porriño Pontevedra, Spain

HIGHLIGHTS

- Fish and biodiesel waste were used as co-substrates in pig manure anaerobic digestion.
- Both co-substrates improved methane yield but caused VFA and ammonium accumulation.
- Shorter HRT and FW < 10% in the feeding allow to control ammonium inhibition.
- Biodiesel waste co-digestion requires feeding shares < 6% and/or fed-batch operation.
- The poorer the co-digester operation, the higher the *Methanosarcina*/*Methanosaeta* ratio.

ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form 26 July 2012
Accepted 28 July 2012
Available online 7 August 2012

Keywords:
Agroindustrial wastes
Ammonia
Co-digestion
Methanosarcina
Volatile fatty acids

ABSTRACT

Co-digestion of pig manure (PM[1]) with fish (FW[2]) and biodiesel waste (BW[3]) was evaluated and compared with sole PM digestion. Results indicated that co-digestion of PM with FW and/or BW is possible as long as ammonium and volatile fatty acids remained under inhibitory levels by adjusting the operating conditions, such as feed composition, organic loading rate (OLR) and hydraulic retention time (HRT). PM and FW co-digestion (90:10 and 95:5, w/w[4]) was possible at OLR of 1–1.5 g COD/L d, resulting in biogas production rates of 0.4–0.6 L/L d and COD removal efficiencies of 65–70%. Regarding BW, good results (biogas production of 0.9 L/L d and COD elimination of 85%) were achieved with less than 5% feeding rate. Overall, operating at the same OLR, the biogas production and methane content in the co-digester was higher than in the only PM digester.

1. Introduction

In the last years, anaerobic digestion of animal wastes has been promoted in order to avoid the uncontrolled emissions of CH_4 during storage (Novak and Fiorelli, 2010). Pig manure (PM[5]) can be an excellent base substrate for anaerobic digestion due to its inherent buffering capacity and high content of a wide range of nutrients required for the development of anaerobic microorganisms. However, PM has a low biogas yield, around 20–30 m^3/ton (Angelidaki and Ellegaard, 2003), and high ammonium concentrations (2–3 g N–NH_4^+/L). Consequently, PM is preferably co-digested with high carbon content wastes, on one hand, to improve the C/N ratio (Hartman and Ahring, 2006), and on the other hand, to increase the biogas production, essential for the plant's economy. It has been shown that bioenergy production in farm biogas plants could be enhanced by 80–400% by using organic wastes and by-products as co-substrates (Braun and Wellinger, 2003; Weiland, 2010). Despite the well-known reported co-digestion benefits, such as optimum humidity and C/N ratio or inhibitory substances dilution (Mata-Álvarez et al., 2000), it is not clear whether some substrates have adverse impact when they are co-digested with another waste (Callaghan et al., 2002). Therefore, it is critical to obtain an optimal mixture of the available co-substrates as well as the optimum operating conditions which allow high biogas yields without compromising the stability of the process (Alvarez et al., 2010).

Fish and shellfish canning industry is an important sector in Galicia (NW of Spain), with around 65% of the total Spanish production and representing 45% of the Galician factories and 67% of the jobs (Garcia et al., 2003). This sector generates different

* Corresponding author. Tel.: +34 881 816020; fax: +34 881 816702.
E-mail addresses: leticia.regueiro.abelleira@gmail.com (L. Regueiro), marta.carballa@usc.es (M. Carballa), jaalvarez@aimen.es (J.A. Álvarez), juan.lema@usc.es (J.M. Lema).

[1] PM, pig manure;
[2] FW, fish waste;
[3] BW, biodiesel waste;
[4] w/w, wet weight basis;
[5] PM, pig manure;

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.07.109

solid wastes with a wide range of characteristics depending on the raw material processed (tuna, mussel, sardine, mackerel, etc.). In general, fish wastes (FW[6]) are protein-rich substrates, although they also contain important lipids. Protein-rich materials have a fast biomethanation, but their degradation products (ammonium) can inhibit the process as well (Chen et al., 2008). Ammonium inhibition is directly related to the concentration of the undissociated form (NH_3) or free ammonia (FA[7]), thus becoming more important at high pH levels. The inhibitory FA concentration varies depending on operational parameters such as origin of inoculum, substrate, pH and temperature (Alvarez and Liden, 2008). The reported FA inhibitory concentrations for mesophilic conditions range from 25 to 140 mg N-FA/L, whereas during the thermophilic digestion of cattle manure, higher values (around 390–700 mg N-FA/L) were tolerated after an initial acclimation period (Guerrero et al., 1997).

Biodiesel fuels have recently drawn much attention given that they have various advantages over petroleum-based fuels (Ito et al., 2005). The biodiesel production is carried out by catalyzed transesterification with alcohol (usually methanol). Beside desired methylesters, this process also generates few other products, including crude glycerol or biodiesel waste (BW[8]), oil-pressed cakes and washing water. BW is easily separated from the aqueous phase and it is composed mainly of glycerol. In many occasions, it also contains a significant fraction of lipids due to inefficient separation systems. It is estimated that 1 kg of crude glycerol is generated per 9 kg of biodiesel produced (Dasari et al., 2005). The important increase in the biodiesel production of the last years has resulted in crude glycerol surplus that implied a dramatic 10-fold decrease in biodiesel waste prices (Yazdani and Gonzalez, 2007), and consequently, crude glycerol is often regarded as a waste stream with an associated disposal cost. This biodiesel residue is readily digestible and can be easily stored over a long period, making it an ideal co-substrate for anaerobic digestion (Ma et al., 2008), whereas its digestion as sole substrate is not viable as no nitrogen would be available for microbial populations (Robra et al., 2010). Lipid-rich materials are known to have high methane production potentials (Hansen et al., 1999), but their degradation products, the long-chain fatty acids (LCFA), are known to be inhibitors of methanogenic microorganisms (Pereira et al., 2004). Besides, operational instabilities related to sludge flotation and washout are also reported (Jeganathan et al., 2006).

The aim of this work was to evaluate the use of fish waste and biodiesel waste as co-substrates to enhance the mesophilic anaerobic digestion of pig manure at laboratory scale. The effect of feeding mixture on process performance and microbial community composition was investigated. The results obtained in the co-digestion systems were compared with those obtained in a reactor treating only pig manure.

2. Methods

2.1. Wastes and inoculum

PM was taken from a sewer of a 150-pig fattener and sow farm, which collects both feces and urine. PM samples were homogenized, sieved to 2 mm and stored at 4 °C until use to minimize decomposition. Different batches of PM were used throughout the experiment (>200 days) due to the impossibility of storing the total amount required.

FW was delivered by a canning industry and it consisted of heads, tails, bones and viscera of tuna fish. FW was homogenized by grinding and stored at −20 °C. BW was taken from a biodiesel factory and was stored at 4 °C without pre-treatment. It contained mainly glycerol produced in the transesterification. One batch of FW and BW was sufficient for the complete study.

Anaerobic granular biomass from an internal circulation reactor treating brewery wastewater was used as inoculum, with an initial in-reactor biomass concentration of about 10 g VSS/L.

2.2. Anaerobic reactors

Experiments were carried out in three continuous stirred tank reactors (160 rpm, Heidolph RZR 2041), two co-digesters and one only-PM digester, constructed in methacrylate and with a working volume of 9.2 L, approximately. Reactors were operated at 35 °C by hot water recirculation. The applied feedstock mixtures were prepared every 2–3 days, diluted with tap water according to the applied organic loading rate (OLR), and stored at 4 °C prior to use. The digesters were fed manually after an equivalent volume of digester mixed liquor was removed. Temperature, pH, stirring speed and biogas production were monitored on-line. Other physico-chemical parameters (solids, chemical oxygen demand (COD), alkalinity, volatile fatty acids (VFA) and ammonium) were measured twice per week.

2.3. Operational strategy

The operation of the two co-digesters was identical. They were started-up with a mixture of PM-FW-BW (84:5:11 in wet weight (w/w) basis), which was the optimum mixture obtained from linear programming (Alvarez et al., 2010), at an OLR of 0.5 g COD/L d and a hydraulic retention time (HRT) of 40 d. After the start-up (day 0–19), the operation of the co-digesters can be divided in three periods according to the feeding blend, HRT and OLR applied. In period I (days 20–59), the reactors were fed with a mixture of PM and FW (90:10, w/w) at OLR of 1 g COD/L d and HRT of 35 d. In period II (days 60–115), the percentage of FW was decreased to 5% and the OLR and HRT were increased to 1.5 g COD/L d and decreased to 30 d, respectively. In addition, three pulses of BW (5 g COD/L) were added to the reactors on days 80, 90 and 100. In the last period (period III, days 116–200), FW was replaced by BW in order to prevent ammonia inhibition, the OLR was increased to 2 g COD/L d and the HRT was reduced to 25 d. In all periods, the feeding mixture was diluted with tap water to attain the proper OLR.

The start up of the only-PM digester with OLR of around 0.5 COD/L d and HRT of 20 d took 36 days. In period I (days 37–89), the OLR was increased up to 1 g COD/L d and the HRT was slightly lowered to 18 d in order to reach the target OLR, since the COD of PM was lower in this period. The OLR was further increased to 1.5 and 2 g COD/L d and the HRT was decreased to 15 and 12 d in periods II (days 90–104) and III (days 105–150), respectively. Since different batches of PM were used along the experiment (with different COD concentrations), dilution with tap water was not always required to attain the established OLR.

2.4. Analytical methods

pH, COD, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (N-TKN), ammonium ($N–NH_4^+$), TA (total alkalinity) and PA (partial alkalinity) were performed following standard methods (APHA, 1995). Biogas production was measured online by Ritter milligascounters (Dr. Ing. Ritter Apparatebau GmbH, Bochum, Germany) and biogas composition was analyzed by gas chromatography (HP, 5890 Series II). VFA (acetic, propionic, i-butyric, n-butyric, i-valeric and n-valeric) were analyzed by gas

[6] FW, fish waste;
[7] FA, free ammonia;
[8] BW, biodiesel waste;