Bioresource Technology 110 (2012) 18–25

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Biochemical methane potential (BMP) of food waste and primary sludge: Influence of inoculum pre-incubation and inoculum source

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ARTICLE INFO

Article history:
Received 5 December 2011
Received in revised form 5 January 2012
Accepted 7 January 2012
Available online 16 January 2012

Keywords:
Anaerobic digestion
Biochemical methane potential
Waste-to-inoculum ratio
Food waste
Pre-incubated inoculum

ABSTRACT

Biochemical methane potential tests were conducted to evaluate the effect of using a blank versus a pre-incubated inoculum in digestion of primary sludge at different waste to inoculum ratios (*S*/*X*). In addition, this study explored the influence of using two different anaerobic inoculum sources on the digestion of food waste: digested sludge from a municipal wastewater treatment plant and from a digester treating the organic fraction of municipal solid wastes. The results revealed that although there was no significant difference in methane yield (on average 114 mL CH_4/g $TCOD_{sub}$) or biodegradability (on average 28.3%) of primary sludge using pre-incubated or non-incubated inocula, the maximum methane production rates using non-incubated inoculum were higher than those using pre-incubated inoculum at all *S*/*X* ratios. Moreover, interestingly the inoculum from an anaerobic digester treating municipal wastewater sludge was superior over the inoculum from anaerobic digester treating food waste in digesting food waste.

1. Introduction

The recycling and treatment of the organic fraction of municipal solid waste is rapidly emerging as an effective waste management strategy that diverts wastes from landfills and recovers energy (Fernandez et al., 2001). In recent years, interest in biochemical methane potential (BMP) tests has increased as reflected by the wide range of research papers dealing with the BMP assays (Raposo et al., 2011). The BMP assay is best suited when used to elucidate what types of substrates, from an array of potential substrates, have the highest biomethane potential (Labatut et al., 2011). In addition, BMP assays can be used to estimate the optimum ratios between co-substrates when co-digestion is intended. Lastly, BMP assay results can be used to determine the extent of anaerobic biodegradability of substrates, and thus, relative residence times required for complete digestion (Labatut et al., 2011).

Although there is no *standard* detailed procedure for the BMP test, the various BMP studies followed very similar procedures; the only two main differences between BMP tests relate to consideration of the methane production from the inoculum and the inoculum source. For methane production from the inoculum, many researchers used the blank assay (Neves et al., 2004; Nallathambi Gunaseelan, 1995) approach described below, while some researchers prescribe the German guideline for fermentation tests (VDI-Handbuch, 2006), which entails pre-incubating the inoculum for about 5 days without substrate before using it, thus eliminating the need for the continued testing of both the seed blank and the sample waste. For the blank assay method, the background methane production from the inoculum (determined in blank assays with medium or water and no substrate) is subtracted from the methane production obtained in the substrate assays (Angelidaki et al., 2009). For the pre-incubated inoculum (German guideline), the inoculum should be "degassed" in order to deplete the residual biodegradable organic material present. Degassing should be protracted until no significant methane production is observed: typically 2–5 days of incubation (Raposo et al., 2011) are prescribed in the method. In some cases, e.g. when the inoculum is taken from a reactor fed with relatively high fat/oil concentration, longer periods of pre-incubation may be required, in order to eliminate all the residual (adsorbed/entrapped) substrate (Angelidaki et al., 2009).

A wide range of biomass has been considered as potential seed materials for methane production in BMP tests (Neves et al., 2004;

Abbreviations: BMP, biochemical methane potential; COD, chemical oxygen demand; CV, coefficient of variation; DOPF, Dufferin Organics Processing Facility; HRT, hydraulic retention time; JWPCP, Joint Water Pollution Control Plant; MMPR, maximum methane production rate; MMPRs, maximum methane production rates; MPR, methane production rate; *S*/*X*, waste to inoculum ratios; SCOD, soluble chemical oxygen demand; SRT, solid retention time; SSO, source separated organics; TCOD, total chemical oxygen demand; TS, total solids; TSS, total suspended solids; TVS, total volatile solids; VFAs, volatile fatty acids; VS, volatile solids; VSS, volatile suspended solids.

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0960-8524/$ - see front matter
doi:10.1016/j.biortech.2012.01.025

Hashimoto, 1989; Chynoweth et al., 1993; Owen et al., 1979). It has been observed that the results of the biodegradation tests could vary with the methodology followed. One of the most important variables, which influence BMP results, is the origin of the inoculum (Nyholm et al., 1984), since it determines the initial activity of the microorganisms used for the test. Furthermore, the inoculum source brings about differences in bacterial populations (Thouand et al., 1995), substrate adaptation (Barkay and Pitchard, 1988; Thouand and Block, 1993), and residual anaerobically-biodegradable substrate.

The concentration of microorganisms that are used in the tests will determine the biodegradation rates (Simkins and Alexander, 1984), the lag time (Chudoba et al., 1992), and the probability that the degradation of the substrate occurs during the test performance (Thouand et al., 1995).

There are different anaerobic biodegradation test methodologies, which seldom state inoculum characteristics. For example, some methodologies (Shelton and Tiedje, 1984; Birch et al., 1989; Pagga and Beimborn, 1993; ISO 11734, 1995) recommend anaerobic municipal wastewater treatment plant inoculum.

The waste to inoculum ratio (*S*/*X*) is an important parameter in batch high solids anaerobic digestion processes as well as in the assessment of anaerobic biodegradability of solid wastes (Neves et al., 2004). Although theoretically, the *S*/*X* ratio has an effect only on the kinetics, and not on the ultimate methane yield, which only depends on the organic matter content (Nallathambi Gunaseelan, 1995; Raposo et al., 2006), it is reported that too high *S*/*X* may be toxic while too low *S*/*X* may prevent induction of the enzyme necessary for biodegradation (Prashanth et al., 2006). This ratio also has an effect on the lag phase, which is shorter for low ratios (Chen and Hashimoto, 1996).

Each substrate has its optimum *S*/*X* ratio, considering the potential amount of volatile fatty acids (VFAs) produced and its capacity to buffer the medium due to the ammonium produced by the hydrolysis of proteins (Lesteur et al., 2010). A small amount of inoculum is preferred because of the endogenous biogas production, which can bias the results (Lesteur et al., 2010). Moreover, the increase in the *S*/*X* can lead to overloads due to volatile fatty acid accumulation (Neves et al., 2004). From another point of view, the inoculum concentration should always be high compared to that of the substrate (in term of volatile solids) and the *S*/*X* should be recognised as one of the major parameters affecting the results of anaerobic assays (Neves et al., 2004). Hashimoto (1989) and Labatut et al. (2011) reported that the minimum *S*/*X* ratio of 2 g VS substrate/g VS inoculum was required when digesting wheat straw at concentrations of 10–40 g VS/L and dairy manure at concentrations of $\geqslant 3$ g VS/L, respectively. However, in the case of more recalcitrant wastes (woody feed stocks and municipal wastes), the rate of methane production in BMP assays was optimum at *S*/*X* of 0.5 g VS substrate/g VS inoculum (Chynoweth et al., 1993). The *S*/*X* proposed by Owen et al. (1979) as a standard was approximately 1 g VS substrate/g VS inoculum.

Based on the above mentioned introduction, it is obvious that there is a wide range of the optimum or recommended *S*/*X* depending on the substrate and inoculum (some reported optimum *S*/*X* of 0.5 g VS substrate/g VS inoculum while other reported optimum *S*/*X* of 5.7 g $VS_{substrate}$/g $VS_{inoculum}$). Moreover the BMP test despite its wide use lacks standardization (some researchers recommended using blank assays while others recommended pre-incubating the inoculum prior use). Therefore, the primary purpose of the current work was to evaluate the two approaches i.e. the widely used blank seed assay versus the pre-incubated inoculum in digestion of primary sludge at different *S*/*X*. The secondary goals of this study were the assessment of the impacts of the seed source and *S*/*X* ratio on BMP results. In this study, three seeds from conventional mesophilic digesters, two from municipal wastewater treatment plant digesters and one from a digester treating the organic fraction of municipal solid wastes, were employed.

2. Methods

2.1. Substrates and inocula

Two substrates were used in this study, food waste i.e. organic fraction of municipal solid wastes and primary sludge from a municipal WWTP. The food waste was obtained from Dufferin Organics Processing Facility (DOPF) in Toronto, Ontario, Canada. The city of Toronto's DOPF receives approximately 25,000 metric tons/year of source separated organics (SSO) material from Toronto's residual Green Bin and the commercial Yellow Bag collection programs. The purpose of the DOPF is to separate the film plastic bin finer and contaminant materials fractions of the SSO from the organic material and convert the organic fraction into a material that is a suitable feedstock for the anaerobic digester (Van Opstal, 2006). The primary sludge was obtained from Joint Water Pollution Control Plant (JWPCP), Carson, California. The JWPCP provides both primary and secondary treatment for approximately 300 million gallons of wastewater per day. The characteristics of both substrates are presented in Table 1a.

Three inocula (anaerobic sludge) were used in this study; the first inoculum was collected from the primary mesophilic anaerobic digester at Guelph's wastewater treatment plant (Guelph, Ontario), the second inoculum was collected from the mesophilic anaerobic digester treating SSO at DOPF in Toronto, Ontario, and the third inoculum was obtained from a mesophilic anaerobic digester treating primary and secondary wastewater at JWPCP, Carson, California. The anaerobic digester at Guelph wastewater treatment plant is completely mixed reactor with solid retention times (SRTs) in the range of 14–18 days, that achieves VSS destruction efficiency of ≈45%. The anaerobic digester in DOPF at Toronto is a completely mixed reactor with a solids recycling system with hydraulic retention time (HRT) of approximately 17 days, SRT of approximately 27 days, VSS destruction efficiency of 62%. The anaerobic digester at JWPCP is a completely mixed reactor with HRTs (SRTs) in the range of 17–20 days, with VSS destruction efficiencies of 49–52%. The characteristics of the three inocula are presented in Table 1b. Inocula from both municipal WWTP were about 2.0% solids (w/w) while the DOPF inoculum was about 5.0% solids.

The primary reason for the selection of seed from digesters treating municipal biosolids and food waste is the difference in soluble COD concentrations between the two digesters. The food waste digester seed had five times higher soluble COD concentration than the Guelph municipal wastewater treatment plant digester. Thus, it is anticipated that the ratio of fermentative bacteria i.e. hydrolyzers, carbohydrates, proteins, and lipids degraders to acidformers and methanogens differs between the two seeds. The other reason for selection of food waste anaerobic digestion inoculum and food waste is the increasing number of these facilities worldwide in light of the push for less land filling and resource recovery.

Table 1a
Substrates characteristics.

Parameter	Units	Food waste	Primary sludge
TCOD	mg/L	113000 ± 2800[a]	42800 ± 180
SCOD	mg/L	60300 ± 350	8000 ± 470
TSS	mg/L	48400 ± 2700	26300 ± 260
VSS	mg/L	27900 ± 1300	20000 ± 250
TVFA	mg COD/L	260 ± 20	1680 ± 220
NH_4	mg/L	1670 ± 40	80 ± 20
pH	–	4.6 ± 0.2	5.0 ± 0.1
Alkalinity	mg $CaCO_3$/L	N.A.	2900 ± 180

[a] Values represents the average ± STD of three samples.