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# Effect of the solid content on anaerobic digestion of meat and bone meal

# Guangxue Wu, Mark Gerard Healy, Xinmin Zhan\*

Department of Civil Engineering, National University of Ireland, Galway, Ireland

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# ABSTRACT

The effect of the solid content on anaerobic digestion of meat and bone meal (MBM) was investigated in batch reactors at MBM solid contents of 1%, 2%, 5% and 10%. There was no significant difference in the specific methane (CH<sub>4</sub>) production potential with respect to the total volatile MBM solids (TVS) applied at these solid contents, which ranged from 351 to 381 ml CH<sub>4</sub>/g TVS. However, the highest CH<sub>4</sub> yield with respect to the removed volatile MBM solids (RVS) was 482 ml CH<sub>4</sub>/g RVS at the MBM solid content of 5%; the CH<sub>4</sub> yields were 384–448 ml CH<sub>4</sub>/g RVS at the other MBM solid contents. The lag time of CH<sub>4</sub> production rose with the increase in the solid content. The longer lag time at MBM solid contents of 5% and 10% was due to inhibition caused by high concentrations of volatile fatty acids (VFAs) and free ammonia in the reactors, but the inhibition was reversible. The production of VFAs during the digestion varied with solid contents: at the solid content of 1%, only acetic acid was detected; at 2%, both acetic and propionic acids were detected; and at 5% and 10%, acetic, propionic, butyric and valeric acids were detected. After 93-day digestion, the volatile MBM solid reduction was 92%, 91%, 79% and 80% at MBM solid contents of 1%, 2%, 5% and 10%, respectively.

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# 1. Introduction

Nowadays, a rapid increase in the meat consumption occurs in many countries. According to the EC Animal By-Products Regulations 2003 (S.I. No. 248 of 2003), Category II animal by-products, such as condemned meat, should be rendered at 133 °C and 3 bar pressure. Consequently, a large amount of meat and bone meal (MBM) is being produced from the rendering industry. In the past, MBM was used as animal food such as for cattle, sheep and goats. However, its use as an animal foodstuff has been banned in the EU to control the spread of transmissible spongiform encephalopathies (EC TSE Regulation, No. 999/2001). According to Purcell (2004), in Ireland, the annual MBM production is approximately 150,000 tons and subsequent to the banning of its exportation, due to lack of proper disposal technologies, MBM is mainly stored inland at an annual cost of €3.75 million.

The net calorific value of MBM is 17,000 kJ/kg (Conesa et al., 2005), which means that MBM has a high energy recovery potential. This should be considered when choosing a MBM disposal technology. Possible technologies for MBM disposal include incineration, landfill and anaerobic digestion. In the EU and the USA, incineration has been intensively investigated and widely used for MBM disposal with the aim of energy recovery (Conesa et al., 2005). However, dioxins and toxic gases such as sulfur dioxide (SO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) may be released during incineration

(Goeran et al., 2002; Conesa et al., 2005), causing air pollution. Landfill of MBM may lead to water pollution and pathogen transmission. In addition, the EU Landfill Directive (99/31/EC) has set targets for diverting the biodegradable fraction of municipal waste from landfill sites. Its targets are reductions of 25%, 50% and 65% of 2001 levels by 2010, 2013 and 2020, respectively. This Directive limits the application of landfill to dispose of biodegradable MBM waste. Anaerobic digestion, which has been widely used for wastewater and solid waste treatment (Callaghan et al., 1999; Alvarez and Liden, 2008), is a sustainable and environmentally friendly technology for MBM disposal. During anaerobic digestion of MBM, the biogas produced, which mainly consists of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>), can be used as fuel or to generate electricity. Therefore, anaerobic digestion of MBM is a sound management strategy, with respect to EU polices on greenhouse gas emissions reduction and biofuel production. This is also in accordance with the national strategy of Ireland on energy demands and waste management.

The applied solid content in association with the substrate loading rate is critical to the cost, performance and stability of anaerobic solid waste digesters (Lissens et al., 2001; Alvarez and Liden, 2008). Its effects on anaerobic digestion of olive mill solid residue, poultry slaughterhouse waste, and ruminal and blood waste have been investigated (Lopez et al., 2006; Rincon et al., 2008). Generally, with increasing the solid loading rate, the total CH<sub>4</sub> yield increases, while efficiencies of both the solid reduction and the solid conversion to CH<sub>4</sub> gas decrease (Bujoczek et al., 2000; Lopez et al., 2006). At high loading rates, methanogenic activities might





<sup>\*</sup> Corresponding author. Tel.: +353 91 495239; fax: +353 91 494507. *E-mail address*: Xinmin.Zhan@nuigalway.ie (X. Zhan).

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be inhibited by high concentrations of long-chain fatty acids, volatile fatty acids (VFAs) and free ammonia (NH<sub>3</sub>). However, Lopez et al. (2006) and Rincon et al. (2008) observed that inhibited CH<sub>4</sub> production could be recovered after the acclimation of methanogens to new environmental conditions. No studies have reported on anaerobic digestion of MBM at various organic loading rates or solid contents.

In this study, anaerobic digestion of MBM at four MBM solid contents (1%, 2%, 5% and 10%) was investigated in batch reactors. The aim of this study was to examine the  $CH_4$  production potential of MBM and the effect of the solid content on anaerobic digestion of MBM.

# 2. Methods

#### 2.1. Substrate characterization

The MBM (dry solid) used in this study was provided by Western Proteins, Co. Mayo, Ireland. Its volatile solids (VS) to total solids (TS) ratio was 75%.

#### 2.2. Batch anaerobic digestion experiment

Anaerobic digestion of MBM was examined using batch experiments at MBM solid contents of 1%, 2%, 5% and 10%. Ten reactors, made from Shocht glass flasks and each with an effective volume of 1175 ml, were used in the trial with two replications at each solid content. Two reactors, operated without the addition of MBM, were used as the control. Each reactor was sealed with screw caps and the caps had two sampling holes: one for liquid sampling and the other for gas sampling.

All reactors were filled with 400 ml of anaerobic seed sludge with a biomass concentration of 5 g/l; the seed sludge was taken from the anaerobic digester in Moycullen Municipal Wastewater Treatment Plant, County Galway, Ireland. Then, 0, 6, 12, 30 and 60 g of MBM solids were added into the reactors, corresponding to MBM solid contents of 0%, 1%, 2%, 5% and 10%, respectively. Finally, tap water was added to all reactors to bring the final volume to 600 ml in each reactor. The pH in all reactors was adjusted to 7.0 by using 1 M HCl or 1 M NaOH and all reactors were purged with nitrogen gas to remove oxygen. The reactors were sealed and placed in an oven at a constant temperature of 35 °C. The reactors were shaken manually for 1 min twice daily. Liquid and gas samples were taken at regular time intervals. The volume of the gas produced was measured by displacement of water in a gas collection bottle at 12-h or 24-h intervals. The CH<sub>4</sub> proportion in biogas was measured to calculate the yield of CH<sub>4</sub>.

#### 2.3. Analytical methods

After liquid samples were taken from the reactors, the pH was measured immediately. The liquid samples were centrifuged at 3900 rpm for 10 min and then at 18,000 rpm for 20 min at 4 °C. The supernatants were tested for soluble chemical oxygen demand (COD) and ammonium nitrogen (NH<sub>4</sub>–N). For the analysis of VFAs, the supernatants were further filtered through 0.45  $\mu$ m cellulose nitrate membrane filter paper (Whatman, England), and then VFAs were measured with high performance liquid chromatography (HPLC, Agilent 1200, Agilent Technology, USA) using a UV index detector and an Aminex HPX-87H column (Bio-Rad, USA). Separation during HPLC tests was achieved using a mobile phase of 1‰ H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.6 ml/min and the column temperature was 65 °C. The detector temperature was 40 °C. The VFAs mix (Sigma–Aldrich, USA) was used as the standard for HPLC calibration.

TS, VS and COD were determined according to standard methods (APHA, 1995). The NH<sub>4</sub>–N concentrations in the water samples were analyzed using a Konelab analyzer (Thermo Clinical Labsystems, Vantaa, Finland). NH<sub>3</sub> in the liquid phase was calculated after Anthonisen et al. (1976):

$$\mathrm{NH}_3 = \frac{\mathrm{NH}_4^+ 10^{\mathrm{pH}}}{10^{\mathrm{pH}} + e^{6344/(273+t)}}$$

where *t* is the temperature in °C. The CH<sub>4</sub> proportion in the biogas was measured using a 7890A gas chromatograph (Agilent Technology, USA) with a thermal conductivity detector and a 45–60 mesh, matrix molecular sieve 5A column (Sigma–Aldrich, USA). Helium gas was the carrier gas at a flow rate of 30 ml/min. The temperatures of the injection inlet, oven and detector were 100 °C, 60 °C and 105 °C, respectively.

#### 3. Results

# 3.1. Methane production

In this study, the deviation between the data tested in each pair of parallel reactors was within 10% so only average data calculated were presented. The methane yields of anaerobic digestion of MBM at the four tested solid contents were calculated by subtracting the amount of methane yielded in the control reactors (without the addition of MBM) from the amount of methane yielded in the reactors with MBM added at corresponding solid contents. The cumulative volume of CH<sub>4</sub> production increased with increasing solid contents, with an overall production of 1.6, 3.2, 8.6 and 16.2 l at solid contents of 1%, 2%, 5% and 10%, respectively. The proportion of CH<sub>4</sub> in biogas was around 40% initially and rose to 85–90% within 7 days. There was no significant difference between the specific CH<sub>4</sub> yield with respect to the applied total MBM volatile solids (TVS) at the four solid contents (Fig. 1). It ranged from 351 to 381 ml  $CH_4/g$  TVS. However, the maximum  $CH_4$  production rate decreased with increasing solid contents in the range of 1-10%. The maximum production rates were 39.7 ml  $CH_4/g$  TVS  $\cdot$  d, 25.6 ml  $CH_4/g$  TVS · d and 13.5 ml  $CH_4/g$  TVS · d at solid contents of 1%, 2% and 5%, respectively. There were two rapid CH<sub>4</sub> production periods at the solid content of 10%: the CH<sub>4</sub> production rates of 9.3 ml CH<sub>4</sub>/g TVS · d during Hours 168-360 and 12.8 ml CH<sub>4</sub>/g TVS · d during Hours 672-984 from the start of the experiment. The steady-state at which no further methane accumulation was observed was reached after 10, 15, 35 and 72 days of operation in the reactors with solid contents of 1%, 2%, 5% and 10%, respectively.

#### 3.2. Hydrolysis and acidification

Hydrolysis and acidification are two important steps before  $CH_4$  production. Soluble COD dynamics in the reactors are shown in Fig. 2. Except at the 10% solid content where two soluble COD concentration peaks were observed, at all other solid contents, there was an initial increase in the soluble COD concentration, and then a gradual decrease with respect to time.

The total VFA concentration peaked at Hours 12, 48, 192 and 192 at 1%, 2%, 5% and 10% solid contents, respectively (Fig. 3). In addition, two peaks were observed at the 10% solid content. The highest total VFA concentration was approximately 12,745 mg acetic acid equivalent/l at the 5% solid content, and 27,242 mg acetic acid equivalent/l (the first peak) and 24,584 mg acetic acid equivalent/l (the second peak) at the 10% solid content. The highest total VFA concentration increased with increasing solid contents. Regarding the acidification process, different profiles of VFAs were detected at the four solid contents. At the solid content of 1%, only

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