



## Anaerobic co-digestion of dairy manure, meat and bone meal, and crude glycerol under mesophilic conditions: Synergistic effect and kinetic studies



Petra J. Andriamanohiarisoamanana<sup>a</sup>, Aya Saikawa<sup>a</sup>, Kumiko Tarukawa<sup>a</sup>, Guangdou Qi<sup>a</sup>, Zhifei Pan<sup>a</sup>, Takaki Yamashiro<sup>a</sup>, Masahiro Iwasaki<sup>a</sup>, Ikko Ihara<sup>b</sup>, Takehiro Nishida<sup>a</sup>, Kazutaka Umetsu<sup>a,\*</sup>

<sup>a</sup> Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

<sup>b</sup> Graduate School of Agriculture Science, Kobe University, Kobe 657-8501, Japan

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### ABSTRACT

Anaerobic digestion is a potential renewable energy, climate independent and robust technology, which is able to treat different kinds of organic wastes and by-products. This study investigated the anaerobic co-digestion of meat and bone meal (MBM) with dairy manure (DM) and crude glycerol (CG). Three sets of batch experiments were conducted at mesophilic condition; one set of anaerobic mono-digestion and two sets of anaerobic co-digestion. In experiment I, each substrate was mono-digested at inoculum to substrate ratio of 1. In experiment II, MBM and DM were co-digested at ratios of 1.0:1.0, 1.0:2.0, 1.0:1.0, and 2.0:1.0, while in experiment III CG was co-digested with MBM at ratios of 1.0:3.0, 1.0:1.0 and 3.0:1.0, at a fixed amount of DM. The results of anaerobic mono-digestion showed that CG produced the highest methane yield (0.48 L/gVS) followed by MBM (0.41 L/gVS) and DM (0.17 L/gVS). In the anaerobic co-digestions, methane yield increased with the increase of MBM content, while it increased together with CG content. The kinetic studies showed that the physico-chemical characteristics of the co-digested substrates influenced hydrolysis rate constant and lag-phase, which increased with the increase of CG content. However, synergistic effect was decreased when MBM content was increased, whereas the opposite was observed to that with CG. Therefore, carbon to nitrogen ratio was an important parameter determining synergistic effect in anaerobic co-digestion, while the physico-chemical characteristics influenced the hydrolysis rate constant and lag-phase.

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### Introduction

Since the 1900s meat and bone meal (MBM) has been utilized in live-stock industries as a source of protein (Swisher, 2006). At the beginning of the 1980s, the demand of rendered animal proteins to animal fed has increased dramatically until the 1990s, when bovine spongiform encephalopathy (BSE) outbreak in many countries, which is associated with MBM contaminated with a protein called “prions” (Swisher, 2006; Conesa et al., 2005). As a consequence, the utilization of MBM in animal feed was restricted in the European Union in 2000 (Mondini et al., 2008), while it was in 2001 for Japan after the first case of BSE outbreak (Sugiura et al., 2014). Thereafter, MBM was considered as a waste and required disposal technologies to cope with the million tons of MBM produced annually (e.g., 3 million tons in Europe (Cyr and Ludmann, 2006) and 4 million tons in the U.S. (Swisher, 2006)). Incineration or melting in a cupola furnace is among the most common disposal technology for MBM, which was widely used (Conesa et al., 2005). However, due to the large amount of MBM produced from rendering plant, the

insufficiency of incinerator capacity was a challenge. Therefore, other alternatives were considered such as the use of MBM in cement manufacturers and in agriculture (Mondini et al., 2008; Cyr and Ludmann, 2006). In May 2013 Japan was declared as a BSE free country (OIE, 2013), and hence Japanese Government allowed the use of MBM as organic fertilizer since June 2014.

Since MBM has a calorific value of 17.1 MJ/kg (dry weight) (Soni et al., 2009), its utilization in anaerobic digestion is of interest to recover energy in the form of methane. The utilization of MBM in anaerobic digestion has been reported by Wu et al. at different total solid contents (Wu et al., 2009a) and thermochemical pretreatments (Wu et al., 2009b). They found that the highest methane yields were observed at a MBM solid contents of 5% (0.38–0.45 L/gVS<sub>removed</sub>) (Wu et al., 2009a), and with alkaline pretreatment (NaOH) at 131 °C (0.46–0.56 L/gVS MBM) (Wu et al., 2009b). However, anaerobic mono-digestion of MBM is not always practicable and is a challenging process due to the accumulation of free ammonia nitrogen (Wu et al., 2009a), which can penetrate into microbial cells and disturb cellular homeostasis (Kayhanian, 1999). Different approaches have been used to overcome ammonia inhibition in anaerobic digestion such as dilution of substrates (Kayhanian, 1999; Hejnfelt and Angelidaki, 2009), the combination of digester with an electrochemical

\* Corresponding author.

E-mail address: umetsu@obihiro.ac.jp (K. Umetsu).

system to extract ammonium (Desloover et al., 2015), the combination of AD process with a microbial electrolysis cell coupled to ammonia stripping and adsorption unit to recirculate effluent (Cerrillo et al., 2016), and optimization of feed composition and carbon to nitrogen (C/N) ratio (Nurliyana et al., 2015; Wang et al., 2012). Among them, the adjustment of C/N ratio through anaerobic co-digestion sounds to be the most environmentally friendly and economically profitable to address ammonia inhibition because it does not reduce the digester performance (in the case of dilution) or required additional investment for another unit (in the case of microbial electrolysis cell or electrochemical system). To the best of our knowledge, anaerobic co-digestion of MBM with low or high C/N ratio substrates has not been yet investigated.

Aside C/N ratio adjustment, co-digestion of different substrates helps to stabilize anaerobic digestion process by supplying optimal moisture content and pH, enhancing buffer capacity, balancing essential nutrients and trace metals, and diluting potential inhibitory or toxic compounds (Esposito et al., 2012). Dairy manure (DM) is a high moisture substrate (more than 87%), and is recognized to be an excellent “carrier” substrate that has been used in different anaerobic co-digestion processes as the base substrate (Andriamanohiarisoamanana et al., 2016; Atandi and Rahman, 2012; Angelidaki and Ellegaard, 2003), as it is widely available (Yabe, 2013) and contains the biomass population to produce methane. It can be, therefore, co-digested with MBM, which is a low moisture substrate (less than 3%). However, the co-digestion of low C/N ratio substrates (DM and MBM) may be challenged by the exceedance of nitrogen for microorganism's growth and, probably, leads to the accumulation of free ammonia nitrogen.

Crude glycerol (CG), which is a by-product of biodiesel making companies, is commonly used as a co-substrate in anaerobic co-digestion. It has a C/N ratio ranging between 248:1 and 275:1 (Castrillón et al., 2013; Chen et al., 2008), and has been used in anaerobic co-digestion with nitrogen-rich substrate to adjust to an optimum C/N ratio of 16–33 (Mata-Alvarez et al., 2014) and to boost biogas production (Wohlgemut et al., 2011; Fountoulakis et al., 2010). Various nitrogen-rich organic wastes have been anaerobically co-digested with CG such as cattle manure (Castrillón et al., 2013), swine manure (Wohlgemut et al., 2011) and sewage (Fountoulakis et al., 2010). A significant improvement of biogas production (four times) was observed by Kato et al. (2010) at an addition of CG at ratio of 6% in a laboratory-scale experiments of anaerobic co-digestion with DM. Similarly, an improvement by about two times was observed by Andriamanohiarisoamanana et al. (2016) when DM was co-digested with CG in a thermophilic farm-scale biogas plant. Because MBM has low C/N ratio, the anaerobic co-digestion of MBM with CG is, therefore, an alternative to adjust C/N ratio to an optimum value, to increase methane production from MBM and also to properly manage the projected 4.6 million tons of CG in 2020 (Viana et al., 2012).

The main objective of this study was to investigate the effects of MBM on the improvement of methane yield and process stability in anaerobic co-digestion of DM and CG under mesophilic conditions. The first specific objective was to determine the methane yield of the anaerobic mono-digestion of DM, MBM and CG (experiment I). The second specific objective was to investigate the methane yield and process stability of anaerobic co-digestion of DM and MBM (experiment II). The third specific objective was to determine the effects of anaerobic co-digestion of MBM and CG using DM as main substrate on process performances (experiment III). To obtain useful information for academic and practical applications, experimental and mathematical approaches were undertaken. Particularly, focus was given on synergistic effect and kinetic studies.

## Materials and method

### Materials

Fresh dairy manure (DM) was obtained early in the morning from the free stall barn of 70 lactating Holstein cows located in Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido,

Japan. DM was stored at 4 °C before its use on the next day. Meat and bone meal (MBM) was obtained from a rendering company in Hokkaido. MBM was kept in a freezer at –20 °C until use in order to prevent any change in terms of characteristics and components. The characteristics and components of MBM are shown in Table 1. Crude glycerol (CG) was obtained from a local biodiesel-making company that transforms used cooking oils into biodiesel fuel for local transportation, especially busses and taxis. Methanol was used for transesterification with potassium hydroxide as a catalyst. The characteristics of DM and CG are reported elsewhere (Andriamanohiarisoamanana et al., 2016).

Inoculum was obtained from an active mesophilic biogas reactor treating primarily dairy manure. Inoculum was collected a day before the start of experiment and kept at 4 °C until use. All substrates and inoculum were characterized by measuring total solids content (TS) and volatile solids content (VS) before the start of the experiments.

### Experimental setup

Laboratory scale batch experiments were conducted using 1 L laboratory-scale batch digesters, made from polypropylene, with working volume of 600 mL. Based on the objectives of this study, three groups of batch experiments were conducted, namely anaerobic mono-digestion of DM, MBM and CG (experiment I), anaerobic co-digestion of DM and MBM (experiment II) and anaerobic co-digestion of DM, MBM and CG (experiment III). Inoculum was pre-incubated at 38 °C for 3 days prior to the start of experiments to acclimatize inoculum to the new environment and to complete the digestion of remaining organic substrate (degassing) before the addition of new substrate.

Before the start of anaerobic co-digestion, anaerobic mono-digestion of each substrate (DM, MBM and CG) was conducted in experiment I. Samples were prepared and fed into digesters (D1–D3) to obtain an inoculum to substrate ratio of 1.0:1.0 (gVS inoculum:gVS substrate). Deionized water was added to bring the final volume to 600 mL. In experiment II, anaerobic co-digestion of DM and MBM were investigated by varying the amount of MBM from 1 to 20 g, while the amount of DM was fixed at 100 g. Before weighting the samples, the VS of DM was adjusted to 8%. 100 g of DM was mixed with 1, 5, 10, and 20 g of MBM to obtain a MBM to DM ratio of 1.0:10.0, 1.0:2.0, 1.0:1.0, and 2.0:1.0 (gVS MBM:gVS DM), respectively, and fed into digesters (D4–D7). The inoculum to substrate (DM + MBM) ratio was fixed at 1.0:1.0, and deionized water was added to bring the target volume of 600 mL. In experiment III, the main focus was on anaerobic co-digestion of MBM and CG, while DM was utilized as the base substrate (Atandi and Rahman, 2012). The amount of CG and MBM was varied to three levels to obtain a CG and MBM ratio of 1.0:3.0, 1.0:1.0 and 3.0:1.0 (gVS CG:gVS MBM), while the amount of DM was fixed at 100 g, which represents a DM and substrate (CG + MBM) ratio of 1.0:1.0. The inoculum to substrate (DM + MBM + CG) ratio was fixed at 1.0:1.0, and deionized water was added to bring the target volume of 600 mL and fed into digesters (D8–D10). Blank test digester (D0) containing only inoculum was conducted in order to adjust the biogas

**Table 1**  
Characteristics of meat and bone meal.

Parameters	Units	Meat and bone meal
Total solids content	% (w/w)	98.46
Moisture content	% (w/w)	1.54
Volatile solids	% (w/w)	68.47
Higher heating value	MJ/kg	17.20
Lower heating value	MJ/kg	16.00
n-Hexane extracts	% (w/w)	11.15
Phosphorus	% (w/w)	4.07
Potassium	% (w/w)	0.48
Nitrogen	% (w/w)	10.52
Carbon	% (w/w)	44.09
Hydrogen	% (w/w)	5.22
C/N ratio		4.19

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