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Effects of a gradually increased load of fish waste silage in co-digestion with cow manure on methane production

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ABSTRACT

This study examined the effects of an increased load of nitrogen-rich organic material on anaerobic digestion and methane production. Co-digestion of fish waste silage (FWS) and cow manure (CM) was studied in two parallel laboratory-scale (8 L effective volume) semi-continuous stirred tank reactors (designated R1 and R2). A reactor fed with CM only (R0) was used as control. The reactors were operated in the mesophilic range (37 °C) with a hydraulic retention time of 30 days, and the entire experiment lasted for 450 days. The rate of organic loading was raised by increasing the content of FWS in the feed stock. During the experiment, the amount (volume%) of FWS was increased stepwise in the following order: 3% – 6% – 13% – 16%, and 19%. Measurements of methane production, and analysis of volatile fatty acids, ammonium and pH in the effluents were carried out. The highest methane production from co-digestion of FWS and CM was 0.400 L CH4 gVS^{-1}, obtained during the period with loading of 16% FWS in R2. Compared to anaerobic digestion of CM only, the methane production was increased by 100% at most, when FWS was added to the feed stock. The biogas processes failed in R1 and R2 during the periods, with loadings of 16% and 19% FWS, respectively. In both reactors, the biogas processes failed due to overloading and accumulation of ammonia and volatile fatty acids.

1. Introduction

Salmon farms in Norway are experiencing reduced production due to the large number of fish which die at these facilities. In 2012 a loss of 27.412 million salmon was reported. Until recently such losses were disposed of as waste, despite it representing a lot of organic material (Statistics Norway, 2013). This waste contains large amounts of fat and protein, and can therefore be used as an energy-rich substrate for biogas production. In turn, the digestate from the anaerobic biogas process contains high levels of nitrogen, making it useful as a fertilizer. In Rogaland in western Norway, fish waste is to some extent utilized in a biogas pilot plant (320 m^3) located at Åna Kretsfengsel (a district prison). The fish waste is ensiled (acidified) to avoid microbial growth, and this pretreatment lowers the pH to approximately 3 (Alwan et al., 1993). The combination of acidity and high levels of fat and protein make the FWS difficult to digest as a sole substrate (Nges et al., 2012). Methane production takes place at pH levels from 6.5 to 8.5, and the optimal levels for methane production is between 7 and 8. (Weiland, 2010). The steps in anaerobic degradation of organic material roughly consist of hydrolysis, fermentation, and methanogenesis, which involve several groups of microorganisms (Gujer and Zehnder, 1983), and, accordingly, the performance of an anaerobic digestion process depends largely on the activity of these microorganisms. In general, the microorganisms involved in anaerobic digestion differ widely with respect to their physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions (Chen et al., 2008), and failure to maintain the balance between different groups of microorganisms is the primary cause of reactor instability (Demirel and Yenigun, 2002). In addition, parameters such as temperature and stirring, hydraulic retention time, and organic loading rate is also of importance for the performance of the process (Appels et al., 2008). Process breakdown induced by accumulation of toxic compounds such as NH_3 and fatty acids, are often the result of overloading with energy-rich substrates (Ortega et al., 2008).

It has been demonstrated that the optimum C/N is between 20 and 30 (Parkin and Owen, 1986), and if the C/N is too low, the process may be inhibited by accumulation of NH_3 produced from protein degradation (Angelidaki and Ahring, 1993; Angelidaki

Abbreviations: FWS, fish waste silage; CM, cow manure; sCSTR, semi-continuously stirred tank reactor; OLR, organic loading rate ($g\ L^{-1}\ d^{-1}$); HRT, hydraulic retention time (d); VFA, volatile fatty acids ($g\ L^{-1}$).

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et al., 2004; Yen and Burne, 2007). Another important parameter is the content of lipids. During anaerobic degradation, lipids are hydrolyzed to long chained fatty acids (LCFA) (Angelidaki and Ahring, 1992) and VFAs (Biebl, 2001). Both LCFAs and VFAs are detected as accumulating intermediates in unstable biogas reactors, and can give rise to unstable processes and biogas production failure (Karlsson et al., 2012).

One way to overcome the problems with anaerobic digestion of protein and lipid rich waste materials (energy- rich materials) is to use a mixture of substrates with different properties. Co-digestion may improve the anaerobic digestion process by creating a better nutrient balance, diluting toxic compounds, and stimulating synergistic effects of microorganisms (Chen et al., 2010; El-Mashad and Zhang, 2010; Lehtomaki et al., 2007), and possibly also increase the stability of the system and the methane production.

In addition, to enable the adjustment of the process parameters, inocula adapted to high concentrations of certain compounds can enhance production of biogas from energy-rich substrates (Goncalves et al., 2009; Toreci et al., 2011). Continuous anaerobic co-digestion of different substrates has been studied extensively (Ashekuzzman and Poulsen, 2011; Lehtomaki et al., 2007; Møller et al., 2004), and a few investigations dealing with co-digestion of fish waste or ensiled fish waste and manure have been carried out, mostly through batch experiments. The results of these investigations show that fish waste in general contains high concentrations of fat and protein, and that there is a large risk for accumulation of fatty acids and NH_3 when these types of substrates are anaerobically digested (Gebauer, 2004; Gebauer and Eikebrokk, 2006; Kafle et al., 2013; Nges et al., 2012). Consequently, the exploitation of the promising waste management and biogas potentials from FWS is limited. The present study has three main objectives: (i) to determine the methane production from co-digestion of FWS and CM; (ii) to ascertain optimal mixing ratios of FWS and CM as a reactor feedstock by evaluating methane productions and effluent composition; (iii) to identify a threshold level for the amount of FWS that can be added to an anaerobic reactor.

2. Materials and methods

In general, the present experiment was designed to study the methane production from co-digestion of CM and FWS in laboratory-scale (8-L) s-CSTR reactors. The amount of FWS in the feed stocks was gradually increased over the 450 days of operation.

2.1. Description and chemical analysis of raw materials

The starter culture for the experimental reactors (R1 and R2), and raw materials for feedstocks, were collected in June 2009 from a biogas pilot plant (320 m^2) located in Åna, Rogaland, Norway. The pilot plant is operated with co-digestion of FWS and CM under mesophilic conditions (37 °C). The raw materials used in the pilot plant is manure from dairy cows and fish waste (category 2), the latter consisting mainly of dead salmon from fish farms located on the western and northwestern coast of Norway. The fish waste (not defatted) is pretreated by ensiling with formic acid under pressure (3 bars) and high temperature (133 °C) for 20 min.

Before the start of the experiment, the raw materials were analyzed for content of DM, VS, fat, protein, NH_4^+, and pH levels (Table 1). Content of DM, VS, and pH levels were determined according to methods specified by the International and European Organization for Standardization (ISO 11465: 1993; NS-EN 15935: 2012; ISO 10390: 2005). NH_4^+ concentrations were analyzed by using an NH_4^+ selective electrode (Thermo Scientific Orion ISE/NH4) in diluted (1:10) samples held at 20 °C, and supplemented with an ionic strength adjustor (ISA; 10 mL of ISA per 100 mL of sample); ISA stabilizes NH_4^+. The analyses were performed on triplicate samples. The amount of fat and protein in the raw materials was measured by Eurofins AS Norway, on single samples. Determination of raw fat was carried out by using the SBR method (NMKL, 1989), and the crude protein was determined by using the Kjeldahl N method. The methods for determination of fat and protein have measurement uncertainties of 9–30% and 10–20%, respectively. The characteristics of the raw materials are shown in Table 1.

2.2. Anaerobic reactor setup and feeding strategy

The reactor tanks used in the experiment are previously described in detail by Bergersen et al. (2012). Three reactors were used, two parallel added FWS in co-digestion with CM (R1 and R2), and one additional control reactor added CM only (R0). Each reactor (total volume 10 L, effective volume 8 L, headspace 2 L) was stirred continuously throughout the experiment (30–40 rpm), operated at 37 °C, and fed manually with 267 mL of substrate daily, with an HRT of 30 days. The amount (volume%) of FWS in the feedstock mixture was increased five times during the experiment, in the following manner: 3% FWS for 125 days, 6% FWS for 48 days, 13% FWS for 93 days, 16% FWS for 94 days, and 19% FWS for 90 days. The volume percentages of FWS in the feed stocks corresponds to total gVS L^{-1} day^{-1}(OLR) of respectively 2, 2.3, 3, 3.5 and 4.3. Semi-continuous feeding of the reactors was initiated four days after adding 8 L starter culture, and the reactors were loaded with 3% FWS from startup. Each feedstock mixture was, ahead of every new period with an increase of FWS, prepared in large (50 L) batches and divided into smaller portions (0.5 L plastic containers). The 0.5 L containers were stored at 4 °C to avoid hydrolysis of the solids. The feed stock materials were analyzed for content of DM, VS and pH levels twice during each period with different amounts of FWS, and no particular degradation was detected.

2.3. Chemical analysis of feedstocks and effluents

The feedstock mixtures were analyzed for content of DM (data not shown), VS (data not shown), carbon and nitrogen, and pH levels at the start of each new period with a change of feedstock composition. Determination of total nitrogen and carbon was performed by Eurofins AS Norway, based on the total Kjeldahl nitrogen content (spectrophotometric analysis) and the content of combustible material (gravimetric analysis).

Reactor effluent samples were analyzed for content of DM (data not shown), VS (data not shown), NH_4^+, and pH levels every week during the experiment (The analysis methods are described in section 2.2). The NH_3 concentrations in the effluents were calculated from NH_4^+ concentrations, pH and temperatures. The VS removal was calculated with respect to DM and VS content in the reactor feed stocks and effluents.

Effluent samples for VFA measurements were collected twice during the last week of each period with different feedstock composition. In the period with loading of 16% FWS in R1, and that with 19% FWS in R2, the VFA levels were measured on day 30 of the first HRT.

Concentrations of VFAs (acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, n-valeric acid, iso-caproic acid, n-caproic acid, and heptanoic acid) were analyzed at Telemark University College (HIT), by gas chromatography (Hewlett Packard 6890) with a flame ionization detector and a capillary column (FFAP 30 m, inner diameter 0.250 mm, film 0.25 μm). The oven was programmed from 80 °C for 1 min, to 180 °C at a rate of 30 °C min^{-1}, and then 230 °C at a rate of 100 °C min^{-1}. The carrier gas was helium, at a flow rate of 24 mL min^{-1}. The injector and detector temperatures were set at 200 and 250 °C, respectively. The samples (50 mL) were prepared by centrifugation (1300 rpm,