



# Mesophilic and thermophilic anaerobic co-digestion of rendering plant and slaughterhouse wastes

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## ABSTRACT

Co-digestion of rendering and slaughterhouse wastes was studied in laboratory scale semi-continuously fed continuously stirred tank reactors (CSTRs) at 35 and 55 °C. All in all, 10 different rendering plant and slaughterhouse waste fractions were characterised showing high contents of lipids and proteins, and methane potentials of 262–572 dm<sup>3</sup> CH<sub>4</sub>/kg volatile solids (VS)<sub>added</sub>. In mesophilic CSTR methane yields of ca 720 dm<sup>3</sup> CH<sub>4</sub>/kg VS<sub>fed</sub> were obtained with organic loading rates (OLR) of 1.0 and 1.5 kg VS/m<sup>3</sup> d, and hydraulic retention time (HRT) of 50 d. For thermophilic process, the lowest studied OLR of 1.5 kg VS/m<sup>3</sup> d, turned to be unstable after operation of 1.5 HRT, due to accumulating ammonia, volatile fatty acids (VFAs) and probably also long chain fatty acids (LCFAs). In conclusion, mesophilic process was found to be more feasible for co-digestion than thermophilic process, methane yields being higher and process more stable in mesophilic conditions.

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## 1. Introduction

Anaerobic digestion is one option to sustainably produce energy from organic matter including biodegradable wastes. In anaerobic digestion process, micro-organisms convert biodegradable waste to biogas, mainly consisting of methane and carbon dioxide, and digestate. Methane can be used to produce heat and/or electricity or vehicle fuel whilst, nutrient rich digestate can be used for example as a soil conditioner. In slaughterhouse, animals are slaughtered and processed into meat products for human consumption. Besides meat products, animal by-products not intended for human consumption are produced. Treatment of these wastes is regulated due to risk of disease spread, mainly Bovine Spongiform Encephalopathy (BSE). In European Union (EU), treatment of slaughterhouse wastes is regulated by the Animal By-product Regulation (ABPR 1069/2009/EC – replacing the ABPR 1774/2002/EC, European Parliament and Council, 2009). According to ABP regulation, animal by-products and their process products and wastes are

**Abbreviations:** CSTR, continuously stirred tank reactor; FM, fresh matter; GC, gas chromatograph; HRT, hydraulic retention time; LCFA, long chain fatty acid; NH<sub>3</sub>, free ammonia nitrogen; NH<sub>4</sub>, ammonia nitrogen; OFMSW, organic fraction of municipal solid waste; OLR, organic loading rate; SCOD, soluble chemical oxygen demand; TKN, total Kjeldahl nitrogen; TS, total solids; TVFA, total volatile fatty acids; uVFA, unionised volatile fatty acid; VFA, volatile fatty acid; VS, volatile solids.

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classified into three categories depending upon the risk they pose towards human, animals and environment (European Parliament and Council, 2009). This regulation also defines the corresponding treatment and utilisation possibilities for these materials. Most of the animal by-products from slaughterhouses are treated by rendering (European Commission, 2005). Rendering is processing of edible and non-edible animal by-products, in most cases with heat (Woodgate and van der Veen, 2004). Raw materials for rendering process includes carcasses, parts of carcasses, heads, feet, offal, excess fat, excess meat, hides, feathers, bones and blood (European Commission, 2005; Woodgate and van der Veen, 2004). In rendering process, raw materials are ground to a uniform size (<50 mm) and subjected to heat treatment (e.g. 133 °C for 20 min at 3 bars) for sterilization. Solid and liquid parts are separated, water is evaporated and fat is separated from protein and bone (Woodgate and van der Veen, 2004). The finished fat (e.g. tallow, lard, yellow grease) and the solid protein (e.g. bone meal, poultry meal) are pressed into cake for processing into crude animal feed. Depending on the raw material characteristics, rendering is carried out as wet or dry processes and operated either as a single batch or multiple continuous process.

Slaughterhouse and rendering wastes are considered as ideal substrates for biogas production, because they usually contain high concentration of organic matter and are rich in proteins and lipids. However, anaerobic digestion of these materials is extremely prone to failure due to production of inhibitory compounds such as ammonia, VFAs and LCFAs (Cuetos et al., 2008; Hejnfelt and Angelidaki, 2009).

Ammonia is produced by the biological degradation of the nitrogenous matter, mostly proteins (Kayhanian, 1999). In anaerobic digestion process, proteins are hydrolysed to amino acids, which are degraded to VFAs (Mata-Alvarez, 2003). In the anaerobic digestion process, ammonia exists in two forms viz. ammonium ions ( $\text{NH}_4^+$ ) and ammonia gas ( $\text{NH}_3$ , free ammonia), the relative amount of each depends on pH (Hobson and Wheatley, 1993; Kayhanian, 1999). Free ammonia has been suggested to be more inhibitory than total ammonia nitrogen (Angelidaki and Ahring, 1994), because it can readily diffuse across the cell membrane (Kadam and Boone, 1996). Besides pH, temperature also affects the dissociation constant of ammonia nitrogen and concentration of free ammonia in the process; the higher is the temperature the higher is the concentration of free ammonia (Kayhanian, 1999). In the literature a wide range of inhibitory levels, 0.045–1.1 g/l, have been reported for  $\text{NH}_3$  (e.g. Angelidaki and Ahring, 1994; Hansen et al., 1998; Kayhanian, 1999). Similarly, for  $\text{NH}_4\text{-N}$ , concentrations of 3.86 and 5.60 g/l were found to decrease methane production by 50% in treating synthetic substrate (pet food) to simulate organic fraction of municipal solid waste (OFMSW) at 35 and 55 °C, respectively (Benabdallah El Hadj et al., 2009), whilst concentration of 6 g/l led to inhibited steady state at 55 °C, but was less inhibitory at 35 °C in CSTRs treating swine manure (Hansen et al., 1998).

Similarly, under anaerobic conditions, lipids are first hydrolysed to LCFAs, which are oxidised to acetate or propionate and hydrogen through the  $\beta$ -oxidation pathway (Cirne et al., 2007; Mata-Alvarez, 2003). Inhibitory concentrations of individual LCFAs vary, as e.g. 30 mg/l of oleic or linoleic acid inhibited acetoclastic methanogenesis, whilst 100 mg/l of stearic acid was not inhibitory (Lalman and Bagley, 2000, 2001). Furthermore, oleic acid was found less inhibitory to acidogenesis than for methanogenesis (Beccari et al., 1996). LCFAs were speculated to be reason for process failures in CSTRs treating poultry slaughterhouse wastes at 35 °C (Cuetos et al., 2010) and pig slaughterhouse waste with manure at 35 and 55 °C (Hejnfelt and Angelidaki, 2009). Moreover, temperature was reported to effect oleic acid inhibition as acetate-utilising methanogenesis was more susceptible to oleic acid toxicity at 55 °C than at 35 °C (Hwu and Lettinga, 1997). Nevertheless LCFA inhibition has been shown to be temporary, but recovery time might be long (Cirne et al., 2007). Studies on anaerobic digestion of rendering wastes are scarce whereas, several studies on the anaerobic digestion of slaughterhouse waste, especially, poultry waste have been reported in the literature. For rendering wastes, methane potentials of 497 and 487  $\text{dm}^3 \text{CH}_4/\text{kg VS}$  were reported for bone flour and fat, respectively, in batch assays at 55 °C (Hejnfelt and Angelidaki, 2009). In a similar study, methane potential of 351–381  $\text{dm}^3 \text{CH}_4/\text{kg VS}$  was reported for meat and bone meal at 35 °C (Wu et al., 2009). For slaughterhouse wastes, methane potentials of 225–619  $\text{dm}^3 \text{CH}_4/\text{kg VS}$  were reported for pig waste at 55 °C (Hejnfelt and Angelidaki, 2009) and 580  $\text{dm}^3 \text{CH}_4/\text{kg VS}$  at 35 °C (Rodríguez-Abalde et al., 2011). Similarly, methane potentials of 460  $\text{dm}^3 \text{CH}_4/\text{kg VS}$  were reported for poultry waste at 35 °C (Rodríguez-Abalde et al., 2011) and 210–910  $\text{dm}^3 \text{CH}_4/\text{kg VS}$  both at 35 and 55 °C; highest potential was obtained for offal (Salminen et al., 2003). On the other hand, methane yields of 520–700  $\text{dm}^3 \text{CH}_4/\text{kg VS}$  were reported during semi-continuous digestion of poultry slaughterhouse wastes in CSTR experiments at 35 °C (Cuetos et al., 2008; Salminen and Rintala, 2002).

Co-digestion of slaughterhouse wastes with other materials containing low nitrogen and/or lipid content is one option to improve the digestion process stability and increase methane yields. Methane yields of 270–500  $\text{dm}^3 \text{CH}_4/\text{kg VS}$  have been reported during the co-digestion of slaughterhouse wastes with other substrates such as manure, sewage sludge and OFMSW (Alvarez and Liden, 2008; Cuetos et al., 2008; Hejnfelt and Angelidaki, 2009;

Luste and Luostarinen, 2010). In many cases, large quantities of slaughterhouse and rendering wastes are generated at the same processing plant. Moreover, treatment and use of these organic wastes, rich in protein and lipids, through anaerobic digestion is considered as a sustainable solution for simultaneous recovery of energy and nutrients. As far as we know, there are no studies on semi-continuous anaerobic digestion of rendering plant wastes alone or co-digestion of rendering plant wastes with slaughterhouse wastes. The objective of the present study was to evaluate the feasibility of anaerobic semi-continuous co-digestion of rendering plant wastes with slaughterhouse wastes in laboratory scale CSTRs at 35 and 55 °C. In addition, methane potentials of 10 different rendering plant and slaughterhouse waste fractions were studied in batch assays at 35 °C.

## 2. Methods

### 2.1. Substrates and inocula

Seven different types of rendering plant wastes and three different slaughterhouse by-products were used as substrates in the experiments. Characteristics of the substrates are presented in Table 1. The rendering wastes viz. melt (sterilized (133 °C, 20 min, 3 bar) mass), biosludge (sludge from wastewater treatment), fat from fat separation well (fat separated with  $\text{H}_2\text{O}_2$  from wastewater of production equipments and rooms), separator sludge (water, protein and fat extracted in final purification by centrifuge from sterilized and solids separated fat), decanter sludge (solids, separated by centrifuge from fat separated by pressing from sterilized mass), fat (sterilized and purified fat) and boneflour (solids separated by pressing from sterilized mass) were collected from a rendering plant (Honkajoki Ltd., Finland). The above mentioned waste fractions were chosen in the study as there is need to develop waste treatment method. Slaughterhouse wastes viz. the contents of stomach and intestines of bovine (without rumen and reticulum) and swine were from a meat producing factory (Saarioinen Ltd., Jyväskylä). Discarded poultry (turkey) was delivered by Honkajoki Ltd., Finland. At the laboratory, the slaughterhouse by-products were macerated (5 mm) by using a meat mincer (Talsa W 22). The well homogenised materials were stored at –20 °C until further use. All the substrates, rendering plant as well as slaughterhouse wastes, are heterogeneous, which needs to be taken into account when considering the results.

Digested sludge from a municipal wastewater treatment plant (Nenäinniemi, Jyväskylä, Central Finland) was used as inoculum in mesophilic experiments. For thermophilic experiments, digested materials from a full-scale biogas plant (Stormossen, Vaasa, Finland) treating putrescible organic fraction of municipal waste was used as inoculum. Characteristics of the inocula are presented in Table 1.

### 2.2. Batch experiments

Biochemical methane potential experiment was carried out in 1 l glass bottles with a working volume of 750 ml. To each bottle, rendering plant wastes (seven materials) or slaughterhouse wastes (three materials) and inoculum (250 ml/bottle) were added at a substrate to inoculum (Nenäinniemi) VS ratio of 0.25. Tap water was added in order to obtain the working volume of 750 ml.  $\text{NaHCO}_3$  (3 g/l) was added as buffer. Thereafter, bottles were flushed with  $\text{N}_2$  for 3 min in order to create anaerobic conditions and sealed with silicon stoppers. Assays containing only inoculum and water were used as controls. The methane production from control assays was subtracted from the sample assays. The prepared assays were incubated statically at  $35 \pm 1$  °C. The

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