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Anaerobic digestion of thermal pre-treated emulsified slaughterhouse wastes (TESW): Effect of trace element limitation on process efficiency and sludge metabolic properties

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ABSTRACT

Slaughterhouse solid wastes, characterized by a high lipid content, are considered a valuable resource for energy production by means of anaerobic digestion technologies. Aim of this study was to examine the effect of trace element limitation on the mesophilic anaerobic digestion of thermally pre-treated emulsified slaughterhouse wastes (TESW). Under two distinct experimental periods (Period I – low and Period II – high trace element dosage respectively) a CSTR with sludge recirculation was operated at increasing organic loading rate (OLR) from 1.5 to 10 g L^{-1} d^{-1}. Under optimum conditions, COD removal was higher than 96%, biogas yield equal to 0.53 L g^{-1} COD feed and the biogas methane content 77%. Trace element limitation however, resulted in a dramatic decline in process efficiency, with VFA accumulation and events of extreme sludge flotation, despite that the soluble concentration of Ni, Co and Mo were between 12 and 28 μg L^{-1}. This is indicative of mass transfer limitations caused by lipids adsorption onto the anaerobic biomass.

1. Introduction

Slaughterhouse wastes are considered a valuable resource for the production of energy by means of anaerobic digestion technologies (e.g. Ware and Power, 2016; Cuetos et al., 2010). They consist mainly of animal fats and proteins which entail a high energy content. Indeed, lipids can yield up to 1.4 m^3 of biogas per kg VS (Labatut et al., 2011), their digestion, however, is accompanied with process instabilities, such as foaming, accumulation of Long Chain Fatty Acids (LCFA), sludge flotation, low biogas yield or even complete digester failure (Lienen et al., 2014). These issues have been addressed in recent review articles (Rasit et al., 2015; Long et al. 2012; Alves et al., 2009; Chipasa and Medrzycka, 2006).

Poor digester performance is often attributed to the (low) solubility and bioavailability of fats (Cirne et al., 2007), their ability to adsorb onto the anaerobic biomass, disintegrating the flocs and causing sludge flotation (Petruy and Lettinga, 1997), as well as to the bactericidal effects of LCFA on hydrolytic, acidogenic and methanogenic microorganisms (Hwu and Lettinga, 1997). LCFA perturb the bacterial cell membrane and impair metabolic regulation, while more extensive solubilization can result in membrane lysis and trigger cell death (Jackman et al., 2016). Finally, encapsulation of the active syntrophic communities by LCFA can hamper the diffusion of substrate and nutrients, thus leading to inhibition of growth and function (Souza et al., 2009).

Since the anaerobic digestion of organic matter depends largely on the activity of methanogenic archaea, both acetotrophic and hydrogenotrophic, it is essential to maintain a balanced environment for these microorganisms, to ensure long-term process efficiency (de Vrieze et al., 2013). Besides, the degradation of lipids proceeds mainly via the acetic acid pathway (Sam-Soon et al., 1991), which is also the major route for protein degradation (Ramsay and Pullammanappallil, 2001). The nutritional requirements of methanogenic archaea include several trace elements. The latter play an important role in their enzymes systems, in respiratory processes and cell structural stability (Glass and Orphan, 2012; Zandvoort et al., 2006). In studies however, dealing with lipid-rich wastes, including slaughterhouse by-products, the applied trace element dosage varies significantly from zero (0) to 80 μg Ni, 500 μg Co, 40 μg Mo and 800 μg Fe per g COD influent (Zhang et al., 2014; Cuetos et al., 2010; Jeganathan et al., 2006; Fernandez et al., 2005; Li et al., 2002; Salminen and Rintala, 2002; Petruy and Lettinga, 1997; Rinzema et al., 1993).

The objective of this study was to examine the effect of trace element limitation on process efficiency and sludge metabolic properties during the continuous anaerobic digestion of thermally pre-treated emulsified slaughterhouse wastes (TESW). The study

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was conducted using a CSTR with biomass recirculation while applying a low or high trace element dosage respectively. Process efficiency was evaluated based on COD removal, biogas production and methane yield, COD and Volatile Fatty Acid (VFA) accumulation and the degree of foaming/sludge flotation.

2. Materials and methods

2.1. Waste origin and pre-treatment

Slaughterhouse solid wastes originating from cattle processing (fat tissues, stomach and rumen without their contents, breasts and reproductive organs, bladder and intestines with their contents) were pre-treated (see Fig. 1a), using a fine-particle crusher (<40 mm) (Bigas Alsina S.A.), mixed with water (1:2.5) and emulsified at 2880 rpm using a KS EMULSIFIER F320 (Karl Schnell). By this procedure the mixture was converted into a paste (particle size < 4 mm) which was subsequently heat treated at 133 °C for 20 min at 3 bar to ensure pressure sterilization. The remaining solids (<10% of the initial waste) were separated using a decanter centrifuge at 4100 rpm (Pieralisi Series 600).

2.2. Anaerobic digester

An anaerobic digester (CSTR) with 42 L working volume was used for the study. The digester temperature was maintained at 39 ± 1 °C using a thermal bath (LAUDA) with hot water recirculation through the reactor double jacket. Mixing was performed with a paddle mixer operated at 40 rpm. The TESW were placed into a completely mixed tank (5 L working volume) at room temperature (20 °C) to avoid solidification of animal fat (Sam-Soon et al., 1991). The TESW were fed into the digester semi-continuously using a peristaltic pump (Watson Marlow) and a timer (from 4 up to 12 feedings per day, depending on the applied OLR). During operation, tap water was added with the incoming waste, to control ammonia concentration inside the digester at 2240 (±340) mg L^{-1}. The reactor effluent was connected to a sedimentation tank (4 L working volume) where the anaerobic sludge was separated and recirculated to maintain MLSS concentration equal to 9 (±2) g L^{-1}. A schematic representation of the experimental setup is given in Fig. 1b.

2.3. Digester operation

The study consists of two (2) distinct experimental periods, the first (Period I) with a low and the second (Period II) with a high trace element dosage. Before each experimental period the laboratory digester was re-inoculated with 42 L fresh anaerobic biomass originated from a full-scale anaerobic reactor treating pressure sterilized animal by-products. The seed anaerobic biomass was characterized by MLSS = 6.4 ± 1.5 g L^{-1}, MLVSS = 5.0 ± 1.2 g L^{-1}, COD soluble = 2.16 ± 0.95 g L^{-1}, PO_4-P = 29 ±10 mg L^{-1}, NH_4-N = 1310 ±790 mg L^{-1} and pH = 7.8 ± 0.2.

During period I (days 0–100) the trace element dosage was maintained equal to 6 μg Co, 6 μg Ni and 10 μg Mo per L reactor and day, independently of the applied OLR. This was based on the TE dosage applied to the full-scale anaerobic reactor treating pressure sterilized animal by-products. Furthermore, from days 60 to 100 the supplementation of trace elements was completely interrupted to examine the effect on process performance. During Period II (days 100–250) the applied trace element dosage was proportional to the organic loading rate (see Electronic Supplementary Material) and it was maintained equal to 4 μg Co, 4 μg Ni and 6 μg Mo per g COD feed, based on the results of a previous study (Qiang et al., 2012). Process performance was assessed according to COD removal efficiency, biogas production rate, methane yield, COD and VFA concentrations inside the digester and the degree of sludge flotation (measured as cm of floating material on top of the liquid phase).

During both experimental periods, trace element supplementation was performed daily using a pipette and a stock solution containing 5 g L^{-1} of each $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$ and $Na_2MoO_4 \cdot 2H_2O$ The chemicals used were supplied from SIGMA ALDRICH.

2.4. Anaerobic sludge metabolic activity

Anaerobic sludge samples obtained from the laboratory digesters at the end of each experimental period (and the seed anaerobic

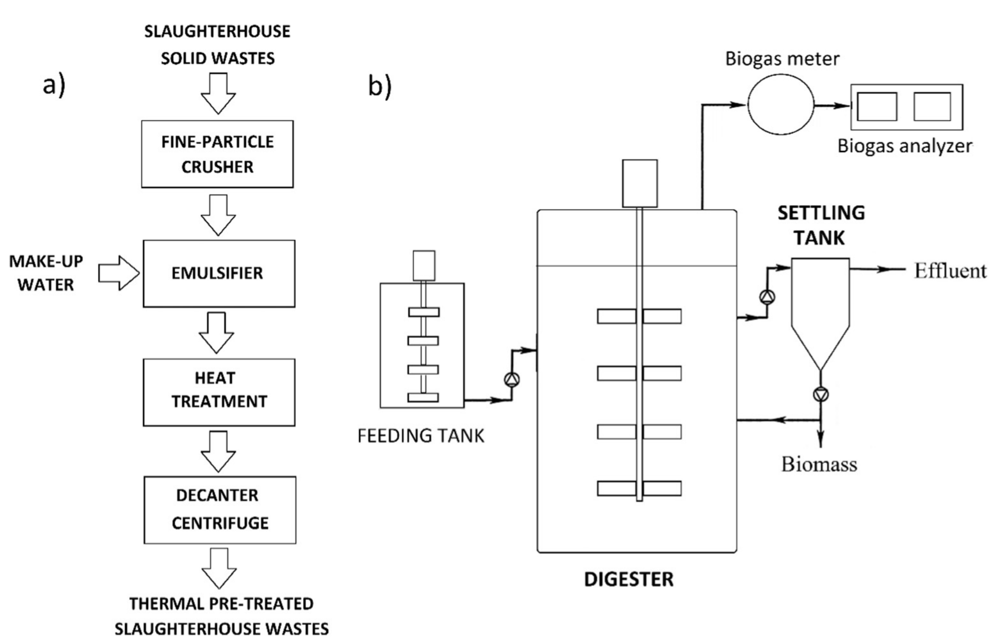

Fig. 1. (a) Flow diagram for the preparation of thermally pre-treated emulsified slaughterhouse wastes (TESW) and (b) experimental setup for the continuous anaerobic digestion of TESW.

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