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Saponification pretreatment and solids recirculation as a new anaerobic process for the treatment of slaughterhouse waste

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HIGHLIGHTS

- Novel anaerobic digestion systems for the treatment of fatty waste are proposed.
- Substrate saponification and solids recirculation both benefits the process.
- Saponification enhances emulsification and bioavailability of fatty residues.
- Recirculation minimizes substrate/biomass wash-out and induces microbial adaptation.

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ABSTRACT

Different configurations of anaerobic process, adapted to the treatment of solid slaughterhouse fatty waste, were proposed and evaluated in this study. The tested configurations are based on the combination of anaerobic digestion with/without waste saponification pretreatment (70 °C during 60 min) and with/without recirculation of the digestate solid fraction (ratio = 20% w/w). After an acclimation period of substrate pulses-feeding cycles, the reactors were operated in a semi-continuous feeding mode, increasing organic loading rates along experimental time. The degradation of the raw substrate was shown to be the bottleneck of the whole process, obtaining the best performance and process yields in the reactor equipped with waste pretreatment and solids recirculation. Saponification promoted the emulsification and bioavailability of solid fatty residues, while recirculation of solids minimized the substrate/biomass wash-out and induced microbial adaptation to the treatment of fatty substrates.

1. Introduction

Besides proper waste management practices, the concept of "waste to energy" is currently being promoted as an opportunity to face the fossil fuel crisis, the global warming and the consequent stringent environmental legislation. In this context, the anaerobic digestion of organic waste plays a crucial role as a technology that combines waste treatment and renewable energy production, in the form of biogas. High strength lipid containing wastes are interesting substrates for the anaerobic digestion process due to its high theoretical methane potential. Considerable amounts of this kind of substrates are generated from food processing industry, from the production of vegetable and animal oils, and from slaughterhouses facilities (Appels et al., 2011). Recently, (Hejnfelt and Angelidaki, 2009 and Palatsi et al., 2011) have characterized individual fractions of Danish and Spanish animal by-products, respectively, and determined its high potential methane yields.

Under anaerobic conditions, lipids are hydrolyzed by extracellular lipases to long chain fatty acids (LCFA) and glycerol. The LCFA are subsequently degraded via the β-oxidation mechanism to acetate and hydrogen, which are further converted to a methane–carbon dioxide mixture, known as biogas (Weng and Jeris, 1976). The rate of lipid hydrolysis depends, among other factors, on the specific type of lipid (triglycerides, phospholipids or sterols), the lipid particles size and the biomass specific surface area (Massé et al., 2003). In the case of particulate fatty substrates, where high amounts of suspended solids (SS) are present, the hydrolysis can be considered as the rate limiting step of the whole anaerobic process (Sayed et al., 1988; Vavilin et al., 2008). Also, released LCFAs have to be adsorbed onto biomass to be degraded (Hwu et al., 1998) and this phenomenon can affect the transport (Pereira et al., 2005) and/or protective functions (Galbaraith and Miller, 1973) of cell membranes, inhibiting the anaerobic activity (Lalman and Bagley, 2001; Palatsi et al., 2012). Lipid and LCFA adsorption

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onto biomass have also been reported to cause sludge flotation and wash-out, process that can take place at concentrations below the LCFA toxicity limit (Hwu et al., 1997).

Several pretreatment techniques have been investigated to reduce the particle size and to promote the solubilization of lipids. Among these, saponification (Battimelli et al., 2009, 2010), enzymatic hydrolysis (Masse et al., 2003) and enzymatic bio-augmentation (Cirne et al., 2006) have been claimed to enhance the hydrolysis rates. Furthermore, new treatment strategies developed to prevent problems related to LCFA inhibition have been proposed. The use of acclimated biomass (Cavaleiro et al., 2008), the addition of adsorbents as biofibers or bentonite (Palatsi et al., 2009) and the application of feeding strategies, based on sequential LCFA accumulation-degradation steps (Cavaleiro et al., 2009) have been suggested as possible alternatives.

Conventional high-rate anaerobic reactors, such as upflow anaerobic sludge blanked (UASB) reactor (Kim and Shin, 2010), expanded granular sludge bed (EGSB) reactor (Pereira et al., 2002) or anaerobic filter (Alves et al., 2001), have been used for the anaerobic treatment of lipid-rich inflows. Also, novel reactor designs as the *Buoyant Filter Bioreactor* (BFBR) and the *Inverted Anaerobic Sludge Blanket* (IASB) reactor, have been proposed for the treatment of lipid-rich wastes and slaughterhouse wastewaters, respectively (Haridas et al., 2005; Alves et al., 2007). However, the treatment efficiency of these technologies is limited by the content of solids in the substrate, opposite to plug flow (PF) and continuously stirred tank reactors (CSTR) that allows higher solids content. Consequently, further process optimization and new technological developments are still required for the treatment of complex lipid-rich wastewater and, specifically, for that containing solid fatty waste substrates. Different modifications in the design of conventional anaerobic reactors have been proposed as well in order to overcome the problems related to sludge flotation and wash-out. Biomass recirculation (Hwu et al., 1997; Pereira et al., 2001) and partial phase separation (Kim and Shin, 2010) were assayed for that purpose.

The objective of the present study is to test and validate a new reactor system configurations that are suitable for the treatment of complex lipid-based solid waste. All limiting aspects on the anaerobic degradation of lipids, such as particulate substrate, slow hydrolysis rates, high suspended solids content, substrate-biomass flotation or wash-out, and possible process inhibition have been considered in the process design. The concept of this system combines (i) the waste pretreatment through saponification, (ii) the anaerobic digestion in a completely mixed anaerobic reactor and (iii) the inclusion of a solids recirculation stage.

2. Methods

2.1. Slaughterhouse fatty waste

The solid waste used as substrate in the present experiments was flesh fat from cattle carcass collected in a slaughterhouse located in Narbonne (France). This substrate was homogenized, sieved at 5 mm to remove the fat-containing membranes and stored at −20 °C. Fresh substrate was characterized in terms of total solids (TS), volatile solids (VS), total chemical oxygen demand (COD) and long chain fatty acids content (LCFA), according to Section 2.4.

2.2. Saponification pre-treatment conditions

Saponification consists on the reaction between a lipid and an alkali, resulting in the production of LCFA salts (soap) and glycerol release. The saponification pretreatment was conducted in batch-mode inside an enclosed glass reactor coupled with a condensates recovery system. The reaction was performed in alkali conditions (sodium hydroxide, NaOH, at 32% w/w concentration) under continuous mixing (1000 rpm) at 70 °C during 60 min. NaOH was added in stoichiometric excess, adopting an equivalent ratio of 0.04 $mol_{NaOH}\ g^{-1}_{CODsubstrate}$, as proposed by Battimelli et al. (2009). The excess of alkali was checked during the pretreatment by monitoring the pH, which was maintained around 12.

Soaps were prepared at four different organic matter concentrations of fatty substrate (5%, 10%, 15% and 30% of VS). The soap concentration was selected taking into account the homogeneity and fluidity at room temperature. The efficiency of the saponification process was assessed by total and free LCFA determination, according to Section 2.4.

2.3. Experimental set-up

Experiments were conducted in three anaerobic continuously stirred tank reactors (CSTR) of 5 L, maintained at mesophilic conditions (35 °C). All reactors were inoculated with mesophilic granular sludge sampled from an UASB reactor of a sugar facility (Marseille, France). Sampled granular seed sludge was broken by high shear stress agitation and added into the CSTR reactors in a high concentration (32.9 ± 0.6 $g_{VSS}\ L^{-1}$), in order to obtain a high initial biomass content. Three different operational strategies were investigated, summarized as follows:

- In the first reactor, or **configuration R1**, saponification pretreatment was applied to the substrate, with the aim to enhance the emulsification and bioavailability of lipids. A recirculation step of the solid fraction of reactor outflow was also coupled to the anaerobic digestion process in order to overcome problems related with biomass/substrate flotation and wash-out. Effluent was manually withdrawn from reactors before each feeding cycle, centrifuged (6500 rpm, 20 min at 20 °C) and the solid fraction reintroduced into the reactor, with a volumetric recirculation ratio around 20% of the outflow (w/w).
- In the second reactor, or **configuration R2**, the saponified substrate (soap) was anaerobically digested, but in this case the recirculation step was not applied in order to evaluate the influence of the solids recirculation, comparing the system performance and efficiency respect to configuration R1.
- In the third reactor, or **configuration R3**, raw waste (not saponified) was directly digested in the anaerobic reactor, including the effluent solid fraction recirculation step, with the aim of quantifying the influence of the saponification pre-treatment on process efficiency, comparing the system performance and efficiency respect to configuration R1.
- The same operational procedure was followed in all tested configurations or reactors.
- Initial sludge starvation (**period 0**). In order to remove the residual biodegradable organic matter contained in the inoculum, CSTRs were maintained in batch conditions without feeding until residual biogas production. After that, the anaerobic activity of the sludge was evaluated by a series of easily biodegradable compound (ethanol) injections. In total, 9 ethanol pulses were performed and 3 increasing concentrations were tested: 0.9 $g_{COD}\ L^{-1}$ (4×), 2.3 $g_{COD}\ L^{-1}$ (3×) and 4.5 $g_{COD}\ L^{-1}$ (2×). At the end of this period, the inoculum activity ($g_{COD\text{-}CH_4}\ g^{-1}_{VSS}\ day^{-1}$) and the minimal biogas production rate ($mL_{biogas}\ min^{-1}$), representing the endogenous biogas production, were determined for each reactor as described by Battimelli et al. (2009).
- Acclimation period (**period I**). The 3 different reactor configurations (R1, R2, and R3) were tested in pulses-feeding conditions. A new cycle (feeding of fatty waste) was started when the system presented a stable response to the tested inflow pulse