



# Semi-continuous co-digestion of solid cattle slaughterhouse wastes with other waste streams: Interactions within the mixtures and methanogenic community structure



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## HIGHLIGHTS

- Co-digestion of slaughterhouse wastes with three other substrates was evaluated.
- Synergy and antagonism found in batch mode were corroborated in semicontinuous mode.
- Digestion of slaughterhouse waste as a single substrate failed.
- Process performance and microbial community was influenced by mixture interactions.
- The process with mixture combination that showed antagonism in batch mode failed.

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## ABSTRACT

The performance of the anaerobic co-digestion process is strongly related to the characteristics of the substrates utilized. In this work, the impact of mixture interactions, i.e., synergy and antagonism, previously observed in batch operation mode were evaluated under semi-continuous co-digestion of slaughterhouse waste (SB) and its different combinations with manure (M), various crops (VC), and municipal solid waste (MSW). The effects on the process performance and the microbial community structure were investigated. The digestion of SB failed at an OLR of  $0.9 \text{ gVS L}^{-1} \text{ d}^{-1}$ . However, stable performance with higher loadings was observed for mixtures that displayed synergy obtained earlier in the batch mode (i.e., SB + M, SB + VC + MSW). Bacterial and Archaeal groups increased for the SB + M and SB + VC + MSW, compared with the digestion of SB alone and that for SB + VC. The combination that showed antagonistic effects (SB + VC) resulted in unstable operation and poor representation of methanogens. It was proved that synergetic or antagonistic effects observed in batch mode due to the different mixture compositions could be correlated to process performance, as well as the development of the microbial community structure during semi-continuous operation.

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## 1. Introduction

Co-digestion technology is considered today as one of the most popular topics within the field of anaerobic digestion (AD). The number of publications dealing with this subject has grown

exponentially in the last five years. Co-digestion is referred to as the simultaneous digestion of a mixture of more than one substrate with complementary characteristics in order to improve the performance of the biological degradation process. The digestion of several materials gives higher methane yields than the expected ones calculated from the results when single materials are treated individually [1]. Some of the reasons linked to the enhancement of methane production are related to a combination of substrates which results in positive interactions in the system, like the dilution of potentially toxic compounds, a better balance in alkalinity, pH, moisture, organic matter, and macro- and micro-nutrients, i.e., carbon/nitrogen ratio, sodium, potassium, phosphorus, etc. [2].

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A recent review on co-digestion of solid waste reported that manure, sewage sludge, and the organic fraction of municipal solid waste have been the most investigated co-substrates for biogas production and therefore gained a lot of attention both within research and also in industrial applications [3]. However, in order to increase the economic profitability of biogas plants dealing with co-digestion, the utilization of energy-rich waste streams becomes more and more important [4].

Residues from the meat industry, i.e., slaughterhouse waste, are rich in proteins and lipids, with very high methane potential [5]. The increasing interest in this type of waste has meant that during the last three years, about 12% of all scientific papers published on the topic of co-digestion have focused on residues from slaughterhouse activities, mainly from swine [6,7] and poultry [8,9] industries, as potential co-substrates for biogas production.

Nevertheless, it was reported that the utilization of slaughterhouse residues might result in unstable process performance [10] and stress conditions for the microbial community due to the formation of potential inhibitory compounds [5]. Because of these technical and microbiological problems, many of the large-scale biogas plants that previously treated slaughterhouse waste are therefore no longer in operation [11].

One possible solution to the above problems is the proper utilization of slaughterhouse waste in the co-digestion processes. So far, several studies investigating the process, have paid special attention to the influence of operation parameters, like organic loading rate [4,10,12,13], hydraulic retention time [10,13], and the effect of the temperature on the performance of the semi-continuous digestion process [7,14].

However, it is important to point out that the co-digestion process is not simply a degradation of a mixture of different materials. Depending on the feedstock composition, the operation conditions, and the microbial community structure developed under the digestion process, the co-digestion may lead not only to positive synergistic effects, as mostly reported in the literature [15–17], but also to antagonistic interactions resulting in lower methane yield than the expected ones [18].

Furthermore, most of the reports found in the literature address in particular the influence of synergy due to the mixture composition on the bio-methane potential determined during the batch operation mode [15–17].

No attention has yet been paid to increase our understanding of synergy or the antagonism found in batch co-digestion assays of slaughterhouse waste with agro-wastes using different mixture combinations [18]. There is a lack of available information regarding the impact of mixture interactions, found in batch experiments, under semi-continuous operation and their possible impact on the microbial community structure. Moreover, no references were found on the negative impact that may occur during the co-digestion process if an appropriate combination of a mixture of slaughterhouse wastes with agro-residues was not selected.

The present study, therefore, is a further development of the work performed previously by Pagés-Díaz et al. [18]. The aim is to evaluate the effect of the mixture interactions found in batch digestion assays on the reactor performance as well as on the methanogenic community structure, while investigating the long-term effects using semi-continuous operation.

## 2. Materials and methods

### 2.1. Substrates and inoculum

Four different raw materials were used in the co-digestion experiments: solid cattle slaughterhouse waste (SB), a mixture of animal manure (M), i.e., pig (50% wet weight (ww)), horse (25%

ww), cow (25% ww), and the organic fraction of the municipal solid waste (MSW). The fourth component was various crops (VC) prepared as a mixture of the residues from the fruit and vegetable waste (70% ww) and straw (30% ww). To be able to take the existing conditions in Cuba in account, the composition of M and VC fractions was determined based on the data obtained from different slaughterhouse enterprises and from the farms found nearby them in Cuba, as it is explained in our previous study [18]. A representative mixture of the cattle slaughterhouse waste was obtained from a biogas plant (Kristianstad, Sweden), and the components of the solid manure fraction (M) were collected from farms outside Borås, Sweden. The MSW was provided by a large-scale biogas plant (Borås Energi och Miljö, Borås, Sweden). The components for the VC fraction were collected from different sites outside Borås. Each individual fraction was initially cut and minced into smaller pieces using a blender (Blender HGB55E, Commercial Torrington, U.S.A.). These fractions were thereafter mixed to obtain appropriate mixture compositions, packed and stored at  $-20^{\circ}\text{C}$  prior to use.

The SB was digested as a sole substrate in the reference reactor. Furthermore, three different mixture combinations were prepared: SB + M (50%:50%), SB + VC + MSW (33%:33%:33%), and SB + VC (50%:50%). These combinations were selected, because they showed either synergy or antagonism during the previous batch operation [18]. Moreover, these mixtures also represent the most probable waste combinations found nearby slaughterhouse enterprises in both Cuba and Sweden. The fractions of the different components were based on the wet weight. These combinations were run in parallel in four different reactors. The chemical characterization data for each individual substrate fractions and for the investigated the mixture ratios are presented in Table 1. More detailed information on the analyses methods used for the characterization of the different substrate fractions is presented in Pagés-Díaz et al. [18]. Thermophilic inoculum (TS 3.8%, VS 2.1%) obtained from a large-scale (3000 m<sup>3</sup>) anaerobic co-digestion plant, treating municipal solid waste (Borås Energi och Miljö AB, Borås, Sweden), was used as the inoculum in each reactor.

### 2.2. Semi-continuous co-digestion experiments

Four identical continuously stirred tank reactors (CSTR), each with a working volume of 3 L, were used to evaluate the digestion of the SB and its co-digestion with different agro-wastes. The temperature was controlled to maintain thermophilic conditions ( $55 \pm 1^{\circ}\text{C}$ ) by circulating water from a water bath into the water jacket of each reactor. The contents of the digesters were continuously mixed by impellers, rotating at 90–110 rpm to prevent the particulate material from floating. Produced gas volumes were determined by an Automatic Methane Potential measuring system (Bioprocess Control, Sweden).

Digester 1 was fed with only the SB. Digesters 2, 3, and 4 were used to evaluate the mixture interactions during the semi-continuous co-digestion of SB + M, SB + VC + MSW, and SB + VC. Each reactor was fed once daily throughout an experimental period of 101 days.

The reactors were all operated at a hydraulic retention time (HRT) of 25 days based on the Grenz time ( $t_{\text{Grenz}}$ ), determined previously in batch assay using the same mixture combinations [18]. The organic loading rate (OLR) was gradually increased, starting from  $0.5 \text{ gVS L}^{-1} \text{ d}^{-1}$  in all of the digesters to the final loading rates of  $0.9 \text{ gVS L}^{-1} \text{ d}^{-1}$  (SB),  $3.0 \text{ gVS L}^{-1} \text{ d}^{-1}$  (SB + M), and  $2.0 \text{ gVS L}^{-1} \text{ d}^{-1}$  (SB + VC + MSW, as well as SB + VC). The increase in the OLR was controlled by monitoring the volatile fatty acids (VFA)/alkalinity (Alk) ratio to achieve stable process conditions. Gas samples were taken every day to monitor the biogas composition. The pH in the reactors was also measured every day.

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