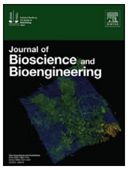

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# Evaluation of methane generation and process stability from anaerobic co-digestion of sugar beet by-product and cow manure

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**The effect of mesophilic anaerobic co-digestion of dried pellets of exhausted sugar beet cossettes (ESBC-DP) and cow manure (CM) on the enhancement of methane generation and process stability were studied with the aim to select the best substrate mixture ratio. A series of batch experiments was conducted using the following five mixture ratios of ESBC-DP:CM: 0:100; 25:75; 50:50; 75:25 and 100:0. Best results were obtained from mixture ratios with ESBC-DP proportions in the range of 25–50%. Mixture ratio of 50:50 showed a specific methane production (SMP) increase of 81.4% and 25.5%, respectively, in comparison with mono-digestion of ESBC-DP and CM. Evolution of the indirect parameter named acidogenic substrate as carbon (ASC) could be used to provide more insight about the process stability of anaerobic digestion. ASC accumulation was observed in reactors with higher ESBC-DP proportions leading to a delay in VFAs consumption and conversion into methane.**

[**Key words:** Anaerobic co-digestion; Methane generation; Sugar beet by-product; Cow manure; Process stability; Acidogenic substrate as carbon]

In recent years, the expanding use of biomass as an energy source forms a major part of the global energy system by increasing its use as a feedstock for biofuel production and contributing in reducing carbon dioxide emissions and other pollutants for the global warming (1). Additionally, using biomass as a resourceful material for renewable energy production could prevent the landfilling of the wastes which creates environmental hazards (2). Agri-food industries generate large amounts of waste biomass, which can be used in waste-to-energy processes (3,4). At this juncture, anaerobic digestion (AD) proved to be a robust process to generate energy from organic waste, where the economy and the environment are in balance with each other (5).

The high carbohydrate content in agri-food wastes is considered as the main component for biogas production by means of AD process. Lignocellulosic agri-food wastes, such as exhausted sugar beet cossettes (ESBC), can be used as a raw material for biogas production since they are composed mainly of carbohydrates, cellulose, hemicelluloses, and lignin material (6,7). ESBC are a by-product of the beet sugar industry, formed from the fibrous residues of sugar beets (*Beta vulgaris*) after several extraction processes (7). The dried pellets (DP) of ESBC used in this study are composed of 85% beet pulp and 15% molasses and have a high lignocellulosic-type organic matter (OM) content (90% volatile solids, VS) and a nitrogen deficiency (6,8).

From other hand, the carbonaceous substrate ESBC has low nitrogen content which is a necessary nutrient for AD. Therefore, in order to achieve efficient biogas production, supplementation of pH buffer and minerals is essential to optimize the pH conditions and nutrient balance (9,10). However, the cost of operation could be considerably increased. It has been reported that co-digestion can be an interesting option for improvement in biogas yields during AD of lignocellulosic wastes due to the positive synergisms established in the digestion process by providing a better nutritional balance (10–15). Livestock wastes such as animal manure (cow manure, CM, in present study) not only contribute nitrogen (nutrient balance) and alkalinity (buffering capacity) but also provide a high microbiological activity, which is able to degrade vegetal fiber as well as diluting the effect of toxic compounds contained in agri-food wastes (7,16,17).

Some studies about AD of sugar beet by-product were found in literature (6–8,18–22), however, few of them were focused on the treatment of exhausted sugar beet pulp as dried pellets (6,8). The pellets of ESBC are usually used as animal feedstock which is not a compromising strategy for sugar beet plants processing due the high energetic cost of this industry. The use of sugar beet by-product for clean energy production such as biogas rich-methane could offset the costs of production of sugar from sugar beet.

Ohuchi et al. (18) studied the thermophilic anaerobic co-digestion of sugar beet tops silage (SBT) with dairy manure (DM) at four SBT silage proportions. The highest methane yield of 422 mL/gVS and VS reduction of 57%, were obtained when the mixture contained the lowest SBT proportion (40%) while the system failure was observed for the highest SBT proportion. Similarly, Umetsu et al. (20) studied the thermophilic anaerobic co-digestion of sugar beet by-products (tops and roots) with DM in both batch and semi-continuous systems. For batch experiment, the authors

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observed that methane production was stopped at 30% (tops and roots) and 15% (roots) beet by-products additions to DM, however, SBT proportions at 40% have shown the maximum methane yield. Subsequently, in semicontinuous operation, the authors observed that the mixture with 40% beet tops have shown the highest cumulative methane production increasing by around 60% over the digestion of DM as a sole substrate.

Aboudi et al. (6) also investigated mesophilic AcoD of dried pellets of exhausted sugar beet cossettes (ESBC-DP) with pig manure (PM) as animal co-substrate. They reported that the PM addition in the proportion of 68% caused a significant increase in methane yields by creating a synergistic effect in the AD medium. In another attempt, Lehtomaki et al. (21) studied the mesophilic AcoD of SBT with different crop residues and CM. They analysed the effect of substrate proportions in the mixtures. They reported that at a crop proportion of 30% (corresponding to 15–19% of SBT), the specific methane production (SMP) increased 32% with respect to the digestion of CM as a sole substrate.

So far no study has been conducted on co-digestion of ESBC-DP and CM. Therefore, the present study was carried out to evaluate the AcoD of ESBC-DP and CM at mesophilic conditions using five different mixture ratios of the substrates with the objective to find out the best combination between the selected substrates to achieve best methane production and process stability.

## MATERIALS AND METHODS

**Substrates and inoculum** The ESBC as DP was obtained from a sugar beet processing plant at Jerez de la Frontera (Cádiz) in the south of Spain, during the summer harvesting period. Samples were stored at 4°C to avoid its degradation at room temperature. The used ESBC-DP had around 20–70 mm of length and 6 mm diameter and its TS content ranged 80–90%. The pellets of ESBC are composed of 85% of beet pulp and 15% of residual molasses. CM was collected from a semi-intensive farm facilities located at El Puerto de Santa Maria (Cádiz) in the south of Spain and was used the same day of its collection. The farm does not have any system for separation at source of urine and faeces.

Mesophilic anaerobic effluent from a laboratory scale semi-continuous reactor fed with ESBC-DP was used as inoculum. The reactor was operated previously, at stable conditions, for about two years and the stabilised methane yield was 280 $mLCH_4/gVS_{added}$.

The physico-chemical characteristics of the substrates and inoculum are summarized in Table 1.

**Experimental start-up and operation** Batch tests were conducted using a series of 2 L working volume stainless steel reactors (dimensions: 160 × 260 × 650 mm). The reactors have a glass cover with several ports including an inlet port for feeding and an output port for biogas collection. The temperature (35 ± 0.5°C) was maintained during all the experiments by a heating plate located at the base of each reactor which was covered by a metal jacket for better heat transfer. Temperature was continuously measured by an inner temperature sensor and controlled by a proportional-integral-derivative (PID) control system. The mixing system consisted in an independent motor agitation and a stirring blade for each reactor and the stirring rate was maintained at 18 rpm (6). At the start of assays, reactors were inoculated at 50% (dry basis) with the mesophilic inoculum commented before. The following ESBC-DP:CM mixture ratios were tested: 0:100, 25:75, 50:50, 75:25 and 100:0. The total solids (TS) content of the mixtures was adjusted to 8% (8). The tests were carried out in duplicate. The initial pH was measured and was adjusted to the required pH (10) by adding an alkali (NaOH, 8M). Reactors were hermetically sealed and then were initially flushed with nitrogen gas to remove any residual oxygen. Tests were run until no further production of biogas was observed (approaching zero).

**TABLE 1.** Physicochemical characteristics of substrates (ESBC-DP and CM) and inoculum.

| Component | Unit | ESBC-DP[a] | CM | Inoculum |
|---|---|---|---|---|
| pH | – | 5.84±0.12 | 6.17±0.2 | 7.32±0.14 |
| TS | g/kg | 874.5±0.12 | 221.3±0.28 | 36.9±0.18 |
| VS | (%TS) | 88.9±0.35 | 77.8±0.24 | 20.8±0.15 |
| DOC | gC/kg | 37.84±0.15 | 12.93±0.42 | 3.84±0.18 |
| sCOD | $gO_2/kg$ | 48±0.26 | 15.1±0.15 | 9.68±0.22 |
| tCOD | $gO_2/kg$ | 120.72±0.24 | 86.25±0.32 | 15.38±0.22 |
| TVFA | gHAc/kg | 5.8±0.12 | 2.6 ±0.48 | 1.3±35.2 |
| Alkalinity | $gCaCO_3/kg$ | 3.3±0.16 | 34.6±0.25 | 42.2±26.8 |
| $N\text{-}NH_4$ | gN/kg | 0.18±0.14 | 2.48±1.38 | 0.29±0.52 |
| TKN | gN/kg (TS) | 15.4±1.02 | 36.1±0.63 | 5.7±0.54 |
| Ratio C/N | – | 33.5±0.2 | 12.5±0.16 | 21.2±0.26 |

[a] ESBC en pellets form lixiviated sample with deionized water.

**Analytical methods** To characterise the substrates and control the process, the following parameters were analysed: pH, total solids (TS), VS, total and soluble chemical oxygen demand (tCOD and sCOD), dissolved organic carbon (DOC), volatile fatty acids (VFAs), ammoniacal and total Kjeldahl nitrogen ($NH_4$-N and TKN) and alkalinity. All analytical determinations were performed according to standard methods (23). The pH was measured directly from the samples using a Crison-Basic20 pH meter (Crison Instrument, Spain). For determinations of sCOD, DOC and VFAs, the samples were previously lixiviated with deionised water, during 2 h, and filtrated by 0.47 mm glass fiber filter according to Álvarez-Gallego et al. (24). Samples for VFAs measurement were filtrated again with 0.22 μm Teflon filter. The parameters tCOD, TS and VS determinations were performed directly without lixiviation. DOC analysis was carried out in an Analytic-Jena multi NC 3100 carbon analyser with chemiluminescence detector (CLD) by infrared-combustion method (5310B) of Standard Method using the oxygen 5.0 at pressure of 4–6 bars. The VFAs analysis was carried out using a gas chromatograph Shimadzu GC-2010 equipped with a flame ionisation detector (FID) and capillary column filled with Nukol. Hydrogen was used as carrier gas with a flow rate of 50 ml/min (6). In addition, $N_2$ as make-up gas and synthetic air as comburent gas were used.

Biogas was collected in a 10 L Tedlar gas bag (SKC, UK) and its volume was measured daily using a high precision wet drum-type gas meter (Ritter TG5). The gas composition was determined by using a gas chromatograph (Shimadzu GC-2014) with a stainless steel column packed with Carbosieve SII (diameter of 3.2 mm and 3.0 m length) and thermal conductivity detector (TCD). Helium was used as a carrier gas with flow rate of 30 ml/min.

**New indirect parameters for the AD performance interpretation** In order to gain a better knowledge of the co-digestion of ESBC-DP with CM and its effect on the effectiveness of AD process, new indirect parameters were analysed, basing on the classical analytical determinations, which provide additional information about the process evolution.

Fdez-Güelfo et al. (25) have established these new indirect parameters to evaluate the AD process performance and especially to understand the relationship between the different microbiological stages of the AD process and the effect on the system stability.

According to the authors, the parameter dissolved acid carbon (DAC) represents the content in carbon associated to VFAs and it can be obtained from the VFA concentration in the medium (considering the relation between the carbon weight and the molecular weight in each VFA). Moreover, the acidogenic substrate as carbon (ASC) parameter is related to the soluble OM (expressed in carbon terms) which has been not transformed into VFAs. Thus, ASC can be obtained from the difference between the DOC and the DAC:

$$ASC = DOC - DAC \qquad (1)$$

The new indirect parameter, ASC, can be used as an indicator to study the linkage between each AD stage and the next ones; i.e., to indicate if the process is balanced and the stages are coupled. Thus, an increase of ASC can be produced when the hydrolysis rate is higher than acidogenesis rate, as it is observed usually at the beginning in a batch AD test. Besides, a decrease in ASC can be expected in the opposite case when most of the OM has been hydrolysed and its conversion into organic acids predominates. Furthermore, a supported increase in DAC can be associated to an imbalance between acidogenesis and methanogenesis, related to higher metabolic rate of acidogenic microorganisms. Finally, decreasing in DAC is normally related to methane production through methanogenic activity. In short, it can be pointed out than accumulation of ASC in the process can be related to problems in the acidogenic stage while DAC accumulations are related to problems in methanogenic stage.

## RESULTS AND DISCUSSION

**Characterisation of ESBC-DP and CM** The characteristics of the two substrates can be observed in Table 1. As can be seen, the used dried pellets of ESBC are a carbonaceous-type material with a high solid content (high VS and COD values). On the contrary, total nitrogen and alkalinity contents of CM are 2.3 and 10.5 times higher than for ESBC-DP. In consequence, the selected wastes are complementary and their mixture can lead to a suitable nutrient balance, offsetting the deficiencies of each one.

In AcoD process, the ratio between the contents of carbon and nitrogen (C/N ratio) in the feedstock is considered as one of the critical parameters for process performance. The range of 20–30 for