



# Performance of a two-stage anaerobic digestion system treating fruit pulp waste: The impact of substrate shift and operational conditions

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## ABSTRACT

Food and beverage industry wastes present high amounts of organic matter, which may cause water quality degradation if not treated. Two-stage anaerobic digestion is a promising and efficient solution for the treatment of this type of wastes whilst producing bioenergy. The composition of fruit pulp waste varies throughout the different harvesting seasons, which may impact the process performance. In this study, a two-stage anaerobic digestion system was operated to assess the effect of substrate shift from peach to apple pulp wastes (obtained from a fruit juice company) on the microbial community activity and performance. During acidogenesis, the sugar conversion was higher than 95% for all operational conditions tested, obtaining a degree of acidification up to 89%. Principal Component Analysis was used to evaluate the relationship between the initial fermentation state of the residues in each operational condition and the obtained effluent. Methanogenic activity resulted in high organic carbon consumption (89%) and high methane productivities, achieving a maximum of 4.33 L<sub>CH<sub>4</sub></sub>/(L.d) for peach waste influent. Overall, the results showed that the microbial community activity was not affected by the substrate shift, converting the sugars into biogas rich in methane (>70% CH<sub>4</sub>). Microbial analysis showed that the communities present in the acidogenic and methanogenic reactors were highly enriched in bacteria and archaea, respectively. The observed stability of the process, also demonstrated in pilot scale, confirmed the robustness of the process and thus, was suitable for implementation in companies producing seasonally different fruit wastes in a continuous operation.

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## 1. Introduction

The increase of urban population and human consumption, the intensification of food industries during the last decades have led to a rise in the production of concentrated organic wastes (Hidalgo et al., 2016). These can cause serious environmental problems if not treated. Anaerobic digestion (AD) has been shown to be a suitable option for the treatment of high strength organic effluents (Ersahin et al., 2011), presenting several advantages when compared to aerobic systems such as energy savings/production, lower production of sludge, less requirements of nutrients and

smaller reactor footprint (Buitrón et al., 2014). A wide range of wastes such as fruit and vegetable waste (Bouallagui et al., 2004; Ganesh et al., 2014), food waste (Voelklein et al., 2016), vinasses (Fu et al., 2017; Peixoto et al., 2012; Solera et al., 2002), parboiled rice, glycerol and sewage (Peixoto et al., 2012), and wastes from food and beverage (F&B) industries can be treated using the AD process. AD performance depends on the activity of microbial communities that convert the organic matter into biogas, rich in methane (Appels et al., 2008). Although most AD processes use single-stage systems, where all stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) occur in the same reactor (Ganesh et al., 2014), it can be advantageous to separate the AD process into two phases: acidogenesis and methanogenesis. Actually, it has been shown that two-stage AD systems are appropriate for treating wastes with high sugar content (Lindner et al., 2016) such as fruit pulp wastes. This separation allows the optimization of the AD process by operating each phase at optimal environmental and operational parameters (e.g. pH and hydraulic retention

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time (HRT)), improving the performance and biogas methane content (Voelklein et al., 2016). Moreover, the separation allows to potentially smooth the oscillations due to substrate change occurred in the acidogenic reactor which may act as a buffer for the methanogenic community, protecting them from pH shocks that may result from acid accumulation when concentrated wastes are used (Capson-Tojo et al., 2017a; Solera et al., 2002).

Fruit pulp waste is a sugar-rich substrate with high methane productivity potential through AD. However, its utilization presents some limitations since each type of fruit has a specific harvesting season, making it difficult to operate the reactor during long periods of time with the same feedstock (Fonoll et al., 2015). Thus, it is important to evaluate the effect of substrate shift on reactor performance and stability. Fonoll et al. (2015), using one-stage anaerobic digesters, studied the effect of a sequential co-substrate change in a co-digestion of mixed fruit wastes (peach, banana or apple waste) and sewage sludge and observed that the change of fruit wastes as co-substrate did not affect the system stability. However, to the best of our knowledge, the effect of mono fruit waste shift on the microbial community activity and reactors performance has not been deeply studied. When treating fruit pulp waste, given the seasonality of fruits, the use of a two-stage AD system may have an additional advantage since the acidogenic reactor is more robust and can tolerate the sequential substrate change and consequently protect the methanogenic reactor from the variability in the wastes' initial composition. The evaluation of this effect and the optimization of this process will allow companies, such as F&B industries, to treat their wastes whilst producing biomethane, in a continuous and stable way, which may be used to cover the energy consumption of the treatment and may generate a surplus of energy for other stages of the industry.

Hence, the main objective of this work was to assess the impact of substrate shift on the performance of a two-stage process (acidogenic and methanogenic reactors), operated under different operational conditions. Simultaneously, the optimal operational conditions to maximise methane productivity were investigated. The performance was assessed taking into account the degree of acidification (DA) and biomethane production. Furthermore, two-stage AD pilot scale reactors were successfully operated in continuous mode at a juice producing company (Sumol + Compal Marcas S.A.).

## 2. Material and methods

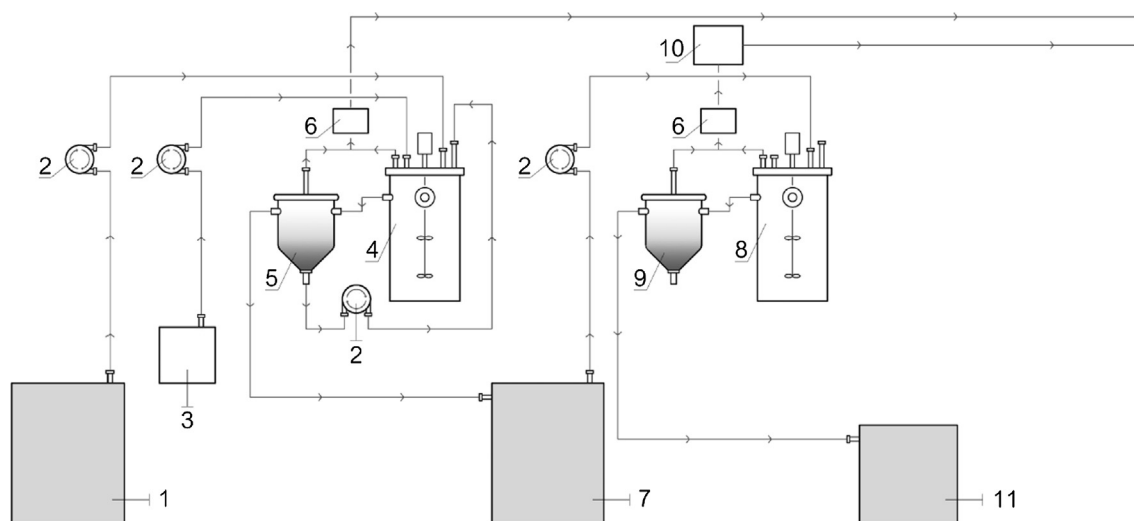
### 2.1. Laboratory scale

#### 2.1.1. Bioreactor set up

Two-stage AD of a F&B industry waste was performed in two 5 L continuous stirred tank reactors (CSTR) (Bioprocess control, Sweden) (Fig. 1). Two settlers were coupled to the acidogenic and methanogenic reactors to clarify the effluent. The settled biomass and other solids were recirculated to the acidogenic reactor, with a recirculation flowrate similar to the feed flowrate, in order to avoid biomass washout and thus, increase the solids retention time (SRT). Both reactors were operated at mesophilic conditions (30 or 37 °C, see below). The pH was automatically controlled in the acidogenic reactor at  $5.50 \pm 0.05$ . The pH of the methanogenic reactor was naturally maintained at  $7.4 \pm 0.2$ . The acidogenic reactor was inoculated with sludge from an anaerobic digester (Municipal wastewater treatment plant) with an initial inoculum/substrate ratio of 0.33 gVSS/gCOD. The methanogenic reactor was inoculated with granular sludge from an anaerobic Biobed Expanded Granular Sludge Blanket (EGSB) reactor treating brewery wastewater with an initial inoculum/substrate ratio of 12.16 gVSS/gCOD. The acidogenic and methanogenic reactors were operated for 235 and 213 days, respectively. The recirculation of biomass in the acidogenic reactor or the utilization of granular biomass in the methanogenic reactor allowed uncoupling the solids and hydraulic retention times. This allowed achieving high levels of biomass, using long SRT and short HRT, increasing the efficiency of the process.

#### 2.1.2. Experimental set up

The conditions used in this work are depicted in Table 1. Two different pulp wastes (peach and apple pulp waste), supplied by a juice producing company (Sumol + Compal Marcas S.A.), were used as substrate. The fruit pulp waste was diluted with tap water to achieve an acidogenic influent chemical oxygen demand (COD) concentration between 21.2 and 51.1 gCOD/L. The acidogenic effluent was used as substrate for the methanogenic operation. To avoid nutrient limitation, the acidogenic influent was supplemented with N (as  $\text{NH}_4\text{Cl}$ ) and P (as  $\text{KH}_2\text{PO}_4$ ) to maintain a COD:N:P ratio of 100:0.5:0.1 between days 0–127 and 100:1:0.2 from day 128 onwards (Condition A5, Table 1).



**Fig. 1.** Two-phase AD set-up: (1) acidogenic influent vessel; (2) pumps; (3) NaOH solution; (4) acidogenic bioreactor; (5) acidogenic settler; (6) gas flowmeters; (7) acidogenic effluent/methanogenic influent vessel; (8) methanogenic bioreactor; (9) methanogenic settler; (10) methane and carbon dioxide gas sensors; (11) methanogenic effluent vessel.

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