Volatile fatty acids production from agroindustrial waste: impact of process pH

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Volatile fatty acids (VFAs) are considered valuable building blocks for the chemical industry since they are precursors of reduced chemicals (alcohols, aldehydes or ketones) in the so-call carboxylate platform (Dahiya et al., 2015). Conventionally, these carboxylic acids have been obtained by petrochemical means, which involves a high environmental impact as well as a high economic cost. As an alternative, VFAs can be generated via anaerobic digestion (AD) from a wide variety of organic wastes such as agroindustrial wastes. VFAs production through a biological process gives rise to a low-cost sustainable production model that contributes to mitigate climate change, boosting a circular economy where waste resources are valorized as bio-products and consequently reducing their disposal associated costs and environmental impact.

Agroindustrial wastes treatment trough AD to produce CH_4 has been widely studied as a traditional bioprocess for wastes valorization. However, the bioconversion into VFAs has to be optimized by promoting hydrolytic and acidogenesis stages along with methanogenesis inhibition. Since methanogenic microorganisms require neutral pH to convert VFAs into methane, pH control is a key factor for the success of the process. In this manner, the novelty of this research relies on assessing agroindustrial wastes as a resource for VFAs production and identifying key microbial population that ensures high bioconversion yields

This study evaluated two operational pH conditions for the anaerobic bioconversion of different agroindustrial wastes into VFAs. Strazzera and co-workers (2018) reported that the hydrolysis of agroindustrial wastes drops the pH and provokes methanogenesis inhibition. In this context, pH adjustment would minimally contribute to process control. For that reason, AD of selected agroindustrial wastes (residual cucumber, tomato and lettuce) was initially performed in three continuous stirred tank reactors (CSTR) with a working volume of 1 L, which were run under equal operational conditions (25°C and Organic Loading Rate - OLR of 3 gVS·d⁻¹L⁻¹) and without pH adjustment. VFAs production in absence of pH adjustment was lower than 6 g·L⁻¹, giving rise to bioconversion efficiencies of 16.8%, 13.2% and 9.0% for cucumber, tomato and lettuce residues, respectively (Figure 1). These low efficiencies were consequence of an excessive pH drop in the bioprocess (4.60, 3.85 and 3.77 for cucumber, tomato and lettuce) that induced not only methanogenesis inhibition but also partial inhibition of the acidogenesis. To prevent acidogenesis inhibition, AD of agroindustrial wastes was carried out under the same operational conditions (25°C, OLR of 3 gVS $d^{-1}L^{-1}$) and with adjusted pH, where pH was maintained between 5.5 and 6.0. This pH was selected because is in the optimal range of the acidogenic stage according to Kwietniewska and Tys (2014). After pH adjustment, the bioconversions of cucumber, tomato and lettuce waste were increased to 52.6%, 40.1% and 49.4% respectively (Figure 1). In this manner, pH adjustment favored the potential use of these agroindustrial wastes for VFAs production purposes, which bioconversion were much higher than those previously reported from anaerobic fermentation of foodwaste (Moretto et al., 2019). Regarding the VFAs profile, it was detected a high content of long-chain VFAs as well as the prevalence of even-numbered carbon ones, which accounted for 86.0, 94.3 and 80.9% for CSTRs degrading cucumber, tomato and lettuce with pH adjustment, respectively (Figure 1). These VFAs are considered precursors for the production of biodegradable biopolymers such as poly-3-hydroxybutyrate, which could reduce by 50% the economic cost of certain biopolymers production (Iglesias-Iglesias et al., 2019).



Figure 1. VFAs measured during the steady state of the reactors fed with cucumber (CSTR-C), tomato (CSTR-T) and lettuce (CSTR-L) residues and their corresponding bioconversion efficiencies when CSTRs were run w/wo pH adjustment.

Once CSTRs reached the steady-state, microbial communities were studied by analyzing 16S rRNA gen for both Bacteria and Archaea. As it can be seen in Figure 2a, pH adjustment promoted the phylum Firmicutes, which is involved in the hydrolysis and acidogenesis steps of the anaerobic digestion process. In CSTRs operated with pH adjustment, Firmicutes was mainly composed of genera *Ruminococcus, Acidamnicoccus, Lactobacillus, Bulleidia* and of those belonging to Clostridiales order (Figure 2b). These bacteria are related to carbohydrates degradation into VFAs with even number of carbons, which may be responsible of the high content of acetic, butyric and caproic acids in the effluents of CSTRs operated with adjusted pH.



Figure 2. Relative abundance of Bacterial and Archaeal OTUs at (a) phylum and (b) genus levels for the reactors fed with cucumber (CSTR-C), tomato (CSTR-T) and lettuce (CSTR-L) residues w/wo pH adjustment. Taxonomic groups with relative abundance lower than 1% were excluded from the legend.

These results indicated that all CSTRs with pH adjustment promoted a robust microbial population involved in the first steps of AD and with high hydrolytic and acidogenic activity.

Thus, this study proves the feasibility of using agroindustrial wastes for VFAs production via AD since high bioconversion efficiencies can be reached by adjusting the pH value to a proper range that boosts a key bacterial community.

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