Indigenous microbiome as a promising strategy to maximize organic waste valorization into added-value chemicals

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Waste management strategies are crucial for sustainable development. Anaerobic fermentation (AF) has demonstrated a great potential for the valorization of organic waste into added-value bioproducts and energy vectors, such as carboxylates (short-chain fatty acids, SCFAs), hydrogen (H_2) and ethanol (EtOH) (Greses et al., 2022). AF is normally carried out by open-mixed cultures collected from conventional anaerobic digestion (AD). Nonetheless, this inoculum presents methanogenic microorganisms that have the capability of degrade these compounds into biogas (CH_4 + CO_2), which results in detrimental for its production. As an alternative, the use of indigenous microbiota grown in agroindustrial waste (AGW) represents a promising strategy. The absence of methanogens and the high affinity of indigenous microbiota for the residue composition could maximize AGW valorization. To prove this hypothesis, this investigation aimed at evaluating the indigenous microbial dynamics related to the AGW valorization into added-value chemicals via AF.

AF of AGW was carried out in a continuous stirred tank reactor (CSTR) in the absence of external inoculum. Operational conditions included a temperature of 25 ºC, an organic loading rate of 3 g VS $L^{-1}d^{-1}$, a hydraulic retention time (HRT) of 20 d, and an adjusted pH between 6 and 6.5. The CSTR was operated until the chemical parameters (i.e. chemical oxygen demand-COD, solids, SCFAs) measured exhibited stability, and no less than 3 HRTs. Microbial dynamics were also analyzed to elucidate its correlation with obtaining target compounds.

As can be seen in Figure 1, the compound profile throughout the experiment evidenced the critical pH effect on obtaining added-value chemicals. In the start-up period, the pH dropped to 4.5 due to the acidic nature of AGW, resulting in a profile dominated by primary metabolites, namely lactic acid (15.5 g L^{-1}) and ethanol (11.3 g L^{-1}). This result was consistent with the results obtained by Mosquera-Toscano et al., 2023, who reported lactic acid as the main compound when performing self-fermentation at a pH lower than 5. During the transition period, when the pH adjustment started, a shift in the chemical distribution became evident. Lactic acid was completely consumed, and ethanol concentration started to decrease. This may be attributed to the initiation of carboxylates synthesis, which requires the consumption of primary metabolites (Tang et al., 2017). Thus, primary metabolites were notably reduced (6.9 g L^{-1}), while carboxylates became the most abundant compounds (24.0 g L^{-1}). SCFAs profile was mainly composed of acetic (7.3 g L^{-1}), butyric (6.6 g L^{-1}), propionic (4.8 g L^{-1}) and valeric (5.0 g L^{-1}) acids. Once the steady-state was reached, the AF resulted in a total carboxylate concentration of 49.5 g COD·L^{-1}, corresponding to a bioconversion efficiency of 65.7 % (according to the COD measured in the feedstock). This value was remarkably high when compared to previous studies dealing with carboxylates production via AF of AGW. For instance, Greses et al., 2023 reported a bioconversion efficiency of 59.6%, under similar conditions and using anaerobic sludge as inoculum. This result confirmed the capability of indigenous microbiota to produce carboxylates at high concentration without requiring the addition of external inoculum. The chemical distribution encountered in the steady-state was composed by butyric (12.2 g L^{-1}) and acetic (8.8 g L^{-1}) as main carboxylates, increasing the accumulation of longer-carbon chain acids such as hexanoic (3.1 g L^{-1}) and valeric (3.9 g L^{-1}). This is because the pH in the reactor was successfully maintained at an average of 6.

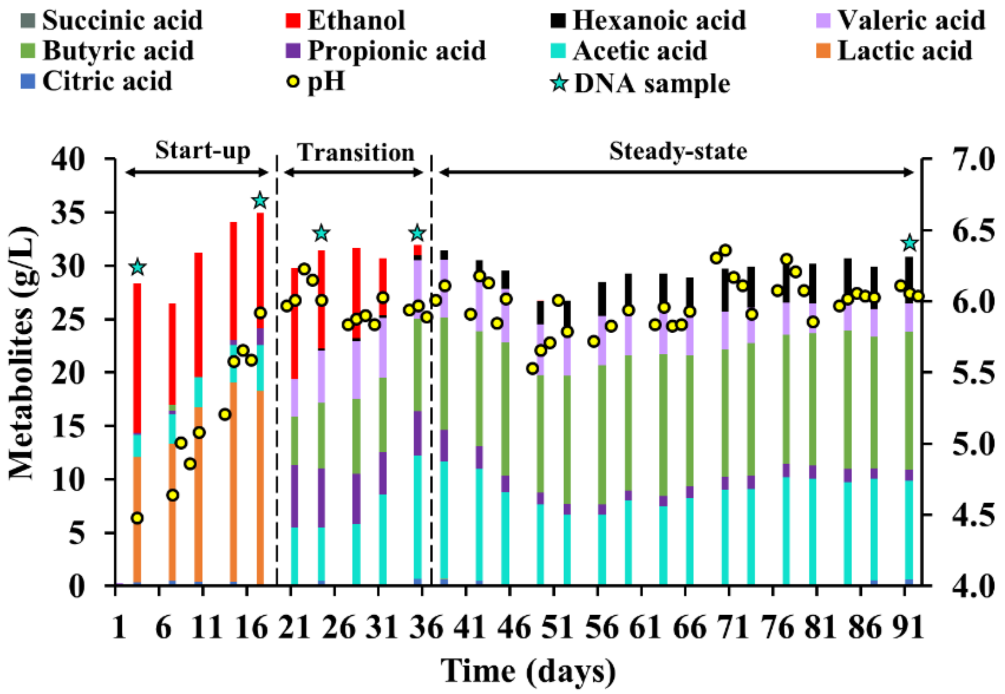

Figure.1 Metabolites distribution during the AF of AGW.

The microbial community analysis revealed Firmicutes as the prevailing phylum (>65 % relative abundance) in the whole process. These results were in agreement with previous studies of AF of AGW that recognized Firmicutes as key phylum in carboxylates production (Greses et al., 2023). However, the pH oscillation clearly affected the bacterial distribution within this phylum. Bacteria belonging to Bacilli class dominated the Firmicutes phylum (100 %) when lactic acid and ethanol were the most abundant compounds in a pH range between 4.5 and 5.9 (start-up period). Nevertheless, during the transition stage, the change in the metabolite profile was associated with a Clostridia class increase (44 %) at the expenses of Bacilli decrease (< 32 %). Once the AF reached the steady-state, members of Clostridia class dominated the bacterial profile (>80 %), being the key players when the compound pool was totally comprised of carboxylates (pH 6-6.5).

These results demonstrated that self-fermentation is a feasible strategy to maximize chemicals production since high yields were reached despite of not using external inoculum. This fact confirmed that there is no need for external inoculum addition, which reduce the costs. Likewise, the effect of pH variations on the chemical profile evidenced the remarkable versatility of the indigenous consortium, that could enable the production of several target metabolites by only altering the operational pH range.

Acknowledgments

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