# Organic waste valorisation into bioenergy and bioproducts through a cascade combination of bioprocesses

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In the current search of renewable sources that can replace the use of traditional fossil fuels, the efforts of the research community are focused on producing energy and chemicals using organic wastes (OW) as a sustainable resource. Anaerobic digestion (AD) is one of the most studied biological processes for OW valorization in which, a complex and synergic microbial community transforms the organic matter present in residues into a source of energy (biogas) through four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The AD of carbohydrate-rich wastes such as vegetable and fruits normally results inthe inhibition of biogas production as a consequenceof an excessive accumulation of short-chain fatty acids (SCFAs) produced during acidogenesis. To overcome this issue, the fermentative (hydrolysis, acidogenesis, acetogenesis) and methanogenic stages are conventionally decoupled in two different reactors to maximize biogas production (Markphan et al. 2020). This traditional technology allows valorising OW into one single product, biogas ($CH_4$ and $CO_2$), since the SCFAs produced in the fermentative reactor are used as substrate in the methanogenic reactor. However, the fermentative stage of AD could produce a wide range of intermediate metabolites with higher market value than biogas, which includes not only SCFAs but also ethanol (EtOH) and hydrogen ($H_2$) (Greses et al., 2021). As these metabolites are valuable green chemicals to replace petrochemical derivatives, they might be considered as products by themselves. To maximize OW valorisation into biochemicals and bioenergy, this investigation assessed a new biotechnological approach based on acascade combination of anaerobic bioprocesses. For such a purpose, a carbohydrate-rich waste (80 % carbohydrates on dry matter basis) was firstly valorised in a thermophilic anaerobic fermentation (AF) operated in continuous mode and devoted to carboxylates (SCFAs) production. Thereafter, the carboxylates-rich liquid stream attained from the AF was separated and only the solid spent residue was fed to a conventional AD reactor to further valorise the OW into biogas.

55ºC-AF, operated at slight acid pH (5.8), resulted in a total SCFAs concentration of 23.4 g SCFAs $L^{-1}$, corresponding to a bioconversion efficiency of 50 % (in Chemical oxygen demand (COD) basis). This high bioconversion evidenced the suitability of the imposed operational conditions since this bioconversion value was higher than those reported in previous studies (Moretto et al. 2019). The SCFAs profile was mainly dominated by butyric (65.6 %) and acetic acid (24.4 %). Normally, AF of OW releases a wide variety of SCFAs, increasing the technology complexity and the economic cost related to their recovery for subsequent uses. Thus, the prevalence of two SCFAs was particularly interesting to simplify the downstream process. Moreover, the metabolisms induced in AF also resulted in the production of EtOH (22 g $L^{-1}$) and $H_2$ (140 L $H_2$/kg $COD_{OW}$). Therefore, this first stage of OW valorisation resulted in two bioproducts (SCFAs and EtOH) and one energy stream ($H_2$) from a single residue.

The subsequent AD of the solid spent residue from the AF showed a methane yield of 300 L $CH_4$ kg $VS_{inf}^{-1}$, corresponding to 54 % of COD removal. This is an outstanding result considering the similar methane yields obtained by direct AD of raw OWs or by a traditional two-stage AD of FWs (300-400 L kg $VS_{inf}^{-1}$) (Negri et al., 2020).The reason for such a high methane yield was attributed to the macromolecular distribution of the solid spent. The content of carbohydrates in the solid fraction of AF effluent (48 % on dry matter basis) rendered this waste particularly suitable to be further valorised in a conventional AD process. Likewise, the carbohydrate content was not high enough to provoke the inhibition of biogas production by SCFAs accumulation, as it occurs in conventional AD of raw OW. It can be thus stated that this cascade approach helps mitigating common inhibitory issues while allowing maximizing resource utilization.

The microbial community analysis performed by 16S rRNA gene sequencing revealed that the slight acid pH (5.8) imposed in AF reactor forced a biodiversity decrease from 6.736 (Shannon index) in the 55ºC-inoculum (pH 7.8) to 2.500 in the 55ºC-AF reactor, suggestinga microbiome specialization in the intermediate metabolites production during the fermentative stage. By contrast, the biodiversity in 35ºC-AD reactor (5.148) was close to the value calculated from the 35ºC-inoculum (6.975). This minor variation was related to the microbiome adaptation to the substrate (solid residue rich in carbohydrates), evidencing that pH exhibited higher influence on the microbiome than the substrate composition. This fact was confirmed by comparing the microorganisms identified in the inocula and reactors (Figure 1). The 55ºC-inoculum showed a variety of phyla conventionally encountered in thermophilic AD, whereas Firmicutes phylum prevailed in 55ºC-AF. This phylum was mainly dominated by acidophilic bacteria involved in EtOH and SCFAs production, such as *Thermoanaerobacterium*. The results obtained from conventional AD denoted a microbial community shift from protein degraders (Proteobacteria, Actinobacteria) towards carbohydrate degraders (*Palubibacter*, undefined SB-1).

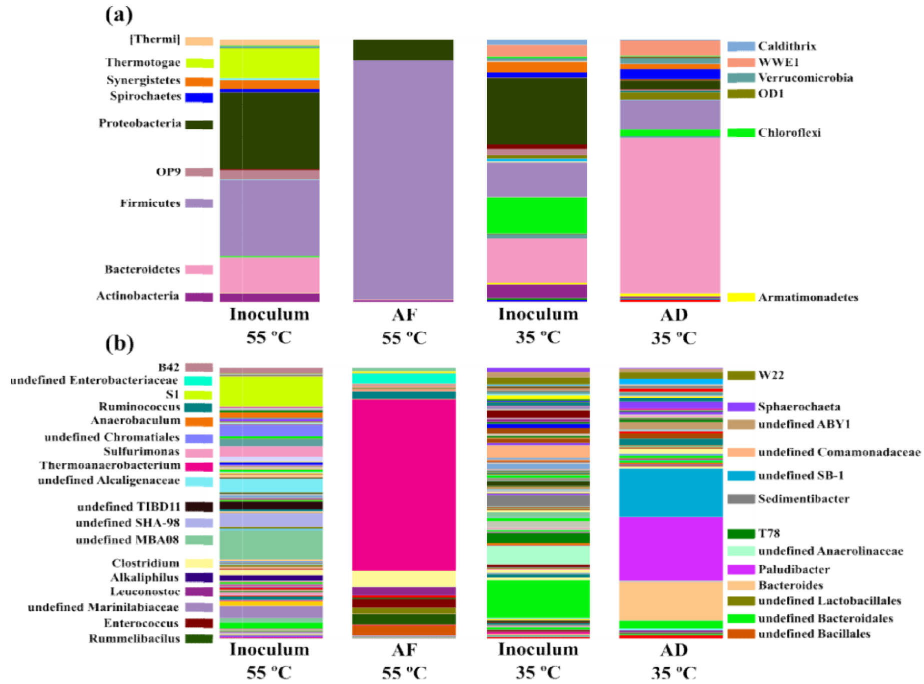


**Figure 1. Bacteria and Archaea identified in the reactors and the corresponding inocula in terms of relative abundance at (a) phylum and (b) genera levels.**

These results highlighted the potential of a cascade combination of anaerobic bioprocesses as suitable multiproduct strategy to valorise OW. Against conventional AD technologies, this non-conventional two-stages AD process attained 2 bioproducts (SCFAs and EtOH) and 2 energy streams ($H_2$ and biogas) from a single OW, helping to decrease the waste burden in treatment facilities, reducing the negative environmental impact of OW disposal and boosting a circular economy model.


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