Microbial community of the anaerobic fermentation of urban waste: effect of the hydrodynamic cavitation pre-treatment

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Volatile fatty acids (VFAs) are valuable building blocks which can be sustainably produced from organic waste through dark fermentation processes. Substrates' pre-treatment is often needed to enhance their solubilization and bioavailability, among which hydrodynamic cavitation (HC) seems very promising (Bhat and Gogate, 2021). In fermentation processes, the VFAs yields and distribution for a given substrate are essentially the results of the microbial community composition and activity (Ramos-Suarez et al., 2021). However, the trend of microbial communities is still uncertain, since they are shaped by factors like the inoculum source, the substrate fed, the operational conditions and the pre-treatment applied (Llamas et al., 2021). At present, some studies have been conducted on the impact of ultrasonic cavitation on fermentation products (Yang and Wang, 2020; Liu et al., 2018), but the knowledge of the effect of HC pre-treatment of a mixture of sewage sludge (SS) and vegetable waste (VW) is still lacking.

This study focuses on the effect of the HC pre-treatment of a mixture of SS and VW on the microbial community of the fermenters.

The substrate used for fermentations was a mixture composed of sewage sludge collected from the local wastewater treatment plant (Venice, Italy) and seasonal vegetable scraps in a 1:1 ratio on a TVS basis. This mixture was pre-treated by HC, performed with a stator and rotor assembly (Three-es S.r.l) for 30 minutes with a power of 8 kW, P of 1.4-1.5 bar, $Q_{mixture}$ of 25-30 L/min, and 1550-1650 rpm.

Fermentation tests were conducted at T=37°C in a fermenter with V=4L. Batch tests were performed until the VFAs concentration stopped increasing. Then, the reactors were fed in a semi-continuous manner, with an OLR of 8 kg_{TVS}/m^3 d and a HRT of 5-6.6. Daily samples were collected to determine VFAs, soluble chemical oxygen demand (sCOD), pH, alkalinity, and cations (APAT, 2003; APHA, 2012).

The samples for microbial analyses were collected at the beginning of the steady-state and the end of the experiment. The *Next Generation Sequencing* of the V4 region of the 16S rRNA was performed following the "double step PCR" protocol. Then, taxonomic identification was performed with the database SILVA 132 (BMR Genomics s.r.l.). Species richness was calculated with the species richness estimator Chao 1 (Chao, 1984), the Shannon H_0 index (Shannon, 1948) and the Pielou's evenness (Pielou, 1975).

The fermenters reached a pseudo-steady state, with a VFAs concentration of 12.94 ± 0.63 $gCOD_{VFA}/L$ for the cavitated and 18.23 ± 0.51 $gCOD_{VFA}/L$ for the not cavitated. The VFAs profile was similar, with the sum of acetic, propionic, butyric and iso-butyric acids accounting for 50.8% and 59.9% of the total for the cavitated and not cavitated, respectively. Heptanoic acid was present in higher percentages in the not cavitated, thus indicating that the HC pre-treatment enhanced the substrates' conversion into VFAs with shorter carbon chains (Moretto et al., 2019).

The microbial analyses revealed that the microbial community was mainly composed by *Bacteria*, while *Archaea* were almost absent (0-0.8%). This indicates that fermentative microorganisms can be favoured only by tuning the operative conditions, without any inoculum pre-treatment or chemical compounds addition.

At the beginning and the end of the steady state, in both reactors, the most abundant phyla were *Firmicutes, Bacteroidetes* and *Proteobacteria*, widely known as the main responsible of the hydrolytic and acidogenic phases of the dark fermentation. *Firmicutes* represented 52.6% and 47.0% of the community in the cavitated and not cavitated, respectively. *Bacteroidetes* were more abundant in the cavitated (25.7%) than in the not cavitated (13.1%) with *Prevotella_7* as the most abundant genus. The genus *Prevotella* is a known protein degrader, producing organic acids and NH_4^+, which played a role in maintaining the pH stability without any control. *Proteobacteria* were less abundant in the cavitated (10.8%) than in the not cavitated (26.7%). The main difference at the beginning of the steady-state was the abundance of the

Proteobacteria and *Bacteroidetes*, which could be ascribable to carbohydrate disgregation due to the HC pre-treatment. This could have disadvantaged *Proteobacteria,* which are known as degraders of different carbon sources (Hartati et al., 2018).

At the end of the steady-state, a variation in the abundance of the main phyla between the cavitated and not cavitated was observed. *Firmicutes* decreased to 12.8% in the not cavitated, while they still represented 47.4% of the cavitated. The percentage of *Proteobacteria* increased, reaching 19.7% and 48.3% for the cavitated and not cavitated, respectively. Such a high increase in the not cavitated could be due to the central role of *Proteobacteria* in carbohydrate disgregation. However, this did not correspond to a variation in the VFAs profile or yields, probably thanks to the diversity of the microbial community.

Species richness was stable between the beginning of the steady-state and the end of the experiment for both fermenters. Lower values of all the species richness indices were observed for the cavitated with respect to the not cavitated (table 1). This could be due to the hydrolyzation of the organic compounds contained in the mixture after the HC pre-treatment, which could prevent the development of the microorganisms involved in the hydrolysis of carbohydrates and proteins, as already observed in Llamas et al., 2021 after enzymatic pre-treatment.

Table 1. Microbial community diversity of the reactors fed with the not cavitated and cavitated mixtures at the beginning (initial) and the end (end) of the steady-state.

	$\text{Not cavitated}_{\text{initial}}$	$\text{Cavitated}_{\text{initial}}$	$\text{Not cavitated}_{\text{end}}$	$\text{Cavitated}_{\text{end}}$
Chao1	272.00	190.00	288.00	207.00
Shannon	4.54	3.85	4.68	3.96
Evenness	0.81	0.73	0.83	0.74

In conclusion, this work gave an insight into the microbial community of mesophilic fermenters fed with a mixture of vegetable waste and sewage sludge. It allowed to identify the variation of the main phyla in response to the pre-treatment and suggested that the stability of the VFAs profile can be maintained thanks to the microbial community diversity.

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