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ORIGINAL PAPER

Microbial Community of the Acidogenic Fermentation of Urban Waste: Effect of the Hydrodynamic Cavitation Pre-treatment

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Abstract

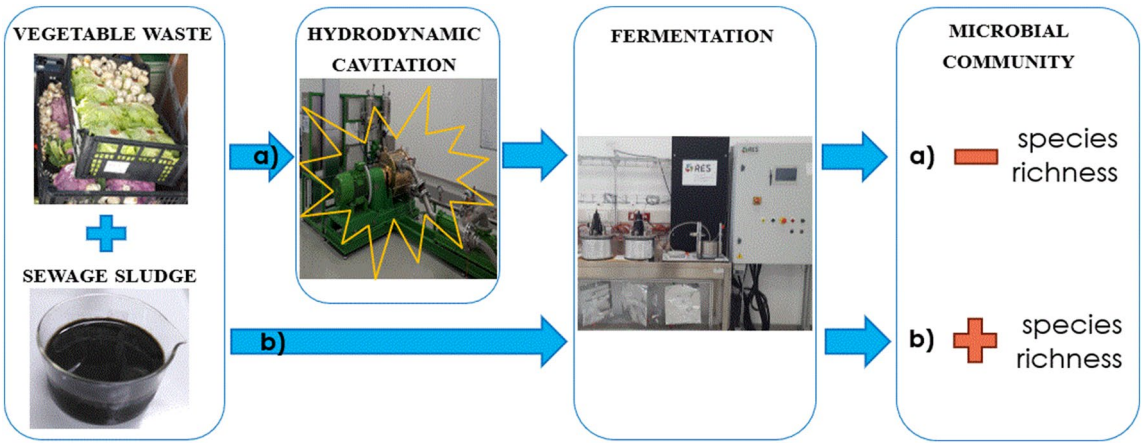
Purpose This study aims at understanding how the acidogenic fermentation microbial community was impacted by the hydrodynamic cavitation (HC) pre-treatment of the substrates' mixture, constituted by waste-activated sludge and vegetable waste 1:1 on a TVS basis.

Methods HC was performed with power = 8 kW, $P = 1.4–1.5$ bar, $Q_{mixture}$ of 25–30 L/min, 1550–1650 rpm, duration: 30 min. Fermentation tests were conducted on cavitated (CAV) and not cavitated (NCAV) mixture at T = 37 °C inside 4 L reactors in batch mode, then switched to semi-continuous with an OLR of 8 kg_{TVS} m^{-3} d^{-1}. Microbial community was characterized by 16S rRNA sequencing at the beginning and end of the pseudo-steady-state. Ecological diversity and clustering among the samples were determined by beta diversity, Venn diagram, and non-metric multi-dimensional scaling (NMDS) analysis.

Results Cavitation was efficient in substrates' hydrolyzation but resulted in a lower microbial diversity of 3.85 (Shannon Index) and VFAs concentration of 12.9 $gCOD_{VFA}$ L^{-1} in the anaerobically fermented cavitated mixture (AF-CAV), respect to 4.54 and 18.2 $gCOD_{VFA}$ L^{-1} in the anaerobically fermented not cavitated mixture (AF-NCAV), respectively. NMDS analysis showed that AF-CAV and AF-NCAV samples formed two different clusters, with VFAs concentration as the only significant factor explaining their difference ($R^2 = 1$, $Pr > r = 0.04167$). Functional redundancy among community members probably allowed to maintain a stable VFAs composition despite the microbial community variation observed at the end of the test.

Conclusion The insights here provided on the effects of HC confirm the fundamental role played by microbial community in acidogenic fermentation processes and underline its importance in evaluating the effect of substrates' pre-treatment.

Graphical Abstract



Keywords Microbial community · Acidogenic fermentation · Cavitation · Vegetable waste · Sludge · NMDS analysis

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Abbreviations

HC	Hydrodynamic cavitation
HRT	Hydraulic retention time
OLR	Organic loading rate
TS	Total solids
TVS	Total volatile solids
UC	Ultrasound cavitation
VFAs	Volatile fatty acids
WAS	Waste-activated sludge
NMDS	Non-metric multi-dimensional scaling

Statement of Novelty

The novelty of this study relies in the identification of the effect of the hydrodynamic cavitation pre-treatment of organic waste on the microbial community of anaerobic fermentation. This allowed to define the pre-treatment applicability to the anaerobic fermentation process and the future research needed for its optimization.

Introduction

Our current practices of energy and chemicals production from non-renewable sources and uncontrolled waste generation are inconsistent with the need for combating climate change and respecting the Planet's biocapacity. On the contrary, the date where humanity depletes the Planet's biocapacity, the *Earth Overshoot Day*, comes sooner every year [1]. To collaboratively develop solutions embracing the economic, social and environmental sustainability, 193 countries agreed to the 17 Sustainable Development Goals (SDGs) developed by the United Nations [2]. Waste bioconversion through acidogenic fermentation (AF) can contribute to at least 5 SDGs by improving the wastewater treatment process (clean water and sanitation, SDG 6) and waste management (sustainable cities and communities, SDG 11), producing affordable and clean energy (SDG 7) and bio-based products (responsible consumption and production, SDG 12), ultimately combating climate change (climate action, SDG 13) [3].

The main product of AF are volatile fatty acids (VFAs), which are key platform chemicals for a plethora of industries, among which chemicals, pharmaceuticals, food and agriculture [4]. Despite being currently obtained from petrochemical compounds through a process entailing considerable environmental impacts, VFAs can be sustainably produced from organic waste through AF [5]. The main organic waste streams of the urban metabolism are represented by waste-activated sludge (WAS) and food waste, which can be fully valorized in an integrated biorefinery approach [6, 7]. Their management through acidogenic co-fermentation can provide environmental improvements respect to the separate WAS anaerobic digestion and aerobic composting of the organic fraction of municipal solid waste (OFMSW) [8]. Moreover, this strategy is reported to improve the process performance thanks to (i) a higher organic material content; (ii) a stronger buffer capacity; (iii) balanced macronutrients and micronutrients; (iv) dilution of toxic and inhibitory compounds; (v) a more diverse microbial community [9, 10].

WAS pre-treatment is needed to enhance its conversion to VFAs during acidogenic fermentation due to its complex floc structure [11]. A promising physical–chemical pre-treatment is hydrodynamic cavitation (HC), a process consisting of the formation, growth and collapse of vapor cavities due to a sharp pressure drop engendered by a sudden constriction [12]. The cavity implosion creates a « hot spot», an extremely reactive microenvironment reaching temperatures in the order of 1000–10,000 K and pressures of 100–1000 bar, and characterized by high-shear microjets and turbulences [13]. The subsequent collapsing events at the solid–liquid interface creates cracks and voids on the solid surface, causing its progressive disgregation [13]. Under harder operative conditions HC can desintegrate also the cellular membrane of microorganisms, thus partially disinfecting the substrate [12]. Both substrate disinfection and disgregation can affect the microbial community composition: the former would entail the loss of the substrates' endogenous microflora, which is often efficient in their anaerobic transformation [14], while the second could advantage acidogenic microorganisms over the hydrolitic ones. HC technology can bring both environmental and economic advantages, thanks to its higher potential of scalability and low cost, which has been proven to be orders of magnitude cheaper than ultrasonic cavitation (UC), where the ultrasounds used to generate the pressure drop entail a higher energy consumption [12]. Literature has mainly focused on UC, reporting an increase in VFAs yields when applied to food waste [15] and WAS [16]. Only one study was conducted on a mixture of WAS and OFMSW, interestingly showing that a milder UC pre-treatment yielded a 24% increase in the BMP, while a stronger one lowered biogas production, probably due to a disinfection effect [17]. At present, HC has been extensively tested on sludge and wastewater [12]. In contrast, only one study has been conducted on a mixture of WAS and vegetable waste, showing no increase in VFAs yields in the AF process [18]. Moreover, the effects of the HC pre-treatment on the microbial community of the AF process are still unknown.

Microbial community composition and activity essentially determine the VFAs yields and distribution for a given substrate [4]. Currently, research efforts in AF have mainly targeted the optimization of operating parameters and substrate pre-treatment to increase its bioavailability. However, in mixed culture fermentation, the process parameters

optimization and the substrate pre-treatment both imply the selection of the most adapted microbial communities [19]. In this context, the effect of the substrate HC pre-treatment on the microbial community developed during AF requires further investigation, with the aim of optimizing organic waste conversion into VFAs. To cover this knowledge gap, the study presented here investigated the effect of the HC pre-treatment of a mixture of WAS and vegetable waste on the microbial community of two semi-continuous AF reactors at the beginning and at the end of the pseudo-steady-state. For this purpose, ecological diversity and clustering among the samples were determined by beta diversity, Venn diagram, and non-metric multi-dimensional scaling (NMDS) analysis.

Materials and Methods

Anaerobic Fermentation Tests

The substrate used for fermentations was a mixture composed of WAS 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 min with a power of 8 kW, P of 1.4–1.5 bar, $Q_{mixture}$ of 25–30 L/min, and 1550–1650 rpm. The effect of the HC pre-treatment was evaluated by comparing the physical–chemical characteristics of the mixture before and after cavitation. The substrates' degree of disintegration (DD_{COD} %) was calculated as in Tonanzi et al. [11].

The anaerobic inoculum consisted of digestate collected from a WWTP, where WAS and OFMSW are anaerobically co-digested. Digestate is one of the most widely used inoculum for anaerobic fermentation, since it contains hydrolytic and fermentative bacteria, while methanogens can be easily inhibited and/or washed out by tuning the operative conditions [20].

Fermentation tests were conducted at T = 37 °C in a fermenter with V = 4 L. Batch tests were performed until the VFAs concentration stopped increasing. Then, the reactors were fed semi-continuously, with an organic loading rate (OLR) of 8 kg_{TVS} m^{-3} d^{-1} and a hydraulic retention time (HRT) of 5–6.6 d. According to the literature, the experimental parameters applied were those giving the best performances [21, 22]. The tests were inoculated with anaerobic digestate (31–34% v/v) to maintain a high F/M ratio and inhibit methanogens [23, 24]. Additionally, methanogenesis was avoided by applying acidic conditions and the short HRT indicated above, without the need to add chemical inhibitors. Daily samples were collected to determine VFAs and pH.

Microbial Community Analysis

The samples for microbial analyses were collected at the beginning of the pseudo-steady state and the end of the experiment. The extracted DNA was used as template for the amplification of the V4 hypervariable region of the 16S rRNA gene using the universal PCR primers Parada–Apprill [25, 26]. PCR reactions and next generation sequencing were performed commercially (BMR Genomics s.r.l., Italy). OTU assignment and taxonomical assignment was carried out using the default pipeline of QIIME2 [27]. Species richness was calculated with the species richness estimator Chao 1 [28], the Shannon H_0 index [29] and the Pielou's evenness [30]. The Operative Taxonomical Units (OTUs) specific to or shared between AF-CAV and AF-NCAVat both time points were visualized through a Venn diagram. The OTU table was used as input for a non-metric multi-dimensional scaling (NMDS) biplot to graphically ordinate the samples and assess the differences between AF-CAV and the AF-NCAV at the beginning of the pseudo steady-state and at the end of the test. The distance between samples was calculated with Bray–Curtis. The correlation between the microbiota composition and the tested factors was investigated by fitting the NMDS ordination scores with the *envfit Vegan* function. The tested factors were: time, hydraulic retention time, organic loading rate, temperature, mixing, pH, total VFAs (gCOD L^{-1}), single VFAs (gCOD L^{-1} and percentage on the total). NMDS analysis was performed with the *metaMDS* function implemented in the R package *Vegan*.

Analytical Methods

All analyses were performed according to the APAT, IRSA-CNR [31], APHA, AWWA, and WET methods [32]. VFAs were determined with an Agilent 1100 SERIES high-performance liquid chromatograph (HPLC) equipped with an Acclaim™ Organic Acid 4 × 150 mm column (Thermo Fisher) running at 20 °C and with a diode array detector (DAD). A gradient elution was performed at a constant flow rate of 1 mL/min, using 2.5 mM methanesulfonic acid and HPLC-grade acetonitrile. The Volatile Free Acid Mix CRM46975 was used as standard (Sigma-Aldrich).

Results and Discussion

Anaerobic Fermentation Tests

As thoroughly detailed in a previous paper, the HC pre-treatment of the mixture determined a 15% decrease in the COD of the solid fraction and a DD_{COD} of 6%, attributable to the transfer of the organic material from the solid to the liquid phase [18]. In fact, the CAV showed a VFAs concentration

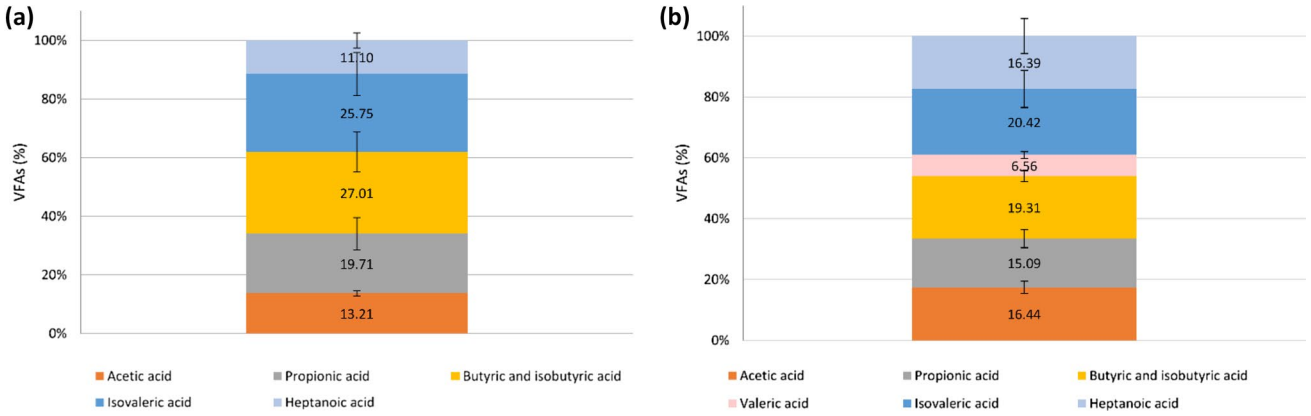

Fig. 1 VFAs profile (COD basis) of the semi-continuous at pseudo-steady-state of **a** AF-CAV and **b** AF-NCAV

Table 1 Microbial community diversity of the reactors fed with the CAV and NCAV mixtures at the beginning of the pseudo-steady-state (initial) and the end of the test (end)

	$AF\text{-}CAV_{initial}$	$AF\text{-}NCAV_{initial}$	$AF\text{-}CAV_{end}$	$AF\text{-}NCAV_{end}$
Chao1	190.00	272.00	207.00	288.00
Shannon	3.85	4.54	3.96	4.68
Evenness	0.73	0.81	0.74	0.83

of 6.8 $gCOD_{VFA}\ L^{-1}$, while NCAV contained 1.7 $gCOD_{VFA}\ L^{-1}$.

The fermenters reached a pseudo-steady-state after 22 and 26 days, with a VFAs concentration of $12.9 \pm 0.6\ gCOD_{VFA}\ L^{-1}$ and $18.2 \pm 0.5\ gCOD_{VFA}\ L^{-1}$ for the AF-CAV and AF-NCAV, respectively, as fully detailed in Lanfranchi et al. [18].

The pH was not controlled but remained stable at values of 5.4–5.5 for both reactors.

As indicated in Fig. 1, the VFAs profile was similar, except for a lower butyric acid concentration and a higher heptanoic acid concentration in the AF-NCAV. The sum of VFAs with five atoms of carbon, i.e., iso-valeric and valeric acids, was similar even though valeric acid was detected only in the AF-NCAV. The higher heptanoic acid concentration observed in the AF-NCAV can indicate that the HC pre-treatment enhanced the substrates' conversion into VFAs with shorter carbon chains, as already observed for thermal pre-treatment [21].

Microbial Community Analysis

Microbial community analysis revealed a similar species richness between the beginning of the pseudo-steady-state and the end of the test for both fermenters. The AF-NCAV showed the highest diversity, with a Shannon Index of 4.54, comparable with the value of 5.09 obtained on similar substrates and higher than the value of 1.57 attained by fermenting fruit and vegetables alone [33, 34]. Lower values of all the species richness indices were observed for the AF-CAV with respect to the AF-NCAV (Table 1). This could be due to the hydrolyzation of the organic compounds contained in the mixture after the HC pre-treatment, as indicated by the DD_{COD} and the rise in VFAs concentrations, which could have prevented the development of some microorganisms involved in the hydrolysis of carbohydrates and proteins, as already observed in Llamas et al. [35] after enzymatic pre-treatment. Moreover, the substrates' HC pre-treatment might have reduced or inactivated their indigenous microbial load, even if a low specific energy (SE) of 2868 kJ $kgTS^{-1}$ and power density (P_D) of 0.08 W/mL were applied [18]. In fact, two main stages have been identified in the sludge cavitation pre-treatment process: in the first stage, the porous flocs are broken down into small particles and release extracellular polymers; in the second stage, the biomass is inactivated. These stages arise after different pre-treatment durations, depending on the parameters applied and the sludge characteristics, with cell disintegration being proportional to supplied energy [36, 37]. For instance, in Chu et al. [38] the second stage arose after 20 min at a P_D of 0.3 W/mL and SE of 96,100 kJ/kgTS, while in Zhang et al. [39] it occurred after 10 min at a P_D of 0.5 W/mL.

Despite the substrate's hydrolyzation, the pre-treatment resulted in a lower VFAs concentration in the AF process. This supports the hypothesis that a higher microbial diversity allows fermenting of different substrates more efficiently [33, 40].

The Venn diagram showed that 69 OTUs were common to all the samples (Fig. 2). A similar, low number of OTUs was in common between the AF-CAV and the AF-NCAV at the beginning of the pseudo-steady-state (29 OTUs) and the end of the test (23 OTUs). This is confirmed by the similar, wide distance between these samples in the NMDS plot (Fig. 3).

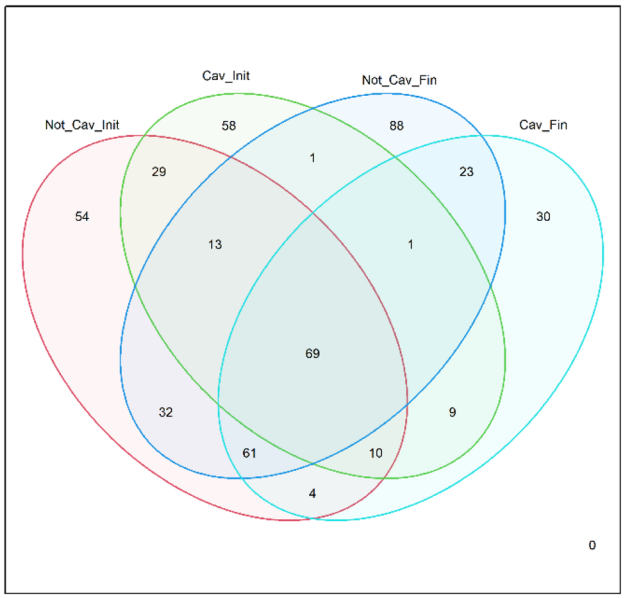

Fig. 2 Venn diagram showing the shared/specific bacterial OTUs between the AF-CAV and the AF-NCAV at the beginning of the pseudo-steady-state and the end of the test

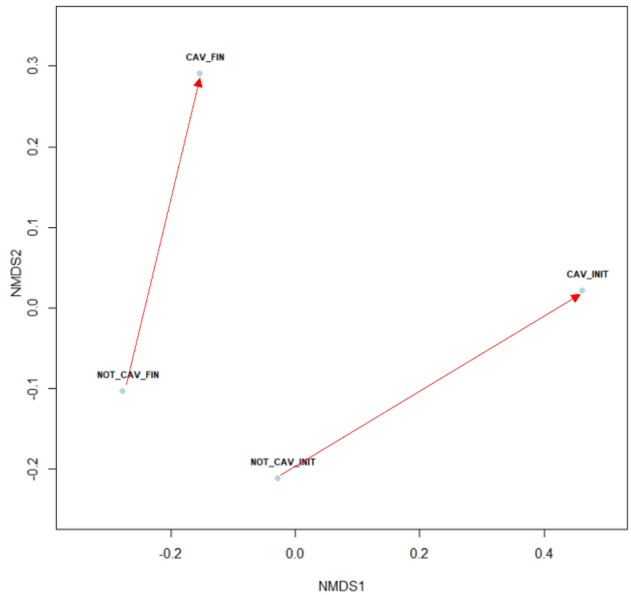

Fig. 3 NMDS biplot showing the samples clustering. The red arrows indicate the distance between the AF-CAV and AF-NCAV, which is explained by the VFAs concentration in gCOD L^{-1} as the only significant factor ($R^2 = 1$, $Pr > r = 0.04167$). (Color figure online)

The number of OTUs specific to the AF-CAV and AF-NCAV at the beginning of the pseudo-steady-state was similar, accounting for 58 and 54 specific OTUs, respectively. Then, the two conditions underwent differentiation, with 30 OTUs specific to the AF-CAV and 88 to the AF-NCAV at the end of the test. This is ascribable to the fact that both fermenters were inoculated with the same digestate at the beginning of the test, thus starting with a similar microbial community that probably differentiated over time. Finally, in the AF-CAV, only nine specific OTUs were in common between the beginning of the pseudo-steady-state and the end of the test, against the 32 OTUs of the AF-NCAV. This can also be observed in the NMDS plot as the wider distance between the AF-CAV's two-time points with respect to those AF-NCAV (Fig. 3) indicating that a change in the microbial community composition has occurred over time.

The NMDS biplot showed that the AF-CAV and AF-NCAV were separated by the y-axis and clustered together in the upper (AF-CAV) and the lower (AF-NCAV) part of the NMDS plot (Fig. 4). The AF-NCAV samples formed a more defined cluster, while the AF-CAV samples clustered together more loosely. This confirmed what was observed in the Venn diagram, where a lower number of OTUs were shared between the AF-CAV at the beginning of the pseudo-steady state and the end of the test, indicating a change in the microbial community composition over time (Fig. 3).

Based on the correlations of the tested factors (listed in paragraph 2.2), the distance between the two clusters, indicated by the red arrows in Fig. 4, can be explained by the only significant factor, i.e., VFAs concentration expressed as $gCOD_{VFA}\ L^{-1}$ ($R^2 = 1$, $Pr > r = 0.04167$).

Altogether, these results suggest that the HC pre-treatment resulted in a lower diversity in the fermentative microbial community, which led to a lower VFAs concentration in the fermented effluent.

Microbial Community Composition

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

At the beginning of the pseudo-steady-state and the end of the test, in both reactors, the most abundant phyla were *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria*, widely known as the main ones responsible for the hydrolytic and acidogenic phases of AF (Fig. 4a) [40, 41].

At the beginning of the pseudo-steady state, *Firmicutes* represented 52.6% and 47.0% of the community in the AF-CAV and AF-NCAV, respectively. The most represented family was the *Veillonellaceae* family in both the AF-CAV (22.0%) and AF-NCAV (33.8%), with *Megasphaera* and *Mitsuokella* as the most abundant genera (Fig. 4b). In the AF-CAV, the other main families were the *Ruminococcaceae* (8.9%), *Erysipelotrichaceae* (6.6%), *Acidaminococcaceae* (4.8%), *Lachnospiraceae* (4.0%) and *Lactobacillaceae* (3.5%). The same main families were observed in

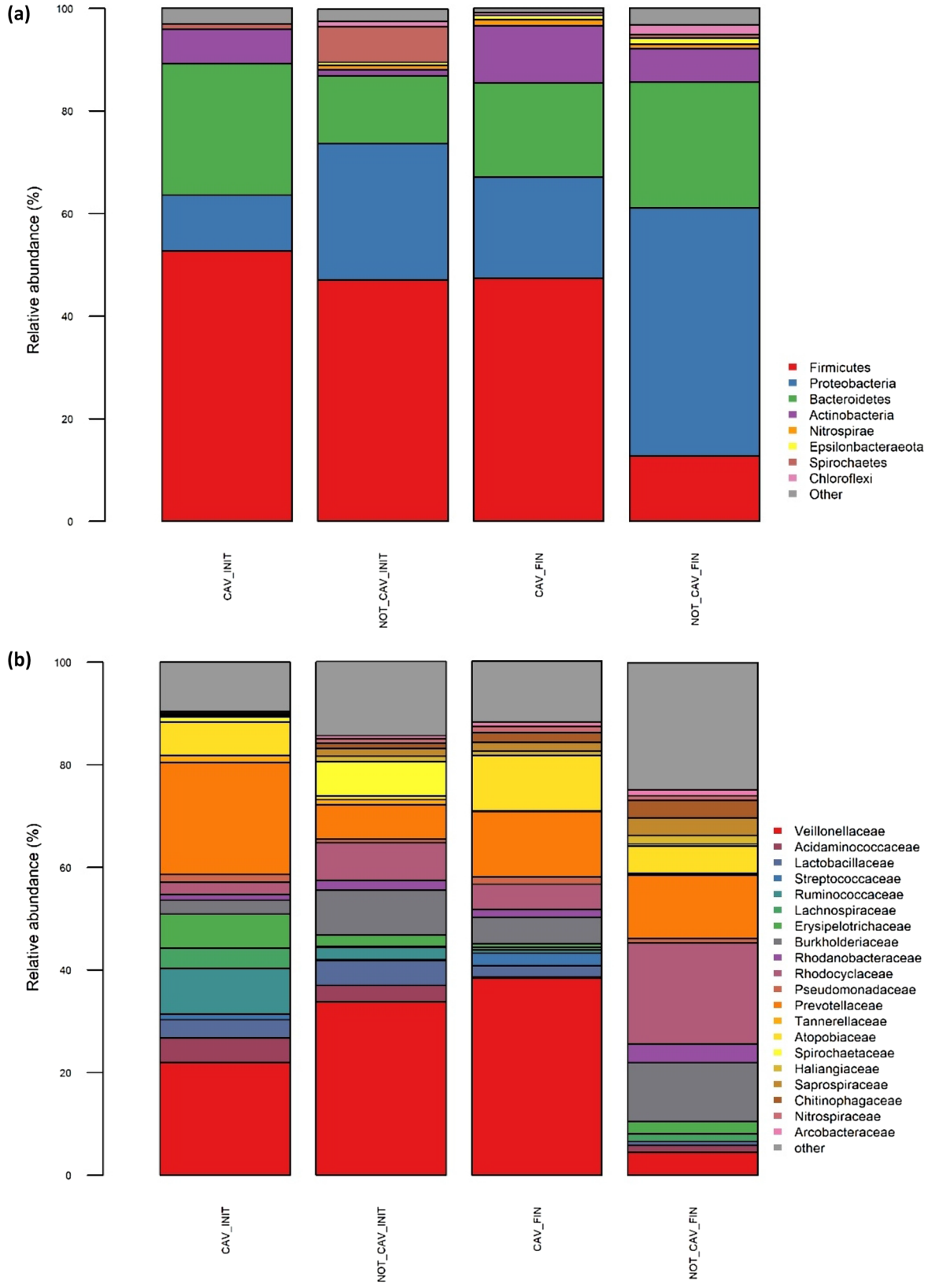

Fig. 4 Relative abundance of the bacterial OTUs at the **a** phylum level; **b** family level. Families are represented in the colour shades of the phylum they belong to (e.g. orange = Firmicutes). Only taxa with a relative abundance ≥ 1% were reported (Colour figure can be viewed at www.springer.com). (Color figure online)

the AF-NCAV, even if with different percentages: *Lactobacillaceae* (4.9%), *Acidaminococcaceae* (3.2%), *Ruminococcaceae* (2.4%), and *Erysipelotrichaceae* (2.2%). The phylum *Bacteroidetes* was more abundant in the AF-CAV (25.7%) than in the AF-NCAV (13.1%), with *Prevotella_7* as the main genus. *Proteobacteria* were less represented in the AF-CAV (10.8%) than in the AF-NCAV (26.7%) and were mainly constituted by the families *Rhodocyclaceae* and *Burkholderiaceae*. The *Rhodocyclaceae* family was mainly represented by the genera *Thauera*, *Dechloromonas* and *Ferribacterium*, while the *Burkholderiaceae* family by the genera *Lautropia* and *Rhodoferax*.

At the end of the test, a variation in the abundance of the main phyla between the AF-CAV and AF-NCAV was observed. *Firmicutes* still accounted for 47.4% of the AF-CAV, while they decreased to 12.8% in the AF-NCAV. The most represented family is always the *Veillonellaceae* family. In theAF-CAV, the most abundant genera of the phylum *Firmicutes* were *Mitsuokella* (20.4%) and *Megasphaera* (16.8%), followed by *Lactobacillus* (2.1%), *Lactococcus* (2.5%), *Clostridium_sensu_stricto_*1 (1.7%) and *Dialister* (1.3%). In the AF-NCAV, the most represented genera were still *Mitsuokella* (2.4%), *Dialister* (1.3%), *Acidaminococcus* (1.1%), *Lactobacillus* (0.8%) and *Megasphaera* (0.7%). Regarding the *Bacteroidetes* phylum, *Prevotella_7* was the main genus also at the end of the test.

The percentage of *Proteobacteria* increased over time, reaching 19.7% and 48.3% at the end of the test for the AF-CAV and AF-NCAV, respectively, maintaining the same dominating families and genera. The *Actinobacteria* phylum, almost totally represented by the genus *Olsenella,* showed an increase in both the AF-CAV (11.1%) and the AF-NCAV (6.4%) at the end of the test.

Discussion of the Microbial Community Composition

The results showed that the microbial community structure at the phylum level is coherent with what is reported in the literature for AF of food waste [34, 40, 41], agroindustrial waste [23], and sewage sludge [42, 43], where the dominant phyla were reported to be those of *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*.

Firmicutes were always the most abundant in the AF-CAV and at the beginning of the pseudo-steady state in the AF-NCAV, with percentages of 47–52.6% similar to those observed in AF of food waste [41], sewage sludge [43] and blackwater with food waste [44]. The dominant family of *Veillonellaceae* is reported to be constituted of propionate-producing microorganisms by H_2 consumption and lactate or succinate degradation, with varying metabolic characteristics according to the species [45, 46]. *Veillonellaceae* were mainly represented by the genera *Mitsuokella* and *Megasphaera*. *Mitsuokella* sp. are fermentative microorganisms with no hydrogen production reported in the literature, known to produce acetic, lactic and succinic acid [47, 48]. To our knowledge, AF literature reports *Mitsuokella* sp. only in a study on the co-fermentation of food waste and WAS [49]. Therefore, the genus *Mitsuokella* seems a peculiarity of the abovementioned work and of the present study. *Megasphaera* sp. are non-spore-forming obligate anaerobes able to produce H_2, acetic, propionic, butyric and valeric acids from glucose and lactic and/or acetic acid [50–52]. Coherently with our study, *Megasphaera* sp. is reported to become dominant at pH 5–6 during food waste acidogenic fermentation, where it played a fundamental role in the conversion of lactic acid to acetic acid [53], and it was also reported to be positively correlated with VFAs production [34].

Bacteroidetes were detected with percentages of 13.1–25.7%, similar to those reported in the AF of food waste [41], sewage sludge [43] and blackwater with food waste [44]. *Bacteroidetes* are known to be involved in the degradation of proteins, which are metabolized to generate NH_3 and carboxylic acids such as acetate, propionate and succinate [54]. In this study, all the samples were dominated by *Prevotella_7*. The genus *Prevotella* is constituted by asaccharolytic, anaerobic and proteolytic microorganisms, known to be implied in the proteolytic degradation of plant residues and able to produce NH_4^+ and organic acids during amino acids fermentation [44, 55]. *Prevotella* is reported to be one of the dominant genera in the AF of fruit and vegetables at pH 5.5, where it displayed a positive correlation with propionic acid [34]. In this study, NH_4^+ production probably contributed to neutralising acids and, therefore, to the pH stability observed without pH control.

Proteobacteria is one of the main phyla in all the samples, coherently with comparable studies in literature, where the abundance of the different classes *Alphaproteobacteria*, *Betaproteobacteria,* and *Gammaproteobacteria* seems to depend on the type of sludge [23, 34, 42, 44, 56]. *Proteobacteria* are more abundant in the AF-NCAV than in the AF-CAV. In fact, during the experiment, *Proteobacteria* relative abundance increased from 26.7 to 48.3% in the AF-NCAV, while it raised from 10.8 to 19.7% in the AF-CAV. The main genera *Dechloromonas, Ferribacterium* and *Thauera* (*Rhodocyclaceae* family) have been identified in AF of food waste and sewage sludge [33, 57–59], where they can have a negative impact on VFAs and H_2 concentration. In fact, *Dechloromonas* sp. are facultatively anaerobic microorganisms with strictly respiratory metabolism that oxidize acetate with O_2, chlorate, perchlorate, or nitrate [60]. *Thauera* strains are common saprophytic microorganisms in wastewater treatment plants and contaminated soils and sediments, where they are able to mineralize and detoxify toxic aromatic compounds and remove nitrate, in some cases using hydrogen as the electron donor [58, 60–63]. *Ferribacterium* sp. are strictly anaerobic microorganisms which

oxidize acetate, benzoate, formate, and lactate using Fe(III) as an electron acceptor, with a process exploited for energy production in bioelectrochemical systems [60, 64]. They can also use nitrate as an electron acceptor in acetate oxidation [60]. The genus *Lautropia* (*Burkholderiaceae* family) is composed of facultative mesophilic aerobes able to reduce nitrates and nitrites and ferment glucose, fructose, maltose, sucrose, and mannitol [60]. The genus *Rhodoferax* (*Burkholderiaceae* family) is composed of electroactive microorganisms which, in a recent study on necromass degradation in groundwaters, have revealed high abundances of the peptides associated with amino acid and fatty acid degradation, carbohydrate metabolism and amino acid uptake [65, 66]. However, their higher abundance in the AF-NCAV at the beginning of the pseudo-steady-state and the end of the test did not result in a lower VFAs concentration and yield and, based on our knowledge, it does not have a straightforward explanation.

The phylum *Actinobacteria* increased from the beginning of the pseudo-steady state to the end of the test in both conditions. It was represented mainly by the genus *Olsenella*, an aerotolerant genus known as lactic acid, acetic acid and H_2 producer from food waste fermentation at pH = 5–6 [34, 53, 67, 68]. The presence of *Actinobacteria* was expected since they were identified in AF of agroindustrial waste [23] and WAS [42], and the increase in the abundance of *Olsenella* was probably due to the progressive specialization of the microbial community to the substrates and operative conditions applied in this study.

Globally, the results denote some differences in the microbial community composition and diversity of the AF-CAV and AF-NCAV, which were minor at the beginning of the steady state and more marked at the end of the test. While it is not possible to attribute the lower VFAs production in the AF-CAV to the lack or presence of specific genera, it can be hypothesized that the lower microbial diversity observed led to a less efficient acidification of the substrates [33, 40].

The same condition showed a variation over time in the relative abundance among phyla, families and genera, which is more marked in the AF-NCAV, likely due to a lower homogeneity of the NCAV mixture than the CAV one. However, the microbial community composition variation between the two conditions and over time did not affect VFAs composition. Metabolic functionality was retained despite changes in the community, denoting a functional redundancy among community members [69].

Conclusions

This work gave an insight into the effect of the hydrodynamic cavitation pre-treatment of a mixture of vegetable waste and waste-activated sludge on the microbial community of a semi-continuous acidogenic fermentation process. Pre-treatment was efficient in substrates' hydrolyzation but resulted in a lower microbial diversity of 3.85 (Shannon Index) and VFAs concentration of 12.9 $gCOD_{VFA}\ L^{-1}$ in the AF-CAV, respect to 4.54 and 18.2 $gCOD_{VFA}\ L^{-1}$ in the AF-NCAV. This can be attributable to the fact that pre-treatment prevented the development of some microorganisms involved in the hydrolysis of organic matter, thus supporting the hypothesis that a higher microbial species richness allows fermenting of different substrates more efficiently. Moreover, substrates' pre-treatment might have reduced or inactivated their indigenous microbial load, despite the low SE and P_D applied (2868 kJ $kgTS^{-1}$ and 0.08 W/mL, respectively).

VFAs concentration was also the only significant factor in the NMDS analysis explaining the distance between the cluster of the AF-CAV and the one of the AF-NCAV ($R^2 = 1$, $Pr > r = 0.04167$). Altogether, these results suggest that HC pre-treatment resulted in a lower microbial diversity, which led to a lower VFAs concentration in the fermented effluent.

The microbial community retained its metabolic functionality despite changing its composition over time, as proved by the stable VFAs concentrations and profile. This denotes functional redundancy among community members: in fact, for both AF-CAV and AF-NCAV species richness was similar between the beginning of the pseudo-steady state and the end of the test. The community composition changed over time, with minor differences at the beginning of the steady state, which became more marked at the end of the test, as shown also by the Venn diagram.

In light of this, despite being effective in substrates' disintegration, HC should be optimized to identify the optimal operating parameters allowing substrates' solubilisation and minimizing disinfection effects. For a better understanding of the HC effects on the microbial community, the fermentation performances with and without inoculation should also be assessed.

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Declarations

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