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Published Version:
Lanfranchi A., Tassinato G., Valentino F., Martinez G.A., Jones E., Gioia C., et al. (2022). Hydrodynamic cavitation pre-treatment of urban waste: Integration with acidogenic fermentation, PHAs synthesis and anaerobic digestion processes. CHEMOSPHERE, 301, 1-9 [10.1016/j.chemosphere.2022.134624].

Availability:
This version is available at: https://hdl.handle.net/11585/895274 since: 2022-10-03

Published:
DOI: http://doi.org/10.1016/j.chemosphere.2022.134624

Hydrodynamic cavitation pre-treatment of urban waste: integration with acidogenic fermentation, PHAs synthesis and anaerobic digestion processes

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Abstract

Urban waste can be valorized within a biorefinery approach, producing platform chemicals, biopolymers and energy. In this framework, hydrodynamic cavitation (HC) is a promising pre-treatment for improving biodegradability due to its high effectiveness and low cost. This paper deals with the effect of HC pre-treatment on the acidogenic co-fermentation process of thickened sewage sludge from a WWTP and seasonal vegetable waste from a wholesale market. Specifically, HC was assessed by testing two sets of parameters (i.e., treatment time of 30 and 50 min; vacuum pressure 1.4 and 2.0 bar; applied power 8 and 17 kW) to determine its effectiveness as a pre-treatment of the mixture. The highest increase in sCOD (+83%) and VFAs (from 1.93 to 17.29 $gCOD_{VFA}\ L^{-1}$) was gained after 50 minutes of cavitation. Fermentations were conducted with not cavitated and cavitated mixtures at 37°C on 4 L reactors in batch mode, then switched to semi-continuous with OLR of 8 $kg_{TVS}\ m^{-3}\ d^{-1}$ and HRT of 5-6.6 d. Good VFAs concentrations (12.94-18.27 $gCOD_{VFA}\ L^{-1}$) and yields (0.44-0.53 $gCOD_{VFA}\ g_{VS(0)}^{-1}$) were obtained, which could be enhanced by pre-treatment optimization and pH control. The organic acid rich broth obtained was then assessed as a substrate for PHAs storage by *C. necator*. It yielded 0.37 $g\ g^{-1}$ of polyhydroxybutyrate, such biopolymer resulted to have analogous physicochemical characteristics of commercial equivalent. The only generated side-stream would be the solid-rich fraction of the fermented effluent, which valorization was assessed through BMP tests, showing a higher SGP of 0.42 $Nm^3\ kgTVS^{-1}$ for the cavitated.

Keywords: organic waste, sludge, cavitation, VFAs, PHAs

Acronym's list:

AC: Acoustic Cavitation

AD: Anaerobic Digestion

BMP: Biochemical Methane Potential

F/M: Food/Microorganisms

FW: Food Waste

FR: Fermentation Rate

GC: Gas-Chromatography

HC: Hydrodynamic Cavitation

HPLC: High Performance Liquid Chromatography

HRT: Hydraulic Retention Time

OAs: Organic Acids

OFMSW: Organic Fraction of Municipal Solid Waste

OL: Organic Loading

OLR: Organic Loading Rate

pCOD: particulate Chemical Oxygen Demand

PHAs: Poly-Hydroxy-Alkanoates

sCOD: soluble Chemical Oxygen Demand

SS: sewage sludge

tCOD: total Chemical Oxygen Demand

TKN: Total Kjeldahl Nitrogen

TS: Total Solids

TVS: Total Volatile Solids

VFAs: Volatile Fatty Acids

VW: Vegetable Waste

WWTP: WasteWater Treatment Plant

1. Introduction

The effects of climate change represent a global menace that requires action on an international level, thus emphasizing the need for closing, among the others, the carbon cycle. In the form of CO_2, carbon is recognized as the main culprit for climate change. The circular economy approach has been identified as a fundamental part of the solution at both the international and european levels. For this reason, it became the core concept of the EU Green Deal, where waste recovery and valorization into marketable products and energy represent a pivotal aspect (EC, 2019).

In the urban context, the two main waste streams are sewage sludge (SS) and food waste (FW), destined to grow with the increasing world population. At present, in Europe, 13 million tonnes (dry matter) of sewage sludge and 78 million tonnes of food waste are generated every year (Collivignarelli et al., 2019).

An innovative approach for the integrated management of SS and FW is represented by their valorization within a third-generation bio-refinery approach. This consists of the combination of several processes to transform waste into high-added value chemical compounds, such as volatile fatty acids (VFAs) and energy (Battista et al., 2020). VFAs are starting molecules for bioenergy production and the synthesis of various products, such as reduced chemicals, derivatives, and biopolymers. At the moment, 90% of VFAs are produced from non-renewable petrochemical compounds through a process that has considerable environmental impacts (Atasoy et al., 2018). A sustainable alternative can be VFAs production through the anaerobic fermentation of organic waste (Holtzapple et al., 2022). Co-fermentation of SS and FW is reported to improve the fermentation 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 (Fang et al., 2020; Vidal-Antich et al., 2021).

The VFAs-rich liquid fraction can be valorized into several routes in the frame of a biorefinery concept (Sivagurunathan et al., 2018). The fermented effluent rich in VFAs is an ideal substrate for the cultivation of poly-hydroxy-alkanoates (PHAs)-storing microorganisms, both with mixed microbial cultures (MMCs) (Valentino et al., 2019b) and with pure cultures of microorganisms such as *R. eutropha*, also known as *C. necator* (Martinez et al., 2016). If the co-fermentation and the PHAs production processes were coupled, the only waste overflow would be the solid-rich fraction of the fermented effluent, which can be finally valorized through AD. The feasibility of this approach has been recently demonstrated with similar substrates, i.e., the organic fraction of municipal solid waste (OFMSW) and SS (Moretto et al., 2019; Valentino et al., 2019a).

The pre-treatment of the substrates is the first process to be performed in a biorefinery in order to i) reduce the size of the substrate; ii) extract simpler chemical compounds, thus favouring the fermentation process; iii) remove the inert material not applicable to the following bioprocesses (Li et al., 2017). Among pre-treatments, cavitation is a promising physico-chemical process consisting of the formation, growth and collapse of vapor cavities due to a sharp pressure drop. The pressure drop can be generated applying a sudden constriction (hydrodynamic cavitation, HC) or by using ultrasound (acoustic cavitation, AC) (Bhat&Gogate, 2021). HC is a more promising technology from both the environmental and economic point of view, since it has a higher potential of scalability and has been proved to be orders of magnitude cheaper than AC (Bhat&Gogate, 2021). For this reason, it is fundamental to fill the gaps of knowledge of this process. At present, both AC and HC have been tested on SS and wastewater, while few studies have been conducted on FW, testing only AC. The sole study carried out on a mixture of SS and OFMSW showed a 24% increase in the BMP (Cesaro et al., 2012). The best operating parameters have been identified only for the pre-treatment of SS and are pressure of 2-4 bars and duration of 15-60 mins (Garuti et al., 2018; Zhao et al., 2019). This study aims at evaluating the effects of HC of a mixture of organic wastes (SS and vegetable waste, VW) on its physico-chemical characteristics (TS, TVS, sCOD, VFAs, and cations) and on the fermentative and AD processes by testing two sets of operational parameters (pressure, power, duration). VFAs production from this mixture was assessed by batch and semi-continuous fermentation tests, while methane production was quantified by Biochemical Methane Potential (BMP) tests. Finally, the suitability of the non-sterilized fermented effluent obtained for PHAs production by *C. necator* was evaluated.

2. Materials and methods

2.1 Substrates and anaerobic inoculum

The substrates used in this study were the biological sludge collected from a wastewater treatment plant (WWTP) located in Northern Italy and the seasonal vegetable waste from the fruit and vegetable wholesale market. The mixture was made from the two substrates in a 1:1 ratio on a TVS basis, at a volumetric fraction of 73-77% of sludge and 27-23% of vegetable scraps, according to Moretto et al. (2020b) and Valentino et al. (2019a). The anaerobic inoculum consisted of digestate collected from a WWTP, where wastewater sludge and OFMSW are anaerobically co-digested. All the three matrices were recurrently collected during the study period and were characterized in terms of total solids and volatile solids (TS and VS), sCOD, VFAs, total COD (tCOD), Total Kjeldahl Nitrogen (TKN), total phosphorus (Ptot), cations (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}), pH and alkalinity, showing stable values (table 1) (APAT, 2003; APHA, 2012).

Table 1. Average chemical-physical characteristics of the vegetable waste, biological sludge, and anaerobic inoculum applied in this study.

		Biological sludge		Vegetable waste		Inoculum	
Parameter	**Unit**	**Mean**	**Std. Dev.**	**Mean**	**Std. Dev.**	**Mean**	**Std. Dev.**
Total Solids (TS)	g kg^{-1}	36.0	±0.3	96	±13	18.2	±0.7
Total Volatile Solids (TVS)	g kg^{-1}	23.5	±0.4	89	±14	12.0	±0.3
VS/TS	%	65.4	±0.7	92	±2	66	±1
sCOD	gO_2 L^{-1}	0.58	±0.10	-	-	0.34	±0.01
pCOD	gO_2 kg^{-1}	26	±5	111	±20	17	±4

tCOD	gO_2 kg^{-1}	26	±5	-	-	18	±4
TKN	g_N kg^{-1}	0.8	±0.1	1.1	±0.2	1.3	± 0.1
Phosphorus (P)	gP kg^{-1}	0.8	±0.1	0.3	±0.1	0.4	± 0.02
COD:N:P	g	26:0.8:0.8		111:1.1:0.3		18:2.4:0.4	
VFA TOT	$gCOD_{VFA}$ L^{-1}	0.81	±0.01	-	-	0.23	±0.21
pH		7.7	±0.9	-	-	8.3	±0.1
Partial alkalinity	$mgCaCO_3$ L^{-1}	325	±48	-	-	2622	±208
Total alkalinity	$mgCaCO_3$ L^{-1}	606	±53	-	-	3091	±287
Na^+	mg L^{-1}	2664	±732	-	-	2313	±235
$N\text{-}NH_4^+$	mg L^{-1}	45	±64	-	-	1091	±194
K^+	mg L^{-1}	418	±35	-	-	918	±293
Mg^{2+}	mg L^{-1}	2438	±937	-	-	2004	±214
Ca^{2+}	mg L^{-1}	6144	±1942	-	-	5110	±165

2.2 Hydrodynamic cavitation pre-treatment of the substrates

The HC reactor used to generate cavitation was a stator and rotor assembly (BioBang®, Three-es S.r.l.). The maximum inlet pressure to the HC reactor was 2 bars. A closed-loop circuit was applied, where the mixture was recirculated for all the duration of the pre-treatment. Electrical power (kW) was set as a fixed parameter, with rotation velocity varying depending on the mixture's viscosity. HC was performed applying two different sets of parameters. Firstly, a power of 8 kW, P of 1.4-1.5 bar, $Q_{mixture}$ of 25-30 L min^{-1}, and 1550-1650 rpm were applied for 30' on the mixture. In the second pre-treatment, the operating parameters were kept at the lower values indicated above for the first 30 mins to avoid pump overloading during the homogenization of the mixture and were then raised. A power of 17 kW, P of 2.0 bar, $Q_{mixture}$ of 80-100 L min^{-1}, and 1450-1550 rpm were applied for the last 20' on the mixture, for a total duration of the HC pre-treatment of 50'.

To assess the effect of the HC pre-treatment, the physical-chemical properties of the mixture before and after cavitation were compared. The substrates' degree of disintegration (DD_{COD} %) was calculated as in Tonanzi et al. (2021). To evaluate the efficiency of the process and compare it with other pre-treatments, the specific energy input (SE) was calculated as in Gallipoli et al. (2014).

2.3 Anaerobic fermentation tests

Fermentation tests were conducted at uncontrolled pH and 37°C using a laboratory fermenter with 4L of working volume, automatically stirred at 14 rpm (RES Italia). The tests were carried out on the mixtures cavitated for 30’ and 50’ and on the not cavitated mixture, which was considered as a blank.

Batch tests were performed; when the VFAs concentration was stable for at least three consecutive measurements, the reactors were fed in a semi-continuous manner. The parameters applied in the batch and semi-continuous tests were defined according to the experimental tests in literature, giving the best performances, and are reported in table 2 (Moretto et al., 2019; Strazzera et al., 2021; Valentino et al., 2019a). The tests were inoculated with the anaerobic digestate (31-34% v/v) in order to maintain a high F/M ratio that allows to inhibit methanogens (González-Fernández and García-Encina, 2009; Greses et al., 2020). The slight variability of the parameters applied among the three conditions tested is due to the seasonal variability of the substrates used.

Table 2. Parameters applied in the fermentation tests.

	Parameter	**Unit**	**Not cavitated**	**Cavitated 30’**	**Not cavitated**	**Cavitated 50’**
Batch tests	OL	$kg_{tCOD}\ m^{-3}$	34.8	33.4	52.1	54.6
		$kg_{TVS}\ m^{-3}$	24.5	18.8	23.5	25
	F/M	$kg_{tCOD}\ kg_{TVS}^{-1}$	9.92	9.51	14.7	14.1
	F/M	$kg_{TVS}\ kg_{TVS}^{-1}$	7	5.4	6.4	6.7
Semi-continuous tests	OLR	$kg_{TVS}\ m^{-3}\ d^{-1}$	8	8	8	8
	HRT	days	6.6	5	6.0	6.4

Liquid samples were collected daily for VFA analysis, soluble chemical oxygen demand (sCOD), pH, alkalinity, and cations.

The VFAs yields were calculated according to Moretto et al. (2019). In the tests with the cavitated mixture, both the net and total yields were calculated, respectively subtracting, and including, the VFAs generated during the HC pre-treatment.

The fermentation rate (FR) was expressed as $gCOD_{VFA}\ gTVS_{(0)}^{-1}d^{-1}$. For batch fermentations, it was calculated following eq 1:

$$Fermentation\ rate_{batch} = \frac{g\ COD_{VFA}}{TVS_{(0)} * days} \qquad (1)$$

where $gCOD_{VFA}$ indicates the VFAs in the volume of the reactor and $TVS_{(0)}$ refers to the quantity of total volatile solids used to inoculate the fermenters at the beginning of the test.

The fermentation rate of the semi-continuous fermentations at steady state was calculated as it follows:

$$Fermentation\ rate_{semi-continuous} = \frac{g\ COD_{VFA}}{TVS_{(0)} * HRT} \qquad (2)$$

Where HRT is the hydraulic retention time of the fermenters.

Also, for the fermentation rate, the net, and the total rate were calculated in the tests with the cavitated mixture.

In order to describe the VFAs profile for the subsequent PHAs storage, the molar ratio between odd-numbered VFAs and their total concentration (the $[C3/(C3+C2)]_{VFA}$ ratio) was determined. The $[C3/(C3+C2)]_{VFA}$ ratio is a pivotal characteristic since it affects the composition of microbially synthesized biopolymers such as the polyhydroxyalkanoates (PHAs), and, in turn, their market applications (Bengtsson et al., 2010).

2.4 PHAs accumulation tests

Production of PHAs was carried out in two bench-scale 2L fermenters (Infors- Minifors 2) by employing a pure culture of *Cupriavidus necator* (DSMZ 545) in a fed-batch culture system. The experiment was carried out according to a dual-phase process as reported previously when assessing other alternative substrates (Martinez et al., 2016). Briefly, 1) cells were first grown under balanced conditions in batch mode and using glucose as substrate; then 2) polymer was accumulated under imbalanced conditions (limiting N) by feeding the obtained VFAs-rich effluent without sterilization (just filtrated at 0.45µm) using a pO_2-stat strategy. Air was fed at 0.5 VVM, and stirring was set at 600 rpm, allowing to maintain pO_2>20% (Samorì et al., 2021). Moreover, two conditions were carried out, using *a)* the actual organic acid-rich effluent of the cavitated 50' and *b)* using a simulated laboratory prepared solution containing just the detected organic acids at the corresponding concentrations. This allowed to assess the presence of any inhibitor in the actual anaerobic effluent matrix. Samples were performed periodically and treated as previously reported elsewhere (Martinez et al., 2016).

2.5 Biochemical Methane Potential (BMP) tests

All the BMP tests were conducted at 42°C in bottles with a working volume of 0.5 L, with an OL of 4,5-5 kg_{TVS} m^{-3} and an F/M of 0.36-0.48 VS/VS. The BMP tests were carried out on the single substrates (VW and SS), on the mixture of organic waste and biological sludge not cavitated, cavitated 30’ and cavitated 50’, and finally on the solid-rich fraction of the fermented effluent. The volume of biogas produced was measured by water displacement. Biogas composition was determined with the portable biogas analyzer MCA 100 Bio-P (ETG risorse e tecnologia s.r.l). The parameters of the BMP tests (SGP, SMP, k_h) were calculated according to the standard BMP methods (Holliger et al., 2020).

2.6 Analytical methods

All analyses were performed according to the APAT, IRSA-CNR (APAT, 2003), and APHA, AWWA, WET methods (APHA, 2012). Volatile fatty acids were determined with an Agilent 1100 SERIES high-performance liquid chromatograph (HPLC) equipped with an Acclaim™ Organic Acid 4x150 mm column (Thermo Fisher) and with a diode array detector (DAD). The Volatile Free Acid Mix CRM46975 was used as standard. The lactic acid content was determined for the fermented effluent after the pH drop with an HPLC-RID with the method described in Martinez et al. (2015). PHAs content and the obtained polymer characterisation were carried out as previously reported by Martinez et al. (2015) and (2021).

3. Results and discussion

3.1 Hydrodynamic cavitation pre-treatment of the substrates

HC pre-treatments did not impact on TS and TVS concentrations, indicating that no mineralization or evaporation phenomena occurred. On the contrary, HC affected the sCOD, pCOD, and VFAs concentrations. The 30’ HC pre-treatment determined a 39% increase of the sCOD and raised VFAs concentration from 1.7 to 6.8 $gCOD_{VFA}$ L^{-1}. These results were enhanced in the 50’ HC, where an 83% increase of the sCOD and a notable increase in VFAs concentration from 1.9 to 17.3 $gCOD_{VFA}$ L^{-1} were observed, with $gCOD_{VFA}$ $gsCOD^{-1}$ of 0.67. The VFAs concentration of the mixture cavitated for 50’ was comparable with the one obtained during the fermentation process. This clearly indicates that a high concentration of VFAs can be obtained only by HC, reaching values similar to or even higher than those obtained through the fermentation processes. After both pre-treatments a 15-16% decrease in the pCOD of the mixture was observed, due to the transfer of the organic material from the solid (pCOD) to the liquid (sCOD) phase (table 3).

The solubilization of the mixture increased with the intensity and the duration of the pre-treatment, with DD_{COD} of 6% for the cavitated 30’ (at SE of 2868 kJ $kgTS^{-1}$) and DD_{COD} of 17% for the cavitated 50’ (at SE of 3734 kJ $kgTS^{-1}$). This confirms that hydrodynamic cavitation can lead to the same or even higher DD_{COD} with a lower SE with respect to acoustic cavitation (Cesaro et al., 2012; Tonanzi et al., 2021). The highest DD_{COD}s of 27% and 72% achieved in the cited studies (SE of 33873 and 90692 kJ $kgTS^{-1}$, respectively) were not reached, but they can probably be obtained by increasing the SE.

A slight decrease in the pH after 30’ HC was observed, while after 50’ HC, the pH was comparable with the not cavitated mixture, despite the considerable increase in VFAs concentration. The pH stability of the mixture cavitated 50’ could be due to the increase in NH_4^+ concentration that contributed to the buffering of the mixture. The observed increase in NH_4^+ concentration after 50’ of cavitation was probably due to cell lysis and was reported also elsewhere (Zhao et al., 2019). The concentrations of the other cations in the liquid phase after HC showed slight decrease concerning Na^+, Mg^{2+}, and Ca^{2+} concentrations, as observed elsewhere (Laurent et al., 2009; Tonanzi et al., 2021). The divalent cations bridging theory states that Ca^{2+} and Mg^{2+} facilitate bioflocculation, whereas Na^+ hinders it, especially at a ratio between monovalent and divalent cations M/D>2 (Higgins and Novak, 1997; Sobeck et al., 2002). In this study, the M/D ratio of the not cavitated mixtures was 0.33 and 0.26. Therefore, the decrease in Na^+, Mg^{2+} and Ca^{2+} concentrations could be attributed to a slight reflocculation phenomenon caused by the higher content in soluble organic matter (Laurent et al., 2009). After HC, an increase in the total alkalinity of the mixtures is observed due to the increase in VFAs concentration. Table 3 reports the impacts of the HC pre-treatments on the mixtures tested.

Table 3. Characterization of the mixture of SS and VW before and after HC pre-treatment.

Parameter	Unit	Not cavitated	Cavitated 30’	Not cavitated	Cavitated 50’
DD_{COD}	%		6		17
Total Solids (TS)	g kg^{-1}	35.8 ± 0.4	37.3± 0.0	49 ± 6	46.1 ± 0.3
Total Volatile Solids (TVS)	g kg^{-1}	27.2 ± 0.9	28.4 ± 0.0	38 ± 8	36.4 ± 0.2
VS/TS	%	76 ± 2	76.0 ± 0.0	79 ± 5	78.9 ± 0.2
sCOD	gO_2 L^{-1}	8.83 ± 0.10	12.28 ± 0.70	14.20 ± 0.29	25.99 ± 0.35
pCOD	gO_2 kg^{-1}	45 ± 5	38.0 ± 0.5	54.8 ± 0.2	47 ± 1

tCOD	$gO_2\ kg^{-1}$	54 ± 4	50.3 ± 0.7	69.0 ± 0.3	73 ± 1
TKN	$g_N\ kg^{-1}$	0.9 ± 0.1	0.8 ± 0.0	0.9± 0.0	0.9 ± 0.0
Phosphorus (P)	$gP\ kg^{-1}$		0.8 ± 0.2	0.7 ± 0.0	0.8 ± 0.0
COD:N:P	g	54:1.2	50:1.1:0.8	69:0.9:0.7	73:1.2:0.8
VFA TOT	$gCOD_{VFA}\ L^{-1}$	1.7 ± 0.2	6.8 ± 0.1	1.9 ± 0.4	17.3 ± 0.0
Formic acid	$gCOD_{VFA}\ L^{-1}$	0.2 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
Acetic acid	$gCOD_{VFA}\ L^{-1}$	1.0 ± 0.0	0.0 ± 0.0	0.0	4.4 ± 0.0
Propionic acid	$gCOD_{VFA}\ L^{-1}$	0.0 ± 0.0	6.5 ± 0.1	0.1 ± 0.1	3.5 ± 0.0
Butyric and iso-butyric acids	$gCOD_{VFA}\ L^{-1}$	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	2.6 ± 0.0
Valeric acid	$gCOD_{VFA}\ L^{-1}$	0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.0	0.4 ± 0.0
Iso-valeric acid	$gCOD_{VFA}\ L^{-1}$	0.4 ± 0.1	0.2 ± 0.0	0.8 ± 0.1	3.5 ± 0.0
Hexanoic acid	$gCOD_{VFA}\ L^{-1}$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Heptanoic acid	$gCOD_{VFA}\ L^{-1}$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 0.0
Iso-hexanoic acid	$gCOD_{VFA}\ L^{-1}$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.1
pH		7.5 ± 0.0	6.6 ± 0.0	6.4 ± 0.0	6.5 ± 0.1
Partial alkalinity	$mgCaCO_3\ L^{-1}$	100± 0	100 ± 0	106 ± 9	108 ± 12
Total alkalinity	$mgCaCO_3\ L^{-1}$	525 ± 106	738 ± 0	738 ± 18	1017 ± 24
Na^+	$mg\ L^{-1}$	2214± 33	2117 ± 27	3391 ± 67	2949 ± 66

$N\text{-}NH_4^+$	mg L^{-1}	279 ± 13	264 ± 35	0.0	344± 33
K^+	mg L^{-1}	948 ± 9	1092 ± 42	822 ± 10	942 ± 25
Mg^{2+}	mg L^{-1}	1942 ± 27	1794 ± 33	3200 ± 11	3072 ± 31
Ca^{2+}	mg L^{-1}	5139 ± 44	4939 ± 85	7783 ± 8	7496 ± 24

3.2 Anaerobic fermentation tests

Table 4 reports the values of process parameters and VFAs concentration obtained at the end of the batch tests. A high VFAs concentration was expected also in the not cavitated, since vegetable waste is quickly biodegradable. However, a higher VFAs concentration was expected from the cavitated due to the disgregation of the sludge flocs after the pre-treatment. This was not observed, therefore indicating that the HC pre-treatment could be further optimized.

Table 4. Final VFAs concentrations and fermentation performances achieved in the batch tests.

Parameter	**Unit**	**Not cavitated (Blank)**	**Cavitated 30'**	**Not cavitated (Blank)**	**Cavitated 50'**
pH		5.3 ± 0.0	5.4 ± 0.2	5.0 ± 0.0	5.4 ± 0.0
VFAs	$gCOD_{VFA}$ L^{-1}	15.75 ± 0.25	11.82 ± 0.45	18.25 ± 0.40	17.34 ± 0.62
Formic acid	$gCOD_{VFA}$ L^{-1}	0.0	0.0	0.0 ± 0.0	0.0
Acetic acid	$gCOD_{VFA}$ L^{-1}	4.21 ± 0.10	3.09 ± 0.05	4.67 ± 0.16	2.66 ± 0.08
Propionic acid	$gCOD_{VFA}$ L^{-1}	3.34 ± 0.06	1.69 ± 0.02	3.77 ± 0.06	1.60 ± 0.05
Butyric and iso-butyric acids	$gCOD_{VFA}$ L^{-1}	4.81 ± 0.09	4.90 ± 0.05	2.84 ± 0.07	5.15 ± 0.13
Valeric acid	$gCOD_{VFA}$ L^{-1}	0.60 ± 0.01	0.00	0.54 ± 0.03	0.29 ± 0.26
Iso-valeric acid	$gCOD_{VFA}$ L^{-1}	2.33 ± 0.07	1.79 ± 0.19	3.69 ± 0.07	2.10 ± 0.06
Hexanoic acid	$gCOD_{VFA}$ L^{-1}	0.00	0.00	0.00 ± 0.00	0.00

Heptanoic acid	$gCOD_{VFA}$ L^{-1}	0.54 ± 0.01	0.60± 0.00	1.82 ± 0.02	4.84 ± 0.69
Iso-hexanoic acid	$gCOD_{VFA}$ L^{-1}	0.00	0.00	0.91 ± 0.02	0.20 ± 0.12
$[C_3/(C_3+C_2)]_{VFA}$	mol mol^{-1}	0.36 ± 0.00	0.28 ±0.02	0.41 ± 0.01	0.43 ± 0.01
$Activity_{tot}$	$gCOD_{VFA}$ $gTVS_{(0)}^{-1}d^{-1}$	2.17	2.30	3.6	4.2
$Activity_{net}$	$gCOD_{VFA}$ $gTVS_{(0)}^{-1}d^{-1}$	-	1.1	-	1.5
Net yield	$gCOD_{VFA}$ $g_{VS(0)}^{-1}$	-	0.38 ± 0.02	-	0.20 ± 0.02
Total yield	$gCOD_{VFA}$ $g_{VS(0)}^{-1}$	0.60 ± 0.01	0.60 ± 0.02	0.60 ± 0.01	0.62 ± 0.02
Test duration	days	20	13	37	37

Then, the reactors were fed in semi-continuous mode for 3.8 HRTs for the cavitated 30'and 2.6 HRTs for its blank and for 4.0 HRTs for the cavitated 50' and 4.2 HRTs for its blank. Figure 1 shows the trend in VFAs concentration and pH during the whole experiment for the cavitated 30' (1a) and its blank (1b) and the cavitated 50' (1c) and its blank (1d). As observed in previous studies, mesophilic conditions resulted in a continuous fermentative activity that easily reached a steady state (Moretto et al., 2019). Moreover, mesophilic temperatures are preferable in the perspective of implementing the process in a full-scale plant since they require less amounts of energy (Valentino et al., 2019a). A pseudo-stability was reached, where a lower VFAs concentration for the cavitated 30' (12.94 ± 0.63 $gCOD_{VFA}$ L^{-1}) with respect to its blank (18.23 ± 0.51 $gCOD_{VFA}$ L^{-1}) was observed. On the contrary, VFAs concentration of the cavitated 50' (17.93 ± 0.70 $gCOD_{VFA}$ L^{-1}) was comparable with its blank (18.27 ± 0.23 $gCOD_{VFA}$ L^{-1}). However, after 2 HRTs, the cavitated 50' underwent a progressive drop of the pH to a value of 3.83 ± 0.02 with a concentration of organic acids (OAs) of 15.0 ± 0.4 $gCOD_{OA}$ L^{-1}, represented for the 83% by lactic acid. The pH drop was likely due to the greater initial sCOD concentration induced by the HC pre-treatment, which also raised the VFAs concentration from 1.93 to 17.29 $gCOD_{VFA}$ L^{-1}. pH control is needed if VFAs are kept as the target molecules for this process. However, the good yield of 0.52 $gCOD_{Lac}$ gVS_{fed}^{-1} obtained at uncontrolled pH suggests that lactic acid production would be the most advantageous way to exploit the mixture cavitated 50'. In fact, as observed in recent works (Pau et al., 2021; Strazzera et al., 2021) and contrary to what was reported in most of the literature (RedCorn et al., 2016; Tang et al. 2016; 2017; Yousuf et al., 2018; Zhang et al., 2017), lactic acid production became consistently higher at pH <4. This is attributable to the fact that at pH<4, VFAs in the fermentation broth are in their undissociated form, thus being more liposoluble and able to diffuse in the medium and penetrate the bacterial cells. In the cytoplasm, at neutral pH, VFAs dissociate, causing a drop in the intracellular pH. Thus, the cellular activities of the microorganisms are compromised, alongside their capability of producing VFAs (Palmqvist & Hahn-Hägerdal, 2000). On the contrary, lactic acid-producing bacteria were not inhibited at the pH reached in this study (3.82) since the pKa of lactic acid is lower (3.10). The enhancement of lactic acid production at low uncontrolled pH would be an advantage from an industrial point of view since it would decrease the overall cost of the process, the 14% of which is represented by alkalinizing agents (usually NaOH, CaOH or lime) required to stabilize pH (López-Gómez et al., 2018; Joglekar et al., 2006). Moreover, the presence of lactic acid was not detrimental to the subsequent PHAs synthesis process, which has already been carried out successfully on mixtures of organic acids containing lactic acid (Dionisi et al., 2004; 2005; Gouveia et al., 2017).

The VFAs concentrations obtained show that good acidogenic performance took place concerning studies on similar substrates, such as those carried out by Valentino et al. (2019a) (19.5 $gCOD_{VFA}$ L^{-1}), Strazzera et al. (2021) (18 $gCOD_{VFA}$ L^{-1}), and Cheah et al. (2019) (3.65 ± 0.67-11.73 ± 2.37 g_{VFA} L^{-1}). The concentrations obtained were lower than 30 ± 3 $gCOD_{VFA}$ L^{-1} achieved by Moretto et al. (2020) by applying a thermal pre-treatment (T=72 °C for 48 h) to a mixture of SS and OFMSW composed in a similar 70:30 volumetric ratio. This is attributable to the long duration of the thermal pre-treatment, which induced a greater solubilization of the organic matter.

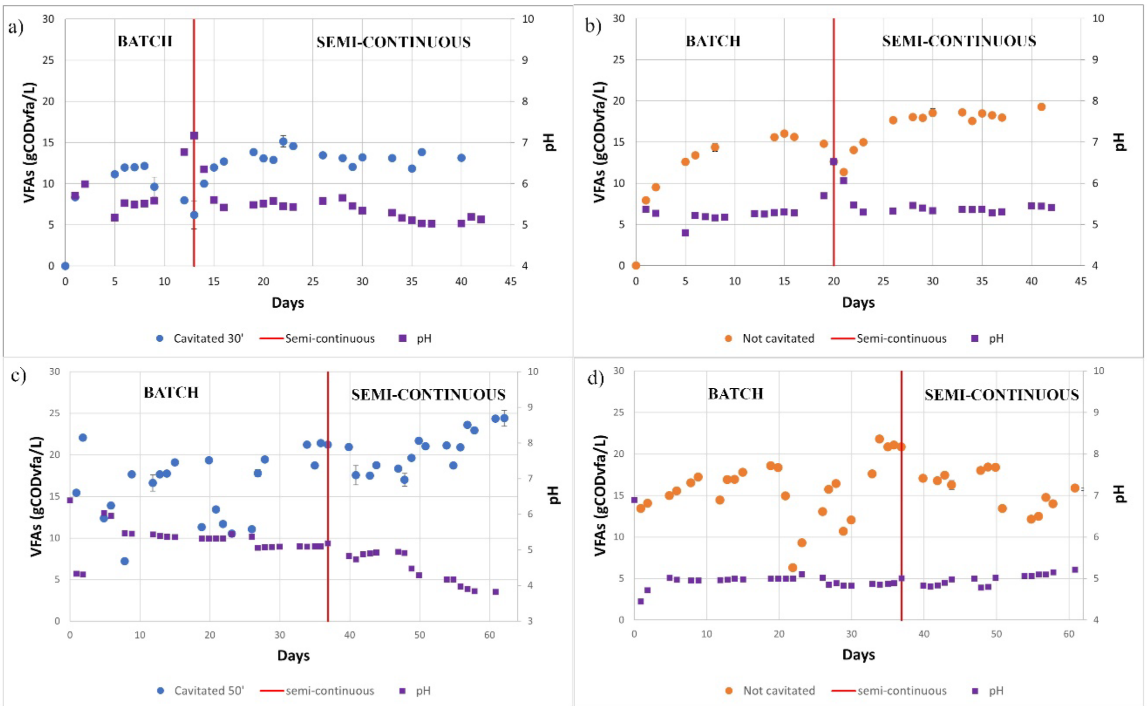

Figure 1. VFAs and pH trend during the whole experiment for a) cavitated 30' and b) blank of cavitated 30'; c) cavitated 50' and d) blank of cavitated 50'.

All the three mixtures tested showed good yields, higher than those obtained by Valentino et al. (2019a) (0.41-0.44 $gCOD_{VFA}\ gVS_{(fed)}^{-1}$) and by Strazzera et al. (2021) (0.38 $gCOD_{VFA}\ gVS_{(fed)}^{-1}$), probably due to the lower protein content of the substrates used in this study respect to OFMSW+SS and synthetic household FW. The slight variation between the yields of the cavitated 30' and its blank and the cavitated 50' and its blank is attributable to the variability of the substrates used (tables 3 and 5). The net fermentation yields were lower for the cavitated mixtures because part of the VFAs was produced during the pre-treatment. The yields obtained were lower than 0.65$gCOD_{VFA}\ gVS_{(fed)}^{-1}$ obtained by applying a thermal pre-treatment (Moretto et al., 2020b). The activity was 17% higher in the cavitated 30' with respect to its blank, while the activity of the cavitated 50' was 8% lower than its blank (table 5).

Therefore, our pre-treatment and fermentation processes could probably undergo further optimization, although the aforementioned studies used a richer substrate, i.e., OFMSW and FW.

Table 5. Final VFAs concentrations and fermentation performances achieved in the semi-continuous tests.

Parameter	**Unit**	**Not cavitated (Blank)**	**Cavitated 30'**	**Not cavitated (Blank)**	**Cavitated 50'**
pH		5.4 ± 0.0	5.5 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
VFAs	$gCOD_{VFA}\ L^{-1}$	18.23 ± 0.51	12.94 ± 0.63	18.27 ± 0.23	17.93 ± 0.70
Formic acid	$gCOD_{VFA}\ L^{-1}$	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Acetic acid	$gCOD_{VFA}\ L^{-1}$	3.01 ± 0.36	1.76 ± 0.04	6.71 ± 1.51	4.35 ± 1.10
Propionic acid	$gCOD_{VFA}\ L^{-1}$	2.94 ± 0.39	2.49 ± 0.81	0.00 ± 0.00	0.26 ± 0.30
Butyric and iso-butyric acid	$gCOD_{VFA}\ L^{-1}$	3.41 ± 0.30	3.31 ± 0.81	1.76 ± 0.27	2.40 ± 0.45
Valeric acid	$gCOD_{VFA}\ L^{-1}$	1.25 ± 0.14	0.52 ± 0.90	0.61 ± 0.04	0.74 ± 0.25
Iso-valeric acid	$gCOD_{VFA}\ L^{-1}$	4.17 ± 0.71	3.31 ± 1.61	1.87 ± 0.26	1.64 ± 0.64
Hexanoic acid	$gCOD_{VFA}\ L^{-1}$	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Heptanoic acid	$gCOD_{VFA}\ L^{-1}$	2.61 ± 0.87	1.36 ± 0.26	5.97 ± 1.68	6.17 ± 1.92

Iso-hexanoic acid	$gCOD_{VFA}$ L^{-1}	0.72 ± 0.75	0.21 ± 0.30	1.35 ± 0.16	0.94 ± 0.17
$[C_3/(C_3+C_2)]_{VFA}$	mol mol^{-1}	0.48 ± 0.04	0.53 ± 0.04	0.22 ± 0.1	0.32 ± 0.04
$Activity_{tot}$	$gCOD_{VFA}$ $gTVS_{(0)}^{-1}$ d^{-1}	0.69 ± 0.11	0.81 ± 0.15	0.60 ± 0.01	0.55 ± 0.02
$Activity_{net}$	$gCOD_{VFA}$ $gTVS_{(0)}^{-1}$ d^{-1}	-	0.54 ± 0.10	-	0.45 ± 0.02
Net yield	$gCOD_{VFA}$ $g_{VS(0)}^{-1}$	-	0.33 ± 0.10	-	0.02 ± 0.02
Total yield	$gCOD_{VFA}$ $g_{VS(0)}^{-1}$	0.52 ± 0.06	0.53 ± 0.07	0.48 0.01	0.44 ± 0.02

The VFAs profiles observed in the semi-continuous tests were similar for the cavitated substrates with respect to their blanks, as illustrated in the supplementary materials (figures S1 and S2). VFAs composition was stable for the not cavitated and for the cavitated 30’, with minor oscillations ascribable to the variability of the substrates used. The VFAs profile was similar, with the sum of acetic, propionic, butyric and iso-butyric acid accounting for 50.8% and 59.9% of the total for the not cavitated and cavitated 30’, respectively. This profile is consistent with the ones obtained at pH=6 in the fermentation process of SS (Moestedt et al., 2020) and FW, except for butyric acid (Feng et al., 2018). In our study, butyric acid reached values comparable with Feng et al. (2018) only in the second batch test, representing 30.4% of the not cavitated and 40.7% of the cavitated 30’. In the semi-continuous process, butyric and iso-butyric acids and propionic acid were detected in similar percentages. The profile obtained in this study is similar to those obtained by Moretto et al. (2019) and Valentino et al. (2019a) on similar substrates, i.e., OFMSW and SS. Acetic, propionic, and butyric acids were identified as the most abundant in both studies, with some differences in the percentages due to the different types of food waste, i.e., OFMSW instead of vegetable waste and the different pre-treatment (thermal). As in this study, non-negligible amounts of valeric (11-13%), iso-valeric (2-3%), hexanoic (8-9%), and heptanoic (4-16%) acids were detected (Valentino et al., 2019a; Moretto et al., 2019). Heptanoic acid was present in higher percentages in the test on the not pre-treated mixture, as in Moretto et al. (2019), thus indicating that the HC pre-treatment enhanced the conversion of the organic substrates into VFAs with shorter carbon chains. Heptanoic acid is known to be present only in mesophilic conditions (Moretto et al., 2019), as those applied in this study. The higher concentration of iso-valeric acid obtained in the not cavitated (20.4%) and cavitated 30’ (25.8%) was found only in few previous studies on SS fermentation, where it was attributed to protein fermentation (Hao&Wang, 2015; Jia et al., 2013; Xiong et al., 2012).

In fact, valeric acid was mainly associated with protein fermentation by reductive deamination of single amino acids or oxidation–reduction between pairs of amino acids via Stickland reaction (Batstone et al., 2002).

The cavitated 50’ kept a stable VFAs profile for the first 2 HRTs, after which lactic acid fermentation took over due to the pH drop. At steady state, the VFAs profiles of the cavitated 50’ and its blank were mainly represented by acetic (26.3% and 36.7%), butyric and iso-butyric (14.8% and 9.7%) and heptanoic acid (37.4% and 32.6%), with non-negligible amounts of valeric (4.4% and 3.4%), iso-valeric (10% and 10.2%) and iso-hexanoic acids (5.8% and 7.4%). The profile obtained differs from the cavitated 30’, probably because of the lower pH and the substrates’ variability. In particular, the relatively high concentration of heptanoic acid indicates that the fermentation process could undergo further optimization.

3.3 PHAs accumulation tests

The OAs-rich effluent of the cavitated 50’ without any further purification nor sterilization (just filtrated) and a laboratory prepared simulating solution (Sim) were used as carbon sources for *C. necator* in the PHB-accumulation phase after cells were grown with glucose (Figs. 2a and 2b). The effluent of the cavitated 50’ was selected as a substrate since it was the cavitated effluent with the highest concentration of OAs at the end of the experiment. A higher concentration of OAs was reported to increase PHAs concentrations (Martinez et al., 2022).

The potential inhibition of OAs towards bacteria was avoided by keeping the concentration almost at zero all along the accumulation phase through a pO_2-stat feeding strategy. Despite the cell dry weight (CDW) concentration profiles were different between both conditions, the biopolymer (PHAs) concentration profiles were similar: in fact, PHAs increased by a similar trend, from about 0.31 g L^{-1} up to 2.1-2.9 g L^{-1}, confirming the equivalency of the two feeding solutions used. The different CDW trends were due to the presence of suspended solids in the actual effluent, these were centrifuged within the cells during sample treatment. During the accumulation phase the cell duplication did occur as a consequence of PO_4^{3-} (0.577 g L^{-1}) and (0.252 g L^{-1}) NH_4^+ occurring in the fed solution. This was effectively confirmed when feeding the simulating solution which did not have suspended solids. The PHB content in *C. necator* at the end of the accumulation phase was 57±5% and 44±2% for the simulated and actual effluents, respectively.

The PHAs yields here obtained with the actual and simulating feeding solutions (0.37 $g_{PHAs}\ g_{Subs}^{-1}$ and 0.25 $g_{PHAs}\ g_{Subs}^{-1}$, respectively, Fig. 2b) were similar to those reached by feeding *C. necator* with a mixture of lactic and acetic acids (0.28 and 0.15 $g_{PHAs}\ g_{Subs}^{-1}$) as reported by Tsuge et al. (2001) and Schwartz et al. (2018), respectively. The PHAs yields were smaller to those obtained with VFAs from cheese whey (0.54 $g_{PHAs}\ g_{VFA}^{-1}$, Domingos et al., 2018).

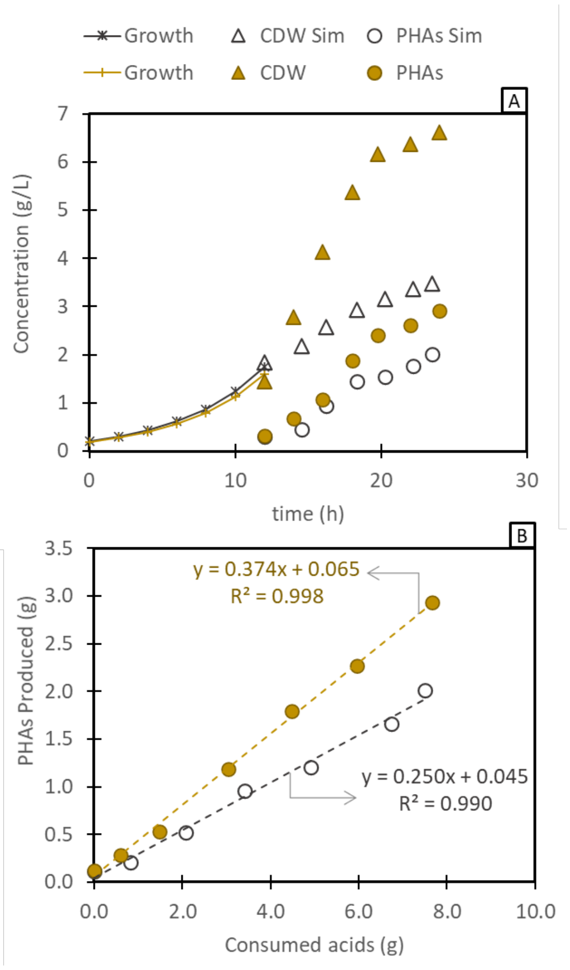

Figure 2. PHAs production from cavitated and anaerobic fermented substrate or laboratory prepared solution (Sim). Graph A shows the experimental concentrations of CDW and PHAs. Graph B shows the PHAs production yields with respect to acids consumed.

The PHB produced by *C. necator* fed with the actual effluent and simulating solution was finally extracted and characterized. Both conditions resulted in the production of an almost homopolymer of hydroxybutyrate (NMR spectres are shown in Figure S2). The molecular weights were 1.34-1.56 and 1.71-1.79 MDa for the actual and simulated effluent, respectively. This was in agreement with what is achievable by feeding the same bacterial strain with conventional carbon sources (e.g., glucose, 1.1 MDa, Samorì et al., 2021). The polydispersity index (PDI) obtained for both conditions resulted significantly lower (1.6-1.8) than the values reported under analogous fermentation conditions (Schwartz et al., 2018). Moreover, T_m and T_c resulted 168-170 °C and 46-47 °C (DSC scans are shown in Figure S3) which are in agreement with commercial PHB characteristics (Schwartz et al., 2018).

3.4 BMP tests

The BMP tests on the solid-rich fermented effluent showed a higher SGP of 0.42 ± 0.01 Nm^3 kg_{TVS}^{-1} for the cavitated respect to the not cavitated (0.34 ± 0.01 Nm^3 kg_{TVS}^{-1}), with a biogas production of 50.3 m^3 10^{-3} kg of substrate for the cavitated and 36.4 m^3 10^{-3} kg of substrate for the not cavitated, respectively. This was probably due to the disgregation of the lignocellulosic fraction of vegetable waste after the HC pre-treatment. The disgregated lignocellulosic fraction was not consumed during the fermentation process, as shown by the absence of improvement in the fermentation yields, but was degraded during the anaerobic digestion process in the BMP test. This improved the overall biogas production from the solid-rich fraction of the fermented effluent. The SGP of the cavitated was only slightly lower than the SGP of 0.44 ± 0.02 m^3 kg_{TVS}^{-1} obtained by Moretto et al. (2020b) in a continuous process. However, it should be considered that the author diluted the solid-rich fermentation effluent with SS, therefore adding degradable organic material to the slowly degradable COD left in the solid-rich fermentation effluent.

4. Conclusions

This work demonstrated that hydrodynamic cavitation is a promising pre-treatment of vegetable waste and sewage sludge, directly producing an interesting concentration of 17.29 $gCOD_{VFA}$ L^{-1} after 50 minutes of pre-treatment, with an SE of 3734 kJ $kgTS^{-1}$ which is consistently lower respect to acoustic cavitation. The solubilization of the mixture increased with the pre-treatment intensity and duration, with a DD_{COD} of 6% and 17% for the cavitated 30' and the cavitated 50', respectively. The fermentation processes showed good VFAs concentrations (12.94-18.27 $gCOD_{VFA}$ L^{-1}) and yields (0.44-0.53 $gCOD_{VFA}$ $g_{VS(0)}^{-1}$), which could be enhanced by pre-treatment optimization and pH control. After the pH drop, the cavitated 50' showed a concentration of organic acids (OAs) of 15.0 ± 0.4 $gCOD_{OA}$ L^{-1}, represented for the 83% by lactic acid. This OAs-rich broth was routed into PHAs production, resulting a performant substrate for PHBs storage with pure cultures of *C. necator*, with a PHB content of 44±2% and a yield of 0.37 g_{PHAs} g_{Subs}^{-1}.

Hydrodynamic cavitation pre-treatment enhanced the conversion into biogas of the solid-rich fermented effluent, with an SGP of 0.42 ± 0.01 Nm^3 kg_{TVS}^{-1} for the cavitated and 0.34 ± 0.01 Nm^3 kg_{TVS}^{-1} for the not cavitated. In conclusion, the feasibility of the integrated management of sewage sludge and vegetable waste in the frame of a biorefinery concept was demonstrated. This paves the way for the optimization of these processes in future research**.**

5. Acknowledgments

This work was supported by the regional project “Ecopolimeri” (ID 10217222) in the frame of the POR 2014-2020 program of Regione Veneto. The Green Propulsion Laboratory of Veritas S.p.A. is gratefully acknowledged for its hospitality. Also, the technical staff of DAIS and RES Italia are gratefully acknowledged for their support.

6. References

APAT-IRSA/CNR, 2003. Metodologie analitiche per il controllo della qualità delle acque. Poligr. e Zecca dello Stato Roma, Ital. 29, 575–581.

APHA/AWWA/WEF, 2012. Standard Methods for the Examination of Water and Wastewater. Stand. Methods 541.

Bao, H., Jiang, L., Chen, C., Yang, C., He, Z., Feng, Y., ... & Wang, A., 2015. Combination of ultrasound and Fenton treatment for improving the hydrolysis and acidification of waste activated sludge. RSC Adv. 5, 48468-48473.

Battista, F., Frison, N., Pavan, P., Cavinato, C., Gottardo, M., Fatone, F., Eusebi, A. L., Majone, M., Zeppilli, M., Valentino, F., Fino, D., Tommasi, T., & Bolzonella, D. 2020. Food wastes and sewage sludge as feedstock for an urban biorefinery producing biofuels and added-value bioproducts. J Chem Technol Biotechnol 95*,* 328–338. Doi: 10.1002/jctb.6096.

Bengtsson, S., Pisco, A. R., Johansson, P., Lemos, P. C., & Reis, M. A., 2010. Molecular weight and thermal properties of polyhydroxyalkanoates produced from fermented sugar molasses by open mixed cultures. J. Biotechnol. 147, 172-179.

Bhat, A. P., & Gogate, P. R., 2021. Cavitation-based pre-treatment of wastewater and waste sludge for improvement in the performance of biological processes: A review. J. Environ. Chem. Eng, 104743.

Bhatia, S. K., Gurav, R., Choi, T. R., Jung, H. R., Yang, S. Y., Song, H. S., ... & Yang, Y. H. (2019). Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) production from engineered Ralstonia eutropha using synthetic and anaerobically digested food waste derived volatile fatty acids. Int. J. Biol. Macromol. 133, 1-10.

Bolzonella, D., Pavan, P., Battistoni, P. & Cecchi, F., 2005. Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. Process Biochem. 40, 1453–1460. https://doi.org/10.1016/j.procbio.2004.06.036

Cesaro, A., Naddeo, V., Amodio, V., & Belgiorno, V. 2012. Enhanced biogas production from anaerobic codigestion of solid waste by sonolysis. Ultrason. Sonochem. 19, 596-600.

Cheah, Y., Vidal-Antich, C., Dosta, J. & Mata-Alvarez, J. 2019. Volatile fatty acid production from mesophilic acidogenic fermentation of organic fraction of municipal solid waste and food waste under acidic and alkaline pH. Environ. Sci. Pollut. Res. 26, 35509–35522. Doi: https://doi.org/10.1007/s11356-019-05394-6

Collivignarelli, M. C., Abbà, A., Carnevale Miino, M., & Torretta, V., 2019. What advanced treatments can be used to minimize the production of sewage sludge in WWTPs?. Appl. Sci. 9, 2650.

Dionisi, D., Beccari, M., Gregorio, S. D., Majone, M., Papini, M. P., & Vallini, G., 2005. Storage of biodegradable polymers by an enriched microbial community in a sequencing batch reactor operated at high organic load rate. J. Chem. Technol. Biotechnol. 80, 1306–1318. https://doi.org/10.1002/jctb.1331

Dionisi, D., Majone, M., Papa, V., & Beccari, M., 2004. Biodegradable Polymers from Organic Acids by Using Activated Sludge Enriched by Aerobic Periodic Feeding. Biotechnol. Bioeng. 85, 569–579. https://doi.org/10.1002/bit.10910

Domingos, J. M., Puccio, S., Martinez, G. A., Amaral, N., Reis, M. A., Bandini, S., ... & Bertin, L., 2018. Cheese whey integrated valorisation: Production, concentration and exploitation of carboxylic acids for the production of polyhydroxyalkanoates by a fed-batch culture. Chem. Eng. J. 336, 47-53.

European Commission, 2019. COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE EUROPEAN COUNCIL, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS The European Green Deal COM/2019/640 final. Retrieved from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52019DC0640

Fang, W., Zhang, X., Zhang, P., Wan, J., Guo, H., Ghasimi, D.S.M., Morera, X.C., Zhang, T., 2020. Overview of key operation factors and strategies for improving fermentative volatile fatty acid production and product regulation from sewage sludge. J. Environ. Sci. 87, 93–111.https://doi.org/10.1016/j.jes.2019.05.027

Feng, K., Li, H., & Zheng, C., 2018. Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste. Bioresour. Technol. 270, 180-188.

Gallipoli, A., Gianico, A., Gagliano, M. C., & Braguglia, C. M., 2014. Potential of high-frequency ultrasounds to improve sludge anaerobic conversion and surfactants removal at different food/inoculum ratio. Bioresour. Technol. 159, 207–214. https://doi.org/10.1016/j.biortech.2014.02.084

Garcia-Aguirre, J., Aymerich, E., de Goñi, J. G. M., & Esteban-Gutiérrez, M., 2017. Selective VFA production potential from organic waste streams: assessing temperature and pH influence. Bioresour. Technol. 244, 1081-1088. Doi: https://doi.org/10.1016/j.biortech.2017.07.187

Garuti, M., Langone, M., Fabbri, C., & Piccinini, S., 2018. Monitoring of full-scale hydrodynamic cavitation pretreatment in agricultural biogas plant. Bioresour. Technol. 247, 599-609.

González-Fernández, C., & García-Encina, P. A., 2009. Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry. Biomass Bioenergy 33, 1065–1069. https://doi.org/10.1016/j.biombioe.2009.03.008

Gouveia, A. R., Freitas, E. B., Galinha, C. F., Carvalho, G., Duque, A. F., & Reis, M. A. M., 2017. Dynamic change of pH in acidogenic fermentation of cheese whey towards polyhydroxyalkanoates production: Impact on performance and microbial population. New Biotechnol. 37, 108–116. https://doi.org/10.1016/j.nbt.2016.07.001

Greses, S., Tomás-Pejó, E., & Gónzalez-Fernández, C., 2020. Agroindustrial waste as a resource for volatile fatty acids production via anaerobic fermentation. Bioresour. Technol. 297. https://doi.org/10.1016/j.biortech.2019.122486

Habashi, N., Alighardashi, A., Mennerich, A., Mehrdadi, N., & Torabian, A., 2018. Improving biogas production from continuous co-digestion of oily wastewater and waste-activated sludge by hydrodynamic cavitation pre-treatment. Environ. Technol. 39, 1017-1024.

Habashi, N., Mehrdadi, N., Mennerich, A., Alighardashi, A., & Torabian, A., 2016. Hydrodynamic cavitation as a novel approach for pretreatment of oily wastewater for anaerobic co-digestion with waste activated sludge. Ultrason. Sonochem. 31, 362-370.

Holliger, C.; Fruteau de Laclos, H.; Hafner, S.D.; Koch, K.; Weinrich, S.; Astals, S.; et al. Requirements for measurement and validation of biochemical methane potential (BMP). Standard BMP Methods document 100, version 1.8. Available online: https://www.dbfz.de/en/BMP (accessed on October 7, 2020).

Jiang, J., Zhang, Y., Li, K., Wang, Q., Gong, C. & Li, M., 2013. Volatile fatty acids production from food waste: effects of pH, temperature, and organic loading rate. Bioresour. Technol. 143, 525–530. Doi: http://dx.doi.org/10.1016/j.biortech.2013.06.025

Laurent, J., Casellas, M., & Dagot, C., 2009. Heavy metals uptake by sonicated activated sludge: Relation with floc surface properties. J. Hazard. Mater. 162, 652–660. https://doi.org/10.1016/j.jhazmat.2008.05.066

Leite, W. R. M., Gottardo, M., Pavan, P., Belli Filho, P., & Bolzonella, D., 2016. Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. Renewable Energy 86, 1324-1331.

Li, Y., Jin, Y., Li, J., Li, H., Yu, Z., & Nie, Y., 2017. Effects of thermal pretreatment on degradation kinetics of organics during kitchen waste anaerobic digestion. Energy 118, 377-386.

Lin, L., Xu, F., Ge, X., & Li, Y., 2018. Improving the sustainability of organic waste management practices in the food-energy-water nexus: A comparative review of anaerobic digestion and composting. Renewable Sustainable Energy Rev. 89, 151-167.

López-Gómez JP, Alexandri M, Schneider R and Venus J, 2018. A review onthe current developments in continuous lactic acid fermentations and case studies utilising inexpensive raw materials. Process Biochem 79, 1–10. https://doi.org/10.1016/j.procbio.2018.12.012.

Luo, J., Huang, W., Zhang, Q., Guo, W., Xu, R., Fang, F., ... & Wu, Y., 2021. A preliminary metatranscriptomic insight of eggshells conditioning on substrates metabolism during food wastes anaerobic fermentation. Sci. Total Environ. 761, 143214.

Martinez, G. A., Bertin, L., Scoma, A., Rebecchi, S., Braunegg, G., & Fava, F., 2015. Production of polyhydroxyalkanoates from dephenolised and fermented olive mill wastewaters by employing a pure culture of Cupriavidus necator. Biochem. Eng. J. 97, 92-100.

Martinez, G. A., Rebecchi, S., Decorti, D., Domingos, J. M. B., Natolino, A., del Rio, D., Bertin, L., da Porto, C., & Fava, F., 2016. Towards multi-purpose biorefinery platforms for the valorisation of red grape pomace: production of polyphenols, volatile fatty acids, polyhydroxyalkanoates and biogas. Green Chem. 18, 261–270. https://doi.org/10.1039/c5gc01558h</div>

Mattioli, A., Gatti, G. B., Mattuzzi, G. P., Cecchi, F., & Bolzonella, D., 2017. Co-digestion of the organic fraction of municipal solid waste and sludge improves the energy balance of wastewater treatment plants: Rovereto case study. Renewable Energy 113, 980-988.

Micolucci, F., Gottardo, M., Pavan, P., Cavinato, C., & Bolzonella, D., 2018. Pilot scale comparison of single and double-stage thermophilic anaerobic digestion of food waste. J. Cleaner Prod. 171, 1376-1385.

Moestedt, J., Westerholm, M., Isaksson, S., & Schnürer, A., 2020. Inoculum source determines acetate and lactate production during anaerobic digestion of sewage sludge and food waste. Bioeng. *7*, 3.

Moretto, G., Ardolino, F., Piasentin, A., Girotto, L., & Cecchi, F., 2020a. Integrated anaerobic codigestion system for the organic fraction of municipal solid waste and sewage sludge treatment: an Italian case study. J. Chem. Technol. Biotechnol. 95, 418-426.

Moretto, G., Russo, I., Bolzonella, D., Pavan, P., Majone, M., & Valentino, F., 2020b. An urban biorefinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas. Water res. 170, 115371.

Moretto, G., Valentino, F., Pavan, P., Majone, M., & Bolzonella, D., 2019. Optimization of urban waste fermentation for volatile fatty acids production. Waste Manag. 92, 21–29. https://doi.org/10.1016/J.WASMAN.2019.05.010

Palmqvist, E., & Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresour. Technol., 74, 25-33.

Pau, S., Tan, L. C., & Lens, P. N. L., 2021. Effect of pH on lactic acid fermentation of food waste using different mixed culture inocula. J. Chem. Technol. Biotechnol. https://doi.org/10.1002/jctb.6982

Peces, M., Astals, S., Clarke, W. P., & Jensen, P. D., 2016. Semi-aerobic fermentation as a novel pre-treatment to obtain VFA and increase methane yield from primary sludge. Bioresour. Technol. 200, 631-638. Doi: http://dx.doi.org/10.1016/j.biortech.2015.10.085

RedCorn, R., & Engelberth, A. S., 2016. Identifying conditions to optimize lactic acid production from food waste co-digested with primary sludge. Biochem. Eng. J. 105, 205–213. https://doi.org/10.1016/j.bej.2015.09.014

Samorì, C., Martinez, G. A., Bertin, L., Pagliano, G., Parodi, A., Torri, C., & Galletti, P., 2022. PHB into PHB: Recycling of polyhydroxybutyrate by a tandem “thermolytic distillation-microbial fermentation” process. Resour. Conserv. Recycl. 178, 106082.

Schwarz, D., Schoenenwald, A. K., Dörrstein, J., Sterba, J., Kahoun, D., Fojtíková, P., ... & Sieber, V., 2018. Biosynthesis of poly-3-hydroxybutyrate from grass silage by a two-stage fermentation process based on an integrated biorefinery concept. Bioresour. technol. 269, 237-245.

Sivagurunathan, P., Kuppam, C., Mudhoo, A., Saratale, G. D., Kadier, A., Zhen, G., ... & Kumar, G., 2018. A comprehensive review on two-stage integrative schemes for the valorization of dark fermentative effluents. Crit. Rev. Biotechnol. 38, 868-882.

Sobeck, D. C., & Higgins, M. J., 2002. Examination of three theories for mechanisms of cation-induced bioflocculation. In *Water Research* (Vol. 36).

Strazzera, G., Battista, F., Tonanzi, B., Rossetti, S., & Bolzonella, D., 2021. Optimization of short chain volatile fatty acids production from household food waste for biorefinery applications. Environ. Technol. Innovation 23, 101562. https://doi.org/10.1016/J.ETI.2021.101562

Tang, J., Wang, X. C., Hu, Y., Zhang, Y., & Li, Y., 2017. Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula. Bioresour. Technol. 224, 544-552.

Tang, J., Wang, X., Hu, Y., Zhang, Y., & Li, Y., 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste manag. 52, 278-285.

Techno-Innovation Alto Adige S.C.p.A. (TIS), 2004. Mappatura delle biomasse avviabili a digestione anaerobica in Alto Adige - Relazione conclusiva -. Ministero delle Politiche Agricole, Agrarie e Forestali Programma Nazionale Biocombustibili. Retrieved from: http://www.provincia.bz.it/agricoltura/download/mappatura-biomasse.pdf.

Tian, X., Ng, W. J., & Trzcinski, A. P., 2018. Optimizing the synergistic effect of sodium hydroxide/ultrasound pre-treatment of sludge. Ultrason. Sonochem. 48, 432-440.

Tsuge, T., Tanaka, K., & Ishizaki, A., 2001. Development of a novel method for feeding a mixture of L-lactic acid and acetic acid in fed-batch culture of Ralstonia eutropha for poly-D-3-hydroxybutyrate production. J. Biosci. Bioeng. 91, 545-550.

Valentino, F., Moretto, G., Gottardo, M., Pavan, P., Bolzonella, D., & Majone, M., 2019a. Novel routes for urban bio-waste management: A combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production. J. Cleaner Prod. https://doi.org/10.1016/j.jclepro.2019.02.102

Valentino, F., Moretto, G., Lorini, L., Bolzonella, D., Pavan, P., & Majone, M., 2019b. Pilot-scale polyhydroxyalkanoate production from combined treatment of organic fraction of municipal solid waste and sewage sludge. Ind. Eng. Chem. Res. 58, 12149-12158.

Vidal-Antich, C., Perez-Esteban, N., Astals, S., Peces, M., Mata-Alvarez, J., & Dosta, J., 2021. Assessing the potential of waste activated sludge and food waste co-fermentation for carboxylic acids production. Sci. Total Environ. 757, 143763. https://doi.org/10.1016/j.scitotenv.2020.143763

Yin, J., Yu, X., Zhang, Y., Shen, D., Wang, M., Long, Y. & Chen, T., 2016a. Enhancement of acidogenic fermentation for volatile fatty acid production from food waste: Effect of redox potential and inoculum. Bioresour. Technol. 216, 996–1003. Doi: http://dx.doi.org/10.1016/j.biortech.2016.06.053

Yousuf, A., Bastidas-Oyanedel, J. R., & Schmidt, J. E., 2018. Effect of total solid content and pretreatment on the production of lactic acid from mixed culture dark fermentation of food waste. Waste Manag. 77, 516-521.

Zhang, W., Li, X., Zhang, T., Li, J., Lai, S., Chen, H., ... & Xue, G., 2017. High-rate lactic acid production from food waste and waste activated sludge via interactive control of pH adjustment and fermentation temperature. Chem. Eng. J. 328, 197-206.

Zhao, Y. H., Zhang, B., Tao, J., Li, Q., & Lv, B. (2019, August). Optimization of Energy Consumption of the Ultrasonic Pretreatment on Sludge Disintegration. In *IOP Conference Series: Materials Science and Engineering* (Vol. 592, No. 1, p. 012198). IOP Publishing.