Ca' Foscari University of Venice

VERITAS

GREENPROPULSIONLAB

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

A. Lanfranchi[1], B. Chouaia[1], G. Tassinato[2], C. Cavinato[1]

[1]Dipartimento di Scienze Ambientali, Informatica e Statistica, Università Ca' Foscari Venezia, 30174, Italy

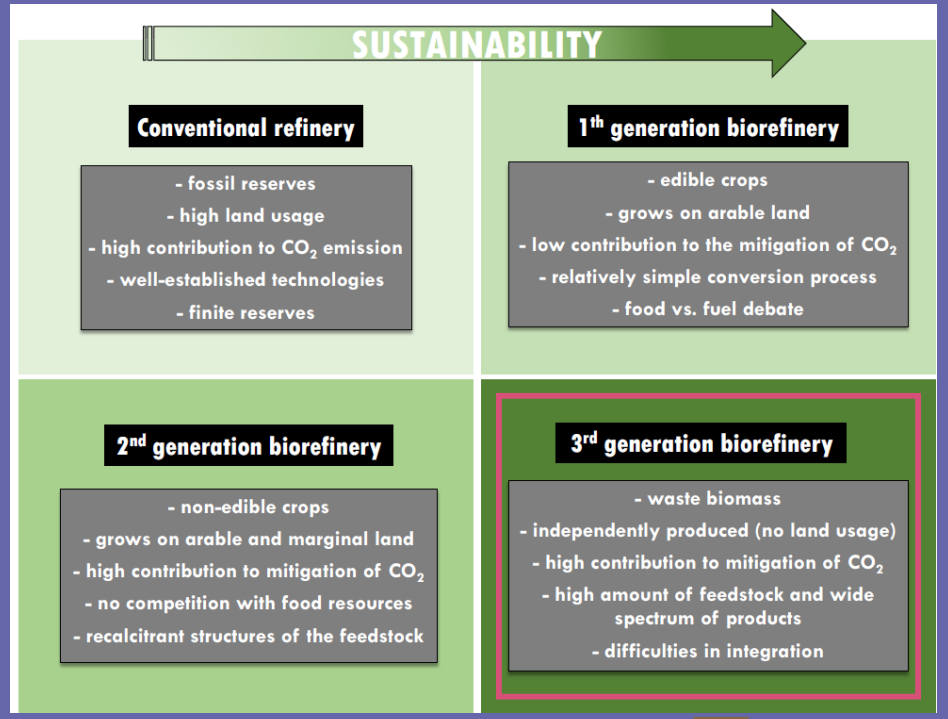
[2]Green Propulsion Laboratory, Veritas S.p.a., Fusina (VE), 30175, Italy

Contacts:
A. Lanfranchi: alice.lanfranchi@unive.it
C. Cavinato: cavinato@unive.it

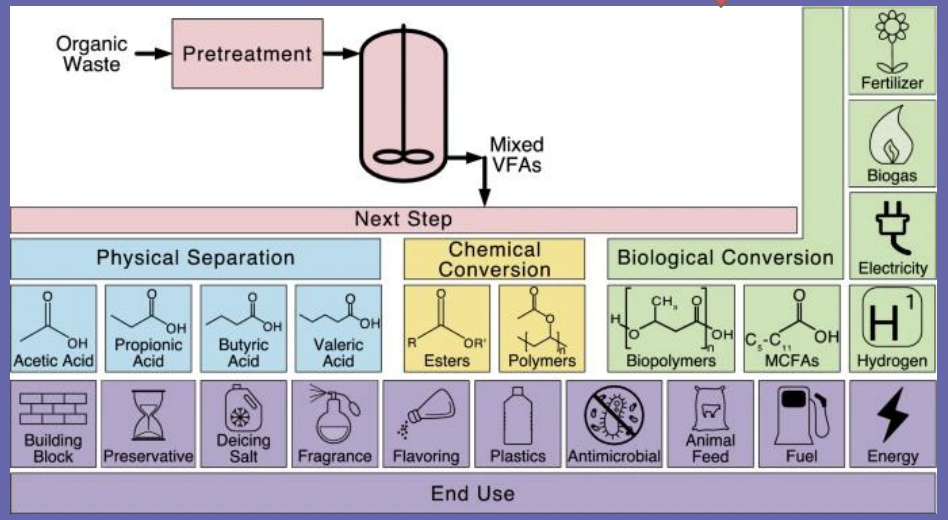
BACKGROUND

VOLATILE FATTY ACIDS (VFAs)

- Aliphatic monocarboxylate compounds C_2-C_7
- **Chemical «building blocks»:** various applications in the food, cosmetics, textile, bioenergy, chemical and pharmaceutical industry
- Global market: 13 mln tonn/year, 8 mld $/year (market value)
- At present, 90% is produced from petrochemical compounds



Evolution of the biorefinery (Strazzera, 2020)



Examples of potential uses and process options of mixed VFAs from acidogenic fermentation (Ramos-Suarez et al., 2021)

MICROBIAL VFAs PRODUCTION FROM URBAN WASTE 1/2

URBAN METABOLISM

VEGETABLE WASTE

SEWAGE SLUDGE

CO-FERMENTATION

Improvement of process performance, thanks to

i) a higher organic material content;

ii) a stronger buffer capacity;

iii) balanced macronutrients and micronutrients;

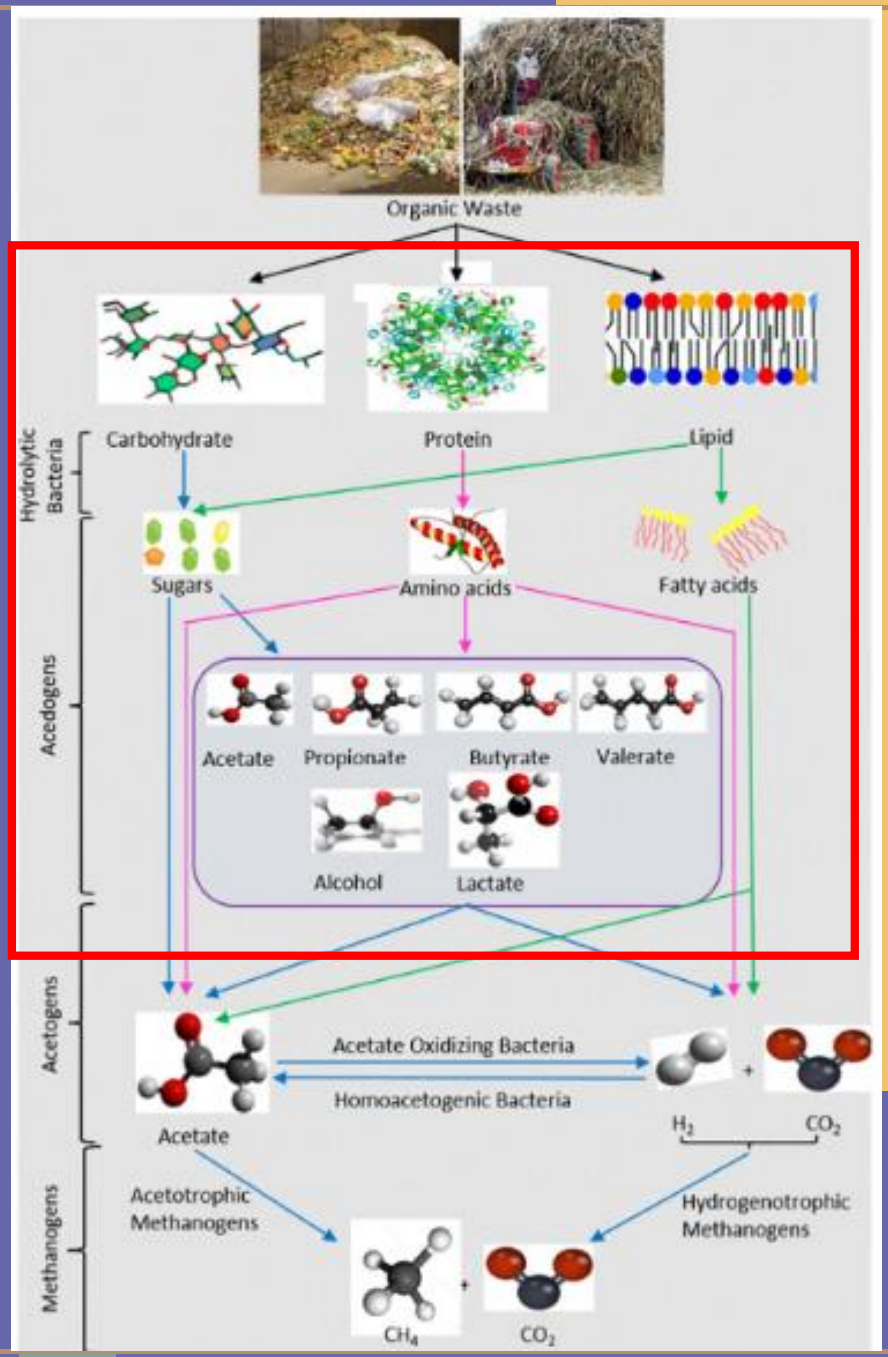
iv) dilution of toxic and inhibitory compounds;

v) a more diverse microbial community

(Fang et al., 2020; Vidal-Antich et al., 2021)

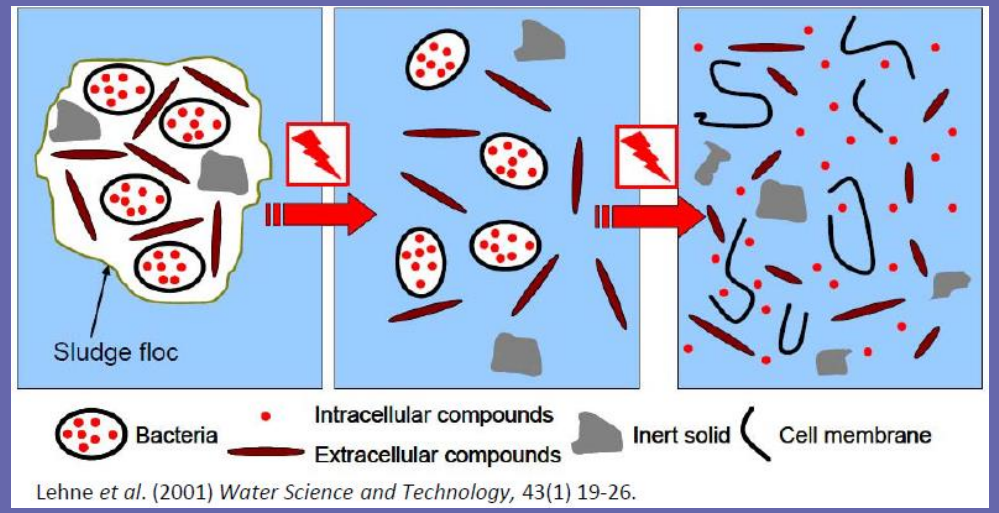
MICROBIAL VFAs PRODUCTION FROM URBAN WASTE 2/2

- VFAs yields and distribution for a given substrate are essentially the results of the microbial community composition and activity (Ramos-Suarez et al., 2021).
- The trend of microbial communities in the anaerobic fermentation process is still uncertain, since they are shaped by several factors like the inoculum source, the substrate fed, the operational conditions and the pre-treatment applied (Llamas et al., 2021).

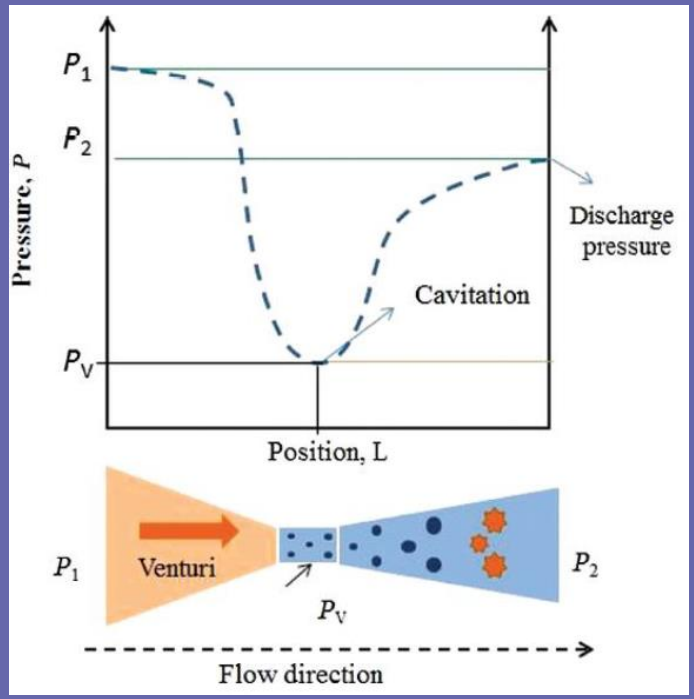


HYDRODYNAMIC CAVITATION PRE-TREATMENT

- Complex floc structure of sewage sludge→ need for pretreatment
- **Cavitation**: physico-chemical process consisting of the formation, growth and collapse of vapor cavities due to a sharp pressure drop. The pressure drop is generated applying a sudden constriction (**hydrodynamic cavitation**) or by using ultrasound **(acoustic cavitation)**.
- **Hydrodynamic cavitation**: higher potential of scalability, cheaper than ultrasound cavitation (Bhat&Gogate, 2021).



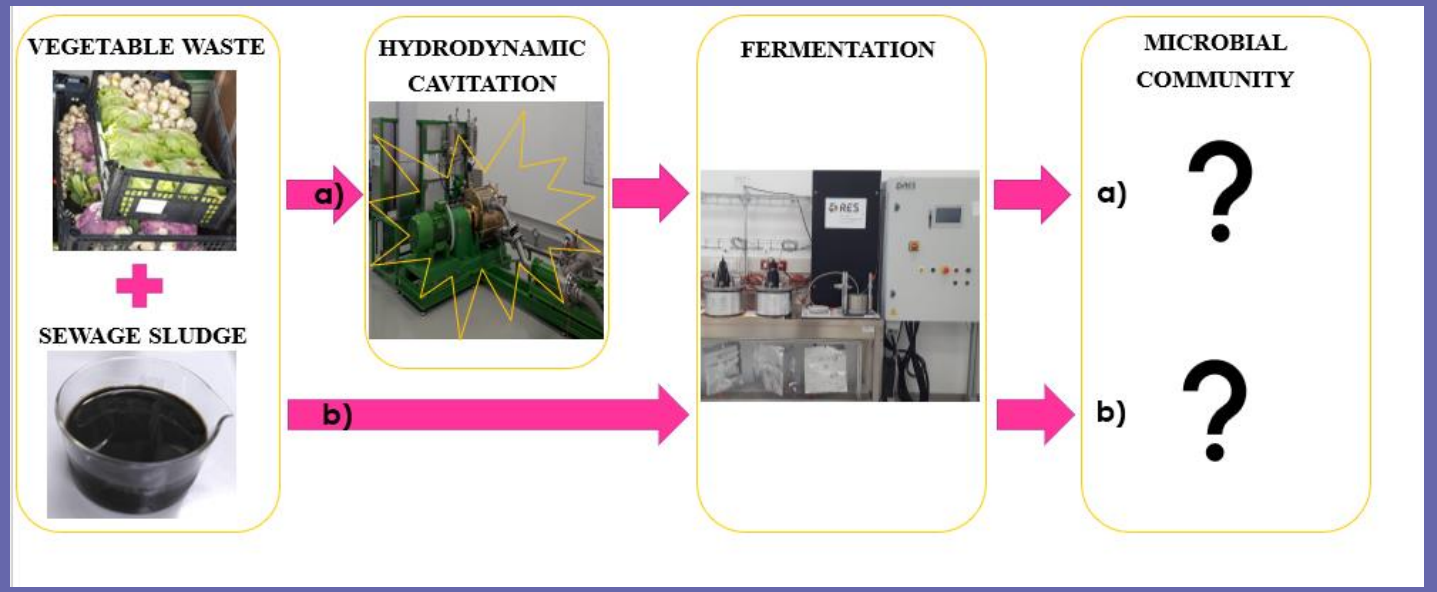
Sludge floc disintegration (Lehne et al., 2001)



Pressure and flux variations in a cavitation engine (Carpenter et al., 2017)

AIM OF THE RESEARCH

Understand the effects of the **hydrodynamic cavitation** pre-treatment of a mixture of vegetable waste and sewage sludge on the **microbial community** of a **semi-continuous anaerobic fermentation system** at the beginning and at the end of the steady state.



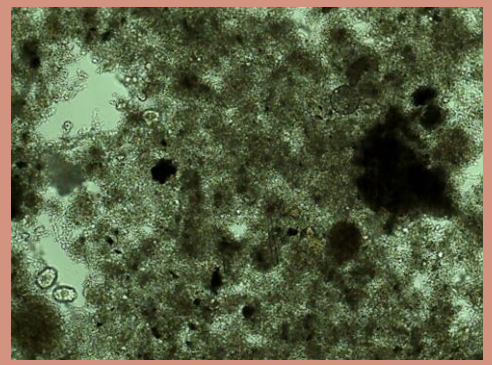
SPECIFIC OBJECTIVES

- Investigate the differences in microbial community diversity (**Chao1, Shannon and Evenness indexes**) between the fermenters fed with cavitated and not cavitated mixture and their variation between the beginning and the end of the steady state
- Assessing the samples clustering and the significant factors determining sample clustering with **non-metric multi-dimensional scaling (NMDS) analysis**

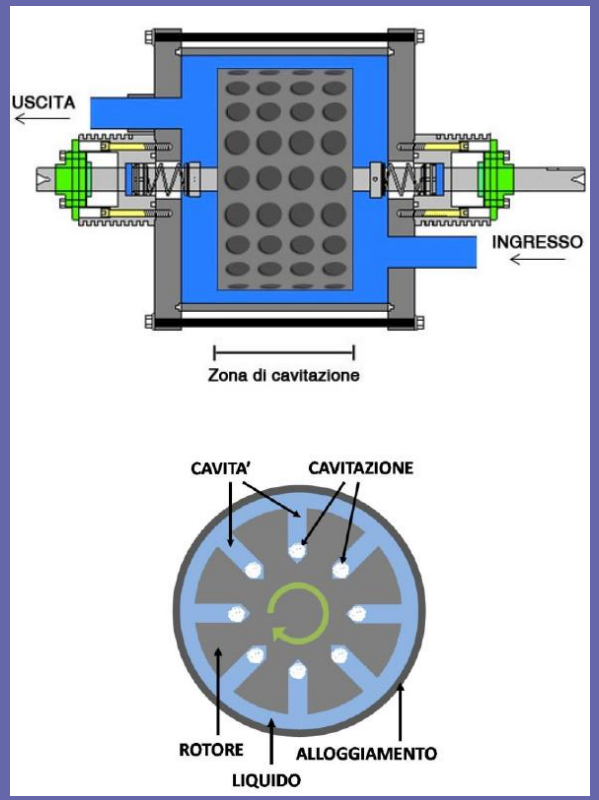
MATHERIALS AND METHODS

HYDRODYNAMIC CAVITATION PRE-TREATMENT

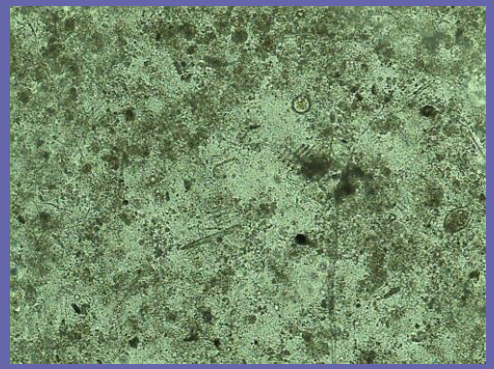
Mixture composition:
70% sewage sludge, **30%** food waste (v/v)



Parameters applied:
power= 8 kW, P= 1.4-1.5 bar, $Q_{mixture}$ of 25-30 L/min, 1550-1650 rpm, duration: 30 minutes



Scheme of the hydrodynamic cavitator used in this study, located in the GP Lab of Veritas S.p.A. (Three-Es s.r.l.)



ANAEROBIC FERMENTATION PROCESS

Working conditions:

- Mesophilic temperature (37°C)
- CSTR reactors with mechanical stirring (14 rpm)
- V= 4 L
- Uncontrolled pH
- Inoculum: anaerobic digestate (31-34% v/v) in order to maintain a high F/M ratio that allows to inhibit methanogens

Process monitoring:

- Daily samples were collected to determine VFAs, soluble chemical oxygen demand (sCOD), pH, alkalinity, and cations.

	Parameter	Unit	Not cavitated	Cavitated 30'
Batch tests	OL	$kg_{tCOD}\ m^{-3}$	34.8	33.4
		$kg_{TVS}\ m^{-3}$	24.5	18.8
	F/M	$kg_{tCOD}\ kg_{TVS}^{-1}$	9.92	9.51
	F/M	$kg_{TVS}\ kg_{TVS}^{-1}$	7	5.4
Semi-continuous tests	OLR	$kg_{TVS}\ m^{-3}\ d^{-1}$	8	8
	HRT	days	6.6	5

MICROBIAL COMMUNITY ANALYSIS

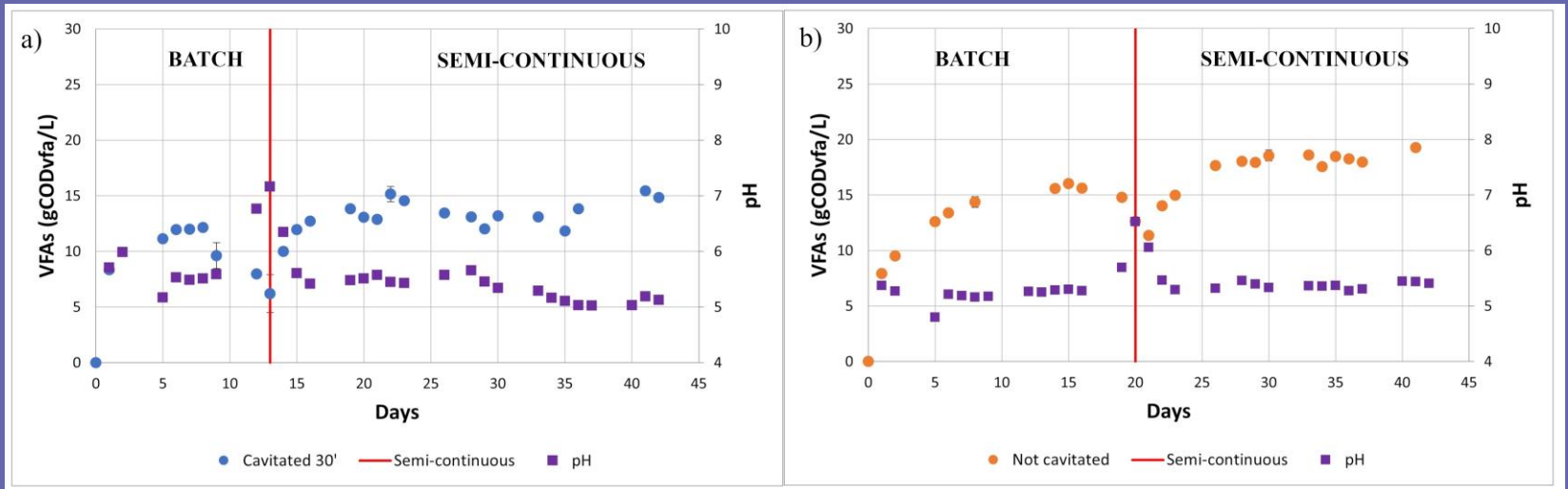
NMDS analysis

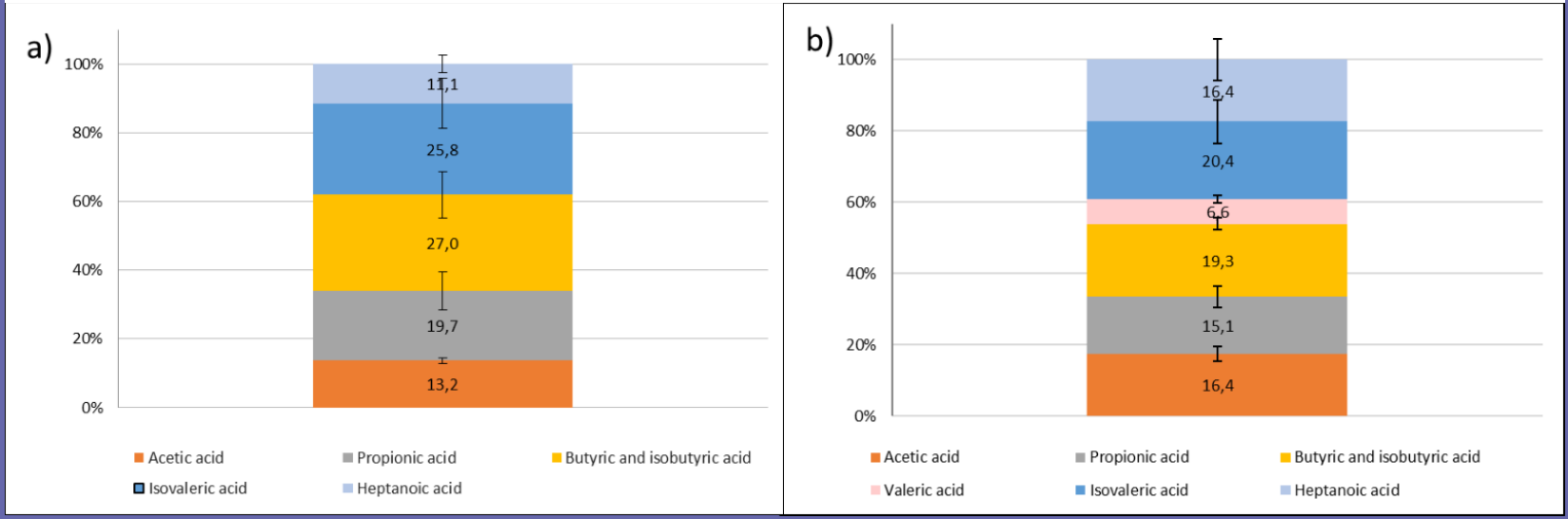
- NMDS creates an ordination of the samples based on a dissimilarity matrix. It orders «points» coming from a multidimensional space (described by many factors) in a space with less dimensions (2 in this study).
- Tested factors: time, HRT, pH, temperature, OLR, sCOD, agitation, total VFAs (gCOD/L), single VFAs (acetic, propionic, etc… gCOD/L)

- Samples for microbial analyses 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)
- Non-metric multi-dimensional scaling (NMDS) biplot was built to graphically ordinate samples and assess the differences among the cavitated/not cavitated at the beginning and at the end of the steady state.

RESULTS AND DISCUSSION

ANAEROBIC FERMENTATION PROCESS





- The fermenters reached a steady state with a VFAs concentration of:

12.94 ± 0.63 $gCOD_{VFA}/L$ for the **cavitated**
18.23 ± 0.51 $gCOD_{VFA}/L$ for the **not cavitated.**

- Similar VFAs profile
- Heptanoic acid was present in higher percentages in the not cavitated→ probably due to the fact that the HC pre-treatment enhanced the substrates' conversion into VFAs with shorter carbon chains.

More details in: Lanfranchi, A., Tassinato, G., Valentino, F., Martinez, G. A., Jones, E., Gioia, C., ... & Cavinato, C. (2022). Hydrodynamic cavitation pre-treatment of urban waste: Integration with acidogenic fermentation, PHAs synthesis and anaerobic digestion processes. *Chemosphere*, *301*, 134624.

MICROBIAL COMMUNITY ANALYSIS 1/3

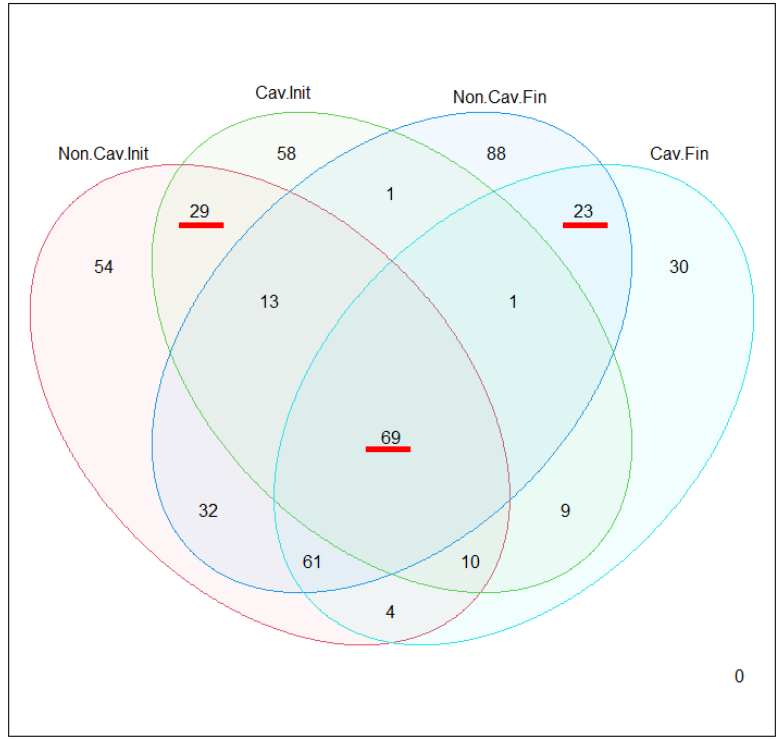
	$Cavitated_{initial}$	$Not\ cavitated_{initial}$	$Cavitated_{end}$	$Not\ cavitated_{end}$
Chao1	190.00	272.00	207.00	288.00
Shannon	3.85	4.54	3.96	4.68
Evenness	0.73	0.81	0.74	0.83

- **Similar species richness** between the beginning and the end of the steady state for both conditions

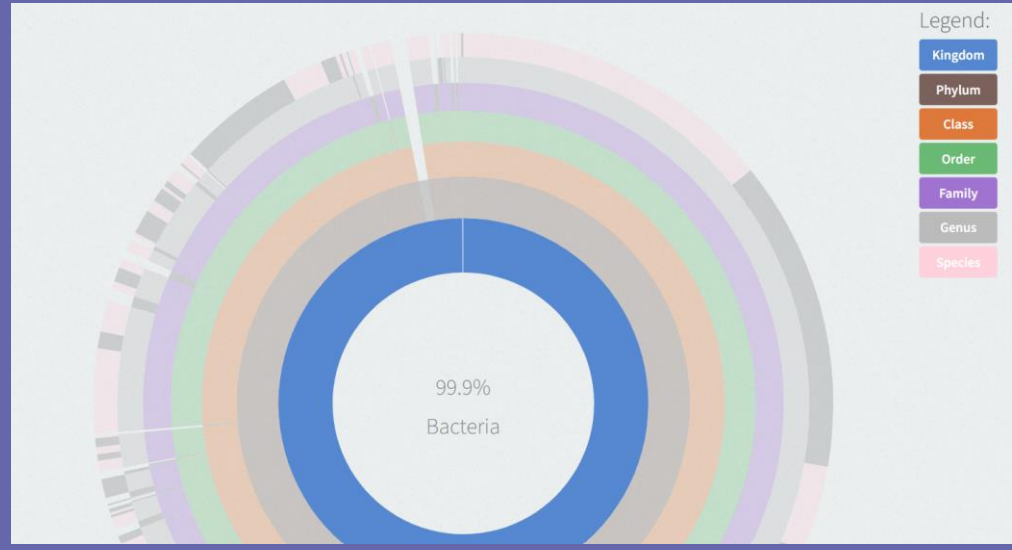
- **Lower species richness for the cavitated→ hydrolization** of organic compounds due to the pre-treatment, which prevented the development of some of the hydrolytic microorganisms involved, as already observed in Llamas et al. 2021 after enzymatic pre-treatment.

- **Venn diagram:**

-**69 OTUs in common** among all the conditions
-Similar number of OTUs in common between the beginning and the end of the steady state
-Higher number of specific OTUs in the final not cavitated (88) respect to the final cavitated (30)



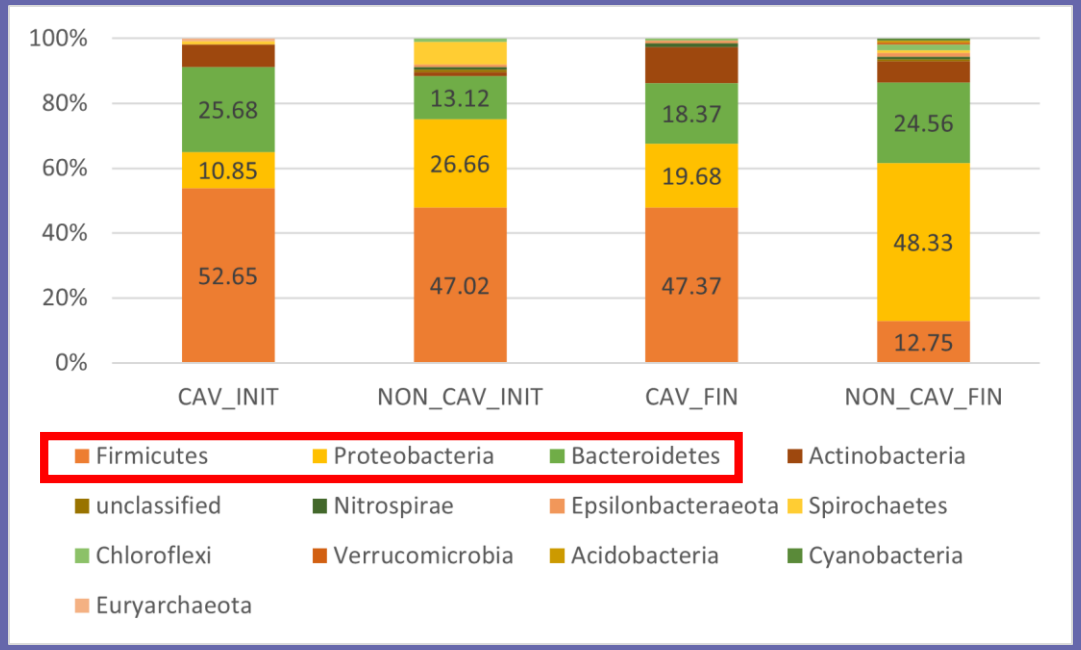
MICROBIAL COMMUNITY ANALYSIS 2/3



- Most abundant phyla: *Firmicutes, Bacteroidetes and Proteobacteria*→ hydrolytic and acidogenic bacteria

- **Beginning of the steady state**: *Proteobacteria* were less abundant in the cavitated (10.8%) than in the not cavitated (26.7%) → carbohydrate disgregation due to the HC pre-treatment. This could have disadvantaged *Proteobacteria*, which are known as degraders of different carbon sources.

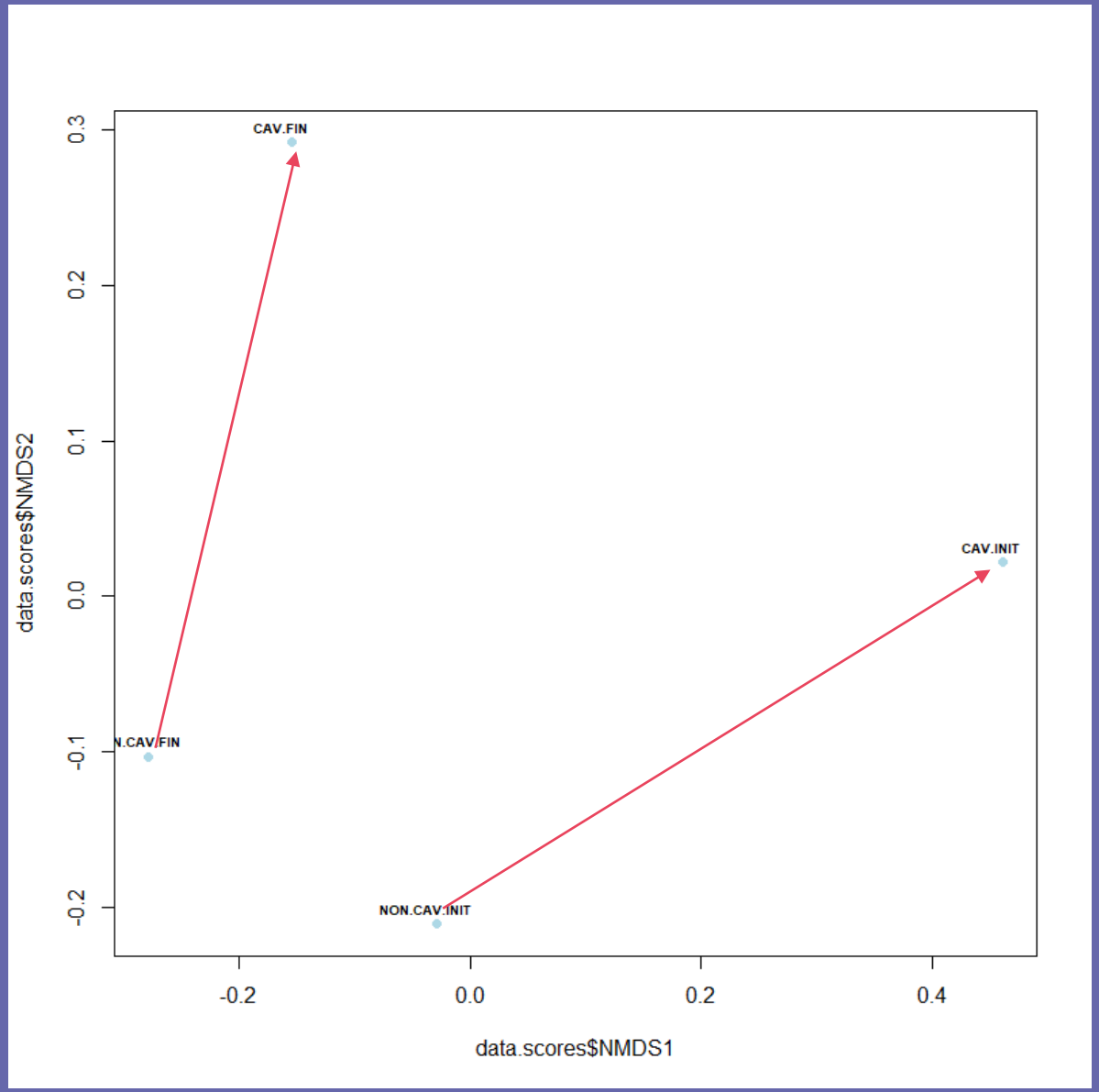
- **End of the steady state**: high increase of *Proteobacteria* in the not cavitated → central role of *Proteobacteria* in carbohydrate disgregation. However, this did not correspond to a variation in the VFAs profile or concentration, probably thanks to the diversity of the microbial community.



Microbial community composition at the order level

MICROBIAL COMMUNITY ANALYSIS 3/3

- The two conditions are separated by the y axis and cluster together in the lower (not cavitated) and the upper (cavitated) part of the NMDS plot.
- Based on the correlations of the tested factors, the distance between the two clusters can be explained by only one significant factor, i.e. **VFAs concentration (gCOD/L)** (r^2=1, Pr>r= 0.04167)
- These results suggest that in this experiment the pretreatment resulted in a lower microbial diversity, which apparently led to a lower VFAs concentration in the fermented effluent.



CONCLUSIONS

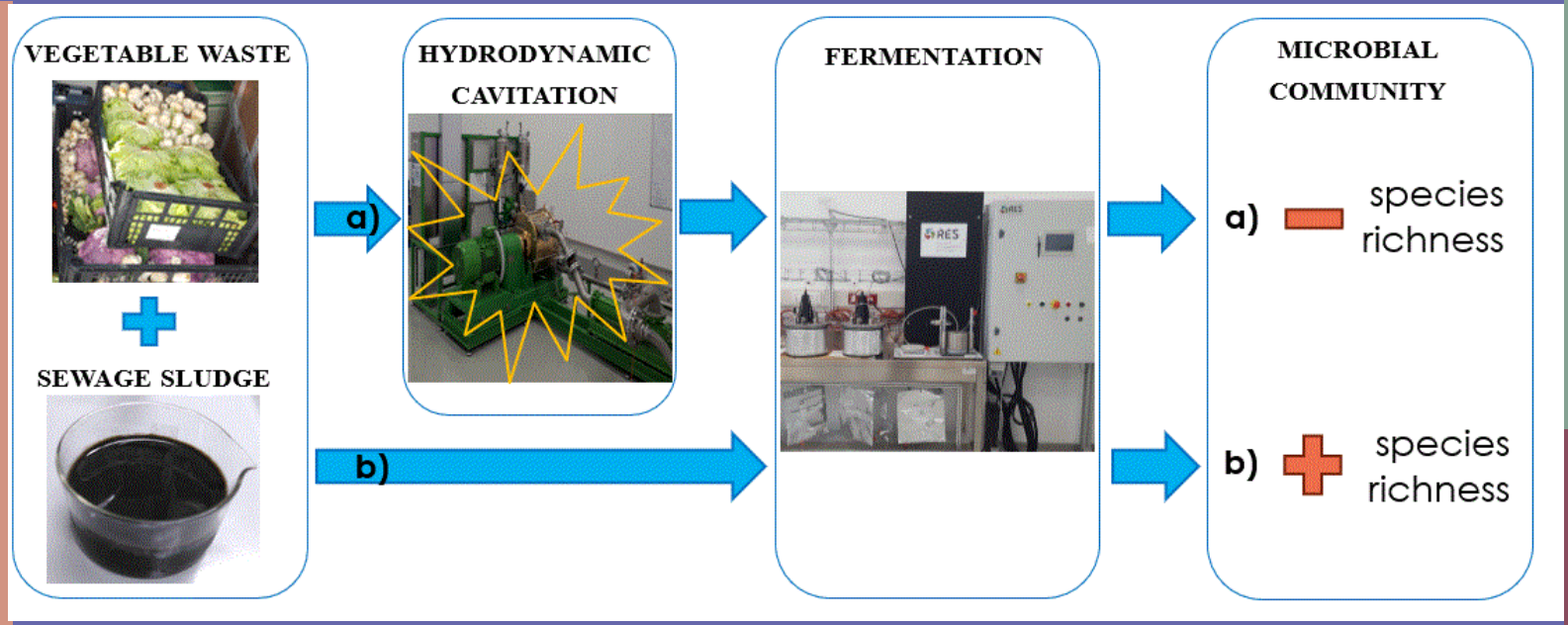
- This work gave an insight into the microbial community of mesophilic fermenters fed with a mixture of vegetable waste and sewage sludge and on the effect of HC pretreatment

- Diminution in microbial species richness of the cavitated :

→ attributable to organic compounds hydrolyzation after the pre-treatment.

→ led to a lower VFAs concentration

- The microbial community composition variation over time did not result in a change in the VFAs profile or concentration, which was kept stable, probably as a consequence of microbial community diversity of the fermenters.



TEAM

Alice Lanfranchi
PhD student
Università Ca' Foscari
Venezia

Bessem Chouaia
Researcher
Università Ca' Foscari
Venezia

Graziano Tassinato, PhD
R&D Manager
Green Propulsion Laboratory
(Veritas S.p.A.)

Cristina Cavinato
Associate Professor
Università Ca' Foscari
Venezia

THANKS
FOR THE ATTENTION

CHARACTERIZATION OF THE SUBSTRATES

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

CHARACTERIZATION OF THE MIXTURE

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
$N–NH_4^+$	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

NMDS

Non-metric multidimensional scaling, or NMDS, is known to be an indirect gradient analysis which creates an ordination based on a dissimilarity or distance matrix. It attempts to represent the pairwise dissimilarity between objects in a low-dimensional space, unlike other methods that attempt to maximize the correspondence between objects in an ordination. NMDS is a rank-based approach which means that the original distance data is substituted with ranks. It is considered as a robust technique due to the following characteristics: (1) can tolerate missing pairwise distances, (2) can be applied to a dissimilarity matrix built with any dissimilarity measure, and (3) can be used in quantitative, semi-quantitative, qualitative, or even with mixed variables.

- Performed with the *metaMDS* function implemented in the R package *Vegan*.
- The correlation between the microbiota composition and the tested factors was investigated by fitting the NMDS ordination scores with the *envfit Vegan* function.

NMDS

```
***VECTORS

                                NMDS1    NMDS2     r2  Pr(>r)
time..days.                   -0.71569  0.69842 0.8469 0.66667
Hydraulic.Retention.Time      -0.40094 -0.91611 1.0000 0.33333
Organic.Loading.Rate           0.40094  0.91611 1.0000 0.33333
Temperatura..C.                0.00000  0.00000 0.0000 1.00000
Agitazione..rpm.               0.00000  0.00000 0.0000 1.00000
sCOD                          -0.29035 -0.95692 0.6338 0.66667
VFA                           -0.72147 -0.69245 1.0000 0.04167 *
pH                             0.40455 -0.91452 0.7873 0.37500
Acetico.perc                  -0.20102 -0.97959 0.9882 0.16667
Propionico.perc                0.04127  0.99915 0.3937 0.79167
Butirrico.e.isobutirrico.perc  0.24427  0.96971 0.9965 0.08333 .
Valerico.perc                 -0.24015 -0.97074 0.9793 0.25000
Isovalerico.perc               0.97653 -0.21540 0.8758 0.29167
Eptanoico.perc                -0.95076 -0.30992 0.6014 0.62500
Iso.esanoico.perc             -0.92680  0.37555 0.6475 0.58333
Acetico.gCOD.L                -0.46695 -0.88428 0.9981 0.16667
Propionico.gCOD.L             -0.88869  0.45852 0.3142 0.75000
Butirrico.e.isobutirrico.gCOD.L -0.58156  0.81350 0.9958 0.16667
Valerico.gCOD.L               -0.28529 -0.95844 0.9853 0.16667
Isovalerico.gCOD.L             0.66253 -0.74904 0.5980 0.66667
Eptanoico.gCOD.L              -0.87666 -0.48111 0.6926 0.58333
Iso.esanoico.gCOD.L           -0.97648  0.21561 0.5873 0.58333
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Permutation: free
Number of permutations: 23
```