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Microbial Population Dynamics during Unstable Operation of a Semicontinuous Anaerobic Digester Fed with a Mild-Treated Olive Mill Solid Waste

Juan Cubero-Cardoso, África Fernández-Prior, Javier Ramiro-Garcia, Angeles Trujillo-Reyes, Belén Caballero-Guerrero, Guillermo Rodríguez-Gutiérrez and Fernando G. Fermoso *

Instituto de la Grasa (IG), Spanish Scientific Research Council (CSIC), Carretera de Utrera, km 1, 41013 Seville, Spain; j.cubero@dqcm.uhu.es (J.C.-C.); mfernandez26@us.es (Á.F.-P.); jramiro@ig.csic.es (J.R.-G.); angeles.trujillo@ugr.es (A.T.-R.); cabaguer@ig.csic.es (B.C.-G.); guirogu@ig.csic.es (G.R.-G.)
* Correspondence: fgfermoso@ig.csic.es; Tel.: +34-954611550

Abstract: This research evaluates process instability together with microbial population dynamics of the startup of an anaerobic digestion of a mild pretreated solid olive oil waste. The pretreatment consisted of a mild thermal treatment called thermo-malaxation and a subsequent dephenolized process of the olive mill solid waste. The anaerobic digestion process of the mild pretreated and partially dephenolized biomass was studied for three Hydraulic Retention Times (HRTs), with 21 days each HRT, with an organic load rate of 1 g VS/L d, carried out at mesophilic temperature (35 ± 1 °C). The average value of methane yield decreased from 204 ± 9 mL CH_4/g VS d on day 21, the last day of the first HRT, to 87 ± 24 mL CH_4/g VS d on day 60, the last day of the third HRT. The alkalinity decreased drastically, indicating instability of the anaerobic digestion process. Although phenolic compounds were partially extracted in the pretreatment, the observed increase in phenolic compounds during reactor operation might be contributed to the methane production decay. Interestingly, volatile fatty acids decreased with time, indicating that not only the methanogenic stage but also the hydrolysis stage was affected. Indeed, the microbial analysis showed that the abundance of hydrolytic bacteria decreased over time. It is also worth noticing that hydrogenotrophic methanogens, while present during the first two HRTs, were not observed at the end of the last HRT. This observation, together with the increase in the relative abundance of acetoclastic methanogens, showed a shift in the methane production pathway from hydrogenotrophic methanogenesis to acetotrophic methanogenesis.

Keywords: anaerobic digestion; solid waste; thermal treatment; phenol; valorization

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1. Introduction

The importance of the olive oil sector in the main producing countries is well known, as is the case of Spain, where 47% of the worldwide consumed olive oil is produced. It should also be borne in mind that the type of cropping system is gradually changing from traditional to intensive or even super-intensive in order to increase production [1]. All this leads to the production of between 5 and 7 million tons of by-products per year in Spain while simultaneously exhausting agronomic resources more rapidly, forcing the use of a larger quantity of fertilizers. This increase makes managing this massive number of by-products even more difficult.

In Spain, the most common system used to extract olive oil is the two-phase extraction system, which produces a semi-solid waste with high humidity (60–70%) [2]. The advantage of this country is the centralization of the industry dedicated to the use of olive mill solid waste, called pomace extractors. The main use is the extraction of pomace oil. Most of it is obtained by extraction with an organic solvent after a drying process that consumes a large amount of energy resources [3]. And once the pomace oil has been extracted, the

final solid is mainly used as biomass for electricity generation. The increase in production, the high drying costs, and the growing importance of the bioactive components present in this type of by-product are proliferating the appearance of new alternatives to improve its management [4]. It should be noted that more and more pomace extractors are using a three-phase extraction system. This process allows oil to be recovered during centrifugation, a liquid fraction rich in bioactive components, and a solid with a lower degree of humidity (50–55%). Therefore, this oil extraction facilitates and reduces the costs of subsequent drying to extract the pomace oil remaining in the solid with solvents. This trend makes it possible to study new ways of management and use [5].

The application of bioprocesses is always an alternative to be considered for the valorisation of this type of agri-food by-product. The limitation in this type of treatment is the presence of inhibitory substances, which for decades have limited the use of by-products from the olive oil industry [6]. The application of the three-phase extraction system makes it possible to obtain a detoxified liquid phase after extracting the phenolic components, as they are precisely the main components responsible for this toxicity. But, it also makes it possible to obtain a solid with a lower phytotoxic content since most of the phenolic compounds have been solubilised in the liquid phase [3]. If the growing appearance of these two phases with a less toxic character is combined with the increasing energy demand, the need would arise to look for bioprocesses that allow us to manage the whole of the olive mill solid waste at the same time as obtaining energy and other products of great interest.

Among all types of bioprocesses, anaerobic digestion can be highlighted, which allows taking advantage of this source of organic matter, generating methane and a stabilised digestate for agricultural use, i.e., a source of energy and biomass for use in agriculture [6,7]. The extraction of phenolic components during the application of the three-phase extraction system would be favourable for the subsequent anaerobic digestion process due to the toxic effect of these compounds on anaerobic microorganisms, particularly the methanogens [8]. In this sense, the necessity arises to study the use of this technology from the two fractions, liquid and solid, which allows a better use while allowing us to manage the growing volumes of this by-product, generating energy and improving the conditions of exhaustion in the olive plantations themselves.

The objective of this study is to broaden the knowledge behind the processes of anaerobic degradation of agri-food residues such as the olive oil solid waste under mesophilic conditions operating in a semi-continuous regime. More specifically, the research focuses on evaluating the instability of the degradation process of a complex biomass as the olive oil solid waste, with the objective of determining the relationship between the decreased methane production and the present microbial population. The implications of understanding this link could be of great importance for designing biological systems capable of maximizing methane production.

2. Materials and Methods

2.1. Materials and Reagents

The reagents that have been used to carry out all the experimentation are the following: $NaHCO_3$ (Panreac, Castellar del Vallès, Barcelona, Spain), Na_2CO_3 (Panreac); Folin–Ciocalteu reagent (Panreac); Methanol 99.9% (Panreac); Gallic Acid (Sigma Aldrich, Steinheim, Germany); $H_2SO_4/AgSO_4$ 10g/L (Panreac); Potassium hydrogen phthalate (Sigma Aldrich); $K_2Cr_2O_7$ (Panreac); $(NH_4)_2Fe(SO_4)$ (Panreac); commercial $K_2Cr_2O_7$ 1N (Panreac); Sulfuric Acid 96% (Carlo Erba Reagents, Carrer dels Filadors, Sabadell, Spain); Sodium hydroxide 99% (Labkem, Barcelona, Spain); and pH buffer solution (Hach, Dusseldorf, Germany).

All samples were characterized by the following determination: Total, Mineral and Volatile Solids (TS, MS, and VS, respectively), Soluble Chemical Oxygen Demand (SCOD), Total Chemical Oxygen Demand (TCOD), pH, and alkalinity. These procedures were conducted in accordance with the guidelines provided by the Standard Methods of APHA [9].

The determination of total phenolic compounds was carried out using a colorimetric technique referred to as the Folin–Ciocalteau method [10]. The individual phenolic compounds were identified and quantified utilizing a Hewlett-Packard 1100 series high-performance liquid chromatography (HPLC) system from Agilent, located in Barcelona, Spain, following method described in Fernández-Prior et al. [11]. Volatile fatty acids (VFA) in the range of C2–C5 were assessed using a Shimadzu GC-2010 gas chromatograph, equipped with a 0.25 mm × 25 m column comprised of 100% ethylene glycol composition, along with a flame ionization detector (FID). The temperature of the oven was gradually raised from 100 to 170 °C at a rate of 5 °C·min^{-1}. A carrier gas mixture of nitrogen (30 mL·min^{-1}), hydrogen (40 mL·min^{-1}), and air (399.8 mL·min^{-1}) was employed at a flow rate of 40.1 mL·min^{-1}, maintained at 456 kPa.

2.2. Thermo-Malaxation Pre-Treatment

The raw material for this study was obtained from semi-solid olive mill waste sourced at the Instituto de la Grasa (CSIC, Seville, Spain) premises. The semi-solid olive mill waste was processed using Pieralisis equipment (Pieralisis, Jesi, Italy) with a milling capacity of 1000 kg/h. The resulting olive mill solid waste was acquired through a two-stage extraction process designed to obtain olive oil. The application of subsequent heat treatment or thermo-malaxation consisted of beating the olive pomace at 60 °C for 90 min. It was then centrifuged in a decanter giving rise to three phases: a first solid phase (SP), a liquid phase (LP), and pomace oil (POO). A fourth phase of suspended solids (SS) was obtained from the storage and sedimentation of the SP. Thanks to the sedimentation time (two months), a new liquid phase free of suspended solids (LFP) was obtained with an increase in HT due to the hydrolysis of the HT precursors, as in a previous study [8]. LPF served as an abundant reservoir of phenolic compounds, subjected to a solid–liquid extraction system to capture these compounds. Subsequently, the phenolic compounds were extracted from the column using a solution composed of ethanol and water in comparable volumes. Phenolic compounds were then concentrated by completely eliminating the ethanol. Finally, a dephenolized liquid fraction (DLP) was obtained. The whole set of samples, SP and DLP, were stored at −20 °C until their use in the different assays. Table 1 provides a summary of the analytical characteristics of the distinct phases utilized within the anaerobic digestion process. These phases are a solid phase (SP), dephenolized liquid phase (DLP), and inoculum, as will be explained in the next section.

Table 1. Physicochemical characterization of the different samples used in the assay.

		DLP	SP	Inoculum
pH		4.9 ± 0.1	4.6 ± 0.1	7.4 ± 0.1
TS	mg/kg	43,135 ± 188	428,811 ± 6716	57,126 ± 437
MS	mg/kg	9403 ± 285	16,827 ± 605	19,988 ± 252
VS	mg/kg	33,732 ± 461	411,984 ± 7023	37,138 ± 388
VS/TS		0.78 ± 0.01	0.96 ± 0.02	0.65 ± 0.01
tCOD	mg O_2/kg	98,148 ± 565	576,024 ± 40,220	77,792 ± 1738
sCOD	mg O_2/L	92,650 ± 1322	60,076 ± 4755	3115 ± 212
Total phenolics	mg gallic acid eq./kg	2396 ± 84	3935 ± 155	106 ± 4
C2	mg O_2/L	-	-	1316 ± 9
C3	mg O_2/L	-	-	1113 ± 8
i-C4	mg O_2/L	-	-	41 ± 1
n-C4	mg O_2/L	-	-	487 ± 4
i-C5	mg O_2/L	-	-	142 ± 1
n-C5	mg O_2/L	-	-	476 ± 4
Total VFA	mg O_2/L	-	-	3576 ± 10
Alkalinity	mg $CaCO_3$/L	-	-	7044 ± 175

Acetic acid (C2), Propionic acid (C3), Isobutyric acid (i-C4), Butyric acid (n-C4), Isovaleric acid (i-C5), and Valeric acid (n-C5).

2.3. Semi-Continous Anaerobic Process Procedure

Microorganisms used in the anaerobic assay were a mixed culture from the anaerobic digester of the "Copero" wastewater treatment plant in Sevilla, Spain. Physicochemical characterization from inoculum is shown in Table 1.

Two glass reactors with a capacity of two litres have been used. 10 g VS/L of inoculum was introduced in the reactors together with water until 1.7 L of working volume at the start of the assay. The reactors were fed every day with 81 mL of SP + DLP + Water, were continuously stirred with a cylindrical magnetic bar, and were thermostated at 35 °C. Biogas was transported by rubber tube until a gas bubbler, with a NaOH solution (3 N), was used for CO_2 removal. Methane production displaced water from a closed tank and was quantified with a graduated cylinder under standard temperature and pressure conditions (25 °C and 1 atm). In the preceding 7 days leading up to this experiment, both reactors were prepared and acclimated using mixtures of a synthetic solution (SS) containing glucose (50 g/L) and sodium acetate (25.2 g/L), with an organic load rate (OLR) set at 1 g VS/L d. Following the acclimation phase, the reactors were operated for 63 days. They were supplied with a combination of solid phase (SP) and dephenolized liquid phase (DLP) after undergoing pre-treatment. During assay, an OLR of 1 g VS/L d was applied over three stages, corresponding with the three hydraulic retention times (HRTs), each spanning 21 days.

2.4. DNA Extraction and Library Preparation

DNA amplification targeted the V4 region of the 16S rRNA using barcoded primers 515f (5′-GTGCCAGCMGCCGCGGTAA) and 806r (5′-GGACTACHVGGGTWTCTAAT). The amplicons were produced via one-step PCR employing the barcodes specified in Ramiro-Garcia et al. [12]. A DNA template (2 µg) was used in the 40 µL PCR reaction, including 10 µL of HF buffer (Thermo Fisher Scientific, Madrid, Spain), 1 µL of dNTP Mix (10 mM; Bioline, London, UK), 1 U of ADN polymerase Phusion™ Plus (Thermo Fisher Scientific), and 10 nM of each barcoded primer. PCR cycles were conducted using an Alpha cycler 1 (PCRmax, Biorad, Watford, UK) following the conditions outlined in Lara M. Paulo et al., [13]. Purification of PCR products was performed using HighPrep™ (Magbio Genomics, Gaithersburg, MD, USA), with elution using 20 µL of Nuclease Free Water (Bioline). Quantification was achieved using a nanodrop (Thermo Fisher Scientific). Subsequently, the purified products were equimolarly combined to form the library pool, which was then subjected to sequencing on the Illumina NovaSeq 6000 platform (Eurofins Genomics, Ebersberg, Germany). Sequence data have been deposited in the European Nucleotide Archive under accession number [PRJEB59213].

2.5. Bioinformatics Analysis

Data from all samples (with a total of 2,984,943 pair-end reads) were analysed using the Silva 138 database [14] to assign taxonomy and NG-Tax [12], a validated pipeline for 16S rRNA analysis, under default parameters. Alpha diversity and beta diversity were calculated using the R packages phyloseq 1.32.0 [15] and picante 1.8.2 [16]. Their plots and the heatmap plot were generated using ggplot 2 23.3.2 [17].

3. Results and Discussion

3.1. Methane Production along Operation Time

The methane production yield was evaluated daily after feeding a mixture obtained after the treatment of olive mill solid waste (SP + DLP) (Figure 1). The anaerobic digestion process exhibits a variable methane production pattern across three HRTs. During the initial HRT, methane production reached 204 ± 9 mL CH_4/(g VS d). Notably, this methane production yield under an OLR of 1 g VS/(L d) closely resembles that achieved for the hydrothermal treatment of olive mill solid waste at 170 °C, accompanied by phenolic compound extraction, which yielded 172 ± 60 mL CH_4/(g VS d) [6]. Between the first and the second HRT, 34% of methane production decreased in the degradation of SP + DLP (Table 2). During the second HRT, the anaerobic digestion process seems to have

been more stable. However, the production decreased by 35% from 135 ± 18 to 87 ± 24 mL CH_4/(g VS d) in the third HRT. The stability of olive mill solid waste in the anaerobic digestion was maintained at an OLR of approximately 1 g VS/(L d) according to Serrano et.al. [18]. Biodegradability was also reduced in the anaerobic digestion process, reaching only 27%. This effect is also shown in solid concentrations in Table 2. During the third HRT, the TS decreased, but the VS increased by 5%. In similar studies with a strawberry extrudate treated with more severe thermal treatments and using solid and dephenolized liquid phase, the same tendency of decrease in methane production is observed with 1 g VS/(L d) [19].

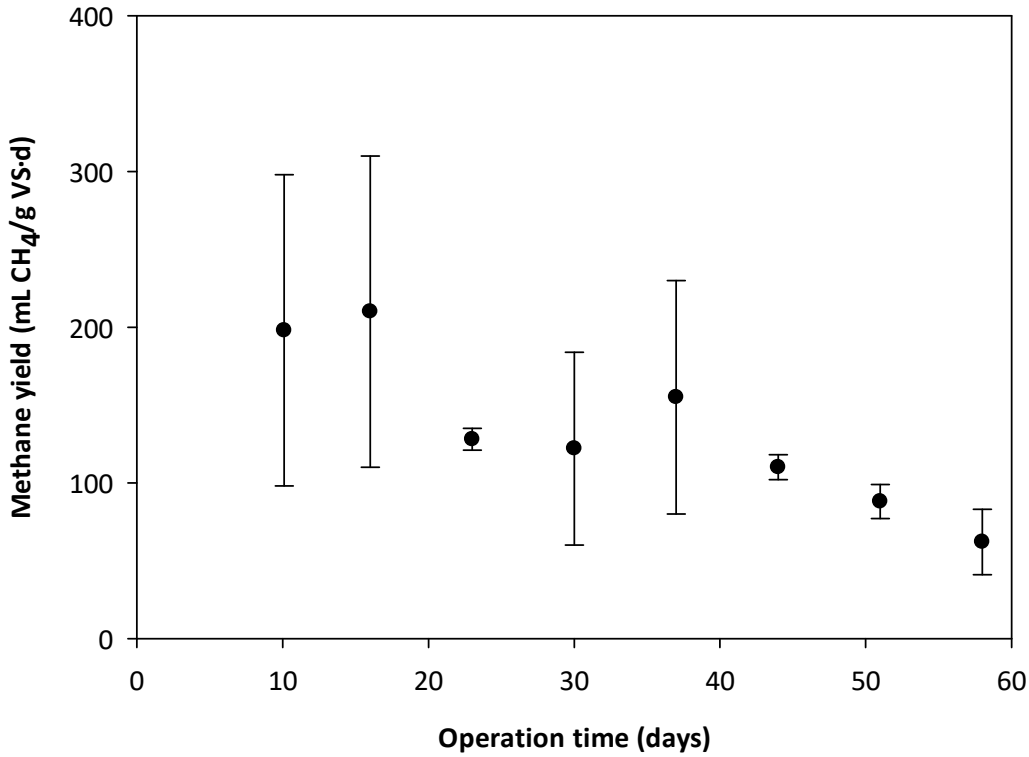

Figure 1. Variation of the methane production yield with their corresponding standard deviations along operation time.

Table 2. pH, alkalinity, TS, VS, sCOD, total VFA, total phenolic compound, methane production yield and biodegradability during the different hydraulic retention times (HRTs).

	1 HRT	2 HRT	3 HRT
OLR (g VS/L d)	1	1	1
Days	0–21	22–42	43–60
pH	7.5 ± 0.1	7.2 ± 0.1	6.8 ± 0.1
Alkalinity (mg $CaCO_3$/L)	7967 ± 2000	4065 ± 747	2270± 709
TS (mg/L)	18,940 ± 2316	14,223 ± 887	12,908 ± 891
VS (mg/L)	9832 ± 628	9823 ± 398	10,389 ± 575
sCOD (mg O_2/L)	3695 ± 1125	1912 ± 540	1968 ± 250
Total VFA (mg O_2/L)	3280 ± 2083	751 ± 500	210± 182
Total phenols (mg gallic acid eq./L)	149 ± 30	243 ± 17	244 ± 8
Methane production yield (mL CH_4/g VS d)	204 ± 9	135 ± 18	87 ± 24
Biodegradability CH_4 (%)	74 ± 40	48 ± 33	27 ± 15

3.2. Control Parameters along Operation Time

pH, alkalinity, sCOD, and VFAs were measured to monitor the anaerobic reactor performance (Figure 2). The high sCOD concentration came mainly from the inoculum and increased in the first days. But during the first HTR, sCOD concentration decreased drastically. Similar to

sCOD, VFAs decreased during the first HRT, giving a relationship of about 90% VFAs/sCOD. The sCOD presented an average value of 1960 ± 250 mg O_2/(L d) along the second and third HRTs. However, the ratio VFA/sCOD decreased from 90 to 10% along the second and third HRTs, when the VFA concentration reached 210 ± 182 mg O_2/(L d) at the end of the third HRT. The pH did not significantly fluctuate along the operation time, i.e., approximately 6.8–7.5, the value recommended for the anaerobic digestion process [20]. But, alkalinity decreased during the operation time, triggering the instability of the process. The alkalinity decreased from 7967 ± 2000 to 2270 ± 709 mg $CaCO_3$/L. Alkalinity around 2000 mg $CaCO_3$/L was similar at 1 g VS/(L d) in similar studies with olive mill solid waste treatment [6,21].

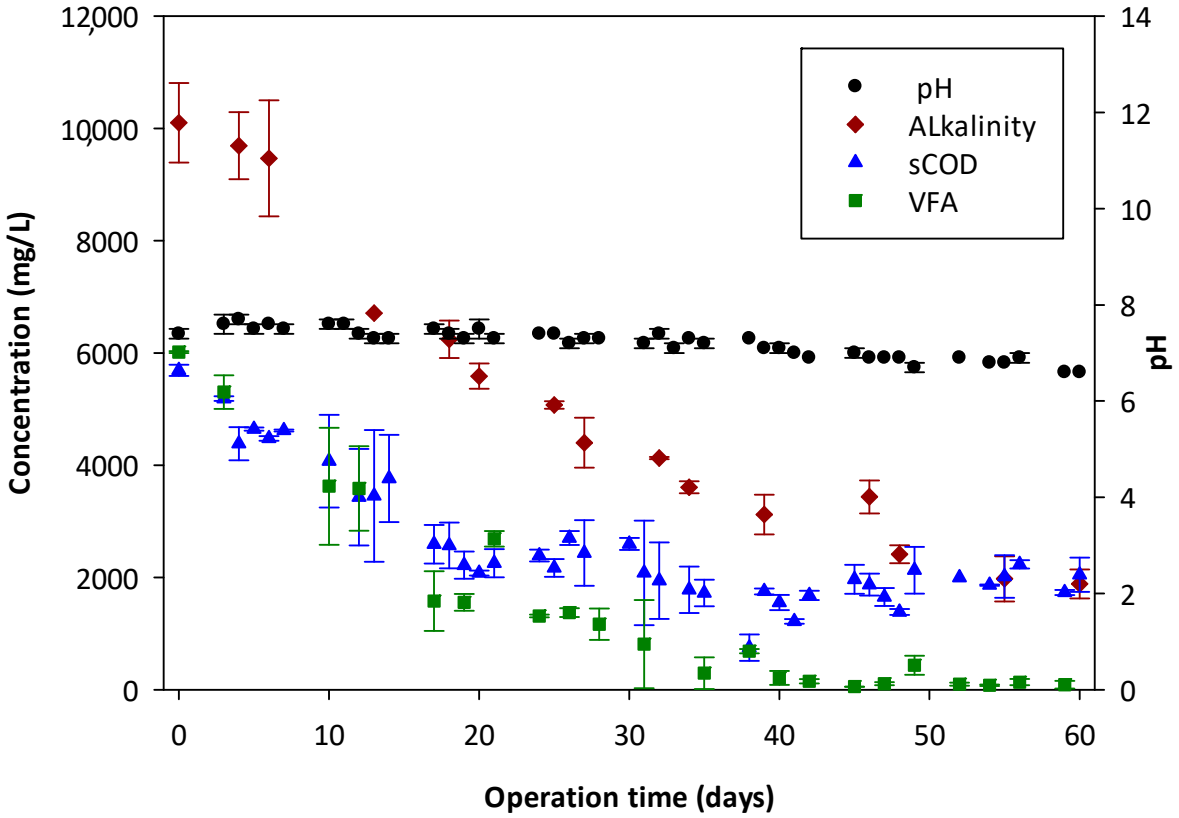

Figure 2. Variation of organic matter measured with sCOD (▲) and VFA (■), and variation of stability measured with pH (●) and Alkalinity (♦) values with their standard deviations along operation time.

Individual VFAs, between C2–C5, were measured along operation time and expressed in mg O_2/L (Figure 3). The inoculum that was used for this test was observed to have a large amount of total VFA with more than 1000 mg O_2/L acetic and propionic acids (Table 1). After two days, a large increase in these acids was observed, reaching more than 6000 mg O_2/L of total VFA (Figure 3). Individual VFAs, mainly Acetic and propionic acids, decreased in the first HRT to less than 2000 and 1000 mg O_2/L, respectively. Individual VFA concentrations were decreased around 704 ± 727 mg O_2/L, propionic acid being the most abundant at the end of third HRT. This parameter is important to evaluate since different studies have reported the inhibitory effect of AGV, such as acetic and propionic acid, with highly varied concentrations [22,23]. High acetic and propionic acid concentrations suggest the instability of the reactor due to the acetogenesis and methanogenesis stages [24].

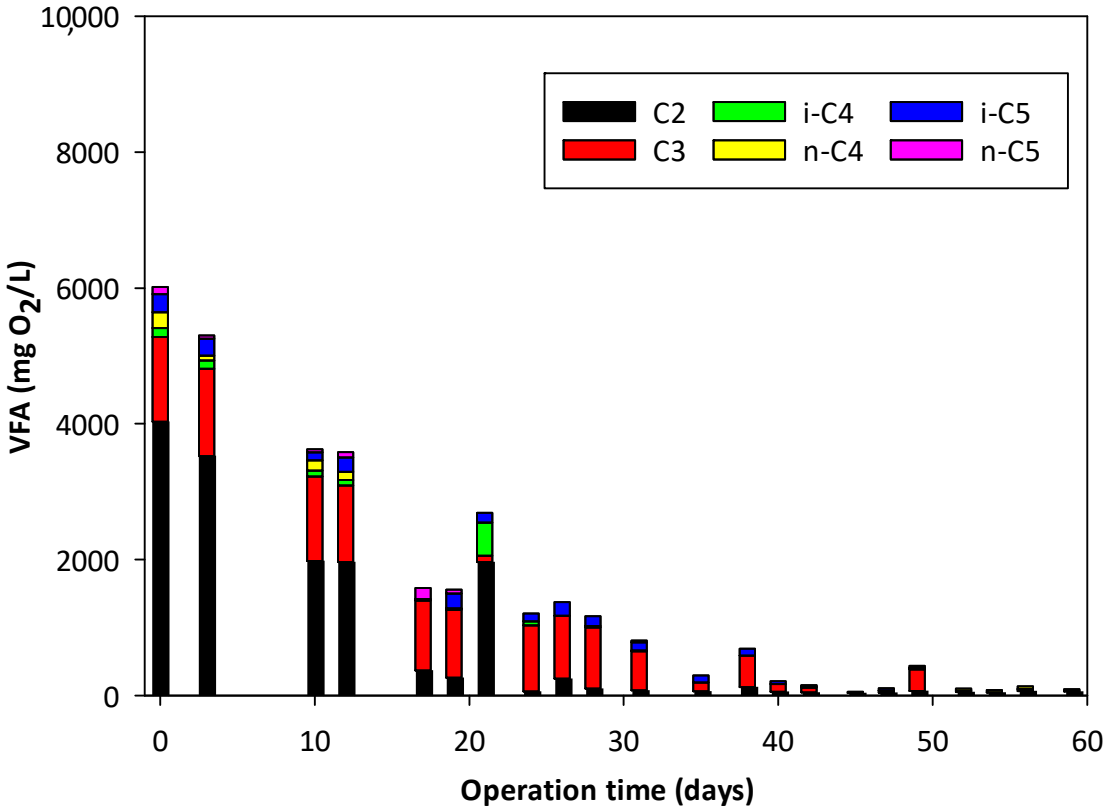

Figure 3. Variation of the individual VFAs values along operation time.

3.3. Variation of the Concentration of Phenolic Compounds along Operation Time

Total and individual phenolic compounds were measured to monitor the anaerobic reactor performance (Figure 4). Total phenolic compound concentration, expressed as mg gallic acid equivalent per litre, increased along the operation time with an average value from 149 ± 30 mg gallic acid/L to 244 ± 8 mg gallic acid/L. According to previous research, phenolic compounds are known inhibitors of the anaerobic digestion process [25]. Microbial growth might be influenced by elevated phenolic compound concentrations [26]. However, the phenolic compound levels observed in these experiments consistently remain below the threshold recognized for inhibitory effects on the anaerobic digestion process, which is typically around 2000 mg/L [27]. Moreover, these phenolic concentrations were even lower than those documented in a study involving olive mill solid waste treated using the identical process as applied in this investigation [21]. Individual phenolic compounds detected with a lower concentration in the reactor were vanillic acid, 4-hydroxybenzoic acid, catechin, and 4-ethylphenol (Table 3). These phenolic compounds were also detected in other studies with the same substrate with other thermal pre-treatment [6,21]. Substrate characterization generally identifies all of these compounds except 4-ethylphenol, an intermediate metabolite indicating incomplete phenolic degradation [6]. Very few simple phenolic compounds remain in the reactors and in very low concentrations. The amount of individual phenolics is far from the amount of total phenolics quantified, which increases with time. This must be due to a polymerization of the phenolics that would remain forming condensed phenols so that although their content is high, their toxicity might be significant. Among simple phenolics, 4-ethylphenol and vanillic acid are the only two that decrease until they disappear, since they are fermentation products. However, catechol and 4-p-hydroxybenzoic acid are intermediate products of the chemical and enzymatic degradation of the main phenolics, and their concentration increases slightly. Since they are compounds with high phytotoxicity, their slight increase could influence the instability of the fermentation [28].

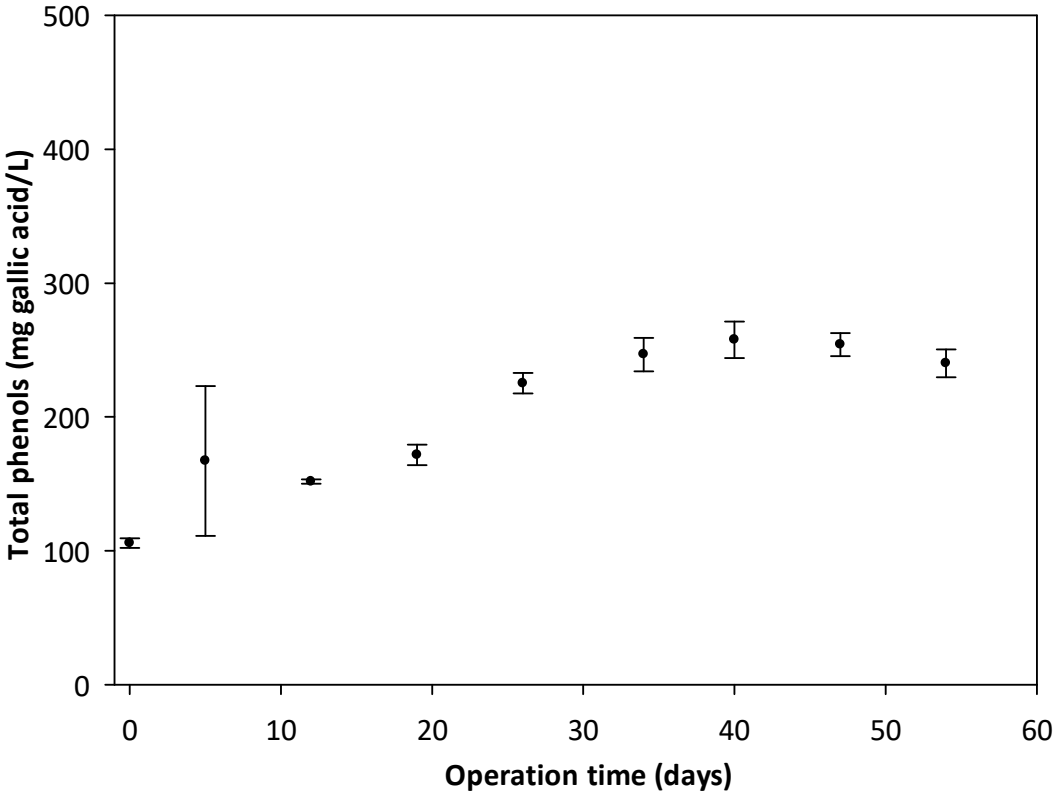

Figure 4. Variation of the total phenolic concentrations with their standard deviation along operation time.

Table 3. Individual phenolic concentration with their standard deviation along operation time.

		Days	12	19	26	34	40	47	54	61
	R.T.	λmax (nm)	Concentration μg/L							
vanillic acid	17.8	254	825 ± 102.5	720 ± 2.5	372.5 ± 12.5	307.5 ± 20	N.D.	N.D.	N.D.	N.D.
4-hydroxybenzoic acid	34.1	254	262.5 ± 10	285 ± 5	260 ± 30	240 ± 15	165 ± 7.5	85 ± 15	395 ± 12.5	285 ± 2.5
catechin	37.6	280	N.D.	2037.5 ± 72.5	2342.5 ±	2832.5 ± 360	2647.5 ± 72.5	4297.5 ± 90	5210 ± 80	4012.5 ± 67.5
4-ethylphenol	49.2	280	traces	traces	traces	traces	traces	traces	traces	traces

R.T., retention time; λmax, maximum wavelength; traces < 0.01 μg/L; N.D., not detected.

3.4. Microbial Population Dynamics along Operation Time

Figure 5 shows the β-diversity and PCoA to determine the general trends of differences and similarities between the biological duplicate of the reactors in inoculum at zero time and the last day of each HRT through cluster analysis. β-Diversity levels include the Weighted Unifrac ASV level, Bray–Curtis ASV level, Weighted Unifrac Genus level, and Bray–Curtis Genus level. Analysing the quantitative data in inoculum at zero time and the last day of each HRT does not show a β-diversity difference between the biological reactor duplicates. During operation time, bacteria analysis showed a difference in β-diversity along each HRT. Also, archaea analysis showed that β-diversity changed slightly in the third HRT (Figure 5).

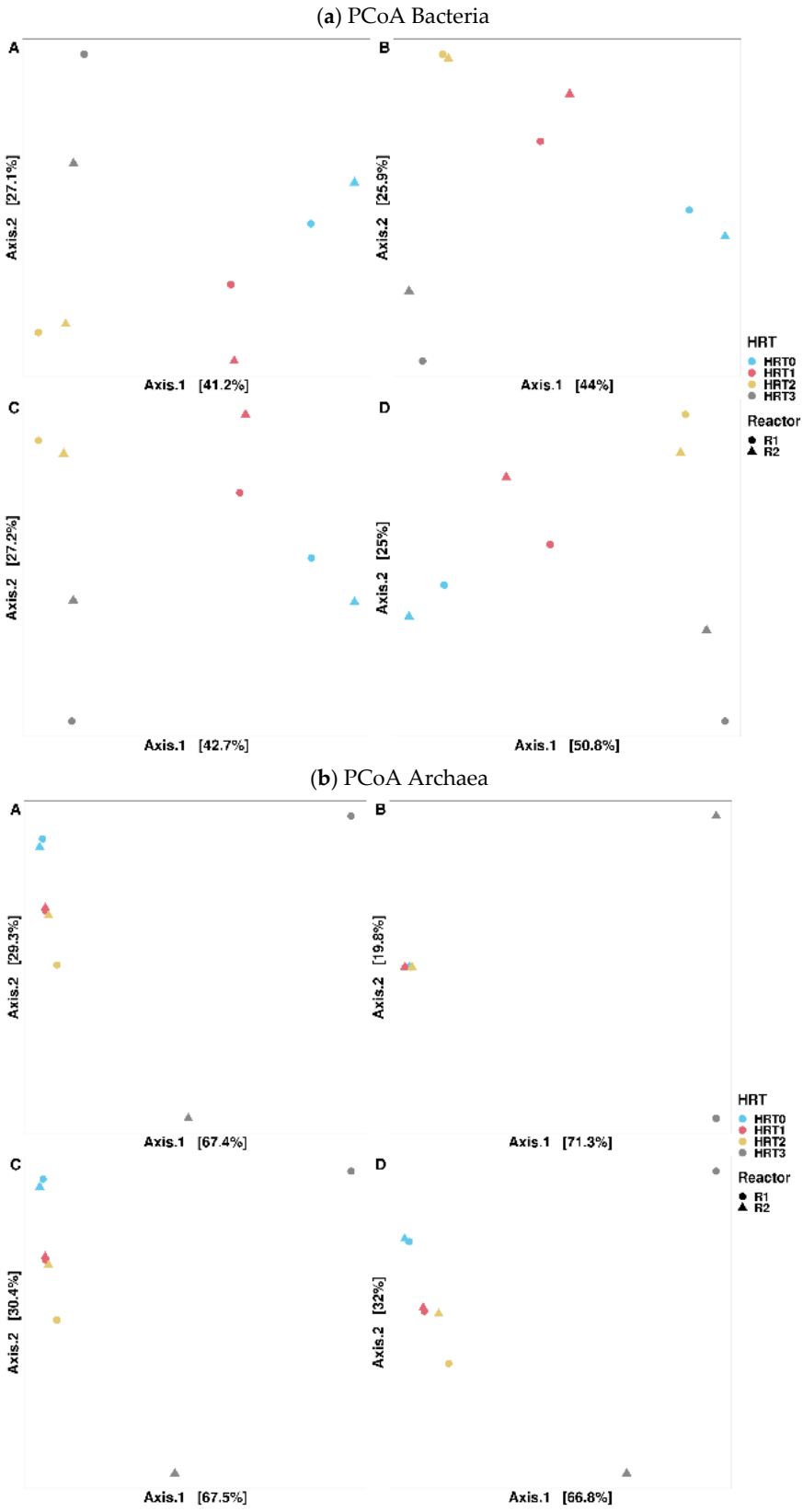

Figure 5. The Beta Diversity PCoA plots for Bacteria (**a**) and Archaea (**b**) are presented, showcasing the inoculum at time zero and the last day of each hydraulic retention time (HRT). These plots are divided into four different levels: A. Weighted Unifrac ASV level. B. Bray–Curtis ASV level. C. Weighted Unifrac Genus level. D. Bray–Curtis Genus level.

The included alpha diversity indices were FaithPD, Shannon, and Simpson, which showed a linear trend (Figure 6). The short measure distance between samples indicates their similarity in the microbial community. Alpha diversity from bacteria has demonstrated a short-range measure, without significant fluctuations, with phylogenetics ranging in FaithPD from 2.0 to 3.6 throughout the digestion progress. Archea fluctuations in FaithPD had slight measure differences, ranging from 0.2 to 0.55. The microorganism of reactors had a similar measure in alpha diversity on the last day of each HRT without having any relevant fluctuations. Regarding the measure ranges in the Shannon and Simpson index, it can be seen that they are similar to other studies on anaerobic digestion [29].

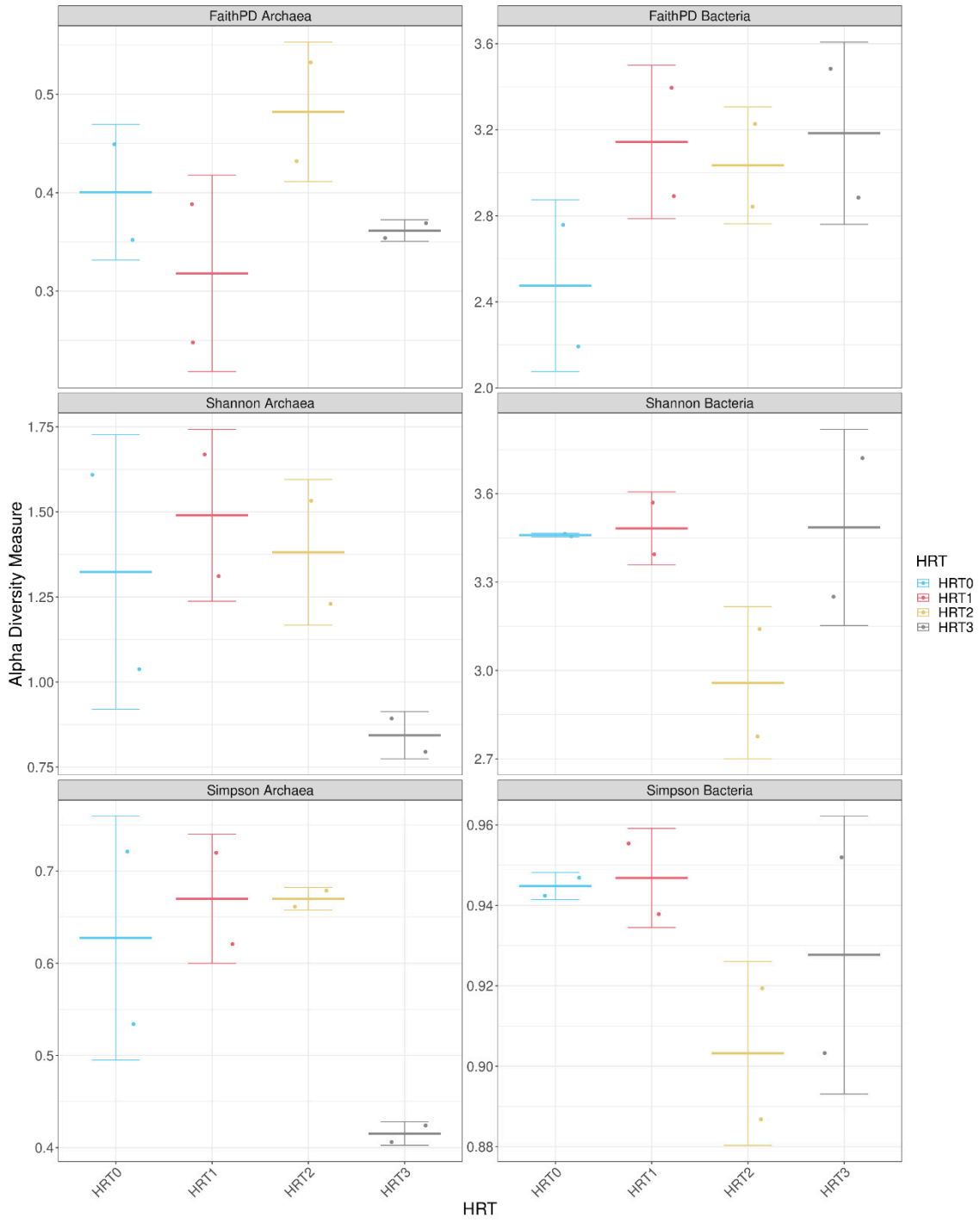

Figure 6. Alpha diversity in inoculum at zero time and the last day of each HRT.

The bacterial microbiome relative composition at the phylum and genus levels is presented in the heatmap abundance (Figure 7). Examination of the bacterial microbiome composition unveiled Bacteroidota as the predominant phylum throughout the experimental period, with various genera undergoing shifts in abundance over time. The *Alistipes* and *dgA-11_gut_group* (family *Rikenellaceae*) genera decreased in relative abundance in favour of genus *Proteiniphilum* (family *Dysgonomonadaceae)* through the experiment, but especially on the last day of the second and third HRT (Figure 7). *Alistipes* has been associated with the production of VFAs and hydrogen through protein and carbohydrate degradation [30]. The decrease in its relative abundance through experimentation would explain imbalances in the AD process, resulting in a reduction of methane production as well as in the accumulation of VFAs [24,31].

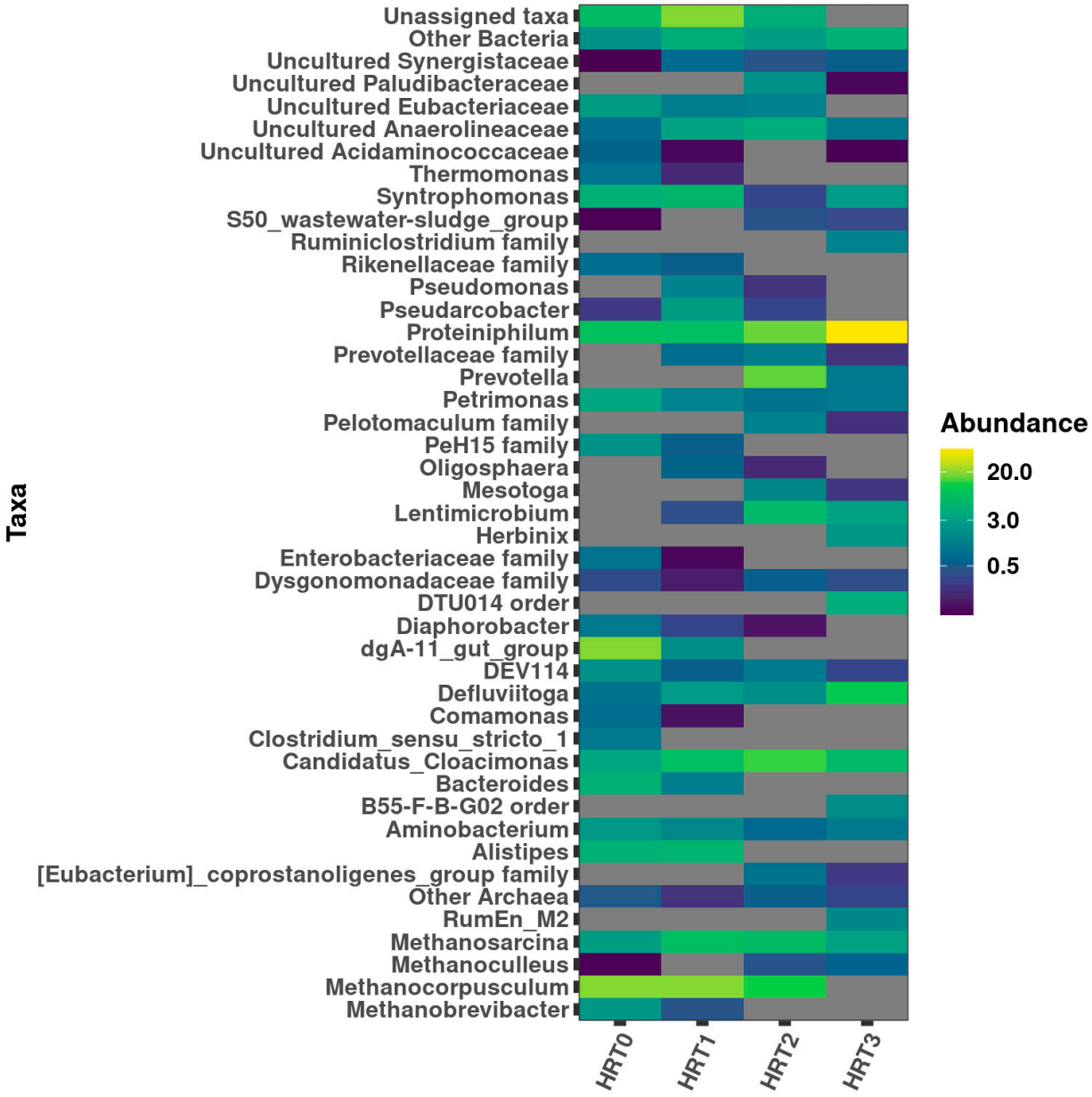

Figure 7. Heatmap abundance samples grouped in inoculum at zero time and the last day of each HRT.

In relation to the archaeal microbial communities, Figure 7 depicts the existence of two primary methanogenic groups crucial for methane production in anaerobic digestion: acetoclastic and hydrogenotrophic methanogens. Acetoclastic methanogens utilize acetate for generating methane and CO_2, whereas hydrogenotrophic methanogens employ H_2 or formate to transform CO_2 into methane [32,33]. The predominant pathway of methane production from in the inoculum at zero time to the last day of the second HRT was the hydrogenotrophic methanogenesis pathway. However, during the experiment, a decline was observed of hydrogenothophic methanogens of the Halobacterota and Euryarchaeota phylums, e.g., the *Methanocorpusculum* and *Methanobrevibacter* genera, until their almost complete disappearance on the last day of the third HRT (Figure 7). The development of an acetoclastic methanogen classified as *Methanosarcina* genus was promoted for all digesters from in the inoculum at zero time to the last day of the second HRT, which would explain the drastic decrease in VFAs at the beginning of the experiment, as well as the stability in methane production (Figures 1–3) [34]. Furthermore, the drastic decrease in methanogens was reflected in a decrease in methane production at the end of the experiment (Figure 7).

4. Conclusions

Olive solid waste thermo-malaxation treated and dephenolizated was evaluated in a semicontinuous anaerobic digestion process. The average value of methane yield decreased from 204 ± 9 mL CH_4/g VS d on the first HRT, to 87 ± 24 mL CH_4/g VS d on the third HRT. Different control parameters were measured to observe the stability of the biodegradation of the complex biomass, with a high phenolic compound concentration, in the anaerobic digestion process. The soluble organic matter, measured by sCOD and VFA, declined over time, showing that the hydrolysis stage was affected. The disappearance of hydrolytic bacteria and increased acetoclastic methanogens confirmed the process's instability.

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