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Understanding the performance of an AnMBR treating urban wastewater and food waste via model simulation and characterization of the microbial population dynamics.

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Abstract

An anaerobic membrane bioreactor (AnMBR) pilot plant treating kitchen food waste (FW) jointly with urban wastewater was run for 536 days. Different operational conditions were tested varying the sludge retention time (SRT), the hydraulic retention time (HRT) and the penetration factor (PF) of food waste disposers. COD removal efficiency exceeded 90% in all tested conditions. The joint treatment resulted in an almost 3-fold increase in methane production (at 70 days of SRT, 24 hours HRT and 80% PF) in comparison with the treatment of urban wastewater only. Mathematical model simulations and Illumina technology were used to obtain in-depth information of this outstanding process performance. Both the PF and SRT factors increased influent biodegradability. The experimental results were accurately reproduced via model simulations modifying only the influent biodegradability. The high SRT and the presence of ground FW in the influent resulted in higher hydrolytic activity. Not only did the *Archaea* population increase 3-fold but *Levilinea* genera was also significantly raised. Three new genera characterised by anaerobic fermentation of amino acids (*Leptolinea*, *Aminomonas* and *Aminobacterium*) were among the ten most abundant of the total sequences identified during the joint treatment, indicating an improvement in the hydrolysis step of anaerobic degradation. Influent biodegradability remained at high values when FW addition stopped.

Keywords AnMBR, food waste, resource recovery, simulation.

INTRODUCTION

Both wastewater (WW) and municipal solid waste (MSW) from household activities are constantly growing due to the ever-expanding worldwide population. Both wastes cause severe environmental

problems, such as contamination of soil, aquatic ecosystems, and air [1, 2]. To protect the environment, stricter regulations have been imposed, such as European Directive 1999/31/CE, which demands a considerable reduction in the organic waste sent to landfills in EU countries, or European Directive 91/271/EEC, which requires specific pollutant concentrations to be met in discharges from urban WWTPs.

Most urban wastewater is now treated via aerobic processes in wastewater treatment plants (WWTP) due to the fact that in most developed countries WW is characterized by low organic matter concentrations [3, 4, 5], which drastically limits the energy recovery potential through anaerobic processes. Aerobic-based wastewater treatment schemes are energy intensive, produce significant quantities of sludge and do not recover the potential resources available in wastewater [6]. In these treatment systems the aeration of the reaction tank for microbial degradation of organic matter consumes a huge amount of energy [7] and can require more than 60% of the total energy consumption of a municipal WWTPs [8]. Moreover, the higher the organic content of the influent, the higher the aeration costs [9]. However, anaerobic treatment schemes can recover energy by converting organic matter into methane-rich biogas, besides having other appealing advantages such as low sludge production, higher pathogen reduction and the possibility of recovering nutrients for reuse in agriculture [10].

The organic matter content of WW is greatly increased by the widespread use of household food waste disposers, as was shown in [11] and [12]. However, to take full advantage of this extra organic matter for energy production, the current aerobic process schemes used in WWTPs should be modified towards more sustainable wastewater treatments such as anaerobic processes (beneficial from an economic, social and environmental points of view). At the present time there is a paradigm shift in which wastewater is no longer considered a waste but a valuable source of raw materials (such as water, energy, nutrients, etc.). This is in line with European Directive

2008/98/CE, which encourages the recovery of resources from household waste and other materials in order to conserve natural resources. The use of anaerobic membrane bioreactors (AnMBR) in WWTPs is thus an evident option. This technology decouples hydraulic retention time (HRT) from sludge retention time (SRT), allowing the application of anaerobic digestion in low strength wastewater treatment, such as typical urban wastewater [13]. Without decoupling SRT from HRT, impractical reactor volumes would be required to operate the biological process at the required SRT to meet the pollutant removal limits (due to the slow growth rate of anaerobic microorganisms). Using membrane technology also provides an almost pathogen-free effluent (of special interest for WW reuse) and high microbial diversity within the biological reactor, since the microorganisms are not lost with the effluent (in contrast to the secondary settler of a conventional WWTP, in which solids are always present in the clarified effluent).

The feasibility of AnMBR technology for the joint treatment of food waste (FW) and urban wastewater has already been demonstrated in [14] and [15]. However, an in-depth insight into the process has not yet been obtained via model simulation, so that few relevant findings on the microbiology of this novel treatment are available. The aim of this work was thus to determine how the different operational conditions can be simulated via a conventional mathematical model in order to compare the simulation results with the experimental data and so determine the effects of the joint treatment of FW and urban wastewater by anaerobic membrane technology.

MATERIALS AND METHODS

Pilot plant

The experimental data given in this paper were collected in the AnMBR pilot plant in the Carraixet WWTP (Alboraya, Valencia-Spain). The influent of the pilot plant comes from the pre-treatment of the full-scale Carraixet WWTP after screening and removal of grit and grease. The process flow

diagram of the pilot plant is shown in Figure 1. The Food Waste (FW) consisted of leftovers from restaurants on the campus of the Universitat Politècnica de València (Valencia, Spain). The substrate was weighed and stored in bags at 4ºC the day before experimental use. A commercial food waste disposer and a 0.5 mm opening size rotofilter were used for the pre-treatment of the FW, which was stored in a co-substrate tank of 0.180 m^3. A three-way valve alternated wastewater and FW inputs to the anaerobic reactor (1.3 m^3). The FW fraction was supplied according to the Penetration Factor (PF, or the percentage of households using food waste disposers) as laid down in the experimental plan. The plant was equipped with two 0.02 um pore size ultrafiltration membranes submerged in separate tanks. A detailed description of the pilot plant, feeding procedure, influent characterization and process results can be seen in Moñino et al. [15, 16].

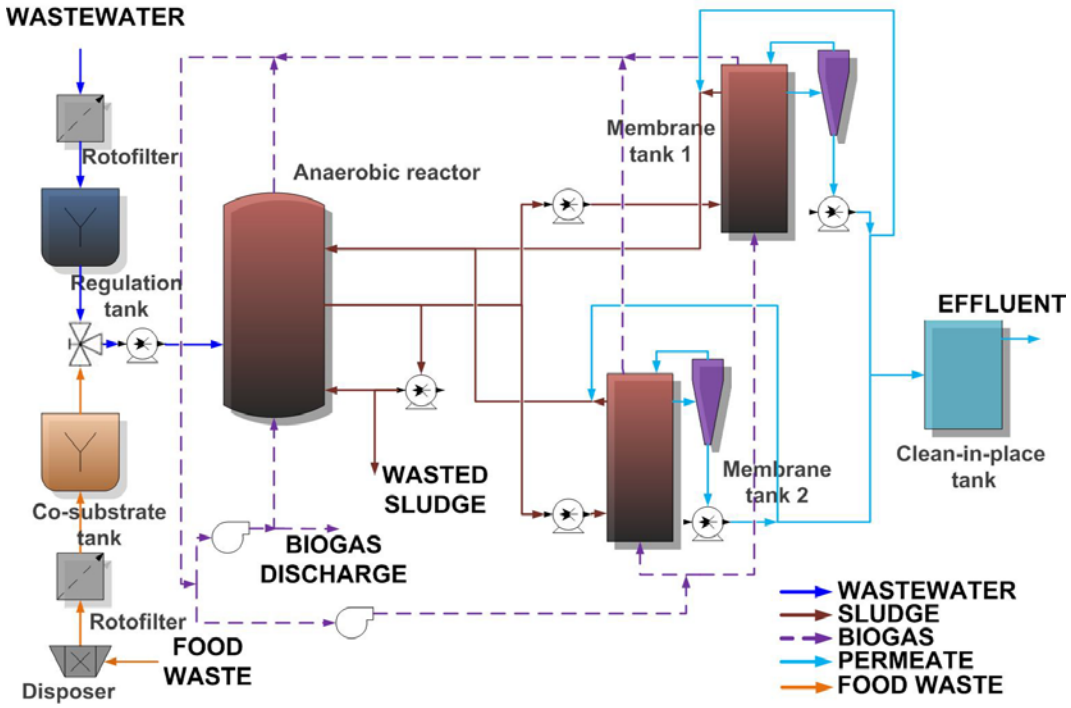


Figure 1. Process flow diagram of the AnMBR pilot plant.

Operating conditions

Six different operational periods were planned, covering a total of 536 days of experiments in the pilot plant. The main operating conditions of each period are shown in Table 1. As can be seen in this table, in four periods (P2 to P5), the AnMBR treated both FW and wastewater at different PF levels (40 and 80%). These two percentages were chosen to consider the effects of different

numbers of households using food waste disposers. In the first and last periods only wastewater was treated. Period 1 was prior to the joint treatment and Period 6 was after FW addition, when a new pseudo steady-state was reached. The data used for the process-modelling was the average process variables from the pseudo steady-state periods. These periods were determined after checking negligible COD accumulation in the COD mass-balance, together with the stabilization of both the reactor solids concentration and methane production.

Table 1. Operational conditions tested in the AnMBR pilot plant.

	P1	**P2**	**P3**	**P4**	**P5**	**P6**
SRT (d)	40±2	41±3	Extended*	70±11	69±6	70±2
T (ºC)	25±2	28±1	28±1	28±1	27±1	28±3
HRT (h)	30±4	18±4	26±3	22±6	24±6	22±4
PF (%)**	0	40±4	40±3	40±4	80±7	0
Duration of the period (d)	31	105	148	125	61	66
Number of days in pseudo steady-state (d)	20	30	25*	40	30	25

* In period 3 (P3), only the sludge required for the daily laboratory analysis was harvested (this period was labelled 'extended SRT'). Operating at this high SRT made it almost impossible to achieve a true pseudo steady-state. For this reason, the last 25 days of the period, in which a relatively stable solid, COD and biogas production was observed, were used to calculate the average process variables for the mathematical model simulation.

** PF is the percentage of households that use food waste disposers.

Analytical Methods

Influent, effluent and AnMBR reactor samples were collected twice a week to monitor the evolution of the biological process. Volatile Solids (VS), COD, sulphide and sulphate concentrations were determined according to Standard Methods [17]. Methane production was recorded and dissolved methane in the effluent was calculated by Henry's Law, as described in [18]. Specific methanogenic activity (SMA) tests were carried out for each period using the Automatic Methane Potential Test System (AMPTS) [Bioprocess Control, Sweden] and performed as described in [19].

Microbial community analysis

Sludge samples were stored at -20℃ and prepared for nucleic acid extraction. For this purpose, the E.Z.N.A Soil DNA Kit (Omega-Biotek) was used following the protocol provided by the manufacturer. The quality and quantity of the nucleic material extracted was determined in a Nanodrop 2000 spectrophotometer (Thermo Scientific) and Qubit 3.0 fluorometer (Life Technologies), respectively. From the extracted DNA samples, 0.2 ng/μL was used for the construction of libraries with universal prokaryotic indexed primers targeting the v4 hyper-variable region of the 16S rDNA [20]. Finally, amplicon sequencing was performed in a MiSeq sequencer within a 2x300 paired-end run by the genomic department of *Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana* (FISABIO) in Valencia (Spain).

The data retrieved from Illumina sequencing was processed for barcode and index removal and then quality trimmed with default parameters of the prinseq-pl algorithm [18]. After joining the filtered reads with fastq-join [19], non-chimeric sequences were phylogenetically classified at genus level within the Ribosomal Database Project Classifier (default parameters). The results were exported to the multimedia and interactive Krona tool [20].

RESULTS AND DISCUSSION

The experimental results from the AnMBR pilot plant (included in Table 2) showed an exceptional increase of methane production, by up to 3-fold, at 70 days of SRT, 24 hours HRT and 80% PF, compared with the treatment of urban WW only (148.7 vs 51.2 L_{CH4}/Kg COD removed). This increase is attributed to the higher biodegradability of the FW (in comparison with urban WW) together with the process operation at high SRT (70 days). The higher the SRT the higher the hydrolysis and degradation of slowly biodegradable organic compounds as a result of the extended contact time between the pollutants and the microorganisms. Both factors also helped to reduce sludge production (from 0.614 kg VS / kg removed COD in P1 to 0.142 in P5), which is currently of special interest due to the stricter environmental constraints on WWTP sludge disposal. The

lowest sludge production (0.015 kg VS / kg removed COD) was observed during AnMBR operation at extended SRT (P3), but under normal operating conditions 0.142 kg VS / kg removed COD could be a representative and achievable reference value. COD and TS legal requirements were achieved in the effluent of the AnMBR plant thanks to the use of the ultrafiltration membranes. COD removal efficiency exceeded 90% in all the tested conditions. The VFA concentration was always lower than the detection limit (10 mg HAc/L), indicating that no process imbalance occurred. Conversely, effluent nutrient concentrations (N and P) exceeded the regulation limits. This effluent could thus be either directly used for agricultural irrigation (note that the ultrafiltration membranes also provide the required disinfection level) or could be given a tertiary treatment to meet the stricter nutrient requirements imposed by the legislation. Indeed, the nutrient concentration in the effluent was higher than in the influent of the AnMBR pilot-plant due to the hydrolysis of the organic matter. In Table 2 it can be seen that N and P effluent concentrations were similar in all the operating conditions with and without FW addition, showing the relatively similar nutrient composition of the FW and WW.

Table 2. Summary of the main experimental results from the AnMBR pilot plant. The data shown are the average values from each pseudo steady-state period.

	P1	**P2**	**P3**	**P4**	**P5**	**P6**
TSS reactor (g/L)	16.58±2	16.25±2	28.94±3	15.48±2	14.41±2	12.83±1
% SSV reactor	69.18±4	68.98±5	68.38±4	70.21±5	69.67±4	69.75±5
Sludge production (kg SV/ Kg COD removed)	0.614	0.316	0.015	0.179	0.142	0.245
Effluent COD (mg/L)	49±3	51.6±4	22.7±2	54.3±5	51.9±5	25.7±3
Effluent AGV (mg HAc/L)	<LD	<LD	<LD	<LD	<LD	<LD
N-NH_4 effluent (mg/L)	49.6 ± 11.1	40.8 ± 5.5	69.6 ± 11.1	69.6 ± 7.7	53.5 ± 4.8	44.5 ± 5.7
P-PO_4 effluent (mg/L)	5.6 ± 0.8	5.0 ± 0.5	7.9 ± 1.3	7.6 ± 1.1	7.9 ± 0.4	5.4 ± 0.8
CH_4 (L/d)	18.2±3	83.7±6	201.7±12	244.1±14	333.7±15	98.8±7
(L/kg COD removed)	51.2±20.4	80.4±22.5	121.1±24.1	114.9±58.5	148.7±57.2	93.9±40.7
SMA (mL CH_4/ g VS d)	10±2	10±2	36±3	49±3	51±4	43±3

*In period 3 (P3), only the sludge required for the daily laboratory analysis was harvested ('extended SRT')

** PF is the percentage of households that use food waste disposers.

<LD Lower than the detection limit (10 mg HAc/L).

Model simulations were performed to reproduce each pseudo steady-state, based on the plant-wide Biological Nutrient Removal Model No. 2 (BNRM2) proposed in [25] and including the sulphate reducing bacteria (SRB) model proposed in [26]. For this purpose the average values were used of all the variables measured (influent, anaerobic reactor and effluent) during each pseudo steady-state. Table 3 gives the main influent variables used for the simulation of each period.

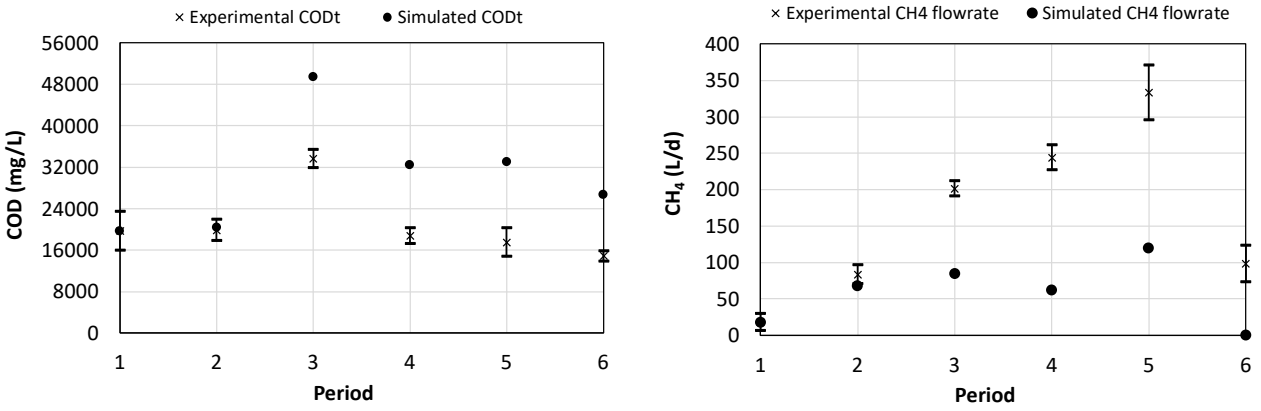
Table 3. Characterization of the influent during each pseudo steady-state period. These influent variable values were used in the model simulations.

Parameter	P1	P2	P3	P4	P5	P6
Total COD (mg/L)	612 ± 64	11512 ± 1023	6283 ± 947	6558 ± 850	11553 ± 1102	606 ± 52
Soluble COD (mg/L)	102 ± 22	3690 ± 339	1619 ± 194	1868 ± 150	3395 ± 353	94 ± 8
Total Nitrogen (mg/L)	58.5 ± 4.5	197.6 ±11	128.0 ± 7	92.0 ±6	198.4 ±13	55.0 ± 4.0
Soluble Nitrogen (mg/L)	43.0 ± 4.3	115.9 ±6	68.0 ± 5	59.0 ±4.2	116.3 ±6	41.0 ± 3
N-NH_4 (mg/L)	35.6 ± 4	28.7 ± 3	49.8 ± 2.7	40.9 ± 2.7	40.8 ± 3	40.0 ± 2.5
P-PO_4 (mg/L)	3.9 ± 0.9	11.9 ± 2.5	10.9 ± 2.8	10.3 ± 3.2	11.0 ± 3.5	3.8 ± 0.6
S-SO_4 (mg/L)	98.0 ± 5.3	331.9 ± 15.4	340.7 ± 15.8	357.6 ± 29.7	373.3 ± 67.6	114.0 ± 9.7
Alkalinity (mg $CaCO_3$ / L)	246 ±32	301 ± 35	307 ± 30	302 ± 27	252 ± 30	300 ±28
VFA(mg HAc/ L)	< LD	612 ± 35	522 ± 30	360 ± 25	722 ± 57	18 ±5

<LD Lower than the detection limit (10 mg HAc/L).

In the first approach to modelling the AnMBR process performance, the default values for all the stoichiometric and kinetic parameters from the BNRM2 model proposed in [25] and for the SRB extension of [26] were used in the simulations of the six pseudo steady-state periods. Figure 2 shows the experimental and simulated total COD in the AnMBR and average methane production. As can be clearly seen in Figure 2, there are considerable discrepancies between the experimental values and the simulated results in Periods 3 to 6, where changes were made to either the influent composition (due to FW addition) or SRT. The mathematical model predicted higher COD accumulation in the anaerobic reactor (percentage error for the simulated data ranged from 46% to

88% in Periods P_3 to P_6) and lower methane production reactor (percentage error for this variable ranged from - 58 % to -100 % in Periods P_3 to P_6).



(a) **(b)**

Figure 2. Experimental versus simulated variables in each pseudo steady-state period prior to model calibration: **(a)** AnMBR Total COD **(b)** Methane production. Note that the 95% interval confidence limits have been included in the experimental values. These confidence limits are calculated as the [average$\pm t_{n-1,\alpha/2}$ (s/n^0.5)], where s is the standard deviation, n the number of values of the variable considered and α the significance level used to compute the confidence level: in this case 0.05.

These discrepancies between the experimental and simulated data suggest a clear difference between the COD biodegradability predicted by the model and the experimental biodegradation observed in the reactor. High biodegradability is directly related to high COD degradation (thus, resulting in low COD accumulation) and high methane production. To address these differences, the anaerobic biodegradability of the influent particulate organic matter was therefore chosen as the calibration parameter. Table 4 shows the initial values of this parameter for each pseudo steady-state (experimentally obtained by means of the classical BOD and COD determinations in the influent, and used in the initial set of simulations) together with the calibrated values obtained via computational analysis to produce the best fit between the experimental data of the process behaviour and the model simulations.

Table 4. Biodegradability of the influent particulate organic matter obtained experimentally and computationally (to get the best fit between the experimental data of the process behaviour and model simulations).

	P1	**P2**	**P3**	**P4**	**P5**	**P6**
SRT (d)	40±2	41±3	Extended*	70±11	69±6	70±2
PF (%)**	0	40±4	40±3	40±4	80±7	0
Initial biodegradability***	44.8	50.1	49.6	49.5	49.2	44.8
Calibrated biodegradability	44.8	52.8	70.8	80.1	86.1	77.5

The biodegradability of the influent particulate organic matter is calculated as Xs/(Xs+Xi), where Xs is the particulate biodegradable organic matter and Xi the particulate inert organic matter.

*In period 3 (P3), only the sludge required for the daily laboratory analysis was harvested ('extended SRT')

** PF is the percentage of households that use food waste disposers.

*** The initial biodegradability was experimentally obtained by means of classical BOD and COD determinations in the influent.

Using the calibrated values of the anaerobic biodegradability of the influent particulate organic matter, a good agreement was seen between the experimentally measured variables of the process and the simulated results (see Table 5). This was the only parameter modified to achieve an accurate fit. It is worth highlighting that the calibrated biodegradability reached values of up to 86%, which is even higher than the experimental anaerobic biodegradability of the FW obtained in [16] (73% (252 ± 11 mL CH_4 / g COD). It therefore seems that the introduction of FW (rich in biodegradable organic matter) and operating at high SRT values result in clearly increased organic matter biodegradation, which could reflect either greater activity of the existing microbial population or a change in the microbial consortia towards a more efficient and hydrolytic species. The fact that this effect remained when FW was no longer added is of special relevance. To get a deeper insight into these microbiological aspects, the microbial population was analysed by Illumina high-throughput sequencing of the 16S rRNA gene.

As pointed out by Kim *et al.* [23], results are required on the characterization of the microbial community structure in a pilot-plant and full-scale anaerobic digestion systems during the treatment of food waste.

Table 5. Experimental from the AnMBR pilot-plant versus Simulated results from the calibrated model.

		Period 1		Period 2		Period 3		Period 4		Period 5		Period 6	
		Exp.	**Sim.**	**Exp.**	**Sim.**	**Exp.**	**Sim.**	**Exp.**	**Sim.**	**Exp.**	**Sim.**	**Exp.**	**Sim.**
AnMBR Effluent	COD_{sol} mgCOD/L	49.0	60.0	51.6	64.7	22.7	34.2	54.3	48.7	51.9	44.6	25.7	24.6
	CH_4 mg/L	46.1	50.2	45.6	58.0	55.5	55.6	54.3	57.8	60.1	56.6	52.9	60.9
	VFA mgCOD/L	0	1.8	2.6	1.7	0	1.2	0	1.3	0	1.4	0	1.3
	$N\text{-}NH_4$ mgN/L	49.6	41.4	40.8	30.3	70.1	56.8	69.1	70.4	53.5	53.6	44.6	47.9
	$P\text{-}PO_4$ mgP/L	5.6	4.4	5.0	5.3	8.2	6.9	7.6	5.6	7.9	8.7	5.4	4.5
	$S\text{-}SO_4$ mgS/L	6.7	3.3	8.4	3.0	6.5	0	11.4	0	11.5	0.0	9.3	0.8
	$S\text{-}HS^-$ mgS/L	93.2	96.4	89.2	90.1	95.0	118.4	97.0	124.2	98.8	123.1	92.6	114.7
Mixed Liquor AnMBR	COD_{total} mgCOD/L	19730	19629.4	19903	19690.2	33650	33463.7	18798	18376.3	17557	17730.4	14880	14962.8
	TSS mg/L	16581	16530.3	16254	16398.5	28943.1	28923.5	15483.7	15516.8	14417	14541.7	12831	12868.9
	VSS mg/L	11476	11417.4	11215	11346	19788	19819	10873	10892	10048	10142.4	8956	8989.1
	NVSS mg/L	5105	5112.9	5039	5052.5	9155.1	9104.5	4610.7	4624.8	4369	4399.3	3875	3879.8
	pH	6.6	6.8	6.9	6.8	6.7	6.7	6.6	6.8	6.6	6.7	6.8	6.8
Gas	CH_4 Flowrate L/d	18.2	18.2	83.7	83.0	201.7	196.2	244.1	243.8	333.7	327.7	98.8	95.6
	Sludge production (Kg SV/KgCOD)	0.614	0.598	0.316	0.347	0.015	0.017	0.179	0.184	0.142	0.155	0.245	0.217

The microbial population in the AnMBR was found to be highly diverse in terms of the different Bacteria and Archaea genera detected. Figure 3 gives the composition at phylum taxonomic level of the communities found in all periods in the form of krona graphs. Figure 4 contains a heatmap quantifying the changes in microbial communities between each two consecutive periods (i.e., from Period 1 to Period 2, from Period 2 to Period 3, and so on). The heatmap is a plot of a data matrix in which the individual values are represented as colours and was used to visually highlight the relevant changes in the microbial genera (increases and decreases) through the color gradient, as well as those genera that do not change appreciably. The main change in the microbial population was detected in the relative abundance of the *Anaerolineaceae* family, belonging to *Chloroflexi* phylum. Four representative genera from this family were found among the most abundant in all the periods: *Levilinea, Bellilinea, Longilinea* and *Leptolinea, Levilinea* being dominant (Table 6 and Figure 4). Another phylum whose relative abundance increased in Periods 4 and 5 was

Synergistetes. The change found inside this phylum was related to the higher relative abundance of *Aminomonas* and *Aminobacterium* in these periods. The relative abundance of these three genera increased as the hydrolytic capacity of the system was enhanced, thus denoting the positive effect of operating at high SRT for FW degradation in the AnMBR. Finally, as can be seen in Table 6, there was a remarkable relative abundance of *Methanosaeta* in Periods 4 and 5.

Table 6. Relative abundances of the dominant genera detected in the AnMBR microbial community

Dominant genera	P1	P2	P3	P4	P5	P6
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales; Anaerolineaceae;Levilinea	16.2	20.0	27.2	25.8	25.7	20.4
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales; Anaerolineaceae;Bellilinea	2.8	1.8	3.4	6.2	6.0	4.5
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales; Anaerolineaceae;Longilinea	2.1	0.8	0.9	4.6	4.2	3.4
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales; Anaerolineaceae;Leptolinea	1.5	0.4	0.5	2.6	2.7	2.2
Bacteria;Synergistetes;Synergistia;Synergistales; Synergistaceae;Aminomonas	1.6	2.3	3.4	5.5	7.2	4.2
Bacteria;Synergistetes;Synergistia;Synergistales; Synergistaceae;Aminobacterium	1.5	n.d.	n.d.	2.3	2.7	1.7
Archaea;Euryarchaeota;Methanomicrobia;Methanosarcinales ;Methanosaetaceae;Methanosaeta	0.2	1.1	0.7	1.9	2.7	2.5

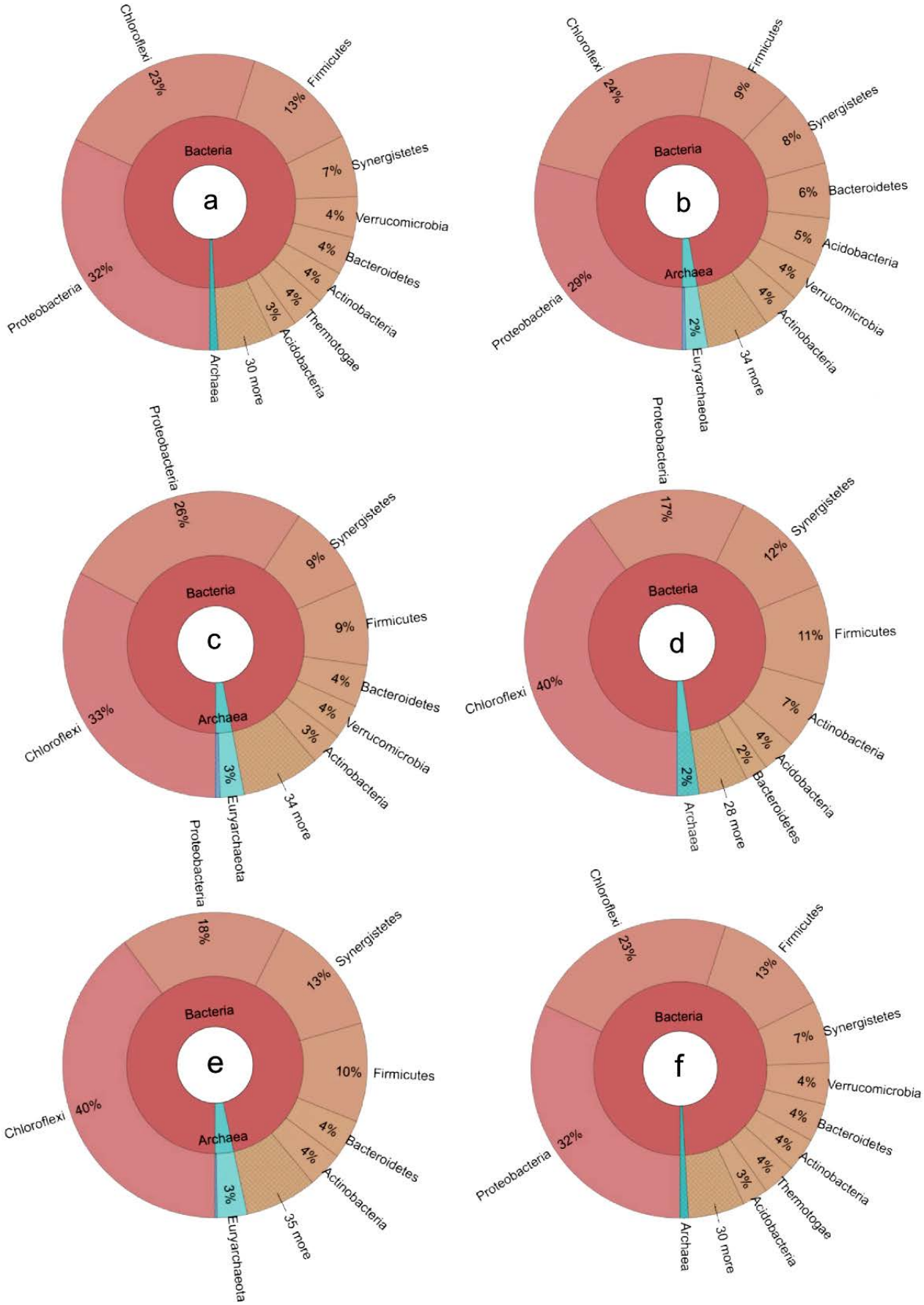


Figure 3. Composition of the microbial communities in Periods 1 (a), 2 (b), 3 (c), 4 (d), 5 (e) and 6 (f) at phylum taxonomic level. A digital version of these krona plots can be found in the online version of this manuscript.

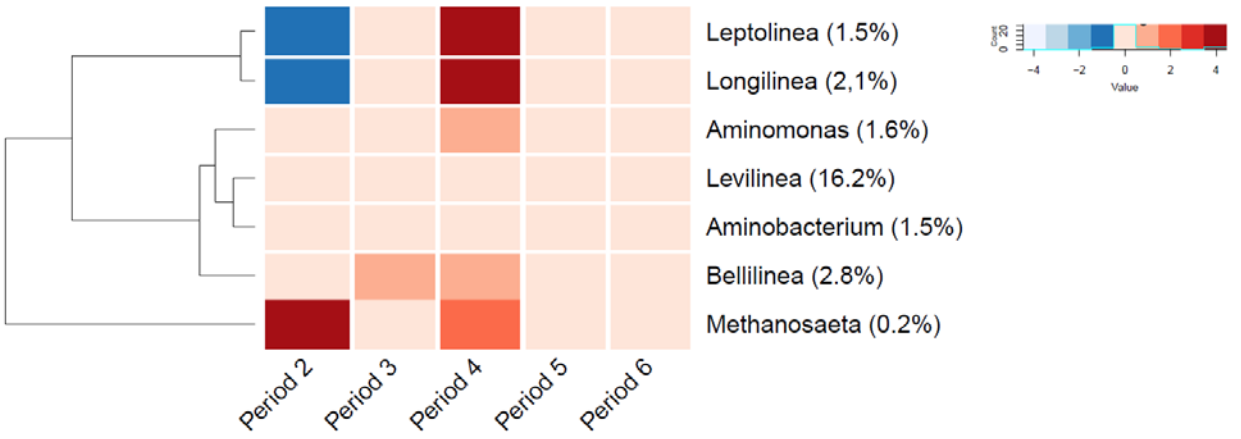


Figure 4. Heatmap quantifying the changes in microbial communities from one period to another. Numbers in parenthesis indicate the relative abundance of the genus in Period 1.

The significant change in the performance of the AnMBR previously described in terms of methane production and influent biodegradability was also observed in the AnMBR microbial community, which shifted towards a more hydrolytic population. This was mainly composed of six genera which are considered the FW-degrading core of the system. The fold changes detected for each of these six genera besides the main methanogen (*Methanosaeta*) are shown in the heatmap plot (Figure 4). Enchained metabolic reactions take place in anaerobic digestion processes and are overdriven by different microorganisms. In the present study, the *Chloroflexi* phylum could have been responsible for the improvement of the hydrolysis and fermentative stages, as no solids were accumulated in the system, but were converted into methane enriched biogas at high rates. As reported by other authors, *Anaerolineaceae* members belonging to the *Chloroflexi* phylum, such as *Levilinea, Bellilinea, Longilinea* and *Leptolinea* have high diverse metabolism capacities but have been poorly described until now. Their crucial role in the degradation of complex polysaccharides has recently been reported by culture-independent analysis [27]. After the hydrolysis of FW from the AnMBR influent, different fermentative genera belonging to the *Anaerolineaceae* family therefore facilitated the conversion of more simplex organic compounds into lactate, hydrogen and mainly acetate. Also, the degradation of the protein content present in this influent was mainly attributed to the *Synergistetes* phylum, as several amino acid fermenters have been described and

affiliated with this taxon [28]. This assumed metabolic potential has also been suggested in other studies that analyzed the microbial diversity in mesophilic anaerobic digesters [29, 30]. Finally, the well described acetoclastic methanogenic capacity of the *Methanosaeta* genus could be attributed to the high SMA values determined in Periods 4 and 5. The higher relative abundance and presumably higher activity and abundance of the fermentative microorganisms found in the digester explain the higher SMA values registered. The rapid conversion of organic compounds from FW into acetate compounds therefore boosted the metabolism of the dominant methanogen found in the system *i.e.*, *Methanosaeta*. The specific methodof this microorganism of breaking acetate up into methane leads to high values of methane production, thus having a positive and highlighting effect over the previously mentioned, which were five times the SMA values. Furthermore, as acetate was expeditiously converted into methane, no VFA accumulation was observed during the whole experimental period (Table 2).

The dominant composition of the microbial community established in the AnMBR between Periods 4 and 5 was found to have a remarkable potential for the biomethanization of the organic matter in the influent. This community was not only observed in the periods in which FW was added to the influent, but was also detected in Period 6, when FW was no longer being added (Table 6 and Figure 4). These results therefore indicate the acclimatization of the biomass to influent during long-term operations and the consequent change in composition and microbial activity from the starting community characterized in Period 1. Thanks to the persistence of the genera that boosted the conversion of organic matter into methane during Periods 4 and 5, the biodegradability values remained over 75% at the end of the experimental period. The role of this microbial-degrading community as hydrolytic microorganisms had a positive effect on the AnMBR performance.

CONCLUSIONS

In this work, the simulation results perfectly matched the experimental data from an AnMBR pilot-plant scale and modified only the anaerobic biodegradability of the influent particulate organic matter. An increase in influent biodegradability occurred when either the FW load or the SRT was raised. This result can be attributed to higher hydrolytic activity within the AnMBR, which was confirmed experimentally via Illumina technology. Adding FW and increasing SRT led to changes in the microbial population within the AnMBR. Hydrolytic and fermentative bacteria were favoured and their activity gave rise to a considerable increase in biodegradation of influent organic matter. The evidence for this is the higher methane production (from 51.2 to 148.7 L_{CH4}/kg COD removed), higher SMA (from 10 to 51 mL CH_4 / g VS day) and lower sludge production (from 0.614 to 0.142 kg VSS / kg COD removed). The FW-degrading microbial population (dominated by *Anaerolineaceae*, *Synergistaceae* and *Methanosarcinaceae*) was not only established during the joint treatment period but also remained in the AnMBR when it again treated wastewater only. Process stability was observed under all the tested experimental conditions, with negligible VFA concentration in the anaerobic reactor and no signals of any type of inhibition.

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