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Semi-continuous anaerobic digestion of the organic fraction of municipal solid waste: digester performance and microbial population dynamics

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ABSTRACT

Anaerobic digestion is an attractive approach for the management of organic fraction of municipal solid waste (OFMSW) and for the recovery of energy from this waste. A semi-continuous digestion of OFMSW was conducted on stirred reactor under mesophilic condition. Three organic loading rates (OLRs) were tested throughout the anaerobic process (1.36, 2.5 and 3.5 g VS/L.d). The achieved results showed that biogas yield decreased with the increase of OLR. Thus, the highest average of biogas yield, 0.51 L/g VS, was recorded at low OLR (OLR_1:1.36 gVS/L.d). Likewise, optimal VS removal, pH, VFA and alkalinity were obtained at OLR_1 compared to the other tested OLR. During the anaerobic digestion, the dynamic of microbial community was also assessed by the high-throughput sequencing technology. *Firmicutes, Bacteroidetes* and *Proteobacteria* were the dominant phyla during the digestion process. This could be related to their roles in hydrolysis of different OFMSW compounds, as well as in acidogenesis and acetogenesis steps. Regarding Archaeal population, the relative abundance of the *Methanoculleus* genus declined, while *Methanosarcina* increased over time. This indicated that acetotrophic and hydrogenotrophic pathways were used for methane production.

1. Introduction

A huge amount of municipal solid waste (MSW) are yielded throughout the world [1]. In Tunisia, the amount of generated MSW was estimated at around 2.4 million tones/year. It increased annually at rate of 2.5% [2]. This increment could be attributed to the increase of urbanization, economic development and population growth. MSW caused a huge problem of solid waste disposal, especially in the developing countries [3]. Generally, to get rid of the most generated MSW in these countries, the authorities resort to the traditional disposal methods such as landfilling, open dumping and open burning [4]. However, these methods are not appropriate because they could lead to a lack of suitable land for landfilling and the emission of pollutants and greenhouse gases. In Tunisia, the organic fraction of municipal solid waste (OFMSW) corresponds to 40–68% of the total amount of waste [2]. The biological processes are attractive alternative approaches to the traditional ones for the OFMSW management [5]. Among these methods, anaerobic digestion (AD) becomes the most interest for waste management throughout the world [6,7]. AD consists of four steps; which are hydrolysis, acidogenesis, acetogenesis and methanogenesis, wherein the organic matter is converted in the absence of oxygen into methane and carbon dioxide [8,9]. Therefore, it results in renewable energy and digestate generation, which could be used as biofertilizer [10]. Although AD process of OFMSW has many advantages, it suffers from some flaws such as low methane production and process instability. The substrate quality as well as the operating parameters such as content of feed waste, the solid retention time (SRT), pH and temperature are among the parameters that could affect the AD process. Thus, the co-digestion of two or more substrates and the substrate pretreatment are recommended to enhance methane yield and process stability [11]. The OFMSW mechanical treatment was among these pretreatment processes which contribute to the rise of the surface area (specific), to a better contact between substrate and inoculum [12] and thus to overcome the rate-limiting step of anaerobic digestion which is the hydrolysis of solid waste [13].

Regarding digester configuration, a two-phase approach is more advantageous for some reasons such as kinetic and operational benefits [14]. However, a single-phase digestion system presents a low cost operation.

During the AD process, the micro-organisms activity is possible at psychrophilic (10–20 °C), mesophilic (30–40 °C), and thermophilic (50–60 °C) conditions. However, the mesophilic condition is the most

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commonly used for operating AD since it is a more stable condition and requires less energy than the thermophilic condition [15]. The mesophilic micro-organisms are more robust and less sensitive to changes in the environmental parameters compared to the thermophilic consortium.

In AD, the microbial communities are strongly influenced by changes in the operating conditions and substrate quality [16,17]; the imbalance of these communities could decrease the reactor performance [18,19]. Therefore, the analysis of the microbial communities' composition as well as their behavior are essential to optimize the digestion process [20].

Some molecular techniques such as the single-strand conformation polymorphism (SSCP), the denaturing gradient gel electrophoresis (DGGE) and the quantitative real-time Polymerase Chain Reaction (qPCR) are used to study the microbial communities structure and dynamic [21]. These, however, suffer from some restrictions to cover the global taxonomic distribution. Recently, the use of metagenomics techniques allowed the discovery of a wide range of new genes and, consequently, the genome sequence of various uncultured bacteria was enabled [22].

In this context, the purpose of the present work was to assess the start-up reactor stability and process performance of conventional single-phase reactor fed with OFMSW collected from Tunisian transfer site. The importance of microbial populations during perturbations of anaerobic digester has long being acknowledged using traditional cultivation methods [23]. However, the literature regarding the dynamics of microbial populations of single-phase reactor treating OFMSW using culture-independent molecular techniques remains scarce. Therefore, the second purpose of this study was to assess the bacterial and archaeal communities' structures and dynamics through high-throughput sequencing technology for the three applied OLR.

2. Materials and methods

2.1. Feedstock and seeding source

The municipal solid waste (MSW) was collected from the transfer site of Sidi Mansour (Sfax, Tunisia). The content of each fraction was determined according to The MODECOM method as described by Chantou et al. [24]. The OFMSW samples were manually sorted from the collected MSW. It contained fruit and vegetable waste, food waste and garden waste. All the samples were shredded with a meat mincer till a particle size of 4–7 mm before to their characterization. The homogenized OFMSW was distributed in several sealed bags and then stored at − 20 °C. Before 24 h of use, each bag was thawed and stored at 4 °C.

The mesophilic anaerobic inoculum used for anaerobic digestion

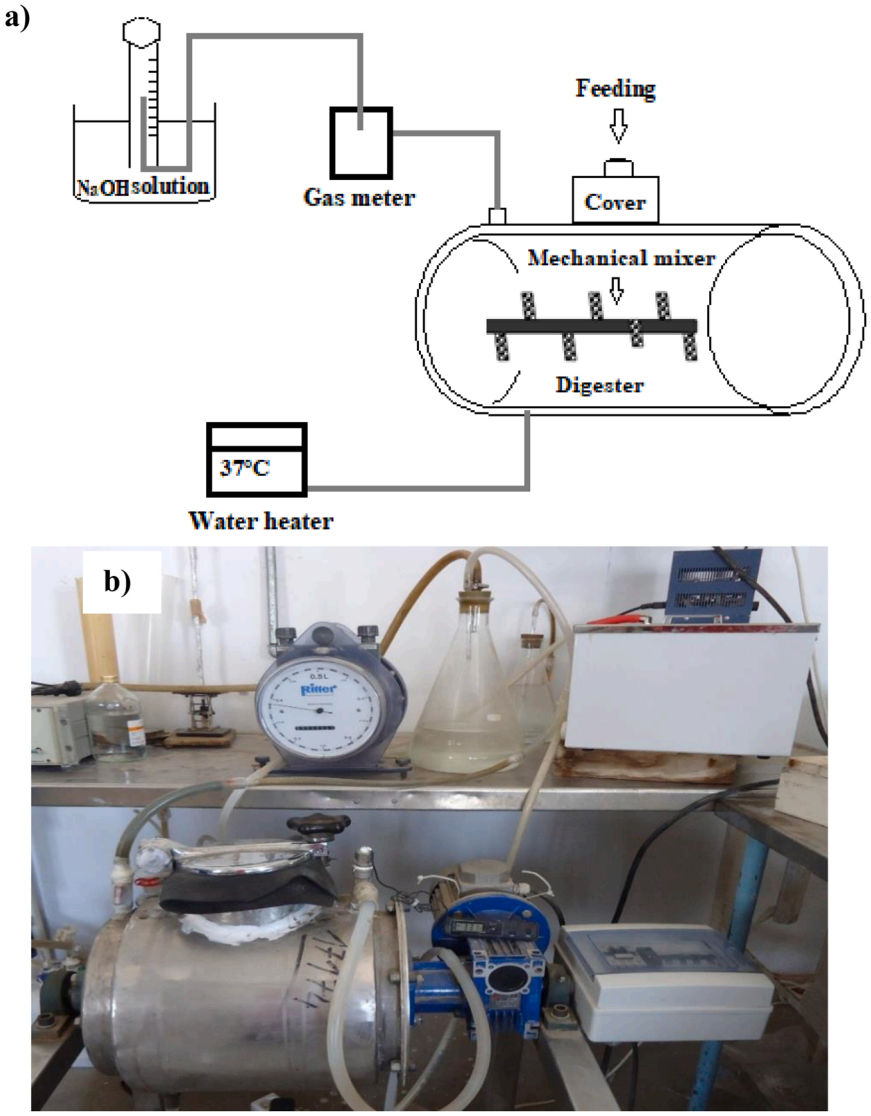

Fig. 1. Anaerobic digestion system of OFMSW (a) Schematic presentation (b) photograph.

experiments was obtained from an anaerobic digester installed in municipal wastewater treatment plant located in the North of Tunisia (Chotrana). This inoculum was firstly fed into the reactor until no biogas was released.

2.2. Experimental equipment and reactor operations

A lab-scale semi-continuously stirred tank reactor (SCSTR) was used during the anaerobic process. The system consists of 15 L (10 L useful volume) stainless steel reactor fitted with devices for mixing and heating. The homogenization of the reactor was performed mechanically with a paddle-shaped stirrer, which was programmed to rotate for 5 min every 60 min. To keep the temperature constant at 37 °C ± 1 °C, the reactor was connected to water bath. Fig. 1 shows a scheme of the process. The feeding and the drainage of digester were achieved through an opening at its top part. The biogas production was measured daily with a gas counter (Ritter Model TG 05).

The experiment was divided in two periods: start-up and operation. During the start-up phase, the system was fed discontinuously with OFMSW by increasing S/I ratio from 0.5 to 2 in order to adapt inoculum to the waste. Regarding the operation period, the reactor was fed manually with OFMSW once a day for 5 days a week.

The produced methane was measured by volume of displaced NaOH solution [11]. it was only determined at OLR_2 and OLR_3, since we encountered a technical problem of Gas Chromatography equipment before this period. The analytical parameters of the feedstock and digestate were determined once a week (VS, pH, VFA and alkalinity).

During anaerobic process, the period of operational problems (agitation, temperature drop, etc.) were not considered in the calculations.

2.3. Analytical methods

Analyses of total solids (TS), and volatile solids (VS) were determined according to standards methods [25], total and soluble chemical oxygen demand (CODs) as described by Knechtel [26]. Total Kjeldahl nitrogen (TKN) and ammonium nitrogen (N-NH_4) were analysed using Kjeldahl-N method.

Total proteins were determined via multiplying organic nitrogen by 6.25. The volatile fatty acids (VFAs) quantification was performed with the high Performance Liquid Chromatography (HPLC) as described by Mlaik et al. [27]. Total alkalinity was measured by adjusting the pH of solution to 4.0 using the titration technique [28]. The flame atomic absorption spectrometry was used to determine the heavy-metal and cations concentrations as reported by Jemli et al. [29].

2.4. Microbial analysis

2.4.1. DNA extraction

Total DNA was extracted from digestate samples (T_i: 0 day, ORL_1: 40th day, ORL_2: 97th day and ORL_3: 130th day) of the anaerobic digester fed with OFMSW using UltraClean Soil DNA Kit (Mo BIO) following the manufacturer's recommendations. The Nano Drop spectrophotometer (2000;Thermo Scientific) was used to purity and the amount of extracted DNA.

2.4.2. 16S rRNA sequencing analysis and data processing

Bacterial and archaeal 16S rRNA genes hypervariable V3-V4 regions were amplified by PCR using prokaryotic universal (Pro341/Pro805R) and specific archaeal (Marc344F/March806R) primers sets [30]. The sequencing of PCR amplicons was performed with Illumina MiSeq (CBS, Sfax-Tunisia) as described in a previous study [31]. Raw data obtained from the Illumina MiSeq sequencing platform were analyzed using the bioinformatics pipeline QIIME software package 1.9 as reported by Caporaso et al. [32]. The Venn diagram was obtained using the Venn Diagram Plotter Program (http://omics.pnl.gov/software/VennDiagramPlotter.php). The α-diversity (Chao estimator, Shannon and Simpson index) was determined using the QIIME software.

2.4.3. Nucleotide sequence accession numbers

The sequences determined in this study were deposited in the GenBank database under accession numbers from MZ145124 to MZ145154 and from MZ145155 to MZ145164 for the bacterial and archaeal 16S rRNA gene reference sequences of OTUs, respectively.

3. Results and discussion

3.1. Substrate characterization

Municipal solid waste (MSW) consists of 46% organic, 19.5% paper, 13.5% plastics, 3.5% glass, 7.5% metals and 10% other types of waste. These results are in line with those of Hoornweg and Perinaz [33], who reported the same percentage of organic fraction. However, this composition was different from that described in a previous Tunisian report [2]. This difference could be attributed to many factors such as the MSW origin and the season during which it was collected [24]. The putrescible fraction represented the highest percentage of MSW [34], and consequently, its physico-chemical characterization was essential. As shown in Table 1, OFMSW exhibited an acid pH of 4.7, owing to considerable amounts of the acid components such as the generation of VFA during storage. The OFMSW is also characterized by a high ratio of VS/TS (0.93), suggesting that most of the components of this substrate were biodegradable. Its organic matter was mainly composed of carbohydrates, proteins and lipids, which contribute to the potential energy of the OFMSW [35]. Besides, the OFMSW was remarked to involve a high moisture content (73.02%), which is a crucial factor for anaerobic biomass growth, metabolic activity and thus for biogas release [36]. The total nitrogen concentration of OFMSW was low, that could have a negative impact on the anaerobic digestion performance [37].

The trace metals play essential roles in maintaining the metabolic osmotic pressure and are the main component of cofactors and enzymes [38]. The OFMSW contained cations such as Mg^{2+} and Ca^{2+} at concentrations of 154 and 194 mg/kg, respectively. In addition, the presence of Fe^{2+} and Zn^{2+} could be explained by the leaching of metal products such as packing box, tableware and some electric appliances.

Some studies reported that there is a difference in the composition of OFMSW in developing countries, which could be linked to the different lifestyles, climates and socio-economic characteristics of their populations [39–41]. Nevertheless, there is some similarity between the OFMSW characteristics in these countries and those of the developed ones, namely the high organic matter and moisture content [41,42].

3.2. Start-up phase

During the startup phase, the S/I ratios effect on biogas yield was investigated. The results showed that the biogas yield decreased while

Table 1
Physico-chemical characteristics of raw OFMSW.

Parameters	Content
pH	4.7 ± 0.20
TS (g/kg)	269.75 ± 8.55
VS (g/kg)	253.31 ± 5.71
Ash (g/kg)	16.44 ± 2.84
Moisture (%)	73.02 ± 0.85
TKN (g/kg)	6.49 ± 0.13
Carbohydrates (g/kg)	10.53 ± 0.27
Proteins (g/kg)	40.61 ± 0.72
Zn^{2+} (mg/kg)	0.35 ± 0.03
Fe^{2+} (mg/kg)	6.04 ± 0.13
Ca^{2+} (mg/kg)	194.01 ± 9.21
Mg^{2+} (mg/kg)	153.79 ± 4.56

increasing the S/I ratio from 0.5 to 2. In fact, biogas yields of 820 and 580 mL/g VS were achieved for S/I 0.5 and 2, respectively (Fig. 2). Similar results were reported by Mlaik et al. [27] during batch anaerobic digestion of OFMSW shredded at different particle sizes. Li et al. [43] found that for mono-digestion of kitchen waste, the methane yield was better at S/I of 1.5 than that of 3. On the other hand, the curve of S/I 2 displayed a different behavior compared to those of 0.5, 1 and 1.5. Therefore, the exponential phase of OFMSW at S/I of 2 was very slow compared to the other S/I ratios. Table 2 summarizes the physico-chemical characteristics of digestate after each applied S/I ratio batches during start-up phase. The pH values ranged between 7.7 and 7.9 for all the tested S/I ratios, which is an optimum range for methanogens growth [44]. This clearly indicates the pH positive effect on biogas yield, which is in line with many studies that reported on the relationship between biogas production and pH [45,46]. Regarding the VFA, its concentrations were between 0 and 0.3 g/L for all tested S/I ratios, which means they were far from methanogens inhibition [47]. During the anaerobic digestion, a direct correlation between alkalinity and VFA accumulation was noticed. Therefore, the VFA/alkalinity ratio was considered as one of the main criteria for monitoring the digester stability. There are three critical values for this ratio [48]: < 0.4 stability of digester; 0.4–0.8 some instability will occur; > 0.8 significant instability. As shown in Table 2, the VFA/Alkalinity ratio increases while increasing the S/I ratio. However, these ratios were often in the range of 0–0.025, which means they did not rise above the critical value of 0.4 even for the highest S/I ratio of 2. As can be noted, VS removal was important, above 50%, for all the tested S/I ratios. These results could demonstrate the stability of the start-up phase for all tested S/I ratios resulted in the well establishment of anaerobic system.

Table 2
physico-chemical composition of digestates (end of batches) during start-up phase of batch anaerobic digestion of OFMSW.

	S/I ratio			
Parameters	**0.5**	**0.75**	**1**	**2**
pH	7.70 ± 0.14	7.94 ± 0.17	8.08 ± 0.12	7.90 ± 0.13
VS removal (%)	64.3 ± 0.91	55.49 ± 0.73	53.7 ± 0.68	50.60 ± 0.48
VFA (g/L)	0.00	0.10 ± 0.14	0.16 ± 0.09	0.30 ± 0.25
Alkalinity (g/L)	6.8 ± 0.35	8.01 ± 0.58	9.30 ± 0.47	11.80 ± 0.84
VFA/alkalinity	0.00	0.012 ± 0.24	0.017 ± 0.19	0.025 ± 0.29

3.3. Semi-continuous anaerobic digestion of OFMSW

3.3.1. Biogas profile

Semi-continuous anaerobic digestion of OFMSW was carried out in stirred reactor. During the whole operating period, three OLRs were applied, which were 1.36 (OLR_1), 2.5 (OLR_2) and 3.5 g VS/L.d (OLR_3). Consequently, HRTs decreased from 125 days to 97 and 82 days for OLR_1, OLR_2 and OLR_3, respectively. Fig. 3a shows the variation of the daily biogas production during mesophilic anaerobic digestion of the OFMSW at different OLRs. Overall, the daily biogas production fluctuated considerably during the anaerobic digestion. In fact, a significant decrease of biogas yield was observed during the transition period from one OLR to another. This could be explained by the stress of microorganisms caused by the increase of OLR [49]. For OLR_1, the maximum and the minimum volumes of biogas were 14.8 and 0.68 L/d, respectively. During this stage, the biogas average volume was 9.2 L/d at 125-day HRT. The average of biogas production were 12.5 and 15.4 L/d, when increasing OLR from 2.5 g VS/L.d to 3.5 g VS/L.d, respectively. It can therefore, be noticed that biogas yield decreased while increasing OLR. In fact, the average of biogas yields were 0.51, 0.48 and 0.44 L/g VS for OLR of 1.36, 2.5 and 3.5 g VS/L.d, respectively. Despite the decline of biogas yields, observed with the increase of the OLRs, reasonable biogas yields (0.51–0.44 L/g VS) were recorded.

For technical reasons, methane yields were only determined at OLR_2 and OLR_3. Fig. 3b shows that both trends of biogas and methane yields were similar. The average of methane yields decreased from 0.29 to 0.26 L/g VS, for OLR_2 and OLR_3, respectively. This result indicates that the increase of OLR leads to an increase in the organic matter and VFA contents, resulting in the inhibition of bacterial activity and thus reduction of the AD performance [50,51]. These methane yields were a bit lower than the range of values, 0.33–0.43 L/g VS as reported by Mu et al. [52]. Campuzano and González-Martínez [53] reported that the methane yield of OFMSW was 0.33 L/g VS during dry anaerobic digestion at an OLR of 5.6 g VS/L.d.

On the other hand, these values were lower than that of the theoretical methane yield of OFMSW, which is 0.57 L/g VS [54]. These results suggest the instability of the anaerobic digestion of OFMSW when the system was operated at OLR_2 and OLR_3. Similarly, Mu et al. [55] found that continuous AD of food waste was not stable even at lower organic loadings.

3.3.2. VS removal

VS is an important parameter to measure the biodegradation of organic matter. It reflects the metabolic activity of microbial population in the anaerobic system [56]. Fig. 4a illustrates the VS removal during anaerobic digestion of OFMSW. Similar to biogas production, the VS

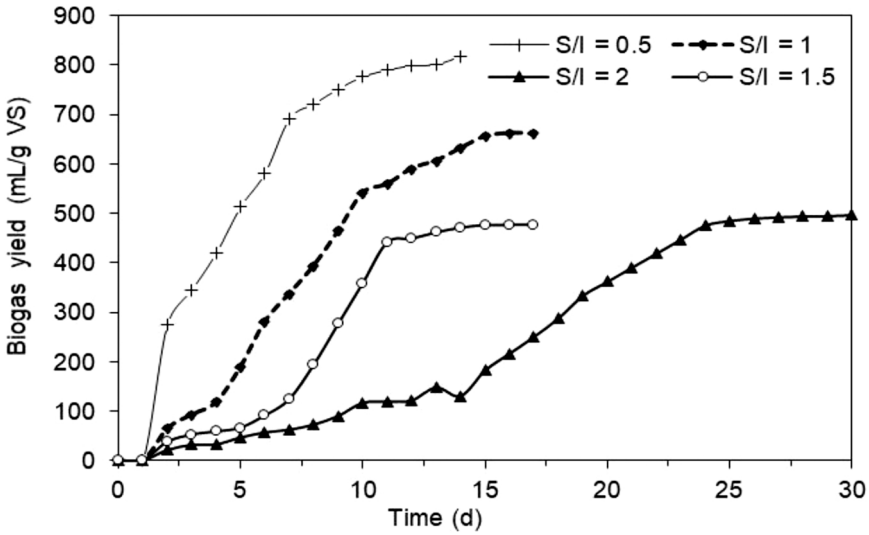

Fig. 2. Variation of biogas yield during start up phase of batch anaerobic digestion of OFMSW at different S/I ratios.

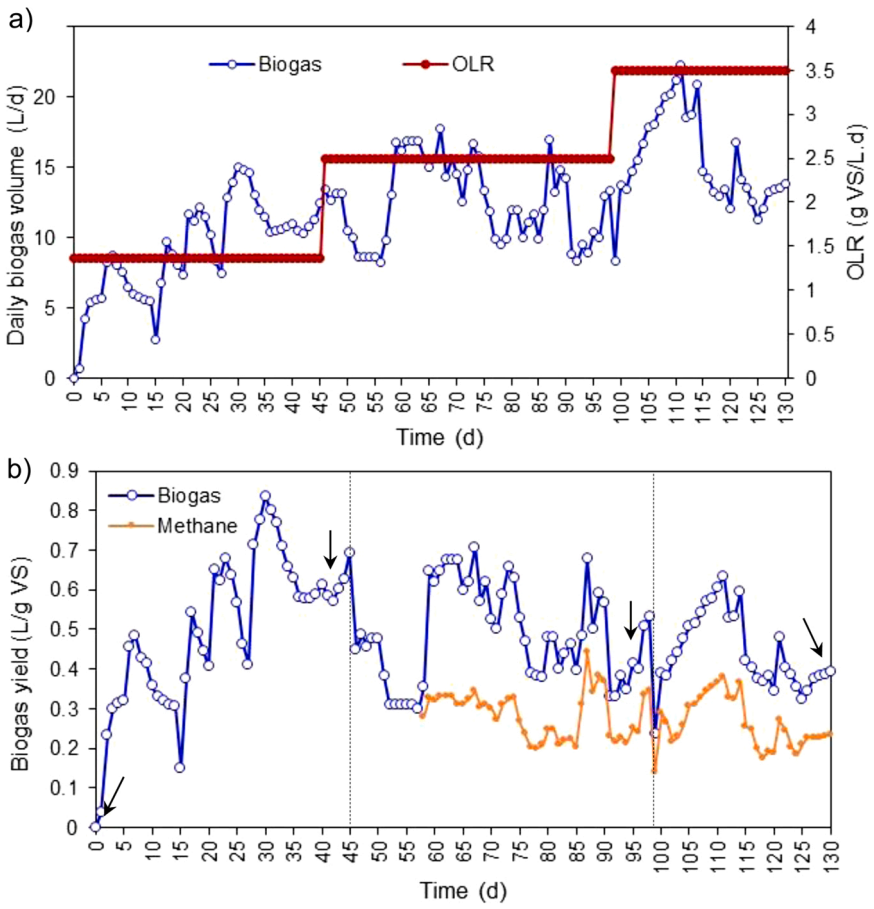

Fig. 3. Variation of biogas volume (a) and biogas and methane yields (b) during semi-continuous anaerobic digestion of OFMSW at different OLRs (Biomass sampling point).

removal curve fluctuated in the course of digestion process. Best results were registered at OLR_1, with an average of 53.4%. However, the average of VS removal was 41.6% at OLR_3. These results confirmed that the digestion process tends to fail, when the OLR was above 1.36 gVS/L. d.

3.3.3. *pH, VFA and alkalinity*

The stability of OFMSW digestion in stirred reactor was assessed through monitoring the change of pH, alkalinity and VFA/ALK ratio. pH is considered as an essential parameter for controlling the anaerobic digestion process [57]. Fig. 4b shows the changes of pH in mesophilic digester fed with OFMSW. During operating OLR_1, the pH average was around 7.3, which is considered as optimum value for methanogens activity [58]. With an OLR of 2.5 gVS/L.d, the average of pH was around 7.7. Operating at OLR_3, an increase of pH was observed until reaching a value of 8.8, which is unfavorable for methanogens growth [19]. This could inhibit the methanogens leading to the decrease of methane yield.

As shown in Fig. 4c, a gradual rise of the VFA concentration in the digester with the increase of OLR was also noticed. This could be attributed to either the increase of VFA concentration in the feed (OFMSW) or the abundance of acidogens in the microbial population. Alkalinity, which reflects the stability of the AD process, was also determined in the digester (Fig. 4b). Results showed that the alkalinity increased throughout the increase of organic loads and varied between 10.1 and 13.3 g $CaCO_3$/L, for OLR_1 and OLR_3, respectively. Many studies reported similar higher alkalinity values during the anaerobic digestion of OFMSW [59,60]. Thus, the biodegradation of OM like proteins could generate NH_3, which contributes to the increase of alkalinity and pH in the digester [59,60]. On the other hand, these values were greater than the optimal range for the anaerobic digestion process, 2–5 g $CaCO_3$/L, which provides a high buffering capacity [61]. Likewise, the VFA/ALK ratio was also determined. The highest values of VFA/Alkalinity ratio were registered at OLR_3. Overall, in most operation periods, this ratio was below 0.34. Although, this ratio was under the critical value for the system stability [48], the malfunction of the digester, which occurred with the increase of alkalinity concentration, was monitored throughout the course of operating OLR_3.

3.4. *Abundance and diversity of microbial populations*

3.4.1. *Procaryotic community*

The change of the microbial diversity during AD process was determined using the high-throughput sequencing of 16S rRNA amplicons.

The number of prokaryotic sequences obtained per sample varied from 23,876 to 57,620 after quality trimming and removal of chimeras. The α-diversity analysis was measured by observed OTUs, Chao1, Shannon, and Simpson indices (Table 3). The results revealed that the highest richness (Observed OTUs) and diversity (Shannon) was observed in the sample collected from digester before applying the semi-continuous feeding (at the end of start-up phase) (T_i), it then decreased during AD. These findings are in agreement with those reported by Kurade et al. [62]. The relative abundance and taxonomic distribution of prokaryotic communities of digestate samples were analyzed at phylum/class levels (Fig. 5). The phyla *Firmicutes*, *Bacteroidetes, Thermotoga* and *Proteobacteria* were the most dominant for all samples (Fig. 5a). These results are to some extent in line with many studies showing that *Bacteroidetes*, *Firmicutes* and *Proteobacteria* were the main phyla in anaerobic digesters [63]. The abundance of these phyla

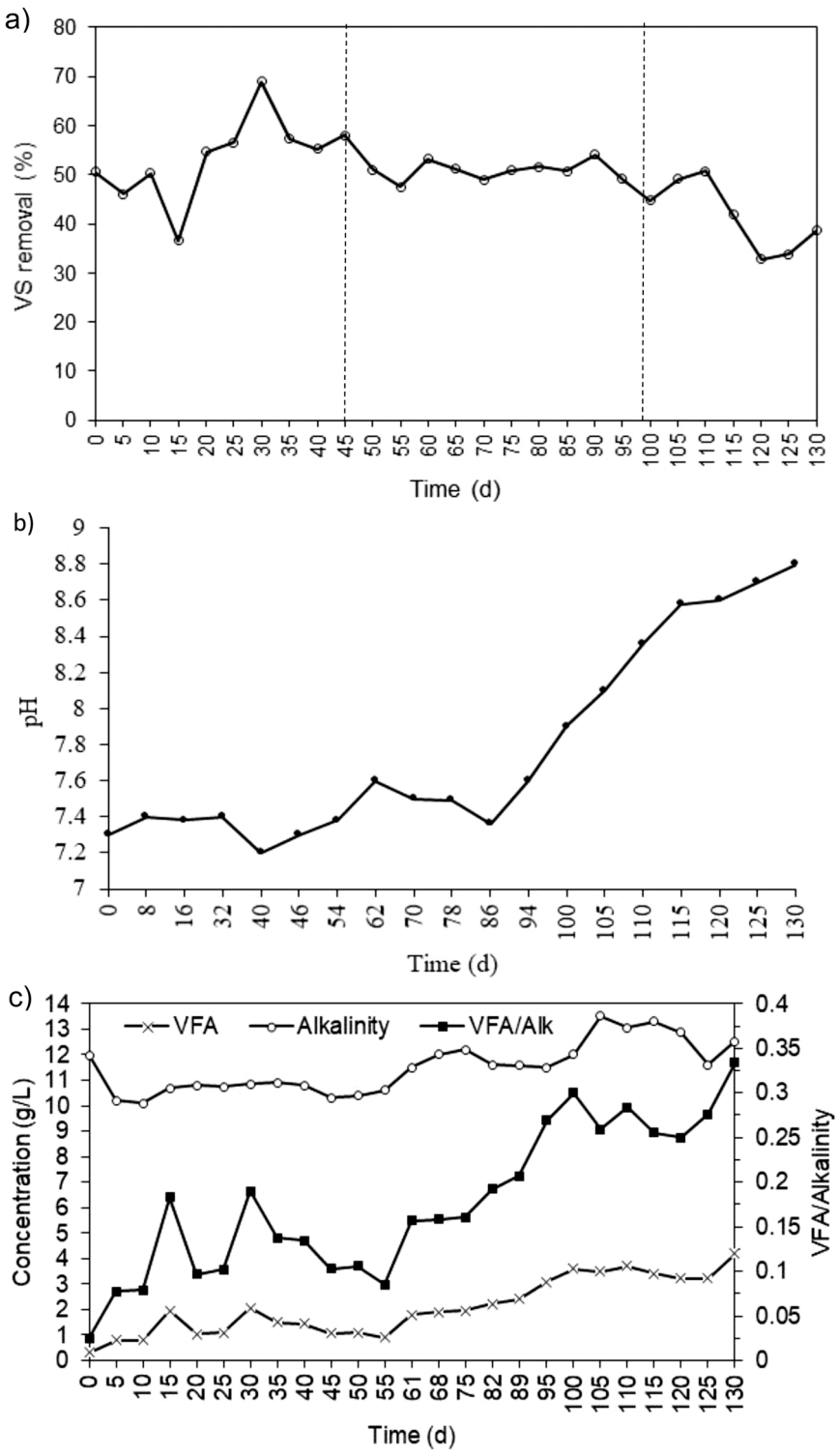

Fig. 4. Time course of VS removal **(a)** pH (b), VFA and VFA/Alkalinity ratio **(c)** during semi-continuous anaerobic digestion of OFMSW in stirred reactor.

could be attributed to their ability to hydrolyze a wide range of substances such as cellulose, proteins and pectin [64]. *Bacteroidetes,* which are detected during anaerobic digestion of organic solid waste [63], contributed to hydrolysis and acidogenesis steps [65].

Although *Firmicutes*, *Bacteroidetes* were the dominant phyla, their relative abundance was remarked to vary during the anaerobic process. OLR_3, for instance, was characterized by a shift in the abundance of *Firmicutes* to *Bacteroidetes* when compared to OLR_2 community distribution. It was previously reported that the *Bacteroidetes* exhibit a low hydrolysis capacity [66]. This could explain the low VS removal and biogas production achieved at OLR_3.

On the other hand, some studies showed that some bacteria affiliated to *Thermotoga* phylum were present with a low frequency during mesophilic anaerobic digestion of OFMSW [67]. Likewise, *Synergistetes*, *Spirochaetes*, *Chloroflexi* were the minor phyla during AD process. The low abundance of *Chloroflexi* could be explained by its role in degrading carbohydrates and thus it could be predominant in anaerobic reactor fed with waste rich in sugar [63].

Table 3
Richness and diversity estimation of microbial communities in samples obtained during anaerobic digestion of OFMSW.

	Samples	Sequences	Chao1 richness estimator	Observed species	Shannon diversity index	Simpson diversity index
Prokaryote 16 S rRNA gene	Ti	41,464	301.00	271	4.59	0.83
universal	OLR_1	23,876	286.83	263	4.27	0.85
primers	OLR_2	40,304	294.08	256	3.92	0.82
	OLR_3	57,620	278.00	260	4.37	0.89
Archaea	Ti	96,853	72.75	72	2.69	0.76
16S rRNA	OLR_1	78,152	40.00	37	1.46	0.55
gene-specific	OLR_2	64,081	43.25	43	1.33	0.52
primers	OLR_3	38,822	35.50	33	1.27	0.52

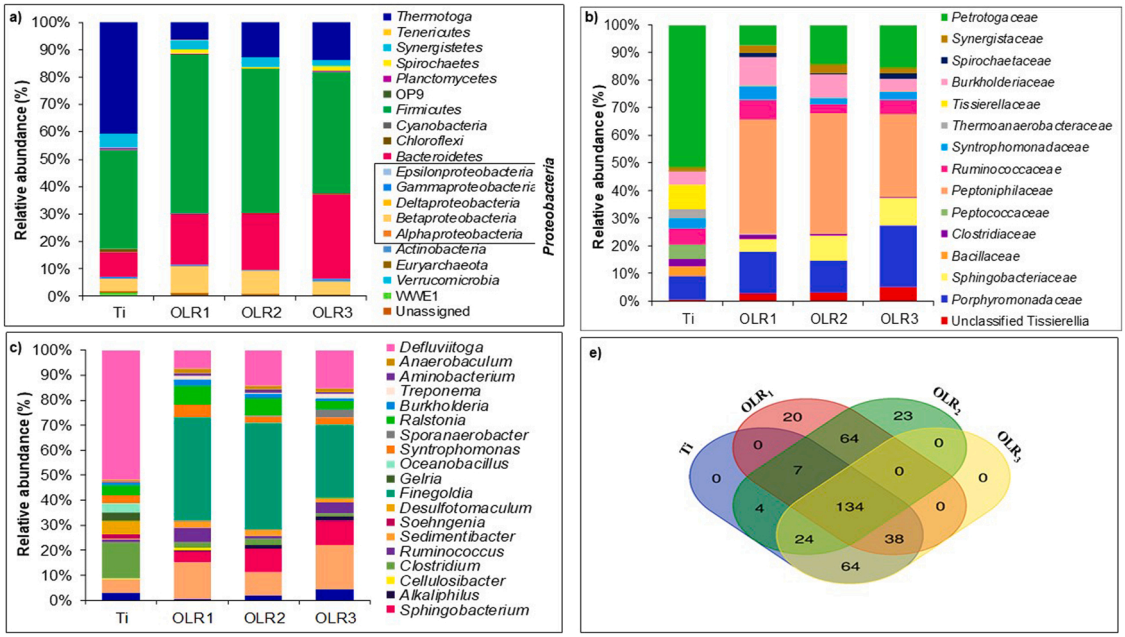

Fig. 5. Taxonomic compositions of bacterial communities at (a) phylum/Class levels (b) Family (OTUs> 1% of relative abundance), (c) genus levels (OTUs> 1% of relative abundance) in each sample derived from stirred reactor at different OLRs and (e) Venn Diagram showing the number of unique and shared operational taxonomic units (OTUs) of the bacterial community.

Fig. 5e illustrates the OTU distribution plotted in the Venn diagram. It could be observed that all digestate samples shared 134 OTUs. However, specific OTUs, 20 and 23 OTUs, were only detected in OLR_1 and OLR_2 samples, respectively.

Among all detected OTUs, the bacterial population were dominated by 31 OTUs (> 1% of all sequences) (Table 4S). Their sequences were affiliated to 15 families (Fig. 5b) and 21 genera (Fig. 5c). The most frequent phylum, *Firmicutes*, was mainly represented by sequences belonging to *Peptoniphilaceae* family, which accounted until 43.5% of the total sequences. Regarding *Finegoldia* genus, its abundance was very low in the beginning of AD (T_i). As time progressed, its dominance was increased, wherein it was above 35% at OLR_2. *Finegoldia magna* was the main detected species within this family (Table 4S). It is known to produce acetate essentially from amino-acids and fructose [68]. It is also noticed that *Porphyromonadaceae* is the most dominant family within the second frequent phylum, *Bacteroidetes*. *Proteiniphilum*, was mostly the major genus of the *Porphyromonadaceae* family. In fact, its dominance increased during the AD, especially at OLR_3 (15.6%). This genus belongs to acetogens, which was able to accelerate the transformation of VFAs to acetate [69]. Chen and Dong [70] showed that *Proteiniphilum* was detected in a mesophilic reactor treating brewery wastewater. *Thermotogae* phylum was represented only by the *Petrotogaceae* family, which was closely affiliated to *Defluviitoga* genus. Among the *Proteobacteria* phylum, *Burkholderiaceae* accounted from 4.8% to 9.9% of the total sequences.

3.4.2. *Archaeal community*

As shown in Fig. 6c, a lower percentage of shared archaeal OTU, 40.35%, between all samples (T_i, OLR_1, OLR_2 and OLR_3) was observed. It is worth noting that the highest number of independent archaeal species was observed in the beginning of the anaerobic process. This demonstrates that the highest diversity of archaeal community was attained before the semi continuous feeding of digester.

As shown in Table 3, the archaeal communities were remarked to be less rich and less diverse than the bacterial communities for all the samples, a trend that was previously observed by Zhang et al. [69]. Additionally, the best archaeal diversity was reached in the beginning of the anaerobic process. When comparing the different OLRs, the results reveal that the higher was the OLR, the lower were the archaeal community diversity and richness (Table 3). These results are in line with those of Jang et al. [71], who reported that the increase of OLR could decrease the diversity of microbial communities. They also indicate that the starting phase of the anaerobic digestion was well established compared to the continuous stage of the anaerobic process.

Fig. 6 shows the distribution of archaeal populations during anaerobic digestion of OFMSW. Archaeal sequences were mainly related to the *Euryarchaeota* phylum (Fig. 6a, Table 5S). Similar trends of phylum abundance for methanogens were reported in previous studies [64,72]. *Methanomicrobia* was the dominant archaeal class of *Euryarchaeota* phylum by more than 90% of the total archaeal populations. At genus level, *Methanosarcina* was the most dominant for all OLRs (Fig. 6b). This could be due to its fast growth on solid waste compared to wastewater

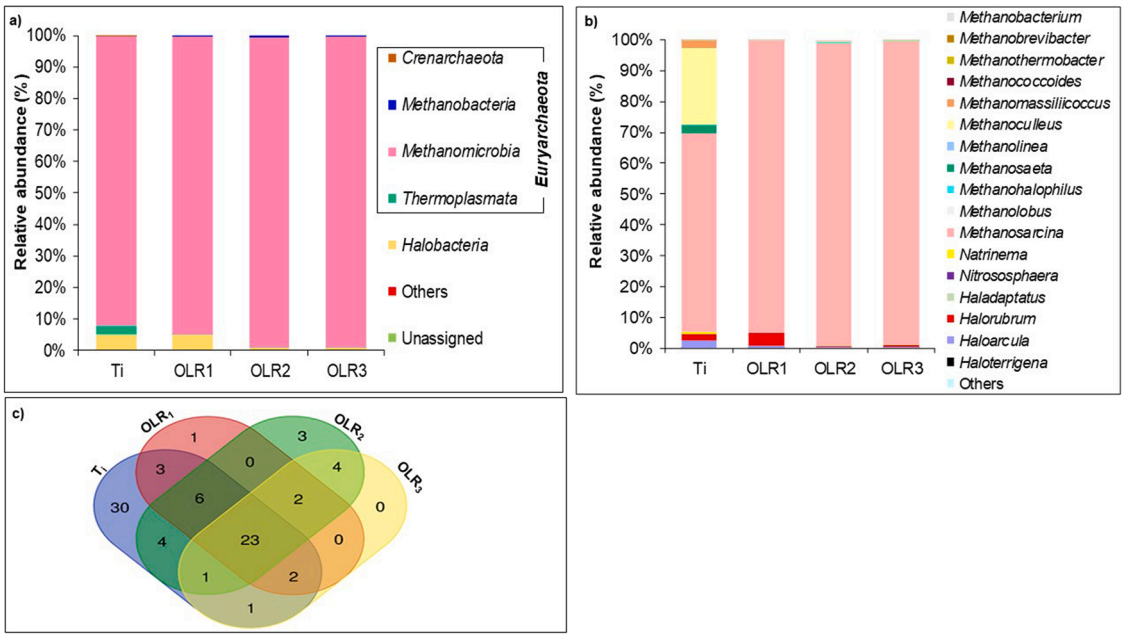

Fig. 6. The relative abundance of micro-organisms based on the taxonomical classification of the archaeal community in each OLR identified at (a) phylum/class levels (b) genus levels and (c) Venn diagram showing the number of unique and common archaeal operational taxonomic units (OTUs).

[73]. Moreover, *Methanosarcina* could play a crucial role in the methanogenesis stage. Therefore, it might produce CH_4 via H_2/CO_2, acetate and methyl compounds [74]. However, *Methanoculleus* was detected frequently at early stage of digestion after inoculum acclimatization (Ti) (Fig. 6b). After that, this genus declined over the time showing the decrease of archaeal population diversity.

3.5. Limitations and future perspectives

The results of the present study confirmed the limitation of single phase of stirred tank solid digester. Thus, the increase OLR to 3.5 gVS/L.d (OLR_3) lead to the failure of anaerobic digestion system of OFMSW. The methane yield and VS removal were very low, while, alkalinity and VFA were increased during OLR_3. On the other hand, the diversity and the dynamics of prokaryotic and archaeal populations were also decreased with the increase of OLR. The limitation of anaerobic digestion of OFMSW in single phase could be overcome by the improvement of reactor configuration and by the application of enzymatic pretreatment of OFMSW [75]. The later could be integrated to anaerobic process to improve renewable energy generation as well as to better reveal the economic feasibility of the full-scale application of OFMSW AD. Another strategy to enhance OFMSW AD is the co-AD process with other organic solid waste [76]. Moreover, the characterization and analysis of anaerobic digestate quality, life cycle assessment and techno-economic analysis [77] must be investigated to further establish a knowledge about bioenergy productivity and agronomic valorization of digestate in Tunisia.

4. Conclusions

The start-up phase of batch anaerobic digestion of OFMSW was efficient for the different tested S/I ratios. The performance of semi-continuous digestion of OFMSW in stirred reactor and the diversity of microbial populations were investigated. The achieved results show that a lower OLR was the optimal operational condition for a maximum biogas production. Nevertheless, the lowest productivity of biogas were obtained at the highest OLR, wherein the monitored parameters, such as pH, VFA and alkalinity, proved that the system was not stable. The metagenomic analysis showed that *Firmicutes, Bacteroidetes, Thermotoga* and *Proteobacteria* were the dominant phyla of the bacterial community. *Methanosarcina*, an acetotrophic methanogen, was the most abundant genus of archaeal community during the anaerobic process. The α-diversity analysis indicated that the archaeal population richness and diversity were the lowest when operating the digester at OLR_3.

The findings of this study proved that the OFMSW anaerobic digestion, applied in a continuous mode, could be efficient only at a low OLR.

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CRediT authorship contribution statement

Najoua Mlaik: Performing manipulation and redaction of the original draft of the manuscript. **Fatma Karray**: Performing microbial analysis and revision of the manuscript. **Firas Feki:** Digester design. **Sami Sayadi** and **Sonia Khoufi:** Revision and editing the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.107941.

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