Journal of Water Process Engineering 49 (2022) 103020

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Performance of UASB reactor treating waste activated sludge: Effect of electro-chemical disintegration on the anaerobic microbial population structure and abundance

Emna Feki [a], Fatma Karray [a], Najla Mhiri [a], Audrey Battimelli [c], Sami Sayadi [b], Sonia Khoufi [a,*]

[a] Laboratory of Environmental Bioprocesses, Centre of Biotechnology of Sfax, BP 1177, 3018 Sfax, Tunisia
[b] Center for Sustainable Development, College of Arts and Sciences, Qatar University, Doha 2713, Qatar
[c] INRA, Université de Montpellier, Laboratoire de Biotechnologie de l'Environnement, 102 avenue des Etangs, 11100 Narbonne, France

ARTICLE INFO

Keywords:
Electro-chemical disintegration
Waste activated sludge
Biogas production
UASB reactor
Microbial community structure

ABSTRACT

The electro-Fenton (EF) disintegration using iron electrodes was performed for the pretreatment of waste activated sludge (WAS). The effect of this electro-chemical pretreatment on anaerobic digestion (AD) performance and microbial population structure was studied. An improvement of biodegradability and bioaccessibility of organic matter was demonstrated. AD of pretreated WAS in an up-flow anaerobic sludge blanket reactor (UASB) resulted to an increase of biogas production by 60 % compared to control reactor without disintegration. PCR-DGGE and real-time qPCR analyses showed that the high abundance of bacteria and the coexistence of Coprothermobacter in the UASB digestate fed with disintegrated sample established a stable bacterial association which is in line with the AD performance. Besides, the increased number of methanogens along the process allowed the improvement of methane production in comparison to control reactor.

1. Introduction

Activated sludge technology is widely used for the treatment of industrial and municipal effluents. This process generates a huge quantity of waste activated sludge (WAS) with approximately 60 g/capita/day [1]. The main method used for the majority of this sludge quantity is the in-situ storage in the WWTP and this can have negative effects on the environment due to the unstable character of this biomass.

In an economic context, the bioconversion and recovery of this organic material would be a judicious solution to reduce their harmful effects. As a bioconversion process anaerobic digestion (AD) can stabilize and reduce the volume of this waste but also can produce a biogas rich in methane [2–4]. The digestion of sludge facilitates their management whatever their final treatment. In fact, anaerobic digestion leads in the first place to a 30 to 50 % reduction in the volumes of sludge produced in the treatment plant [5]. The other major advantage lies in the production of methane, which can be converted to heat and electrical energy. Part of this heat can also be used on site, to maintain the temperature of digesters or to heat buildings. This technique is used worldwide for treating and stabilization of sludge prior to final disposal [6].

The AD process of activated sludge is characterized by the limit rate of hydrolysis step. During this step, the complex macromolecules are solubilized under the action of extracellular enzymes excreted by strict anaerobic bacteria (*Clostridium* for the degradation of cellulose, starch) or optional aerotolerant (*Bacillus* for the degradation of proteins). Particulate compounds are split into monomers (or dimers) small enough in size to be transported across the cell membrane. Once in the cell, these simple molecules can be used as an energy source for metabolism [7]. When considering the methanization of complex wastes containing solid fractions, for example cellulose, hydrolysis should be considered the limiting step [8]. Disintegration of sludge has been referred as possible method to enhance the solubilization and bioavailability of organic matter AD biosystem [9]. However, in the context of the development of

Abbreviations: AD, Anaerobic Digestion; BMP, Biochemical Methane Potential; DOM, Dissolved Organic Matter; HRT, Hydraulic retention time; NEOM, Non Extractable Organic Matter; PEOM, Poorly Extractable Organic Matter; REOM, Readily Extractable Organic Matter; SCOD, Soluble Chemical Oxygen Demand; SEOM, Slowly Extractable Organic Matter; SPOM, Soluble fraction from Particular Extractable Organic Matter; TCOD, Total Chemical Oxygen Demand; TKN, Total Kjeldahl Nitrogen; TS, Total Solids; UASB, Up-flow anaerobic sludge blanket reactor; VS, Volatile Solids; VSS, Volatile Suspended Solids; VFA, Volatile fatty acid.

* Corresponding author.
E-mail address: soniakhoufi@cbs.rnrt.tn (S. Khoufi).

https://doi.org/10.1016/j.jwpe.2022.103020
Received 23 February 2022; Received in revised form 14 July 2022; Accepted 17 July 2022
Available online 2 August 2022

renewable energy production, it is necessary to carry out a pretreatment in order to hydrolyze the activated sludge before its anaerobic digestion. The integration of a disintegration/hydrolysis step could be an interesting alternative in order to remove the economic brakes [10]. Research works are underway to develop technologies for the pretreatment of sludge before anaerobic digestion. These methods are essentially based on the destruction of the flocs and the lysis of microbial cells. Indeed, various physico-chemical techniques have been developed which are based on mechanical, chemical, oxidative (O_3, H_2O_2) or thermal treatments [11]. Currently, there is a specific interest to the use of advanced oxidation processes (AOP) as effective, economical and clean methods to disintegrate WAS by the means of hydroxyl radicals ($HO^{\bullet}$) attack [12,13]. Chemical, electro-chemical (electro-Fenton), sono-chemical and photo-chemical processes (photo-Fenton) are the main AOP methods used for the disintegration of sludge [11]. Compared with Fenton reaction, electro-Fenton (EF) has certain advantages as the generation of reagents in situ (H_2O_2 and Fe^{2+}) via an electrochemical process which is beneficial for enhancement of organics degradation efficiency as well as the decrease in the cost and reduction in the risks associated with reagent transportation. In the case of generation of Fe^{2+} through the reduction of ferric ions on the cathode reduces the production of iron sludge and achieve the diversification of organics degradation pathway, such as Fenton oxidation, anodic oxidation, flocculation, and electric adsorption. However, EF processes have some disadvantage with respect to H_2O_2 in-situ production. The generation of H_2O_2 is slow because oxygen has low solubility in water and the current efficiency is low at acid pH. In addition, the efficiency of the EF process depends on electrode nature, pH, concentration of reagents, concentration of pollution, current density, and temperature [14]. Recently, electrolysis and ultrasound treatment coupled to biological treatment were demonstrated to be efficient to improve biodegradation and stabilization of WAS [15,16].

In this general context, the effect of electro-Fenton (EF) pretreatment on the degree of WAS disintegration and on anaerobic digestion process conducted in UASB reactor was studied. Performance of the digester treating raw and disintegrated sludge was investigated. The microbial community structure and abundance of digested samples were analyzed by denaturing gradient gel electrophoresis (DGGE) and real-time qPCR methods.

2. Material and methods

2.1. Biomass samples

Raw WAS sample obtained from a final clarifier of Saltnia urban wastewater treatment plant (Sfax, Tunisia) was stored at 4 °C. Disintegrated WAS was obtained after pretreatment using an electro-Fenton system. Table 1 summarizes the characteristics of raw and disintegrated sludge used herein.

Anaerobic inoculum sludge (inocula) was collected from a mesophilic digester installed in the laboratory. The average concentration of volatile suspended solids (VSS) was 9.25 g/L.

Table 1
Physico-chemical analysis of raw and disintegrated WAS (data shown as the mean ± SD, $n = 3$).

Parameters	Raw WAS	Disintegrated WAS
pH	6.95 ± 0.2	7.20 ± 0.8
Conductivity (mS/cm)	3.72 ± 0.3	13.72 ± 0.1
TS (g/L)	19.45 ± 1.4	14.28 ± 2
TVS (g/L)	12.67 ± 1.2	10.34 ± 1.3
TSS (g/L)	15.16 ± 0.9	10.50 ± 0.5
VSS (g/L)	7.27 ± 1.3	3.00 ± 0.6
TCOD (g/L)	20.41 ± 4	26.00 ± 1.2
SCOD (g/L)	1.73 ± 2	4.10 ± 0.3
NTK (mg/L)	914.20 ± 10	920.00 ± 3
Proteins (mg/L)	150.12 ± 15	542.36 ± 22
VFA (mg/L)	84.26 ± 8	924.80 ± 26
BMP (mL/g VS)	80 ± 12	135 ± 24

2.2. Sludge disintegration system

Sludge disintegration was performed using an electro-Fenton system equipped by two iron electrodes, an electrolysis reactor and an electric generator ASF type 400/40.10. EF treatment was conducted at the following operational conditions: current density 2.5 A/dm^2; treatment time 1 h; H_2O_2 concentration 1.8 g/L; working volume 300 mL and initial pH 3. The content of reactor was homogenized by magnetic stirrer (100 rpm) and performed at room temperature.

2.3. Biochemical methane potential (BMP) tests

BMP assays were performed at mesophilic conditions (37 ± 1 °C) to determine the methane potential of sludge samples. Bottles having capacity of 120 mL were used as anaerobic reactors and VS substrate/VS inocula ratio equal to 0.5 was maintained in each bottle. In order to enhance the start-up of fermentation, a gas mixture of 75 % N_2 and 25 % CO_2 was used for purging batch reactors. The daily methane production of fermentations was determined against a control batch containing only inocula.

2.4. UASB reactor

The semi-continuous AD was carried out in an up-flow anaerobic sludge blanket (UASB) reactor which is a heterogeneous system. This reactor having a working volume of 7 L was used to study the anaerobic treatment of raw and disintegrated WAS. The digester had a double membrane in PVC thermostated at 37 °C and controlled by water bath to maintain a constant temperature of the reaction medium. Different hydraulic retention times (HRT) were maintained during fermentations (20, 14, 10 and 7 days). A peristaltic pump was used for the feeding of reactor and no adjustment of sludge pH was done before feeding. A liquid displacement system was used to measure biogas production. Methane percentage was determined by passing the produced biogas through KOH solution (20 g/L). The ratio between CH_4 volume and original biogas volume gives the percentage of methane. This operation was done 2 times per week. Before starting fermentation experiments, an acclimation period was carried out by feeding the reactor with a mixture of anaerobic digestate and raw sludge at the same proportion (v/v).

2.5. Fractionation of organic matter

Organic matter of raw and pretreated sludge was characterized by a method based on chemical extractions and fluorescence spectroscopy [17].

2.5.1. Sequential chemical extraction

Sequential chemical extraction of organic matter present in samples was performed according to the methodology developed by Jiminez et al. [17]. This methodology can correlate between the bioavailability of organic matter in sludge and the chemical accessibility [18]. The chemical fractionation of WAS organic matter (OM) resulted to 5 fractions: the dissolved organic matter (DOM); the soluble and extractable OM (SPOM); the easily extractable OM (REOM); the slowly extractable OM (SEOM) and the poorly extractable OM (PEOM).

2.5.2. Fluorescence spectroscopy analysis

Fluorescence spectroscopy analyses were performed according to the protocol described by Jiminez et al. [17]. The fluorescence spectra of the liquid extracts were recorded on a Perkin Elmer LS55 using wavelength excitation ranging between 200 and 600 nm and a scanning monochromator speed of 1200 nm/s. According to He et al. [19], the spectra are decomposed into seven zones (zones I to VII) where each one is

associated to a specific biochemical family: Tyrosine (zone I); Tryptophan (zone II); Tyrosine, Tryptophan and microbial products (zone III); fulvic acid (zone IV); glycol protein (zone V); lignocellulose and melanoin (zone VI) and humic acid (zone VII). In fact, the simplest molecules are located in zones I to III while zones IV to VII represent the complex molecules.

2.6. PCR-DGGE analysis

Total community DNA of two samples of anaerobic digestates from UASB reactor treating raw and pretreated WAS was determined. Digestates were extracted using the EZ-10 Spin Column Soil DNA Mini-Preps Kit (BIO BASIC INC.). Specific primers set (341FGC/907rR) were used to amplify V3-V5 hypervariable regions of the rRNA 16S sequences. The thermal cycling program was: first denaturation at 95 °C (3 min), denaturation at 94 °C (30 s), annealing at 55 °C (45 s) and extension at 72 °C (45 s). A final extension at 72 °C (10 min) was then performed. A 50 μL reaction mixture containing 1X Invitrogen Taq DNA Polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each primer and 1 U Taq DNA Polymerase (Invitrogen) was used to perform PCR reactions. 1 % agarose gel stained with ethidium bromide (EtBr) was used to visualize PCR products. DGGE analyses were performed according to the protocol described by Kumar et al. [20].

2.7. Sequence analysis

Predominant DGGE bands were excised from the gel eluted overnight in 35 mL of MilliQ water and reamplified by PCR using primers devoid of the GC clamp (341F and 518R). PCR products were sequenced using the Big Dye® Terminator cycle Sequencing kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). 16S rRNA gene sequences were initially compared with the GenBank and RDP databases using the online sequence analysis resources "BLAST" (Basic Local Alignement Search Tool) and "Seqmatch" (Ribosomal Database project II; Release 10) [21]. The sequences were checked for possible chimeric structure using chimera check on DECIPHER database (http://decipher. cee.wisc. edu/FindChimeras.html) [22]. Multiple alignments were generated with the MUSCLE program and dendrograms were constructed with MEGA program version 7 [23] on the basis of evolutionary distances that were calculated by the Neighbor-Joining method with Jukes-Cantor model. Statistical evaluation of the tree topologies was performed by bootstrap analysis with 1000 resamplings [24].

2.8. Nucleotide sequence accession numbers

The DGGE band sequences of the present work were deposited in GenBank under accession numbers MH727093, MH890540 to MH890546.

2.9. Quantification of 16S rRNA, and mcrA gene by quantitative real-time PCR (qPCR)

The abundances of bacterial and methanogen communities in digestate samples were assessed by qPCR. Archaeal and bacterial 16S rRNA genes were quantified using the primer sets 344F/519R and 331F/797R, respectively. To enumerate the methanogenic communities, mlas/mcrA-rev primers sets were used for mcrA gene amplification. QPCR was performed in triplicate on a Bio-Rad CFX-96 real-time system (Bio-Rad) using the same reaction components and qPCR protocol condition as previously described [25,26]. Using the standard curves, the abundance of each microbial group was reported as DNA copy numbers of corresponding gene per gram of wet sludge.

2.10. Analytical methods

Methods used for the physico-chemical analysis (COD, TS, VS, TSS, VSS, TKN and pH) are described in previous study [27]. Conductivity was measured using a conductimeter (CONSORT). Protein content was determined by Bradford method [28]. A mixture of 800 μl of the diluted sample and 200 μl of Biorad reagent was maintained at ambient temperature and in the dark during10 min. A calibration curve prepared with concentrated bovine serum albumin (BSA) solution was used to determine protein concentration. Optical density of samples was measured at 595 nm.

For the determination of total volatile fatty acids (VFA), filtered and acidified (pH 3) samples were analyzed by high performance liquid chromatography (HPLC: SHIMADZU 10 AVP).

3. Results and discussion

3.1. EF disintegration effects on sludge properties

3.1.1. Physico-chemical characteristics

Table 1 gives the characteristics of WAS before and after EF treatment. Results showed an effect of EF on different parameters as pH, conductivity, COD, VFA. pH of sludge increased and reached a value of 7.2 after 1 h. Also, an increase of the conductivity was shown 13.72 mS/cm due to the liberation of mineral salts during electrolysis. The VSS/TVS ratio about 0.57 of untreated sludge indicated the high proportion of organic matter flocs. The decrease of this ratio to 0.29 with the drop of TSS to 10.5 g/l proved the sludge disintegration. Effect of EF over sludge solubilization was also noted by the increase of soluble COD, proteins and VFA. However, a decrease in total COD was noted due to the mineralization of OM which makes the measured soluble COD low compared to the real concentration released during treatment. After 30 min of treatment, the VFA concentration increased from 84.26 to 924 mg/L. This shows that the disintegration of WAS by EF pretreatment causes the VFAs release resulting from cell lysis [29]. This was also noted by Xu et al. [30] after electrochemical treatment (Ti/RuO2 anode). So, the increase of VFA content in pretreated sample with a concentration less than 2–3 g/l will obviously promote their anaerobic digestion.

3.1.2. Biodegradability and bioaccessibility

The biodegradability of OM does not only depend on its composition but also on its accessibility and complexity. The integrated methodology, successive chemical extractions - 3D fluorescence spectroscopy, was used to characterize the accessibility and complexity of OM. The effect of the pretreatment on the distribution of different OM categories in the extractable fractions was determined.

The percentages of fluorescence of zones I, II and III were important in the most extractable fractions of the raw and pretreated sludge (Fig. 1). In fact, the fluorescence percentage of zone I increased from 11 % to 38 % in the dissolved fraction (DOM) and from 15 % to 25 % in the soluble fraction (SPOM) after pretreatment. The enhancement of OM solubilization in zone I was also observed in zone III. In the raw sludge, a fluorescence percentage of 10 % in all the fractions was registered. This percentage increased to 20 % after EF pretreatment in all fractions except in PEOM where the percentage is low than 2 %.

For zones IV, V and VI which are respectively analogues of fulvic acids, glycol proteins and lignocellulose show an increase of fluorescence percentages in SPOM, REOM and SEOM after pretreatment but a significant decrease from 60 to 16 % was observed in PEOM. Whereas for the zone VII fluorescence analogous to humic acid [17] shows a high percentage of fluorescence (80 %) in the PEOM fraction of the pretreated sludge in comparison to the raw sludge (10 %). In fact, this zone contains the most complex organic matter and it is not accessible for biological degradation [31]. This finding demonstrates that under the effect of EF treatment there is a conversion of the simple non-extractable molecules, detected in crude sludge, into soluble extractable molecules. According to these results, the EF pretreatment improves bio-accessibility of OM through the release of non-extractable compounds to the soluble and

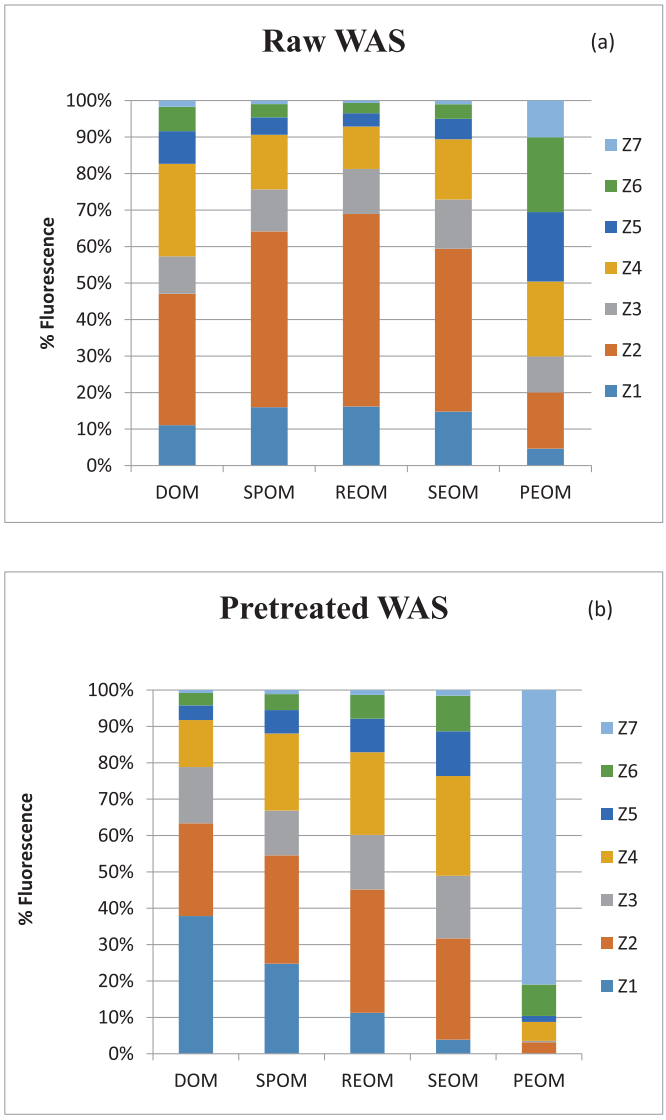

Fig. 1. Percentage of fluorescence zones in the different OM fractions of raw (a) and pretreated (b) sludge.

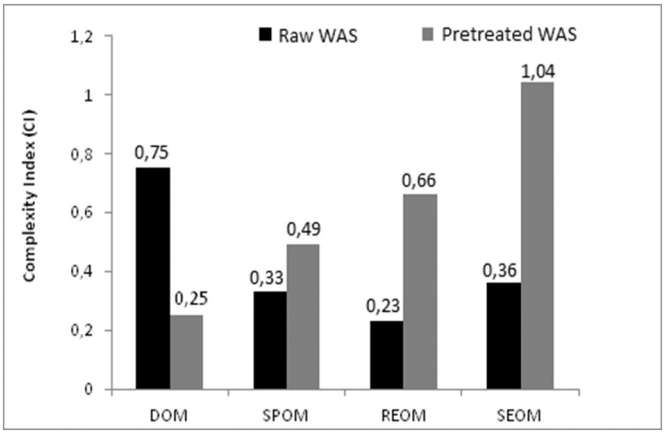

Fig. 2. Complexity index in the different OM fractions of raw and pretreated sludge in the different OM fractions.

extractable fractions.

Fig. 2 shows the complexity index (CI) of each fraction before and after pretreatment. CI is the ratio between the sum of percentages of zones IV, V VI and VII and the sum of percentages of zones I, II and III, and gives an idea about the biodegradability of organic fractions. The results in Fig. 2 show that the index of the DOM fraction of raw sludge decreases from 0.75 to 0.25 after pretreatment. This shows that the OM solubilized during the pretreatment is composed of simple and biodegradable molecules. Increasingly higher index was recorded in the other fractions of the pretreated sludge, indicating the increase of complex molecules concentration with the decrease of accessibility degree. On the other hand, low index was measured in the SPOM, REOM and SEOM fractions of the raw sludge which shows that the organic matter present in these fractions is biodegradable but not easily accessible. Under the effect of pretreatment, this part of organic matter becomes more accessible to microorganisms, which clearly explains the improvement of biogas yield of pretreated sludge.

3.2. Biochemical methane potential (BMP)

BMP assays were performed to determine the methane potential of raw and pretreated WAS samples. Table 1 gives the results of methane yield of samples. The low methane yield of raw sample (0.080 L CH_4/g VS) confirms the hydrolysis limitation due to the resistance of flocs to anaerobic degradation. The increase of anaerobic degradability of pretreated sludge was confirmed in this study as a significant improvement of methane yield about 68 % was registered which is in correlation with the degree of COD solubilization and the released VFA (Table 1). At this step of study, batch fermentation results showed the positive effect of EF disintegration on the anaerobic digestion of WAS.

3.3. Semi-continuous anaerobic digestion of disintegrated sludge

3.3.1. Reactor performance

Table 2 summarizes the operating conditions applied to UASB reactor during fermentation experiments. The schematic configuration of UASB reactor is given in Fig. 3. The daily biogas production was in correlation with the applied OLR during the two fermentations (Fig. 4). However, low biogas yields were noted during the first period of fermentations which then improved with fermentation time and reached 0.5 and 0.8 L/g VS respectively at the end of the fermentation of raw and disintegrated sludge (Fig. 4, Table 2). In the case of raw sludge, no significant increase of biogas yield was registered during the increase of OLR from 0.35 to 0.5 g VS/L. d that showing the low degradability of raw sludge. Based on this result, the fermentation of pretreated sludge

Table 2
Fermentation time, organic loading rate (OLR), biogas yield, methane percentage and COD removal during semi-continuous fermentation of raw and pretreated WAS in UASB reactor (data shown as the mean ± SD, n = 3).

Samples	Fermentation time (days)	OLR (g VS/L·d)	Biogas yield (L/g $VS_{introduced}$)	CH_4 (%)	COD removal (%)
Raw WAS	0–35	0.20 ± 0.02	0.11 ± 0.03	48.00 ± 4.00	12.00 ± 1.20
	91–100	0.35 ± 0.01	0.48 ± 0.03	54.00 ± 2.00	18.00 ± 2.00
	101–130	0.50 ± 0.02	0.50 ± 0.02	56.00 ± 1.00	30.00 ± 3.40
Pretreated WAS	0–35	0.50 ± 0.02	0.16 ± 0.03	58.00 ± 3.00	53.00 ± 3.50
	101–130	1.60 ± 0.02	0.30 ± 0.03	66.00 ± 2.00	84.00 ± 5.20
	131–190	2.00 ± 0.03	0.74 ± 0.03	68.00 ± 1.00	94.00 ± 2.40
	191–250	2.50 ± 0.03	0.80 ± 0.03	67.00 ± 3.00	96.00 ± 1.50

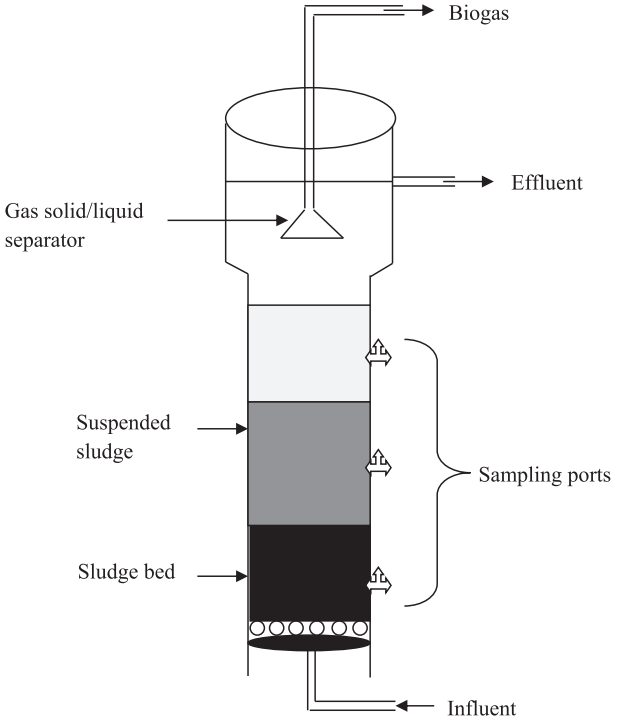

Fig. 3. Schematic configuration of an up-flow anaerobic sludge blanket (UASB) reactor.

was started by applying OLR in the range of 0.5 g VS/L·d. This period was considered as an acclimation phase for the new substrate which explains the low biogas yields. Nevertheless, by increasing the OLR a progressive increase of biogas yield was observed indicating the improvement of consortium activity to convert the hydrolyzed sludge to biogas. This result demonstrated the improvement of biogas yield of UASB reactor by using EF pretreated sludge as substrate which confirms the results of batch fermentation (Table 1). Moreover, an enhancement of biogas yield was observed during the semi-continuous fermentation in comparison to batch condition. Also, high methane percentages were registered during the fermentation of pretreated sludge in comparison to raw sample fermentation (Table 2).

The COD of reactor effluent was determined during fermentations. Table 2 gives the COD removals. A maximum COD removal about 30 % was obtained in the case of raw WAS. After crossing the bed sludge reactor, high removal of COD was registered in the case of pretreated sludge and reached 96 % at an OLR of 2.5 g VS/L·d. This COD removal is considered high compared to other results obtained by Xu et al. [32] 49.2 % and Li et al. [15] 12.5 %. The high COD removal at a TRH 7 days could be explained by the high biodegradability of EF disintegrated sludge and the purification performance of UASB reactor. This result confirms that the anaerobic UASB reactor is a promising digester design for the treatment of WAS [15,33,34].

The present study has shown the effectiveness of EF process as disintegration pretreatment for a significant improvement of sludge biogas yield about 60 %. This finding is considered important in comparison to other studies using electrolysis methods and advanced oxidation processes before anaerobic digestion of WAS (Table 3). The destruction of sludge structure and the enhancement of methane production about only 10 % was revealed by using the indigenous iron activated peroxidation pretreatment [35]. Similarly, electrochemical disintegration of WAS before AD resulted in a 18 and 20 % methane production improvement respectively in the case of pretreatment using boron-doped diamond [36] and carbon [37] electrodes. Charles et al. [38] studied the enhancement of waste activated sludge anaerobic digestion using electrolysis process with an ion exchange membrane.

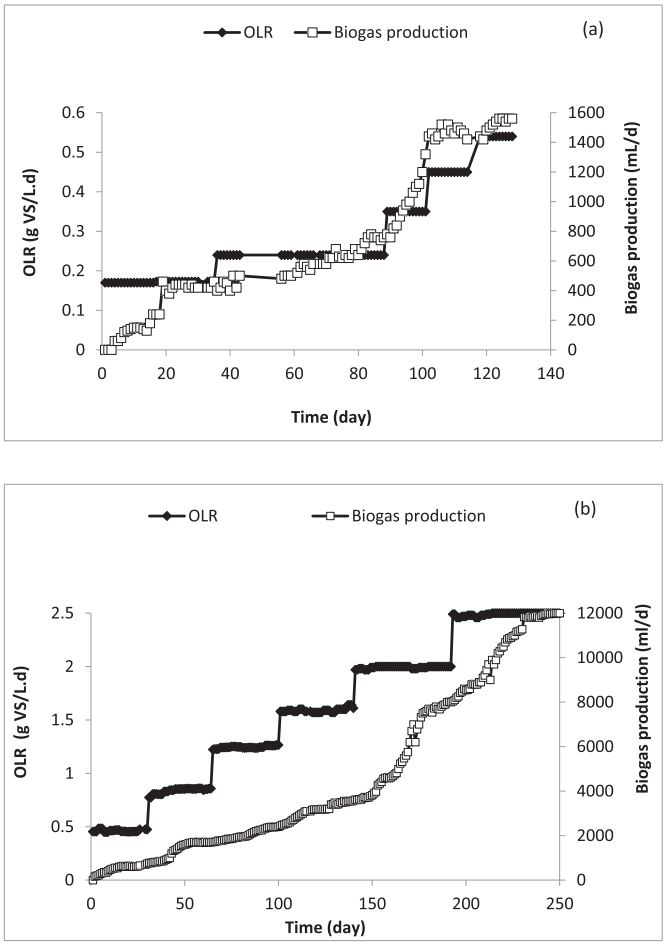

Fig. 4. Evolution of OLR and biogas production during fermentation of raw (a) and pretreated (b) sludge in UASB reactor.

Table 3
Electro-chemical pretreatment methods for the enhancement of WAS anaerobic digestion.

Pretreatment method	Methane improvement (%)	References
Fenton peroxidation process	10	Zhou et al. [35]
Electrochemical with boron-doped diamond electrodes	18	Arinas et al. [36]
Electrochemical with carbon electrode	20	Zeng et al. [37]
Electrolysis process with an ion exchange membrane	31	Charles et al. [38]

Their study mentioned that methane yield during anaerobic digestion at 20 days retention time was 31 % higher than that of untreated sludge. This comparison of results supports the conclusion that electrolysis pretreatment coupled to Fenton reaction before anaerobic digestion has the potential to significantly improve digester performance, resulting in high methane potential. Besides, the UASB digester performance could be explained by the balance between the microbial communities. In order to understand the interaction between these communities, a molecular study of sludge bacterial biodiversity in this reactor was investigated.

3.3.2. Bacterial community structure

Bacterial community structure of digested sludge samples (A: raw and B: pretreated) was monitored using DGGE method. The number and

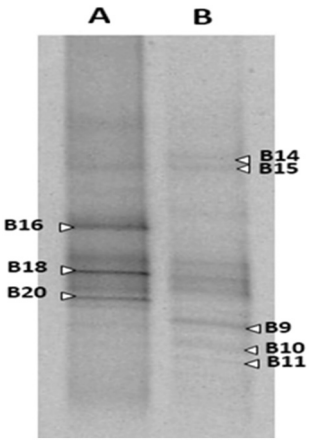

Fig. 5. Denaturing gradient gel electrophoresis (DGGE) profiles of PCR-amplified bacterial 16S rRNA gene fragments obtained from digester treating raw (A) and EF pretreated (B) sludge.

Table 4
Blast results on bacterial 16S rRNA sequences of selected DGGE bands from two sludge samples.

Band	Length (bp)	Phylogenetic affiliation (Phylum or class/ family)	Best match/accession number	Percentage of similarity (%)
B9	186	*Firmicutes Thermodesulfobiaceae*	*Coprothermobacter proteolyticus* DSM 5265/(NR_074653)	100
B10	187	*Nitrospirae Nitrospiraceae*	*Thermodesulfovibrio yellowstonii* DSM 11347/(NR_074345)	100
B11	205	*Actinobacteria Demequinaceae*	*Demequina lutea* SV45/(NR_044222)	95
B14	202	*Firmicutes Lachnospiraceae*	*Oribacterium asaccharolyticum* ACB7/(NR_125571)	98
B15	181	*Firmicutes Lachnospiraceae*	*Clostridium lavalense* CCRI-9842/ (NR_044289)	100
B16	161	*Firmicutes Lachnospiraceae*	*Clostridium symbiosum* ATCC 14940/ (NR_118730)	99
B18	196	*Alphaproteobacteria Rhodobiaceae*	*Parvibaculum lavamentivorans* DS-1/ (NR_074262)	97
B20	190	*Chloroflexi Caldilineaceae*	*Caldilinea tarbellica* D1-25-10-4/ (NR_117797)	97

intensity of migrating bands showed a distinct difference between sample A and B (Fig. 5, Table 4). DGGE profiles show only two common bands in the two samples (bands B15 and B18). Bands B16 and B20 were observed only in sample A whereas bands B9, B10, B11 and B14 were detected in sample B. In fact, 8 bands were excised from the DGGE gel and sequenced. A dendrogram was constructed in order to present the relationship of all the partial 16S rDNA sequences representing the excised DGGE bands (Fig. 5). All sequences were belonging to Firmicutes, Proteobacteria (Alpha-proteobacteria class), Nitrospira and Chloroflexi phyla (Fig. 6). These taxonomic groups are usually detected on anaerobic sludge digestion in UASB reactor [39]. Two sequences (Band B15 and B16) assigned to Clostridium species of the Lachnospiraceae family, are dominant in landfills, sludge and anaerobic reactor [40]. Therefore, in the hydrolysis steps of AD, members of Clostridium produce a wide variety of extracellular enzymes to convert cellulose, xylans, proteins, and lipids into fermentable components [41] and participate in acetogenesis step to produce precursors of methane production for methanogens [42]. Some species belong to the family Lachnospiraceae are involved into hydrolysis, acidogenesis and acetogenesis [43].

Band B14 sequence related to Oribacterium species, strictly anaerobic strains, were isolated from the human oral cavity and sinus pus [44]. Band B20 is affiliated to the genus Caldilinea of the Caldilineaceae family among the chloroflexi phylum. The sequence (Band B20) present 93 % of similarity with *Caldilinea tarbellica* and *Caldilinea aerophila* species, filamentous, thermophilic, anaerobic bacteria isolated during the anaerobic digestion of raw WAS. Some studies demonstrated that some species belong to the family Caldilineaceae are involved into acidogenesis step in the fermentation reactors [38]. Band B9 was closely related to the genus Coprothermobacter which can be classified in the phylum Firmicutes or phyla Dictyoglomi and Thermotoga [45]. The sequence of the band B9 has high similarity 99 % with *Coprothermobacter proteolyticus*, formerly *Thermobacteroides proteolyticus* that was the first isolated from digestate of a thermophilic co-digestion of tannery wastes and cattle manure [41,46]. A *Coprothermobacter platensis* was also isolated from a mesophilic digester treating a protein-rich wastewater [47].

Band B10 was related to the genus Thermodesulfovibrio which uses sulfate and other sulfur compounds [48]. These results indicated the presence of sulfate-reducing bacteria into the UASB reactor. Bands B11 and B18 sequences were related to Demequina and Parvibaculum genera, respectively. These findings illustrated the coexistence of diversified anaerobic bacteria community involved into anaerobic digestion steps in the UASB reactor [49].

Comparison of results, demonstrated that the AD system treating EF pretreated WAS favored the growth of Coprothermobacter. Previous works reported that Coprothermobacter growth is often related to the presence of proteinaceous substrate [50]. Therefore, the proliferation of Coprothermobacter in digester is related to proteinaceous material solubilization induced by the EF pretreatment. The latter caused the solubilization of organic matter highlighted by the release of simple molecules as VFA (Table 1). The high substrate availability in the feed pretreated by EF led to the increase of biogas potential and performance of fermentation which suggest the establishment of syntrophic association between anaerobic bacteria implicated into different steps of AD. According to these results, EF process could be integrated to anaerobic system of WAS for high bioenergy recovery.

3.3.3. Bacterial, archaeal and methanogens communities' abundance

The abundance of bacterial, archaeal and methanogens communities in digestate samples was reported as DNA copy numbers of 16S rRNA or mcrA genes per gram of wet sludge. Before treatment, the abundance of total bacteria and archaea were 195.6 × 108 and 4.29 × 108 whereas, after treatment, their abundances were 139.5108 and 5.47 × 108 DNA copies g-1, respectively. According to the 16S rRNA copy number in bacteria (3.82) and archaeal (1.62) genomes [51], the qPCR data were also expressed in relative percentage. Higher proportion of bacteria ranging between 96.22 and 97.85 % was obtained in comparison to archaea (accounting for 2.14–3.77 % of the total prokaryotic population) for the two samples. Assuming that one copy of the mcrA gene, methanogens accounted for 0.47 % (raw WAS) to 1.92 % (pretreated WAS) of the archaeal community. These results proved that the increased number of methanogen along the process allowed the improvement of methane production in the reactor.

4. Conclusion

The positive effect of EF treatment on the WAS disintegration was demonstrated. The EF pretreatment led to the improvement of biodegradability and bioaccessibility of organic matter. The anaerobic digestion study of pretreated sludge in UASB reactor confirmed the enhancement of biosystem performance. The anaerobic bacteria community analyzed by PCR-DGGE method indicated that EF changed the

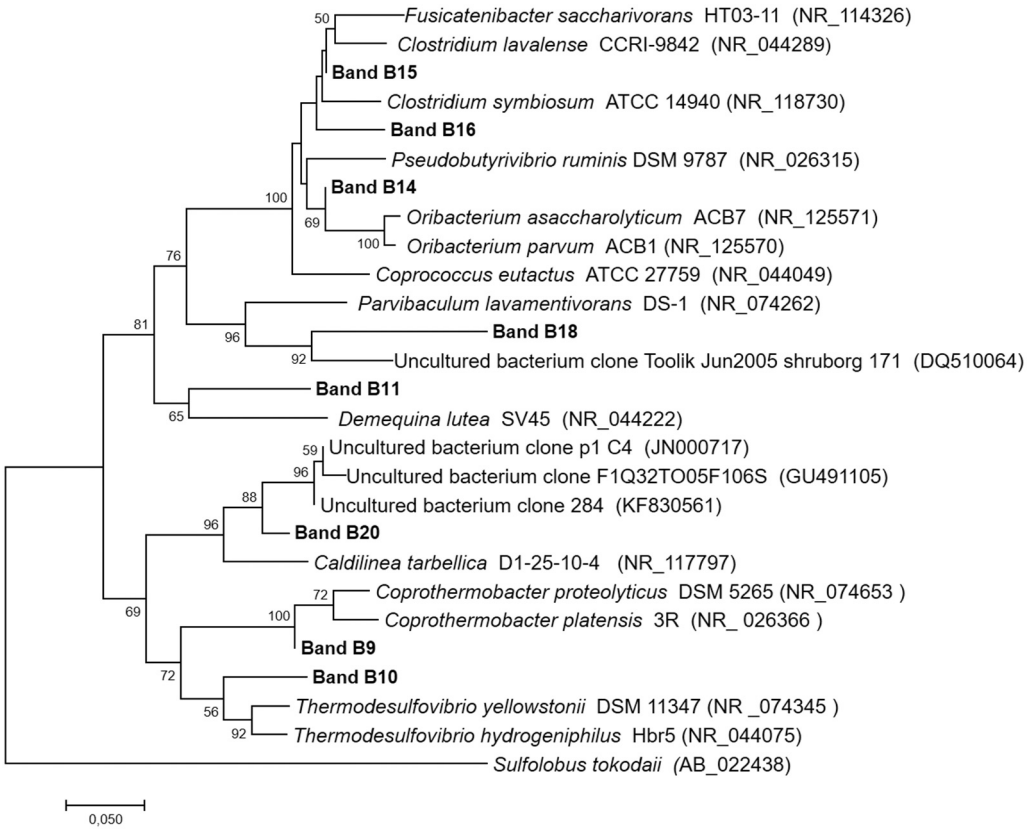

Fig. 6. Phylogenetic tree based on 16S rRNA gene excised DGGE bands compared to representatives of the field Bacteria and environmental clones.

microbial community to be enriched by the Firmicutes, Proteobacteria, Nitrospira and Chloroflexi phyla. These results illustrate a richness of microbial community, thus showing the balance of the anaerobic biosystem with the integration of EF disintegration. Bacterial, archaeal and methanogens communities' abundance was also shown to be improved in UASB reactor treating disintegrated sludge which explains the purification performance and high biogas production. In order to evaluate the techno-economic feasibility of the developed integrated process, a pilot-scale study is needed in the future. The techno-economic feasibility is dependent on the degree of sludge disintegration and methane potential but also on the energetic and environmental benefits. For this reason, carbon footprint analysis and life cycle assessment should be examined in a future work.

Declaration of competing interest

All authors declare that they have no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgements

This research was supported by a grant provided by the Tunisian Ministry of Higher Education and Scientific Research (Contract programme, 2015–2018) and the International Foundation for Science (Research Grant Agreement N° W/5412-1).

References

[1] E. Paul, Y. Liu, Sludge production: quantification and prediction for urban treatment plants and assessment of strategies for sludge reduction, in: Biological Sludge Minimization and Biomaterials/Bioenergy Recovery Technologies, 2012, pp. 81–116.
[2] H. Tong, Y.W. Tong, Y.H. Peng, A comparative life cycle assessment on mono- and co-digestion of food waste and sewage sludge, Energy Procedia 158 (2019) 4166–4171.
[3] Q. Zhang, J. Hu, D.J. Lee, Y. Chang, Sludge treatment: current research trends, Bioresour. Technol. 243 (2017) 1159–1172.
[4] M. Anjum, N.H. Al-Makishah, M.A. Barakat, Wastewater sludge stabilization using pre-treatment methods, Process Saf. Environ. Prot. 102 (2016) 615–632.
[5] Y. Qin, A. Higashimori, L.J. Wu, T. Hojo, K. Kubota, Y.Y. Li, Phase separation and microbial distribution in the hyperthermophilicmesophilic-type temperature-phased anaerobic digestion (TPAD) of waste activated sludge (WAS), Bioresour. Technol. 245 (2017) 401–410.
[6] X.J. Xu, W.Q. Wang, C. Chen, P. Xie, W.Z. Liu, X. Zhou, X.T. Wang, Y. Yuan, A.J. Wang, D.J Lee, Y.X. Yuan, N.Q.Ren, Bioelectrochemical system for the enhancement of methane production by anaerobic digestion of alkaline pretreated sludge. Bioresource. Technol. doi: https://doi.org/10.1016/j.biortech.2020.123000.
[7] W. Parawira, M. Murto, S. Read, B. Mattiasson, Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste, Process Biochem. 40 (9) (2005) 3412–3418.
[8] H. Ennouri, B. Miladi, S.Z. Diaz, L.A.F. Gûelfo, R. Solera, M. Hamdi, H. Bouallagui, Effect of thermal pretreatment on the biogas production and microbial communities balance during anaerobic digestion of urban and industrial waste activated sludge, Bioresour. Technol. 214 (2016) 184–191.
[9] R. Cano, S.I. Pérez-Elvira, F. Fdz-Polanco, Energy feasibility study of sludge pretreatments: a review, Appl. Energ. 149 (2015) 176–185.
[10] L. Appels, J. Baeyens, J. Degreve, R. Dewil, Principles and potential of the anaerobic digestion of waste-activated sludge, Prog. Energ. Combust. 34 (6) (2008) 755–781.
[11] G. Zhen, X. Lu, H. Kato, Y. Zhao, Y.Y. Li, Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: current advances, full-scale application and future perspectives, Renew. Sust. Energ. Rev. 69 (2017) 559–577.
[12] R. Dewil, D. Mantzavinos, I. Poulios, M.A. Rodrigo, New perspectives for advanced oxidation processes, J. Environ. Manag. 195 (2) (2017) 93–99.
[13] R.R. Ana, C.N. Olga, F.R.P. Manuel, M.T.S. Adrian, An overview on the advanced oxidation processes applied for the treatment of water pollutants defined in the recently launched directive 2013/39/EU, Environ. Int. 75 (2015) 33–51.
[14] R. Teguia, D. Guy, B. Noumi Domga, Tannery wastewater treatment by electro-Fenton and electro-persulfate processes using graphite from used batteries as free-cost electrode materials, Case Studies in Chem. Environ. Engin. (2022), https://doi.org/10.1016/j.cscee.2022.100190.

[15] Q. Yu, X. Chen, J.Y. Zhang, Sequential pretreatment for cell disintegration of municipal sludge in a neutral bio-electro-Fenton system, Water Res. 135 (2018) 44–56.
[16] X. Li, T. Zhu, K. Zhang, L. Lv, Y. Shen, T. Chai, Y. Wang, M. You, Y. Xie, Enhanced sludge degradation process using a microbial electrolysis cell in an up-flow anaerobic sludge blanket reactor with ultrasound treatment, Chem. Eng. J. 306 (2016) 17–21.
[17] J. Jimenez, E. Gonidec, C. Rivero, E. Latrille, F. Vedrenne, J.P. Steyer, J. Andreś, Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: towards ADM1 variables characterization, Water Res. 50 (2014) 359–372.
[18] M. Muller, M. Antonini, Y. Dudal, E. Latrille, F. Vedrenne, J.P. Steyer, Combining chemical sequential extractions with 3D fluorescence spectroscopy to characterize organic matter, Waste Manag. 31 (2014) 1916–1923.
[19] X.S. He, B.D. Xi, Z.M. Wei, Y.H. Jiang, Y. Yang, D. An, J.L. Cao, H.L. Liu, Fluorescence excitation emission matrix spectroscopy with regional integration analysis for characterizing composition and transformation of dissolved organic matter in landfill leachates, J. Hard. Mater. 190 (2013) 293–299.
[20] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (7) (2016) 1870–1874.
[21] J.R. Cole, Q. Wang, E. Cardenas, J. Fish, B. Chai, R.J. Farris, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, T. Marsh, G.M. Garrity, J.M. Tiedje, The ribosomal database project: improved alignments and new tools for rRNA analysis, Nucleic Acids Res. 37 (2009) 141–145.
[22] E.S. Wright, L. Safak Yilmaz, D.R. Noguera, A search-based approach to chimera identification for 16S rRNA sequences, Appl. Environ. Microbiol. 78 (2012) 717–725.
[23] K. Tamura, S. Kumar, G. Stecher, MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets, in: MBE Advance Access, 2016. Brief Communication.
[24] J. Felsenstein, Confidence limits of phylogenesis, Evolution 39 (1985) 783–791.
[25] M. Ben Abdallah, F. Karray, N. Mhiri, N. Mei, M. Quéméneur, J.L. Cayol, G. Erauso, J.L. Tholozan, D. Alazard, S. Sayadi, Prokaryotic diversity in a tunisian hypersaline lake, chott El jerid, Extremophiles 20 (2) (2016) 125–138.
[26] M. Ben Abdallah, F. Karray, N. Kallel, F. Armougom, N. Mhiri, M. Quemeneur, J. L. Cayol, G. Erauso, S. Sayadi, Abundance and diversity of prokaryotes in ephemeral hypersaline lake chott El jerid using illumina miseq sequencing, DGGE and qPCR assays, Extremophiles 22 (2018) 811–823.
[27] E. Feki, S. Khoufi, S. Loukil, S. Sayadi, Improvement of anaerobic digestion of waste activated sludge by using H2O2 oxidation, electrolysis, electro-oxidation and thermo-alkaline pretreatments, Environ. Sci. Pollut. Res. 22 (19) (2015) 14717–14726.
[28] M.M. Bradford, A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1968) 248–254.
[29] W. Chen, X. Gao, H. Xu, Y. Cai, J. Cui, Influence of extracellular polymeric substances (EPS) treated by combined ultrasound pretreatment and chemical re-flocculation on water treatment sludge settling performance, Chemosphere 170 (2017) 196–206.
[30] J. Xu, H. Yuan, J. Lin, W. Yuan, Evaluation of thermal, thermal-alkaline, alkaline and electrochemical pretreatments on sludge to enhance anaerobic biogas production, J Taiwan. Inst. Chem. E. 45 (2014) 2531–2536.
[31] Q. Aemig, C. Chéron, N. Delgenès, J. Jimenez, S. Houot, J.P. Steyer, D. Patureau, Distribution of polycyclic aromatic hydrocarbons (PAHs) in sludge organic matter pools as a driving force of their fate during anaerobic digestion, Waste Manag. 48 (2016) 389–396.
[32] S. Xu, L. Zhang, S. Huang, G. Zeeman, H. Rijnaarts, Y. Liu, Improving the energy efficiency of a pilot-scale UASB-digester for low temperature domestic wastewater treatment, Biochem. Eng. J. 135 (2018) 71–78.
[33] S. Chong, T.Kanti Sen, A. Kayaalp, H.Ming Ang, The performance enhancements of upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment –a state-of-the-art review, Water Res. 46 (2012) 3434–3470.
[34] T. Abbasi, S.A. Abbasi, Formation and impact of granules in fostering clean energy production and wastewater treatment in upflow anaerobic sludge blanket (UASB) reactors, Renew. Sust. Energ. Rev. 16 (2012) (2012) 1696–1708.
[35] X. Zhou, Q. Wang, G. Jiang, Enhancing methane production from waste activated sludge using a novel indigenous iron activated peroxidation pre-treatment process, Biores. Technol. 182 (2015) 267–271.
[36] C.B. Arenas, R. González, J. González, J. Cara, G. Papaharalabos, X. Gómez, E. J. Martínez, Assessment of electrooxidation as pre- and post-treatments for improving anaerobic digestion and stabilisation of waste activated sludge, J. Environ. Manag. 288 (2021), 112365.
[37] Q. Zeng, F. Zan, T. Hao, S.K. Khanal, G. Chen, Sewage sludge digestion beyond biogas: electrochemical pretreatment for biochemicals, Water Res. 208 (2022), 117839.
[38] W. Ng, B. Charles, R. Cord-Ruwisch, L. Cheng, G. Ho, A. Kayaalp, Enhancement of waste activated sludge anaerobic digestion by a novel chemical free acid/alkaline pretreatment using electrolysis, Water Sci. Technol. 67 (2013) 27–31.
[39] P.C. Burrell, C. O'Sullivan, H. Song, W.P. Clarke, L.L. Blackall, Identification, detection, and spatial resolution of Clostridium populations responsible for cellulose degradation in a methanogenic landfill leachate bioreactor, Appl. Environ. Microbiol. 70 (4) (2004) 2414–2419.
[40] B.P. Tracy, S.W. Jones, A.G. Fast, D.C. Indurthi, E.T. Papoutsakis, Clostridia: the importance of their exceptional substrate and metabolite diversity for biofuel and biorefinery applications, Curr. Opin. Biotechnol. 23 (3) (2012) 364–381.
[41] S. Jaenicke, C. Ander, T. Bekel, R. Bisdorf, M. Dröge, K.H. Gartemann, S. Junemann, O. Kaiser, L. Krause, F. Tille, M. Zakrzewski, A. Puhler, A. Schluter, A. Goesmann, Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454-pyrosequencing, PLoS ONE 6 (1) (2011), e14519, https://doi.org/10.1371/journal.pone.0014519.
[42] B.C. Si, J.M. Li, Z.B. Zhu, Y.H. Zhang, J.W. Lu, R.X. Shen, X.H. Xing, Z. Liu, Ch. Zhang, Continuous production of biohythane from hydrothermal liquefied cornstalk biomass via two-stage high-rate anaerobic reactors, Biotechnol. Biofuels 9 (2016) 254, https://doi.org/10.1186/s13068-016-0666-z.
[43] M.V. Sizova, P.A. Muller, D. Stancyk, N.S. Panikov, M. Mandalakis, A. Hazen, T. Hohmann, S.N. Doerfert, W. Fowle, A.M. Earl, E. Karen, Oribacterium parvum sp. nov. and Oribacterium asaccharolyticum sp. nov., obligately anaerobic bacteria from the human oral cavity, and emended description of the genus Oribacterium, Int. J. Syst. Evol. Microbiol. 64 (2014) 2642–2649.
[44] M.C. Gagliano, C.M. Braguglia, A. Gianico, G. Mininni, S. Rossetti, Thermophilic anaerobic digestion of thermal pretreated sludge: role of microbial community structure and correlation with process performances, Water Res. 68 (2015) (2015) 498–509.
[45] F.A. Rainey, E. Stackebrandt, Transfer of the type species of the genus thermobacteroides to the genus thermoanaerobacter as thermoanaerobacter acetoethylicus comb. Nov., description of coprothemobacter gen. Nov., and reclassification of themobacteroides proteolyticus, Int. J. Syst. Bacteriol. 43 (1993) 857–869.
[46] C. Etchebehere, M.E. Pavan, J. Zorzopulos, Coprothermobacter platensis sp. Nov., a new anaerobic proteolytic thermophilic bacterium isolated from an anaerobic mesophilic sludge, Int. J. Syst. Bacteriol. 48 (1998) 1297–1304.
[47] Y. Sekiguchi, M. Muramatsu, H. Imachi, T. Narihiro, A. Ohashi, H. Harada, S. Hanada, Y. Kamagata, Thermodesulfovibrio aggregans sp. nov. and Thermodesulfovibrio thiophilus sp. nov., anaerobic, thermophilic, sulfate-reducing bacteria isolated from thermophilic methanogenic sludge, and emended description of the genus Thermodesulfovibrio, Int. J. Syst. Evol. Microbiol. 58 (11) (2008) 2541–2548.
[48] K.W. Finster, R.A. Herbert, K.U. Kjeldsen, P. Schumann, B.A. Lomstein, Demequina lutea sp. Nov., isolated from a high Arctic permafrost soil, Int. J. Syst. Evol. Microbiol. 59 (4) (2009) 649–653.
[49] H.M. Pervin, P.G. Dennis, H.J. Lim, G.W. Tyson, D.J. Batstone, P.L. Bond, Drivers of microbial community composition in mesophilic and thermophilic temperature-phased anaerobic digestion pre-treatment reactors, Water Res. 47 (2013) 7098–7108.
[50] M.C. Gagliano, C.M. Braguglia, M. Petruccioli, S. Rossetti, Ecology and biotechnological potential of the thermophilic fermentative coprothermobacter spp. FEMSMicrobial Ecol. 91 (5) (2015) https://doi.org/10.1093/femsec/fiv018.
[51] D.L. Sun, X. Jiang, Q.L. Wu, N.Y. Zhou, Intragenomic heterogeneity of 16S rRNA genes causes overestimation of prokaryotic diversity, appliedEnviron. Microbiol. 79 (2013) 5962–5969.