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Bacterial Community Structure in Anaerobic Digesters of a Full Scale Municipal Wastewater Treatment Plant – Case Study of Dubai, United Arab Emirates

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ABSTRACT

A highly complex microbial community involved in anaerobic sludge digesters plays vital roles in sludge treatment. The data on microbial ecology is important to accomplish efficient operation of the anaerobic digesters. This study is aimed at monitoring the bacterial community of three full-scale anaerobic digesters of a full-scale municipal wastewater treatment Plant in Dubai, United Arab Emirates. Fluorescent *in-situ* hybridization technique was applied to identify the bacterial groups and quantitative polymerase chain reaction to compare the richness of bacterial and archaeal domain. Results of the fluorescent *in-situ* hybridization technique analysis showed that the phylum *Proteobacteria* was most abundant followed by cytophage-*Flavobacterium* group of *Bacteroides*, *Firmicutes* and *Actinobacteria*. Among proteobacterial subclass *Delta-* and *Alpha-* were dominating than *Gamma-* and *Beta-proteobacteria*. The genus *Desulfobacter* and *Desulfobacterium* were the dominant groups hybridizing 70-76% of total 4', 6'– *diamidino* – 2 *phenylindole* stained cells. The quantitative polymerase chain reaction results showed that Bacterial domain was dominating in all three digesters compared to the archaeal domain.

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KEYWORDS

Anaerobic digester, Bacterial community, Fluorescence in situ hybridization, Oligonucleotide probes, Quantitative polymerase chain reaction.

INTRODUCTION

Gaining knowledge on the association between microbial community and wastewater treatment efficiency is critical for the effective operation of wastewater treatment plants. Several studies had led to quantification and classification of important microorganisms capable of the treatment of wastewater biosolids over the past few years [1-3]. Anaerobic digestion is a widely used method for wastewater biosolids treatment, which reduces the impact of the organic pollutants on the environment. Anaerobic degradation of this biological waste is carried out by various bacterial species present in the digesters including hydrolytic, acid forming, acetogenic, and methanogenic archaea that produce Carbon dioxide (CO_2) and Methane (CH_4) as by products [4]. Each step is driven by a group of microorganisms. To confirm a steady process, it is vital to uphold equilibrium in reaction rate among the four steps [5]. The first step is hydrolysis in which the complex substance is hydrolyzed into monomers and dimers such as glucose and amino acids. Two phyla that consist mostly of the hydrolytic bacteria are *Firmicutes* and *Bacteroidetes* mainly in the genera *Streptococcus*, *Acetivibrio*, *Enterobacterium* and *Clostridium* [6]. The second stage is acidogenesis in which acid forming bacteria ferment the hydrolytic products into volatile fatty acids, acetate and hydrogen. The phyla that contain many known species of acidogens are *Firmicutes*, *Bacteroidetes*, *Chloroflexiand Proteobacteria* [7]. *Lactobacillus* in the phylum *Firmicutes*, *Anaerolinaceae* in the phylum *Chloroflexi*, *Bifidobacterium* in the phylum *Actinobacteria* and a few thermophilic bacteria in the phylum Thermotogaecontain non-hydrolytic acidogens [8]. In the third stage, some of the acid phase intermediate products that cannot be directly used by methanogens are converted into acetate and hydrogen, which can then be used by methanogens. The hydrogen released during acetogenesis exhibits toxic effects on acetogens hence this process takes place in a symbiotic relationship between acetogens and autotrophic methanogens [9]. The acetogens belong to the genera *Syntrophomonas* and *Syntrophobacter* (in the phylum *Firmicutes* and *Proteobacteria*) [10]. The last stage is methanogenesis in which most commonly observed methanogenic genera such as *Methanolinea*, *Methansaeta*, and *Methanospirillum* produce methane using the by-products of previous stages [11].

In the anaerobic digesters along with methanogens and acetogens, sulfate-reducing bacteria are also found. In the presence of sulphate they multiply which often requires hydrogen and acetate, which are the substrates utilized by methanogens [12]. A competition occurs between the two bacterial groups for hydrogen, as both the groups need hydrogen. In such situation sulfate reducing bacteria reap hydrogen and acetate more effortlessly than methanogens [12]. The hydrogen sulphide produced by sulfate reducing bacteria on the degradation of sulphate exhibits inhibitory effects at low levels on methanogens and acetogens than on acidogens. Synergistic relationships exist between acetogens and methanogens for methane production. As a result of digestion, microorganisms metabolize fatty acids and alcohols during which Syntrophic bacteria produce Adenosine Triphosphate (ATP) [13]. Methanogens then utilize these compounds after being converted into acetate and hydrogen. *Syntrophomonas* genus produces acetate, hydrogen and CO_2 upon oxidation of organic acids, which are used by methanogens [9]. This syntrophic association of methanogens and acetogens play a role in the oxidation of propionate, which is likewise a vital phase of methanogenesis process [4]. Another kind of symbiosis is seen between methanogens and bacterial group, which is mostly sulfate reducing bacteria belonging to δ sub division of *Proteobacteria* [14].

To enumerate the presence and relative richness of microbial populations in the sample Fluorescence In Situ Hybridization (FISH) technique is commonly used. FISH is a taxonomic method, which is used for identifying the presence of various phylogenetic groups in an environmental sample. It also provides the direction visualization of the microbial cells. Therefore, hybridization with rRNA-targeted probes has dramatically increased the efficiency of characterization of uncultured microorganisms in a given sample [15].

One of the major wastewater treatment plants in Dubai, UAE is the Jebel Ali Wastewater Treatment Plant (JAWWTP) whose efficient operation and maintenance is indispensable for the city of Dubai. A detailed understanding of microbial community structure and functions is vital for the sustainable management of biosolids generated at various stages of wastewater treatment processes. This study is aimed at monitoring the bacterial community in the anaerobic digesters of a full-scale municipal wastewater treatment Plant in Dubai over a period of five months. FISH technique was employed on the samples with previously published probes for identifying the bacterial community structure of the anaerobic digesters. Series of probes targeting phyla, groups and subgroups were used. For comparing the abundance among bacteria and archaea domain real-time quantitative Polymerase Chain Reaction (qPCR) was used.

MATERIALS AND METHODS

A total of 15 sludge samples were collected from three full-scale anaerobic digesters (1, 3, and 5) from JAWWTP, UAE on a monthly basis. Out of three, digester no. 3 is the oldest and digester no. 5 is the newest. All three anaerobic digesters were operating at a mesophilic temperature 32-37 °C. The capacity of each digester was 7,433 m^3. All three digesters were fed with 60% of raw sludge and the 40% of activated sludge. The operating physiochemical parameters of anaerobic digesters at the time of sample collection are described in Table 1.

The samples were collected from the anaerobic digesters into autoclaved plastic bottles. The bottles were placed in an icebox and brought to the laboratory within an hour. The collected samples were stored at 4 °C until DNA extraction and fixation of biomass for FISH analysis. The samples were fixed with paraformaldehyde within 24 hours. After fixation the samples were stored at −20 °C.

Table 1. Anaerobic sludge digester operational parameters

Parameters	Digester-1	Digester-3	Digester-5
Digester capacity [m^3]	7,433	7,433	7,433
pH*	7.13-7.5	7.27-7.55	7.36
Temperature [°C]	34	34	34
Digester feeding per day [m^3]	2,248	2,148	2,552
Solid retention time (days)	16	16	14
Up flow velocity [m^3/hr]	120	120	120
Hydraulic Retention Time (HRT) (days)	3.3	3	2.91
Organic loading rate [kg oDS/m^3d]	6.84	5.84	6.61
Dry solid* [%]	2.91-3.34	2.56-5.74	2.79-3.54
Volatile solids* [%]	70.27-70.95	43.75-70.15	54.54-67.49
Volatile fatty acid*	165-195	168-205	145.5-195
Alkalinity*	3,014-3,451	2,992-3,512	2,893-3,190
Dissolved sulfide* [mg/L]	37.2-38	32.4-37.2	26.4-27.6

* Minimum to maximum range observed over the sampling period

The DNA is extracted from the samples obtained from the anaerobic sludge digesters within 24 to 48 hours. Total community DNA was extracted from the samples using the PowerSoil DNA Isolation Kit (MO BIO Labs. Inc., Solana Beach, CA) according to the

manufacturer's guidelines. The qPCR was performed to relatively quantify and compare the abundance of bacteria with archaea using comparative Cycle Threshold (CT) method (ΔΔCT). The qPCR amplification was performed in 20 µl reactions. Each reaction contained 1 µl of 20× reaction mixture (5 µl of 10 µM forward primer, 5 µl of 10 µM reverse primer, 5 µl of 5 µM probe and 85 µl of PCR grade water), 10 µl of the TaqMan master mix, 1 µl of DNA sample and 8 µl of PCR grade water. Reactions were performed in duplicates with one control. The reactions were run on an Applied BiosystemsStepOnePlus™ Real-Time PCR System. The following PCR program was used for all samples: An initial denaturation at 95 °C for 10 minutes followed by 40 cycles (denaturation at 95 °C for 15 seconds and annealing/extension at 60 °C for 1 minute). The details of respective primer and probe are given in Table 2.

Table 2. Characteristics of the probe and primer

Primer	Target	Function	Sequence	Reference
ARC	Archaea domain	F primer	ATTAG ATACC CSBGT AGTCC	[16]
		Taqman probe	AGGAA TTGGC GGGGG AGCAC	
		R primer	GCCAT GCACC WCCTC T	
BAC	Bacterial domain	F primer	ACTCC TACGG GAGGC AG	[16]
		Taqman probe	TGCCA GCAGC CGCGG TAATA C	
		R primer	GACTA CCAGG GTATC TAATC C	

The composition of the bacterial communities in this study was determined by using various oligonucleotide probes [17]. Slides were washed with acid alcohol, dried and coated with poly-L-lysin by placing them in the Coplin jars containing the poly-L-lysine solution. The slides were then dried. Approximately 1 ml of the sample obtained from the sludge digesters were fixed in formaldehyde. An aliquot of 1-3 µl of formaldehyde fixed-cell samples were applied to the wells on poly-L-lysin-coated slides allowed to air dry and dehydrated in a series of ethanol solution (50%, 80% and 96%, 3 minutes each). The slides were air dried, and in each well 10 µl of hybridization mixture (containing 9 µl of hybridization solution and 1 µl of oligonucleotide probe) was added (Table 3). The slides were incubated at 37 °C for 4 hours in a moisture chamber for hybridization. Slides were rinsed with 1 ml of the pre-warmed (48 °C for 30 minutes) washing solution. The slides were washed by placing the slides in chambers containing 30 ml of respective washing solution. The slides were then air dried and visualized under Fluorescent Microscope, OlympusBX-51 Series connected to a digital camera DP-72.

Table 3. Sequence of oligonucleotide probes used in this study

Probe name	Sequence (5'-3')	Target	FA [%]	Rank	Reference
LGC354a	TGGAAGATTCCCTACTGC	*Firmicutes* (gram positive with low G + C%)	35	Phylum	[18]
LGC354b	CGGAAGATTCCCTACTGC				
LGC354c	CCGAAGATTCCCTACTGC				
Gam42a	GCCTTCCCACATCGTTT	*Ɣ-proteobacteria*	35	Class	[19]
Bet42a	GCCTTCCCACTTCGTTT	*β-proteobacteria*	35	Class	[19]
SRB281	TCAGACCAGCTAACCATC	Various *δ-proteobacteria*	10	Class	[20]
ALF1b	CGTTCGYTCTGAGCCAG	*α-proteobacteria*	20	Class	[19]
HGC69a	TATAGTTACCACCGCCGT	*Actinobacteria* (high G + C grampositive bacteria)	25	Phylum	[21]
129	CAGGCTTGAAGGCAGATT	*Desulphobacter*	15	Genus	[22]
221	TGCGCGGACTCATCTTCAAA	*Desulphobacterium*	35	Genus	[22]
CF319a	TGGTCCGTGTCTCAGTAC	*Cytophagagroup* of the *Bacteroides*	35	Genus	[23]
EUB338I	GCTGCCTCCCGTAGGAGT	Bacterial domain	25 and 35	Domain	[24]
EUB338II	GCAGCCACCCGTAGGTGT				
EUB338III	GCTGCCACCCGTAGGTGT				

[*] Probes EUB338I, EUB338II, and EUB338III were equimolarly mixed together to obtain the EUB-mix, the probes LGC354a, LGC354b, and LGC354c were equimolarly mixed together to obtain the LGC-mix

RESULTS AND DISCUSSION

The JAWWTP consists of five full-scale anaerobic digesters designated as AD1-5. For this study sludge samples were obtained from AD 1, 3 and 5. The three digesters in order of age, newest to oldest, is AD 5, AD1 and AD 3. The relative abundance of bacterial groups was examined by performing FISH with bacteria-specific probes (EUB338 mix) and 8 different bacterial group-specific probes (Table 2).

Under optimal hybridization conditions, specific groups of bacteria were observed and detected using the corresponding probes. Figure 1 shows representative epifluorescence micrographs of the targeted bacterial cells in the anaerobic digester sludge samples. Most of the bacterial community got hybridized with EUBmix probe (targeted at eubacterial domain). The percentage of cells hybridized by the probe EUBmix ranged between 54-89% of total 4', 6'-diamidino-2 phenylindole (DAPI) stained cells, in all the three digesters throughout the study period. EUBmix probe targeted cells of various morphologies like *cocci*, rods and filaments. A few *diplococci* and short rods could be observed (Figure 1).

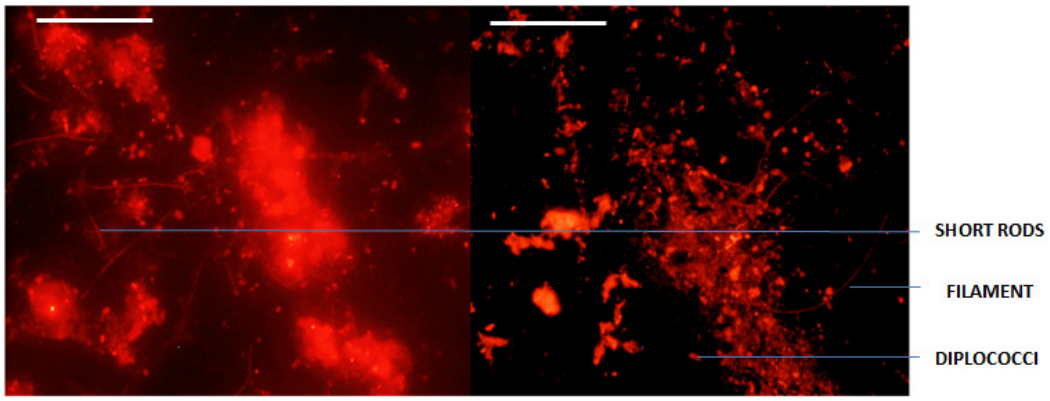

Figure 1. Epifluorescence micrograph showing in situ hybridization with probe EUBmix – Cy3, scale = 10 μm and applies to all photomicrographs (original magnification: 1,000×)

Results from the FISH analysis for each digester throughout the sampling months are shown in Figure 2.

The samples were investigated for the population of different phyla. It was observed that *Actinobacteria* (24.27-25.24% of the total bacteria) constituted the lowest population in digester 3 and 1, when compared to *Firmicutes* (30.57-36.68%) and *Cytophaga-Flavobacterium* (CF) group of *Bacteroidetes* (37.19-31.32%). Conversely, *Actinobacteria* (32.88%) was higher than *Firmicutes* (30.2%) in digester 5. CF group was dominating in digester 5 and 3 compared to *Firmicutes* and *Actinobacteria*. Among the probes targeted, the proteobacterial subclasses *Delta-* and *Alphaproteobacteria* (between 38.5% and 44.4%, respectively) was dominating than *Gamma-* and *Betaproteobacteria* (between 24.8% and 35% respectively) in all digesters. Delta appeared to occur more than Alpha in all digesters whereas Gamma was dominating than Beta in digester 3 and vice versa in digester 5. They were equally dominating in digester 1.

The members of the genus *Desulfobacter* (72.15%) and *Desulfobacterium* (73.3%) of class *Deltaproteobacteria* occurred in high numbers consistently not only in digester 1 but also in other two digesters throughout the study period, except in three samples (Figure 2). The prior presence of a large amount of Desulfovibrio and Desulfobacterium group and a smaller proportion of other SRB could be attributed to the immediate reduction of sulphate [25]. Results obtained in this study were different from the study of Raskin *et al.* [25] and Griffin *et al.* [26], wherein low levels of *Desulfobacter* and *Desulfobacterium* were observed in the anaerobic digesters. Griffin *et al.* [26] reported

that the low levels of feed sulphate were responsible for low population of SRB, making the survival of the SRB's difficult consequently resulting in high methane production.

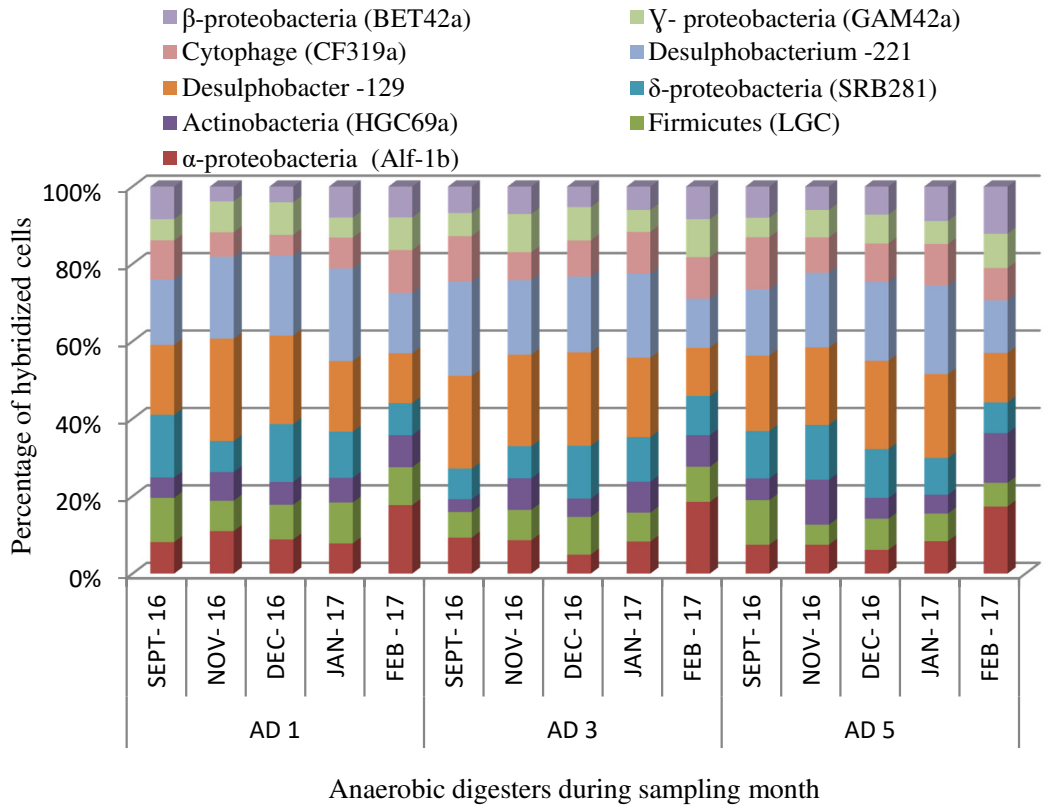

Figure 2. FISH targeted cells for each of the anaerobic digesters for different sampling period

Since the SRB can compete with methanogenic bacteria for hydrogen and acetate, the high concentration of SRB might reduce the overall methane yield. Some previous studies have reported SRB can grow in sulfate-restricted environments [27] due to their aptitude to syntrophically grow with methanogens in the absence of sulphate [28]. A study conducted by Raskin *et al.* [29] reported less fraction of Desulfobacter and a comparatively high fraction of Desulfobacterium in methanogenic reactors. But the average of cells hybridized by Desulfobacterium was less compared to this study (Figure 3).

At the phylum level, the most abundant bacterial groups were found to be *Proteobacteria* followed by CFB group of *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. Abundance levels though slightly different but almost similar levels of population were observed in previous study [30]. However, different results were obtained in the study of Zhao *et al.* [31], who observed that Firmicutes was predominant phyla, representing 92.3% of overall sequences in anaerobic sludge. Sundberg *et al.* [2] reported Firmicutes as dominant *phyla* and *Proteobacteria*, as less compared to *Bacteroidetes* and *Actinobacteria*. Another study conducted by Nelson *et al.* [3] utilizing meta-analysis of accessible sequences in public databases from anaerobic digesters showed *Proteobacteria* and *Chlorofexi* as the dominant groups which are to some extent consistent with the results of this study, where *Proteobacteria* was dominating in all three digesters throughout the study period.

Many groups of bacteria, like *Alpha-*, *Beta-*, *Gamma-*, and *Delta-proteobacteria* are well-known glucose, butyrate, propionate, and acetate-utilizing microbial communities in the sludge. And hence, *Proteobacteria* are the important microbes in the process of anaerobic digestion [32]. Among *Proteobacteria*, *Delta-* and *Alphaproteobacteria* were

predominant class that is in concurrence with a previous study [2], where *Deltaproteobacteria* constituted up to 7% of the total bacteria while other groups of *Proteobacteria* only contribute less than 1% of the total population.

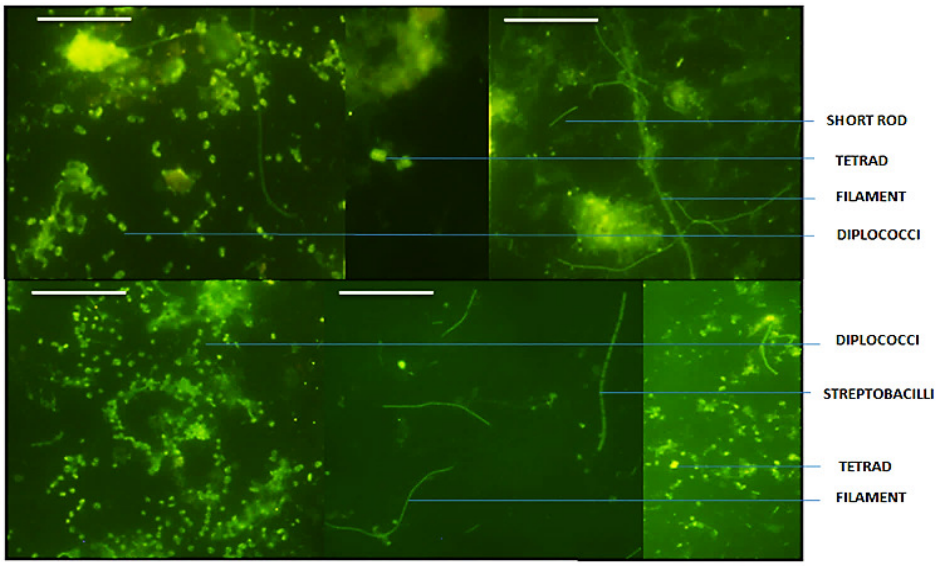

Figure 3. Epifluorescence micrograph showing in situ hybridization with probe; 129 – FITC (a) and 221 – FITC (b) (scale bar = 10 μm)

The probe SRB281 targeted rods, which were dominating, and these rods occurred in *diplobacillus* and *streptobacillus* arrangement (Figure 4) but single cell rods were dominating, probably *Syntrophobacter* species. A few of the *cocci* targeted by the probe SRB281 was in tetrad arrangement. *Deltaproteobacteria* comprises of sulphate reducers and syntrophic bacteria (*Syntrophobacter*) that metabolise propionate, a main intermediate in the anaerobic digestion process, in the relationship with hydrogenotrophs [33].

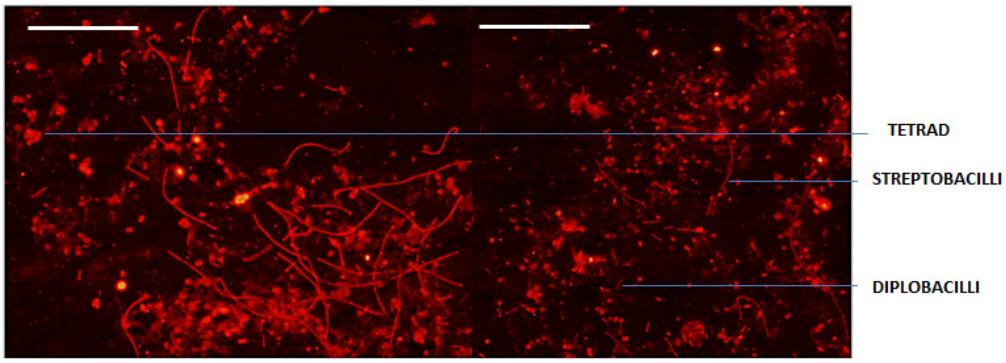

Figure 4. Epifluorescence micrograph showing in situ hybridization with probe SRB281 – Cy3, (scale bar = 10 μm)

Significant population of *cocci* arranged in tetrads were reported by Seviour [34], while fewer single cell rods as identified by the probe Alf1b probably belonging to alpha-subclass of *Proteobacteria* were also observed [35] (Figure 5a). In the samples targeted by Gam42a several *cocci* and comparatively less short rods probably *Enterobacterium* and filaments were seen as reported in a pervious study [35]. Very few diplococcus and tetrad arrangements of *cocci* were observed (Figure 5b). The probe Bet42a identified filaments, rods and *cocci*. The rods were arranged in chains (*Streptobacilli*) and few *cocci* were arranged as *diplococcus* (Figure 5c).

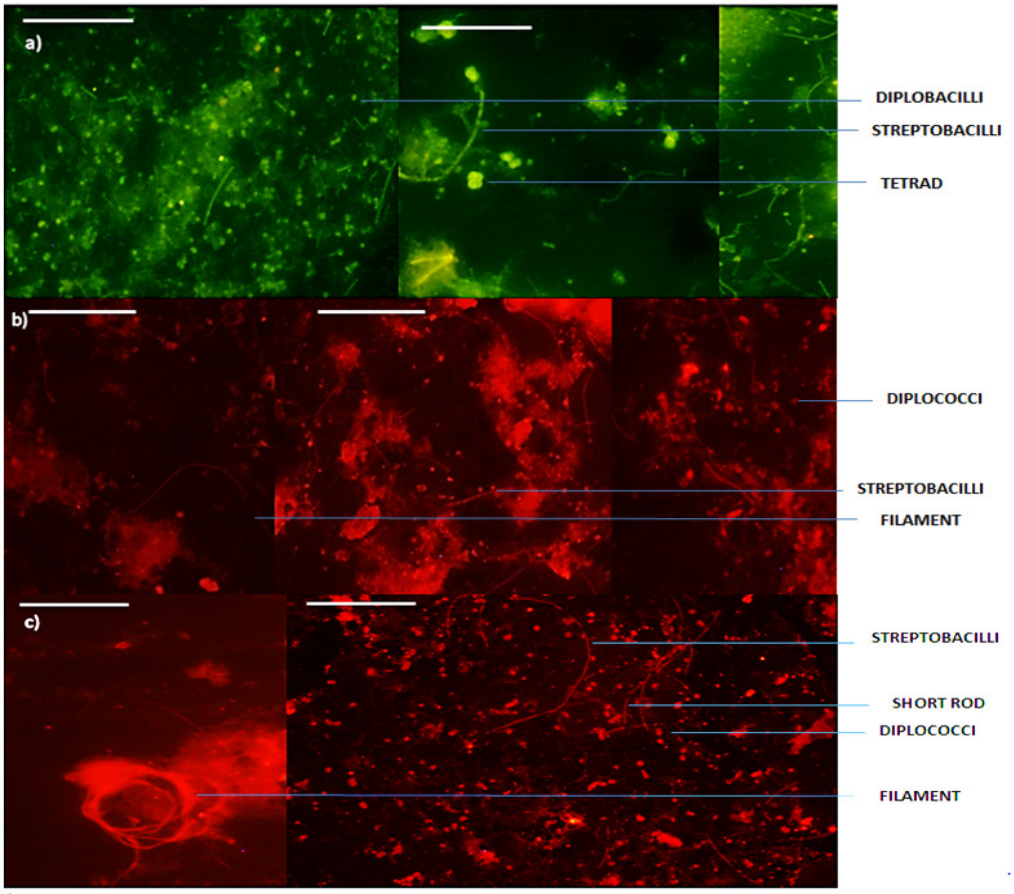

Figure 5. Epifluorescence micrograph showing in situ hybridization with probe; Alf-1b – FITC (a); Gam42a – Cy3 (b) and Beta42a – Cy3 (c) (scale bar = 10 μm)

The second most dominant phyla were *Cytophaga-firmicutes* group of *Bacteroidetes* in the anaerobic digesters. The *Bacteroidetes* comprises of fermentative bacteria, which is assumed to have a critical role in fermenting the organic compounds and acids into CO_2 and hydrogen (H_2) [36]. The probe CF319a targeted *cocci*, short and chains of rods and few filaments (Figure 6), similar morphology has been reported by in a previous study [36].

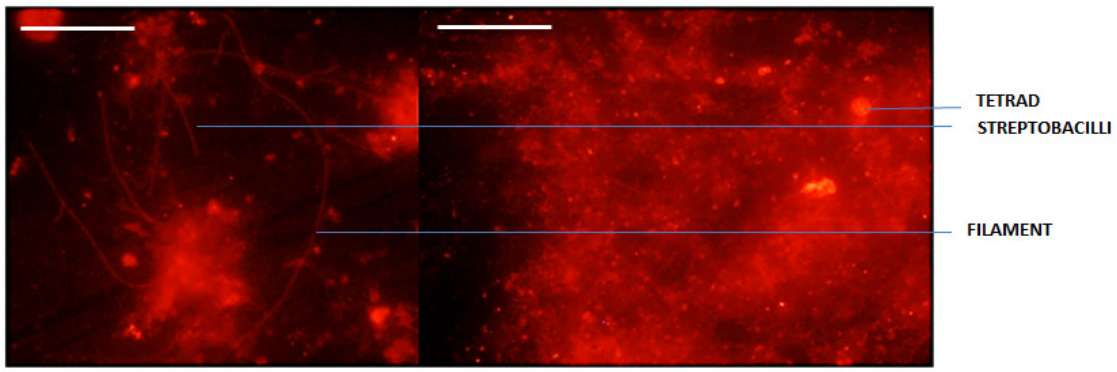

Figure 6. Epifluorescence micrograph showing in situ hybridization with probe CF319a (scale bar = 10 μm)

Most of the members belonging to the *Firmicutes phylum* are syntrophic bacteria that can break down various volatile fatty acids, acetate, valerate, butyrate, isobutyrate and propionate. They are often detected in anaerobic digesters [37].

Syntrophomonadaceae group, which belongs to *Firmicutes*, utilises butyrate for the production of acetate during digestion [38]. In this study, the members of *phylum Firmicutes* were not targeted. LGC mix probe targeted mostly *cocci* and rods. The rods were found to be in both single celled and in chains (*Streptobacilli*) (Figure 7). The single celled curved rods could probably be *Syntrophomonadaceae* and similar morphology was observed in another study [39]. Quite a few numbers of tetrads were also identified.

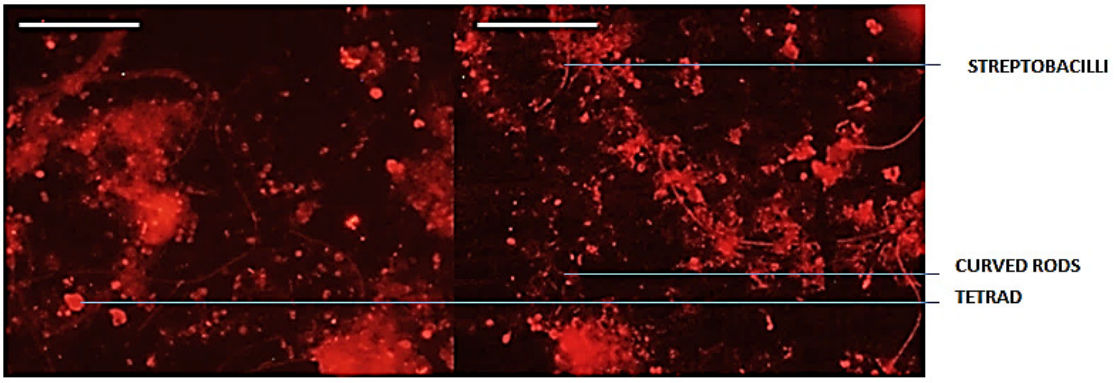

Figure 7. Epifluorescence micrograph showing in situ hybridization with probe LGCmix (scale bar = 10 μm)

The *phyla Actinobacteria* were least dominant in all the digesters, probe HGC69a identified filaments, *cocci* and few rods. Filaments were slightly dominant than *cocci*. *Streptococci* and few *Streptobacilli* were also observed (Figure 8).

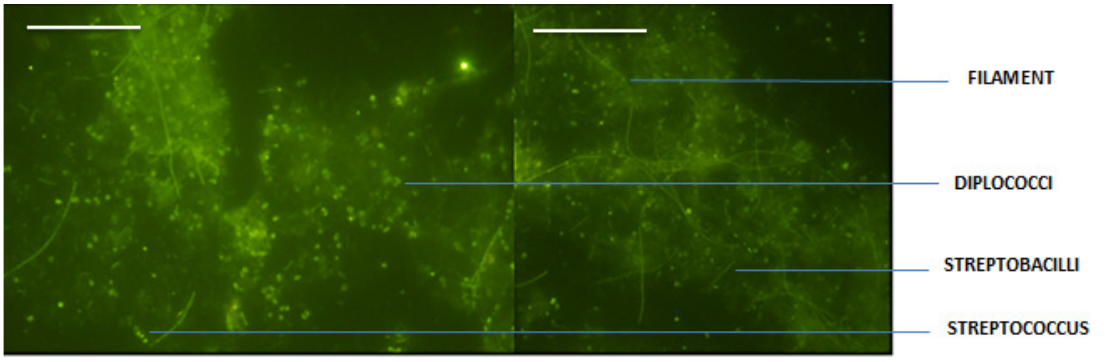

Figure 8. Epifluorescence micrograph showing in situ hybridization with probe HGC69a – FITC (scale bar = 10 μm)

Microbial community structures in full-scale anaerobic reactors have been reported earlier employing metagenomics sequencing approach [1]. This study revealed that *Proteobacteria* was the most dominant phylum, followed by *Cytophaga* group of *Bacteroidetes*, *Firmicutes* and *Actinobacteria*, which is consistent with the previous study [2]. Additionally, certain other studies also have reported the bacterial community structure with some disparities in the predominance of population [2]. These variations in the predominant populations may be related to various influent characteristics and operational conditions, which have been reported to strongly influence the microbial community structure [40].

The bacterial structure of each sample was almost consistent in all months barring a few variations in the taxonomic profile. Only during one occasion a considerable change was detected, that was in the month of February, wherein *Alphaproteobacteria* class was dominating in all digesters compared to other groups (Figure 2) and also the percentage of cells hybridized by the probe Gam42a was highest compared to other sampling months.

The abundance of bacteria and archaea was tested using qPCR comparative CT method (ΔΔCT). The relative quantification was performed for the sludge samples collected in three consecutive months, namely November, December and January. The low CT value indicates high population of taxa in the target sample, as the CT values are inversely proportional to the concentration of target.

The qPCR results indicated that in all three anaerobic digesters the members of domain bacteria were higher than the domain archaea except in two samples where in the population of both bacteria and archaea were almost equal (Figure 9). The CT value of the bacterial domain across all digesters throughout the sampling period ranged between 14.71 and 20.37, whereas for the archaeal domain it ranges between 18.49 and 22.55.

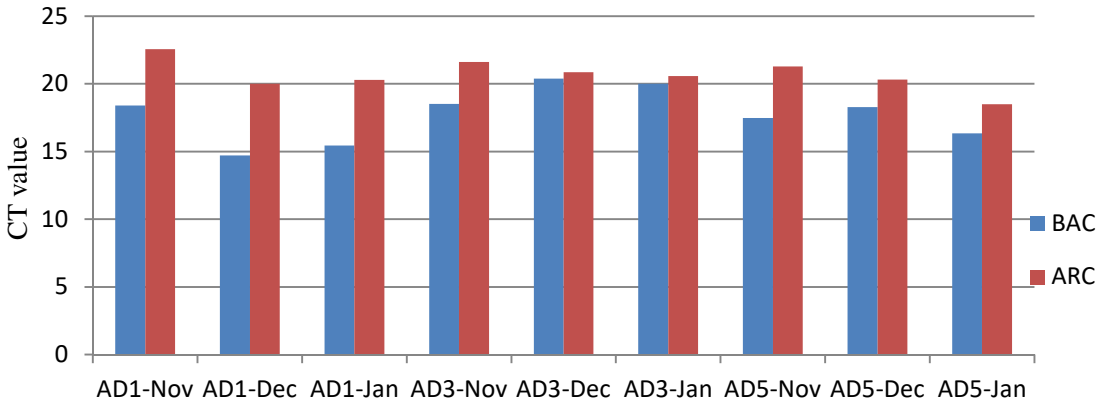

Figure 9. CT values of probe BAC and ARC for each of the digester in different sampling periods

Regueiro *et al*. [40] studied the microbial community of six full-scale anaerobic digesters with different biomasses and reported the dominance of bacterial population in all biomasses compared to the archaeal community. Also, the high diversity of bacterial community was observed by Regueiro *et al*. [40] compared to the archaeal community. In this study, FISH analyses also had shown similar results, the number of cells hybridized by EUBmix ranges between 54-89%, whereas cells hybridized by ARC915 ranges between 27.24-39.19%. The dominance of the bacterial community compared with archaeal community found in this study is in agreement with prior studies [41, 42].

CONCLUSIONS

To improve the digestion process in any sludge, the knowledge of microbial community involved and their function is vital. Therefore, this study aimed at understanding the microbial community structure of full-scale anaerobic digesters of a full-scale municipal wastewater treatment plant in the UAE by employing FISH and qPCR, wherein qPCR was mainly used to study the abundance of the bacterial and archaeal domain through comparative C_T method.

FISH analysis indicated that *Proteobacteria* was most abundant phylum followed by CF group of *Bacteroidetes*, *Firmicutes* and *Actinobacteria* in all digesters. In digester 1 and 3, almost similar trends of bacterial community structure was observed at different time periods. The genus *Desulfobacter* and *Desulfobacterium* were the most dominant single genera in all the digesters except in three samples, hybridizing with 70-76% of cells against total DAPI stained cells. These are sulfate reducing bacteria, which are usually found in anaerobic digesters along with acetate forming bacteria, and methane-forming bacteria. The second most dominant were *Deltaproteobacteria* targeted by the probe SRB281 and *Alphaproteobacteria* targeted by Alf1b.

Deltaproteobacteria comprises of sulphate reducers and syntrophic bacteria. The probe SRB281 identified more rods mostly single-celled which could be probably genus *Syntrophobacter*. The third most dominant group of the bacteria was CF group of *Bacteroidetes*. The *Bacteroidetes* consists of fermentative bacteria, which are capable of hydrolysing and fermenting the organic substances and acids into CO_2 and H_2.

The methane production can be achieved through a step wise process where each step is carried out by different microorganisms in a full-scale anaerobic digester.

qPCR results showed that domain bacteria was more dominant than archaea in all digesters throughout the study period except in two samples where they were present in equal amounts. The members of archaea are only responsible for methanogenesis but the members of the bacteria account for other stages, which take over most of the functions. The diverse bacterial community structure is just an example of their role.

This study provides insights into the microbial community structure ofanaerobic digesters of a full-scale municipal wastewater treatment plant in the UAE. Future work could focus on using high-throughput next-generation sequencing methods for in-depth understanding of the microbial community structure. Also, FISH analysis with newly designed probes targeting genus and species level is likely to provide more details on the microbial functional diversity.

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NOMENCLATURE

Greek letters

ΔΔCT	Comparative CT

Abbreviations

AD	Anaerobic Digester
ATP	Adenosine Triphosphate
CF	Cytophaga-Flavobacterium
CT	Cycle Threshold
DAPI	4', 6'-diamidino-2 phenylindole
FA	Formamide
FISH	Fluorescence *In Situ* Hybridization
JAWWTP	Jebel Ali Wastewater Treatment Plant
qPCR	Quantitative Polymerase Chain Reaction
VFA	Volatile Fatty Acid

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