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Effect of Seasonal Variation on Anaerobic Treatment of Organic Municipal Solid Waste-II: Population Dynamics of Bacteria and Archaea Communities

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Abstract

This study was designed to investigate the effect of seasonal variation on population dynamics of bacteria and archaea communities during anaerobic treatment of organic municipal solid waste. The waste was subjected to anaerobic treatment inside one-stage 250 L-capacity batch-type mesophilic poly-tank reactors with used volume of 230 L, substrate concentration of 5.53% total solids, rumen juice as the source of microbial inoculum and a retention time of 84 days. The first Anaerobic Digestion (AD_H) treatment was conducted during the dry season (between February and April, 2016) while the second Anaerobic Digestion (AD_C) treatment, a repeat of the first process, was conducted during the rainy season (between July and October, 2016). To monitor performance of the anaerobic treatment process, populations of selected bacteria and archaea groups and biodegradation of the feed where estimated with time using standard methods. Inside the AD_H system, the population of Acetoclastic Methanogens (AMA), Hydrogenotrophic Methanogens (HMA), Strict Anaerobic Bacteria (SAB) and Facultative Anaerobic Bacteria (FAB) ranged from not-detected to 8.76×10^6 CFU/ml, not-detected to 7.93×10^6 CFU/ml, 1.45×10^6 CFU/ml to 4.18×10^7 CFU/ml and 1.2×10^6 MPN/ml to 7.5×10^7 MPN/ml respectively with time. Inside the AD_C system, the population of AMA, HMA, SAB and FAB ranged from not-detected to 1.46×10^6 CFU/ml, not-detected to 1.05×10^6 CFU/ml, 9.2×10^5 CFU/ml to 1.85×10^7 CFU/ml and 2.4×10^6 MPN/ml to 1.2×10^8 MPN/ml respectively with time. Biodegradation of the feed inside the AD_H system and the AD_C system, increased to 97.21% and 75.86% respectively after 84 days. Microbial populations inside the AD_H-system increased significantly more than the microbial populations inside the AD_C-systems with time, leading to a significantly ($p<0.05$) better performance of the AD_H-system than the AD_C-system with respect to biodegradation of the feed. Therefore, seasonal variation appears to have influenced the population dynamics of microbial communities during anaerobic degradation of the waste.

Keywords: Municipal solid waste; Anaerobic treatment; Seasonal variation; Microbial community

Introduction

Anaerobic conversion of biomatter to biogas usually includes four stages namely hydrolysis, acidogensis, acetogenesis and methanogenesis, in which various microbial groups play distinct roles [1]. Successful biogas production is based on a stable and adaptable microbial community structure which depends on the type of substrate used and several physico-chemical conditions in the bioreactor. Monitoring those and the dynamics of microbiota is important for planning and optimizing the biogas process, avoiding critical points and reaching the maximum methane yield [2]. The study of microbial communities represents another area of research in the field of anaerobic digestion [3-5]. It is well known that, a deeper knowledge into microbial community dynamics, would provide information in order to, for example, predict system performance under a given set of conditions, or design engineered systems to foster the development of specific communities and to optimize the process for gas production [6].

Engineered systems offer a controlled environment in which complex microbial communities can be studied using cultural and modern culture-independent techniques that can provide an unbiased view of community composition [5]. The research for understanding microbial ecology to improve the efficiency and robustness of AD systems is still on-going [4,6]. Culture-independent molecular techniques have been used to characterize AD-associated microbial communities under a range of process configurations, conditions, feedstocks and using different inocula [6-14]. These studies have provided substantial insight into the biogas producing microbial communities as it relates to the process performance. The aim of the current study was to investigate the effect of seasonal variation on population dynamics of bacteria and archaea community structure during anaerobic treatment of organic municipal solid waste.

Materials and Methods

Anaerobic digestion set-up

The anaerobic digestion process was set-up as described by Stanley et al. [15]. One-stage 250 L-capacity anaerobic digesters (AD) were configured for batch-type mesophilic reactors with useful volumes of around 230 L, rumen juice as the source of microbial inoculum and a retention time of 84 days. The first AD_H process was conducted in duplicate during the dry season (between February and April, 2016) while the AD_C process was conducted in duplicate during the rainy season (between July and October, 2016).

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Preparation, characterisation and anaerobic digestion of the substrate

Preparation and pre-treatment of the rumen juice has been described by Stanley et al. [15]. Collection and pre-treatment of Organic Fraction of Municipal Solid Waste (OFMSW) as well as preparation of the substrate have been described by Stanley et al. [15]. The feed for anaerobic digestion was formulated to arrive at the desired substrate concentration (%) shown in Table 1 as described in Stanley et al. [15]. After preparation, samples of the substrate were collected to determine some of its physical, chemical and microbiological properties using standard methods and the result is presented in [15].

Collection of samples and determination of physico-chemical parameters

To monitor the anaerobic treatment process of the waste, slurry samples from the AD were collected at weekly and bi-weekly intervals (during a period of 84 days) to determine important physicochemical and microbiological parameters which could influence process performance with respect to biodegradation of the feed [1]. Daily online bio-digester Process Temperature (PTM) was measured using digital thermometers with probes (SCT-lilliput, Scichem Tech) which extended into the AD. Weekly process pH was determined using a digital hand-held pH meter (SCT-lilliput, Scichem Tech) as described by Ogbonna et al. [15]. Total organic carbon (TOC), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined using Standard Methods [16]. Substrate Degradability (SD) and Degree of Digestion (DD) of the feed were determined using values of BOD and COD and TOC respectively as described by Schnurer and Jarvis [1]. Total sulphate (SO_4^{2-}) was determined using the Nephelometeric protocol described in Standard methods [16]. Total nitrate (NO_3^-) was determined using the spectrophotometric protocol described in Standard methods [16].

Enumeration of bacteria and archaea populations

Microbial populations were evaluated by counting select groups based on metabolic capacity and oxygen sensitivity respectively. The metabolic groups selected included the populations of Sulphate Reducing Bacteria (SRB), Nitrate Reducing Bacteria (NRB), Cellulolytic Bacteria (CEB), Proteolytic Bacteria (PRB), Butyrate Oxidizing Bacteria (BOB), Propionate Oxidizing Bacteria (POB), AMA and HMA respectively. They were enumerated anaerobically using the agar roll-tube technique described by Holdeman et al. [17,18]. The oxygen-sensitive groups selected included the populations of FAB and SAB. The population of FAB were enumerated using the MPN (n=3) method described in Lozano et al. [19]. The MPN result was interpreted with appropriate MPN tables from Oblinger and Koburger and reported in MPN/ml of digester sample [20]. The populations of SAB were enumerated using the agar roll-tube method described by Holdeman et al. respectively [17,18]. The media were prepared as described for the cultivation of methanogenic and non-methanogenic anaerobes as well FAB [18].

During incubation (at 30° C between one and two weeks), the cultures inside the tubes were monitored for microbial growth. The growth of AMA and HMA were monitored under UV-(black) light for the presence of blue-auto fluorescent colonies due to the exhibition of factor 420 blue-fluorescence specific to methanogens [1,21]. The growth of SRB was monitored for black deposits on or around colonies due to the reduction of SO_4^{2-} to sulphide [22]. The growth of NRB was monitored for red coloration/deposits on or around colonies due to the reduction of NO_3^- to nitrite after the addition of few drops of Griess reagent [23]. The growth of CEB and PRB was monitored for the development of colonies with zone of clearance around them. However, the growth of POB, BOB and SAB was respectively monitored for the development of colonies [18]. Colonies were counted and recorded as observed.

Medium for acetoclastic methanogenic archaea (AMA): The growth medium for AMA which was prepared using a modified basal medium of Wolfe, was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 0.6 g of NaCl, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2.6H_2O$, 0.01 g of $CaCl_2.2H_2O$, 10 ml of oligoelement and vitamin solutions [18], 1.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 15 g of agar powder and the amounts of substrate used per L of medium was 6.0 g of sodium acetate, final pH was 7.2.

Medium for hydrogenotrophic methanogenic archaea (HMA): The growth medium for HMA which was prepared using a modified basal medium of Wolfe, was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 0.6 g of NaCl, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2.6H_2O$, 0.01 g of $CaCl_2.2H_2O$, 10 ml of oligoelement and vitamin solutions [18], 1.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 15 g of agar powder and the amounts of substrate used per L of medium was 6.0 g of sodium acetate, final pH was 7.2.

Medium for propionate oxidizing bacteria (POB): The growth medium for POB which was prepared using a modified basal medium of Wolfe, was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 0.6 g of NaCl, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2.6H_2O$, 0.01 g of $CaCl_2.2H_2O$, 10 ml of oligoelement and vitamin solutions [18], 1.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 15 g of agar powder and the amounts of substrate used per L of medium was 5.0 g of sodium propionate, final pH was 7.2.

Medium for butyrate oxidizing bacteria (BOB): The growth medium for BOB which was prepared using a modified basal medium of Wolfe, was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 0.6 g of NaCl, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2.6H_2O$, 0.01 g of $CaCl_2.2H_2O$, 10 ml of oligoelement and vitamin solutions [18], 1.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 15 g of agar powder and the amounts of substrate used per L of medium was 4.0 g of sodium butyrate, final pH was 7.2.

Medium for sulphate reducing bacteria (SRB): The growth medium for SRB which has been modified from Moissl-Eichinger

System	Substrate	PW:FW	DS (Kg)	WC (Kg)	*WS (Kg)	*RJ (kg)	*WA (kg)	Total (kg)	%TS
AD_H	OFMSW	1:4	12.69	3.21	15.90	6.36	207.74	230.00	~5.53
AD_C	OFMSW	1:4	12.74	3.16	15.90	6.36	207.74	230.00	~5.53

PW=Paper waste, F=Food waste, DS=Dry solid, WC=Water content, WS=Wet solid, RJ=Rumen juice, WA=Water, AD_H=Anaerobic digester operated during the dry season (between February and April, 2016), AD_C=Anaerobic digester operated during the rainy season (between July and October, 2016),*=Feed components, OFMSW=Organic fraction of municipal solid waste.

Table 1: Composition of the Feed for Anaerobic digestion.

was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 1.0 g of Na_2SO_4, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.2 g of $MgSO_4$ $7H_2O$, 0.01 g of $CaCl_2.2H_2O$, 0.02 g of $FeSO_4$ $7H_2O$, 10 ml of oligoelement and vitamin solutions [18], 1.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 15g of agar powder, 0.2 g of sodium thioglycolate and the amounts of substrates used per L of medium were 4ml of sodium lactate and 0.4 g of ascorbic acid, final pH was 7.2 [22].

Medium for nitrate reducing bacteria (NRB): The growth medium for NRB which has been modified from Labat and Garcia was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 2.0 g of $NaNO_3$, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2$ $2H_2O$, 0.01 g of $CaCl_2.2H_2O$, 10 ml of oligoelement and vitamin solutions [18], 1.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 15 g of agar powder, 0.2 g of sodium thioglycolate and the amounts of substrates used per L of medium were 2 ml of sodium lactate and 4 g of sodium acetate, final pH was 7.2 [23].

Medium for cellulolytic bacteria (CEB): The growth medium for CEB which was prepared using a modified basal medium of Wolfe, was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 2.0 g of NaCl, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2.6H_2O$, 0.01 g of $CaCl_2.2H_2O$, 12.5 ml of oligoelement and vitamin solutions [18], 2.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 5.0 g of cellulose powder (CMC), 0.5 g of sodium thioglycolate and 15 g of agar, final pH was 7.2.

Medium for proteolytic bacteria (PRB): The growth medium for PRB which was prepared using a modified basal medium of Wolfe, was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 2.0 g of NaCl, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2.6H_2O$, 0.01 g of $CaCl_2.2H_2O$, 12.5 ml of oligoelement and vitamin solutions [18], 2.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 3.0 g of fish powder, 3 g of meat powder, 0.5 g of sodium thioglycolate and 15 g of agar, final pH was 7.2

Medium for strict anaerobic bacteria (SAB): The growth medium for SAB which was prepared using a modified basal medium of Wolfe, was composed of the following in 1L-capacity Erlenmeyer flasks with butyl rubber corks: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 2.0 g of NaCl, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2.6H_2O$, 0.01 g of $CaCl_2.2H_2O$, 12.5 ml of oligoelement and vitamin solutions [18], 2.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 5.5 g of D-glucose, 3.0 g of sodium acetate, 0.5 g of sodium thioglycolate and 15 g of agar, final pH was 7.2.

Medium for facultative anaerobic bacteria (FAB): The growth medium for FAB was composed of the following in 1 L-capacity Erlenmeyer flask with butyl rubber cork: 1 L of sterile distilled water, 1.0 g of NH_4Cl, 2.0 g of NaCl, 5.0 g of $NaHCO_3$, 0.3 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.16 g of $MgCl_2.6H_2O$, 0.01 g of $CaCl_2.2H_2O$, 12.5 ml of oligoelement and vitamin solutions [18], 2.0 g of yeast extract, 1.0 ml of resazurin solution (1% w/v), 5.5 g of D-glucose, 3.0 g of sodium acetate, 0.5 g of sodium thioglycolate, 6 g of agar, final pH was 7.2.

Metagenomic analysis of archaea community structure

DNA extraction and PCR amplification: To determine the dynamics of microbial community structure during anaerobic digestion of the waste inside the AD_H and AD_C systems, slurry samples were collected at day 1, 14, 35, 56 and 84 respectively and stored at -20°C before further analysis. Total DNA was extracted and purified (in Lahor Research Institute, Nigeria) using the Zymo Research (ZR) fungal/bacterial DNA extraction 96-well format according to the manufacturer's instruction. The quality of DNA was then verified by agarose gel electrophoresis [12]. Extracted DNA was stored at -20°C until further use. Bacteria and archaea 16S ribosomal RNA genes were amplified by polymerase chain reaction (94° C for 3 min, followed by 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min and a final extension at 72°C for 7 min) using primers 8 F-FAM (5'-AGA GTT TGA TCM TGG CTC AG-3')/1492R (5'-GGT TAC CTT GTT ACG ACT T-3') and Arc 109 F-FAM (5'-ACK GCT CAG TAA CAC GT-3')/Arc915R (5'-GTG CTC CCC CGC CAA TTC CT-3') respectively [12]. The 5'-ends of primers 8F and Arc109 F were labelled with 6-carboxyfluoresceinphosphoramidite (FAM). PCR reactions were performed in a 25 µL mixture containing 12.5 µL of quick load one taq one step PCR master mix (2x), 1.25 µL of forward primer (20 µM), 1.25 µL of reverse primer (20 µM), 5.0 µL of nuclease free water and 5.0 µL of template DNA.

Sequencing/analysis of sequences: After the quality of the PCR products were verified by agarose gel electrophoresis, the PCR products were cleaned using the ExoSAP protocol as instructed by the manufacturer. Next, the cleaned PCR products were subjected to labelling (or sequencing) reactions using the ABI V3.1 Big dye kit according to manufacturer's instructions. Next, the labelled products were cleaned with the Zymo Seq clean-up kit as instructed by the manufacturer (ZR). Next, the ultra-pure (or cleaned) DNA samples were loaded (or injected) on ABI 3500 XL analyzers with a 50 cm array using POP7. Following this, sequence data generated were analyzed with the Geneious package (version 9.0.5) and phylogenetic trees were then constructed using neighbour joining.

Result and Discussion

Population dynamics of digester bioindicator microbes

Inside the AD_H operated during the dry season (between February and April, 2016), the average population of CEB increased from 1.82 × 10^5 CFU/ml (at day 1), peaked at 8.89 × 10^6 CFU/ml (around day 49) and then decreased progressively to 4.74 × 10^6 CFU/ml (around day 84). Inside the AD_C operated during the rainy season (between July and October, 2016), the population of CEB increased from 4.10 × 10^4 CFU/ml (at day 1), peaked at 4.98 × 10^6 CFU/ml (around day 56) and then decreased progressively to 3.15 × 10^6 CFU/ml (around day 84) as shown in Figure 1a. The population of PRB inside the AD_H

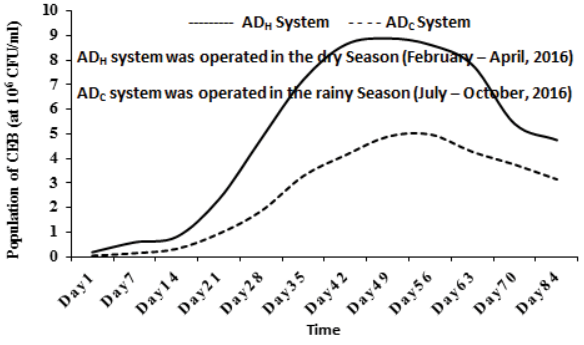

Figure 1a: Population dynamics of cellulolytic bacteria (CEB) during anaerobic treatment of organic municipal solid waste.

system increased from 3.33×10^5 CFU/ml (at day 1), peaked at 8.95×10^6 CFU/ml (around day 35) and then decreased progressively to 2.13×10^6 CFU/ml (around day 84). Inside the AD_C system, the population of PRB increased from 5.0×10^4 CFU/ml (at day 1), peaked at 4.87×10^6 CFU/ml (around day 42) and then decreased progressively to 2.46×10^6 CFU/ml (around day 84) as shown in Figure 1b. In this study, the population dynamics of CEB and PRB were used as bioindicators of hydrolysis and acidogenesis inside the bio-digesters (AD_H and AD_C) [1]. Their population dynamics suggested that hydrolysis and acidogenesis may have increased peaked and then decreased inside the bio-digesters with time.

Inside the AD_H system, the population of BOB increased from 3.70×10^4 CFU/ml (at day 1), peaked at 6.99×10^6 CFU/ml (around day 49) and then decreased progressively to 4.82×10^6 CFU/ml (around day 84). In the AD_C system, the population of BOB increased from 8.0×10^3 CFU/ml (at day 1), peaked at 3.24×10^6 CFU/ml (around day 63) and then decreased progressively to 2.87×10^6 CFU/ml (around day 84) as shown in Figure 2a. Inside the AD_H system, the population of POB increased from 5.10×10^5 CFU/ml (at day 1), peaked at 9.99×10^6 CFU/ml (around day 49) and then decreased progressively to 7.64×10^6 CFU/ml (around day 84). Inside the AD_C system, the population of POB increased from 4.0×10^3 CFU/ml (at day 1), peaked at 5.25×10^6 CFU/ml (around day 70) and then decreased slightly to 4.84×10^6 CFU/ml (around day 84) as shown in Figure 2b. The population dynamics of BOB and POB were used as bioindicators of anaerobic oxidation (or acetogenesis) inside the AD_H and AD_C systems [1,19,23]. As the result indicated, acetogenesis may have increased, peaked and then decreased with time during the anaerobic treatment process inside the AD_H system and the AD_C system respectively.

Inside the AD_H system, the population of AMA peaked at 8.76×10^6 CFU/ml (around day 56) and then decreased slightly to 8.63×10^6 CFU/ml (around day 84). Inside the AD_C system, the population of AMA peaked at 1.46×10^6 CFU/ml (around day 63) and then decreased slightly to 1.21×10^6 CFU/ml (around day 84) as shown in Figure 3a. Likewise, the population of hydrogenotrophic methanogens (HMA) inside the AD_H system peaked at 7.93×10^6 CFU/ml (around day 56) and then decreased slightly to 7.42×10^6 CFU/ml (at around day 84). The population of hydrogenotrophic methanogens (HMA) inside the AD_C system increased, peaked at 1.05×10^6 CFU/ml (around day 49) and then decreased progressively to 7.20×10^5 CFU/ml (at around day 84) as shown in Figure 3b. The population dynamics of AMA and HMA were used as bioindicators of methanogenesis in both AD_H and AD_C systems. As the result indicates, methanogenesis seemed to have increased, peaked and the decreased with time inside the AD_H system and the AD_C system respectively [1].

Inside the AD_H system, the population of SRB increased, peaked at 6.9×10^4 CFU/ml (around day 70) and then decreased slightly to 6.1×10^4 CFU/ml (at around day 84). Inside the AD_C system, the population of SRB increased and peaked at 9.9×10^5 CFU/ml (around day 84) as shown in Figure 4a. Inside the AD_H system, the population of NRB increased progressively to 2.33×10^6 CFU/ml (around day 84). Inside the AD_C system the population of NRB increased, peaked at 3.75×10^6 CFU/ml (around day 63) and then decreased slightly to 3.19×10^6 CFU/ml (around day 84) as shown in Figure 4b. The population dynamics of SRB and NRB were used as bioindicators of process instability inside both AD_H and AD_C systems with time. [1,24,25]. As the result shows, the population of SRB and NRB inside both AD (AD_H and AD_C) increased with time during the anaerobic treatment process. This may have been as a result of their consumption of SO_4^{2-} and NO_3^- contained in the feed [24,26].

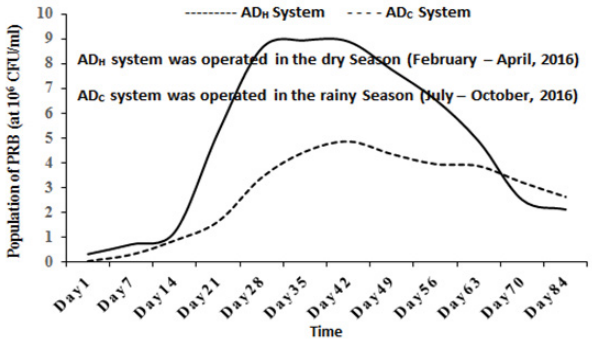

Figure 1b: Population dynamics of proteolytic bacteria (PRB) during anaerobic treatment of organic municipal solid waste.

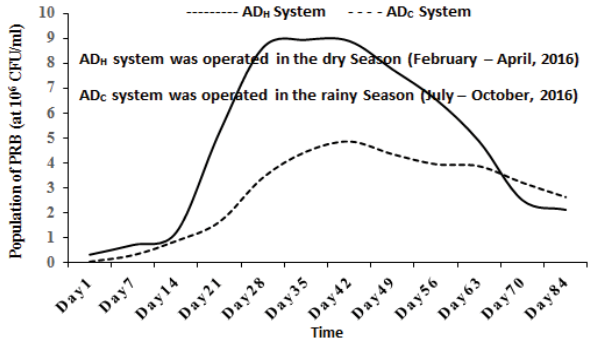

Figure 2a: Population dynamics of butyrate oxidizing bacteria (BOB) during anaerobic treatment of organic municipal solid waste.

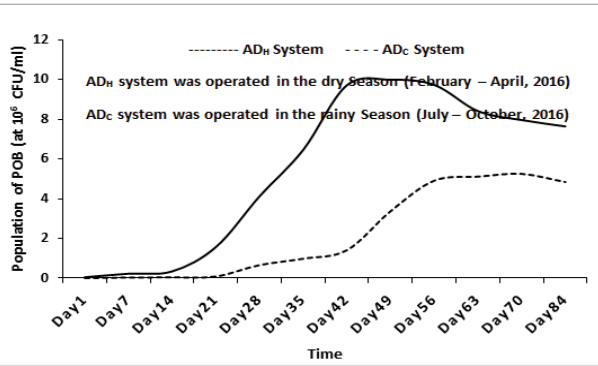

Figure 2b: Population dynamic of propionate oxidizing bacteria (POB) during anaerobic treatment of organic municipal solid waste.

In terms of oxygen sensitivity (or O_2 requirement), the population of SAB inside the AD_H system increased from 1.45×10^6 CFU/ml (at day 1), peaked at 4.18×10^7 CFU/ml (around day 63) and then decreased slightly to 4.06×10^7 CFU/ml (around day 84). Inside the AD_C system, the population of SAB increased from 9.2×10^5 CFU/ml (at day 1) and peaked at 1.85×10^7 CFU/ml (around day 84) as shown in Figure 5a. The population of FAB inside the AD_H system increased from 1.2×10^7 MPN/ml (at day 1), peaked at 7.5×10^7 MPN/ml (around day 21) and then decreased progressively to 1.2×10^6 MPN/ml (around day 84). Likewise, inside the AD_C system, the population of FAB increased from 1.9×10^7 MPN/ml (at day 1), peaked at 1.2×10^8 MPN/ml (around day 35) and decreased progressively to 2.4×10^6 MPN/ml (around day 84) as shown in Figure 5b.

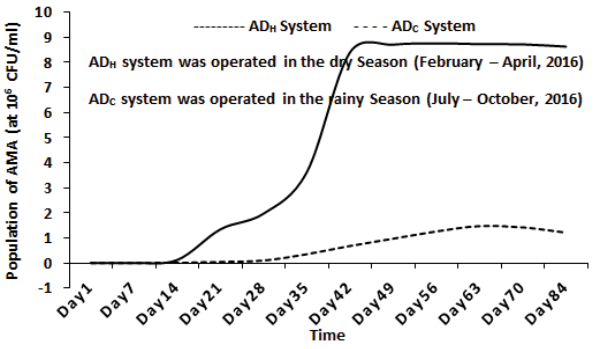

Figure 3a: Population dynamics of acetoclastic methanogenic archaea (AMA) during anaerobic treatment of organic municipal solid waste.

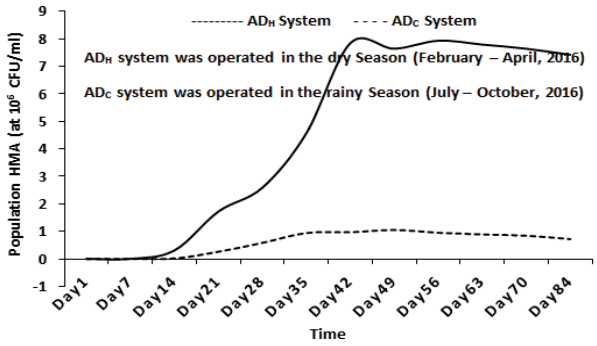

Figure 3b: Population dynamics of hydrogenotrophic methanogenic archaea (HMA) during anaerobic treatment of organic municipal solid waste.

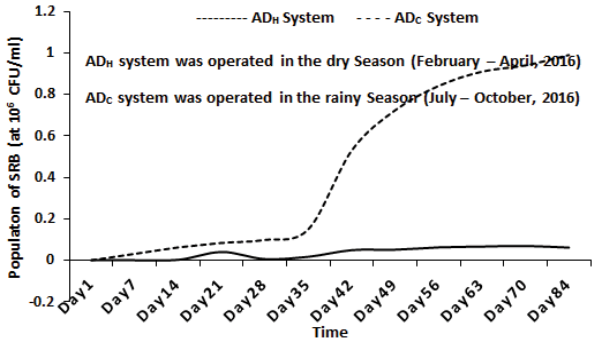

Figure 4a: Population dynamics of sulphate reducing bacteria (SRB) during anaerobic treatment of organic municipal solid waste.

Bacterial and archaeal population dynamics inside the AD appear to resemble the sigmoid pattern of growth usually observed with microbial batch cultures [27]. This was expected since the anaerobic treatment processes were conducted using the batch technology [1]. As the result suggested, hydrolysis, acetogenesis and methanogenesis appear to have progressed much better inside the AD_H operated during the dry season when compared to the AD_C operated during the rainy season of 2016 as shown in Figures 1-3 respectively. This is because the population of bacteria and archaea groups which may be responsible for these stages of anaerobic digestion of the waste were higher inside the AD_H system than those recorded inside the AD_C system with time [1,28]. In terms of oxygen requirement, the population of SAB was significantly higher inside the AD_H system compared to the AD_C system with time (Figure 5a). However, the population of FAB inside the AD_C system was significantly higher at some point than the population of FAB inside the AD_H system (Figure 5b). In fact, the period when the population of FAB peaked inside both systems appeared to have correlated with the same period when the process pH became significantly acidic. According to literature, acidogenesis which is the second phase of anaerobic digestion process, is usually influence by the activities of mostly FAB groups who are the fermenters [1,28]. Therefore, it may be possible to attribute the lower (or acidic) pH recorded inside the AD_C system over time to its significantly higher number of FAB. This increased acidic nature inside the AD_C system may have contributed to its reduced performance with respect to biodegradation of the feed compared to that observed inside the

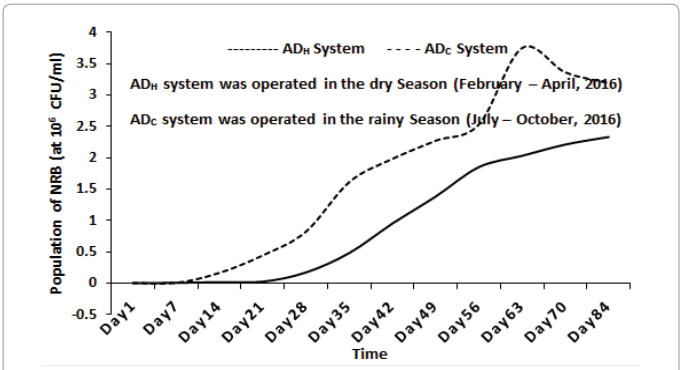

Figure 4b: Population dynamics of nitrate reducing bacteria (NRB) during anaerobic treatment of organic municipal solid waste.

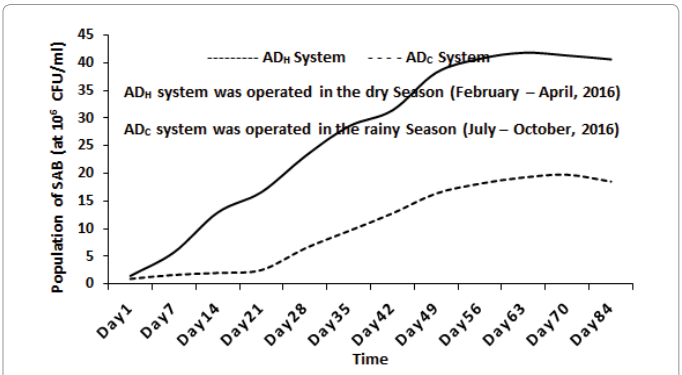

Figure 5a: Population dynamics of strict anaerobic bacteria (SAB) during anaerobic treatment of organic municipal solid waste.

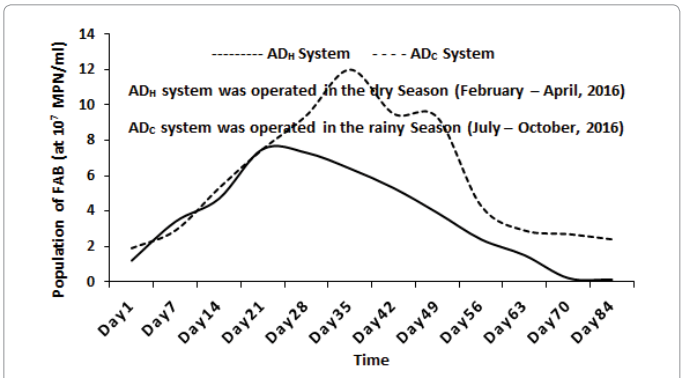

Figure 5b: Population dynamics of facultative anaerobic bacteria (FAB) during anaerobic treatment of organic municipal solid waste.

AD_H system with time. This is because at lower (or acidic) pH, the methanogens are most affected negatively, consequently leading to a reduced biomass conversion efficiency especially if acidity increases above their carrying capacity [1,28].

Archaea community dynamics

At day 1, the archaea community inside the AD_H system operated during the dry season of 2016 appears to have been dominated by *Methanobrevibacter* species followed by other uncultured archaeon clones. Inside the AD_C system operated during the rainy season of 2016, the archaea community appears to have been dominated by uncultured archaeon. However, one uncultured *Methanobrevibacter* species appears to have been present at the time. Around day 14, archaea related uncultured methanogenic archaeon clones, *Methanosphaera stadmanae*, uncultured archaeon clones were represented inside the AD_H system. Inside the AD_C system, archaea related to *Methanosphaera stadmanae*, uncultured archaeon clones and uncultured methanogenic archaeon clones were represented. Around day 35, the archaea community inside the AD_H system was represented by archaea related to *Methanosphaera stadmanae*, uncultured methanogenic archaeon clones and uncultured archaeon clones. Inside the AD_C system, archaea related to uncultured archaeon clones, uncultured methanogenic archaeon clones and *Methanosphaera stadmanae* were represented. Around day 56, archaea related to uncultured archaeon clones, uncultured methanogenic archaeon clones and *Methanosphaera stadmanae* were also represented inside the AD_H system. Inside the AD_C system, the archaea community appears to have been dominated by uncultured archaeon clones and uncultured methanogenic archaeon clones respectively. Around day 84, the dominant archaea inside the AD_H system were related to uncultured archaeon clones. Inside the AD_C system, archaea related to uncultured archaeon clones appeared to have dominated the archaea community, however, others related to uncultured methanogenic archaeon clones and *Methanosphaera stadmanae* were present.

There was a shift in archaea community structure during anaerobic treatment of OFMSW inside the AD_H system and the AD_C system with time as indicated in Figures 6 and 7 respectively. Generally, the archaea community inside both AD_H and AD_C systems appears to have been dominated by uncultured archaeon and uncultured methanogenic archaeon clones as seen in Figures 6 and 7 respectively. Some of the methanogenic archaea that were also observed inside both AD_H and AD_C belonged to *Methanobrevibacter* and *Methanosphaera*. Methanogenic archaea species belonging to these generae have been implicated in biogas (i.e., methane) production inside anaerobic digestion systems

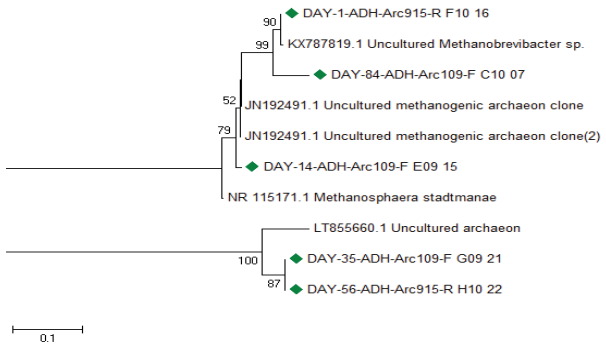

Figure 6: Archaea community structure inside the AD_H system with time.

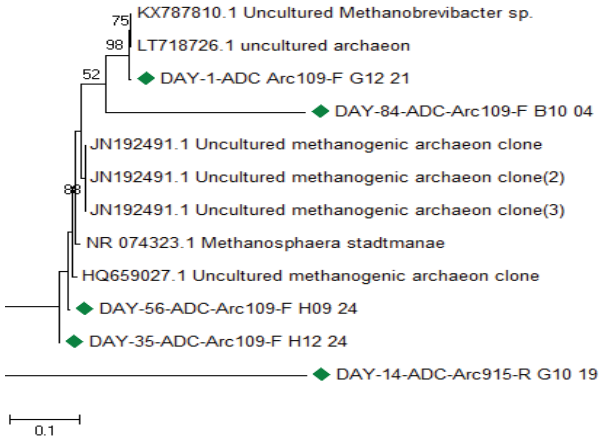

Figure 7: Archaea community structure inside the AD_C system with time.

treating various forms of organic wastes in previous studies [1,14,29-35]. Species belonging to these two methanogenic archaea generae have also been implicated in hydrogenotrophic methanogenesis while utilizing either acetate, formate or alcohol, etc. as substrate [1,29,34,35]. This indicates that hydrogenotrophic methanogenesis may have been active inside the bio-digesters (i.e., AD_H and AD_C systems) with time. It also implies that the dominant methanogenic pathway may have been hydrogenotrophic methanogenesis rather than acetoclastic methanogenesis during anaerobic treatment of the organic solid waste inside both AD_H and AD_C systems [1]. GenBank accession number(s) for the nucleotide sequence(s) isolated during anaerobic treatment of OFMSW inside the bio-digesters (AD_H and AD_C) are presented in Table 2.

Biodegradation of the feed

Degradability (i.e., BOD to COD ratio) of the feed inside the AD_H operated during the dry season (2016) and the AD_C operated during the rainy season (2016) decreased from 0.75 and 0.81 to 0.06 and 0.38 respectively after 84 days (Figure 8a). The DD of the feed (which is a function of TOC) inside the AD_H system and the AD_C system increased to 97.21% and 75.86% respectively after 84 days (Figure 8b). The DD of the feed inside the AD_H operated during the dry season (2016) was significantly ($P<0.05$) higher than the DD of the feed inside the AD_C operated during the rainy season (2016). This may be attributed to the fact that the populations of bacteria and archaea inside the AD_H operated during the dry season (2016) may have adapted better to their environment compared to the much lower bacterial and archaea populations observed inside the AD_C operated during the rainy season of 2016 [1].

Dynamics of COD/SO_4^{2-} and COD/NO_3^- ratios

Inside the AD_H operated during the dry season (2016), COD/SO_4^{2-} ratio ranged from 362.20 to 98.18 after 84 days (Figure 9a). The COD/SO_4^{2-} ratio inside the AD_C operated during the rainy season (2016) ranged from 12,845.40 to 101.47 after 84 days (Figure 9a). COD/SO_4^{2-} ratio less than 3.0 is said to increase the competitive advantage of SRB in biogas producing anaerobic environment over the methanogens with respect to acetate and hydrogen consumption thereby limiting methane production [1,36]. However, in this study, COD/SO_4^{2-} ratios inside both AD (AD_H and AD_C) were significantly greater than 3.0. Therefore, the SRBs may not have contributed significantly to process instability

Nucleotide Sequence	Accession Number
SUB3878683 DAY_1_ADC_Arc109-F_G12_21	MH177267
SUB3878683 DAY-1-ADH-Arc915-R_F10_16	MH177268
SUB3878683 DAY-14-ADC-Arc915-R_G10_19	MH177269
SUB3878683 DAY-14-ADH-Arc109-F_E09_15	MH177270
SUB3878683 DAY-35-ADC-Arc109-F_H12_24	MH177271
SUB3878683 DAY-35-ADH-Arc109-F_G09_21	MH177272
SUB3878683 DAY-56-ADC-Arc109-F_H09_24	MH177273
SUB3878683 DAY-56-ADH-Arc915-R_H10_22	MH177274
SUB3878683 DAY-84-ADH-Arc109-F_C10_07	MH177275

Table 2: Gen Bank accession number(s) for the nucleotide sequence(s) isolated during anaerobic digestion of organic municipal solid waste inside the anaerobic digesters (AD_H and AD_C).

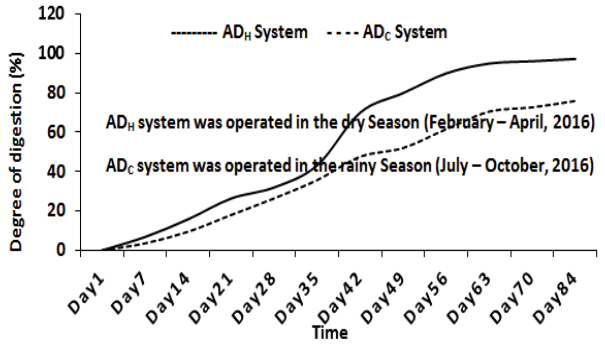

Figure 8a: Degradability of the feed inside the biodigester (AD_H) operated during the dry season and the biodigester (AD_C) operated during the rainy season of 2016.

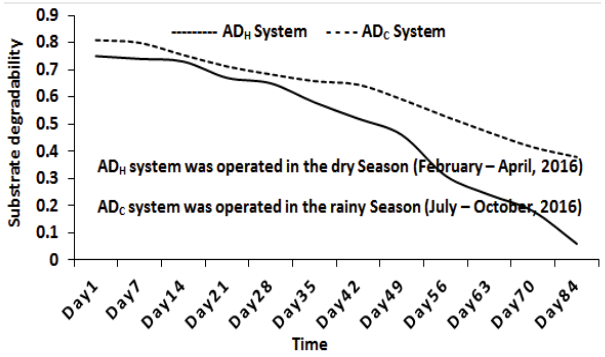

Figure 8b: Degree of digestion of the feed inside the biodigester (AD_H) operated during the dry season and the biodigester (AD_C) operated during the rainy season of 2016.

inside both AD_H and AD_C at any point in time. Furthermore, COD/NO_3^- ratio inside AD_H operated during the dry season (2016) ranged from 74.15 to 271.35 after 84 days of the digestion process (Figure 9b). The COD/NO_3^- ratio inside the AD_C operated during the rainy season (2016) ranged from 73.49 to 4,670.91 after 84 days of the digestion process (Figure 9b). Generally, lower COD/NO_3^- ratios promote process instability because the condition favors the population of NRB which usually out-compete the population of methanogens (under this condition) with respect to acetate consumption thereby limiting methane production [37,38]. However, in this study, COD/NO_3^- ratio inside both AD_H and AD_C systems was significantly higher than the threshold (between 2.0 and 5.0) required for an anaerobic treatment process to be considered unstable [37]. Therefore, the NRBs may not have contributed significantly to process instability inside both AD_H and AD_C at any point in time.

Temperature and pH dynamics

During the dry season when the first AD_H of the waste was conducted, ambient temperature ranged from 30.3°C to 33.6°C with time. However, during the rainy season when the same AD_C process was repeated, the ambient temperature ranged from 26.3°C to 30.5°C with time. Consequently, PTM_H inside the AD_H operated during the dry season and the AD_C operated during the rainy season ranged from 29.7°C to 39.3°C and 26.8°C to 30.8°C respectively with time. This shows that the temperature recorded during the dry season (between February and April, 2016) was relatively higher than temperature observed during the rainy season (between July and October, 2016). Several authors have shown that the anaerobic digestion process of organic matter performs much better at higher temperatures to some degree [12,28,39,40]. This could be one of the reasons why the populations of microbes inside the AD_H system (operated during the dry season, 2016) performed better than the populations of microbes inside the AD_C system (operated during the rainy season, 2016) with respect to biodegradation of the feed. The process pH (which ranged from 6.67 to 5.32 with time) inside the AD_H operated during the dry season (2016) was less acidic than the process pH (which ranged from 6.40 to 4.60 with time) inside the AD_C operated during the rainy season (2016). This suggests that the AD_H operated during the dry season (2016) may

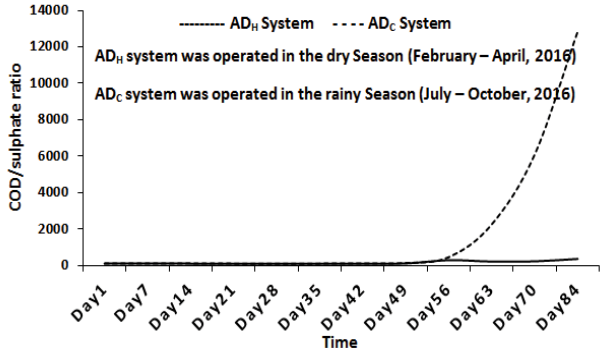

Figure 9a: Dynamics of COD/sulphate (SO_4^{2-}) ratio during anaerobic treatment of organic fraction of municipal solid waste (OFMSW) inside the anaerobic digester (AD_H) operated during the dry season and the digester (AD_C) operated during the rainy season of 2016.

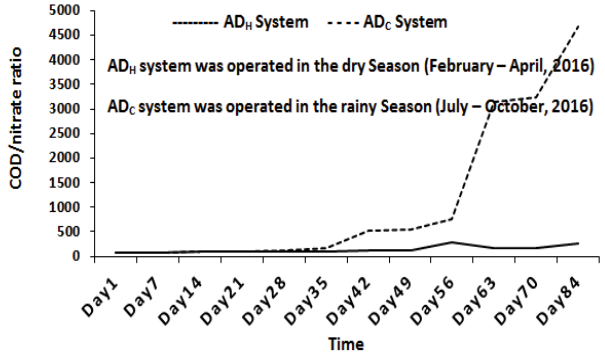

Figure 9b: Dynamics of COD/nitrate (NO_3^-) ratio during anaerobic treatment of organic fraction of municipal solid waste (OFMSW) inside the anaerobic digester (AD_H) operated during the dry season and the digester (AD_C) operated during the rainy season of 2016.

have been more favorable to the microbial populations taking part in the digestion process compared to the AD_C operated during the rainy season (2016) with time [28]. It could be another reason why the AD_H system (operated during the dry season, 2016) performed better than the AD_C system (operated during the rainy season, 2016) with respect to biodegradation.

Conclusion

The aim of the study was to investigate the influence of seasonal variation on the dynamics of bacterial and archaeal populations/community structure during anaerobic treatment of OFMSW. From the study, it was observed that there was a temporal shift in the populations of bacteria and archaea community during anaerobic treatment of organic municipal solid waste. However, the microbial populations inside the AD_H system appeared to have increased significantly more than the microbial populations inside the AD_C systems with time. The presence of methanogenic species which belong to known genera such as *Methanobrevibacter* and *Methanosphaera* suggests that hydrogenotrophic methanogenesis may have been active inside the bio-digesters. It was also observed that the AD_H process conducted between February and April (2016), during the dry season performed significantly ($P<0.05$) better than the AD_C process conducted between July and October (2016), during the rainy season with respect to biodegradation of the feed. This is probably due to its higher process temperature, less acidic process pH and higher microbial populations with time. Seasonal variation appeared to have influenced the population dynamics of bacteria and archaea communities during anaerobic treatment of organic municipal solid waste.

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