



EFFECTS OF TOXICANTS ON THE PERFORMANCE OF A TWO-PHASE ANAEROBIC DIGESTION PROCESS TREATING RAW PRIMARY SLUDGE

Neriamara Martins Dias – neriamara.dias@gmail.com

Departamento de Engenharia Agrícola, Universidade Federal de Viçosa (UFV).

Av. PH. Rolfs, s/ no, Campus Universitário

CEP: 36.570-900 | Viçosa – MG

Ana Martin-Ryals – martinr2@illinois.edu

Agricultural Engineering Sciences Building, University of Illinois at Urbana-Champaign (UIUC).

Lance Schideman – schidema@illinois.edu

Illinois Sustainable Technology Center (ISTC).

Abstract: *This study investigates the current state of anaerobic digestion, discusses the main limitations and bottlenecks that can inhibit anaerobic digestion processes and cause upset or failure, and examines the performance of a two-phase anaerobic digestion process treating raw primary sludge. A brief review of anaerobic digestion as a process, as well as parameters that must be monitored in order maintain effective process performance: pH, temperature, C/N ratio, retention time, organic loading rate, bacterial competition, nutrient content, toxicants, solids content, and mixing/agitation is initially outlined. The discussion then focuses on ammonia, a specific inorganic toxicant and its mechanism of toxicity in anaerobic digestion. Finally, the performance of a bench-scale two-phase anaerobic digestion process operating at mesophilic and ambient temperature to treat raw primary sludge is examined in relation to pH, total and free ammonia concentration, and methane production. It is concluded that methane production in the bench-scale anaerobic digestion system was negatively affected by fluctuations in pH which led to an increase in free ammonia concentration inhibiting the newly adjusted ambient temperature microbial community. In short, this investigation highlights the importance of pH control for achieving and maintaining optimal anaerobic digestion performance.*

Keywords: Ammonia inhibition; Anaerobic degradation; Primary sludge.

EFEITOS DE COMPOSTOS TÓXICOS NO DESEMPENHO DOS PROCESSOS DE DIGESTÃO ANAERÓBIA DE DUAS FASES NO TRATAMENTO DE LODO PRIMÁRIO BRUTO

Resumo: *Este trabalho investiga o estado atual da digestão anaeróbia, discute as principais limitações e os gargalos que podem inibir processos de digestão anaeróbia e causar transtorno ou falhas e examina o desempenho do processo de digestão anaeróbia de duas fases no tratamento de lodo primário bruto. Uma breve revisão da digestão anaeróbica como um processo, bem como os parâmetros que devem ser controlados a fim de se manter a eficácia do desempenho do processo: pH, temperatura, a relação C / N, tempo de retenção, carga orgânica, da competição bacteriana, teor de nutrientes, compostos tóxicos, teor de sólidos, e mistura / agitação é inicialmente delineado. A discussão, em seguida, concentra-se especificamente na amônia, uma substância inorgânica tóxica, e*



seu mecanismo de toxicidade na digestão anaeróbia. Finalmente, o desempenho do processo de digestão anaeróbia de duas fases em escala de bancada operando às temperaturas ambiente e mesofílica para o tratamento de do lodo primário bruto é analisado em relação ao pH, concentração de amônia total e livre, e a produção de metano. Concluiu-se que a produção de metano no sistema de digestão anaeróbia em escala de bancada foi negativamente afetada por flutuações no pH que levaram a um aumento na concentração de amônia livre, inibindo a comunidade microbiana à temperatura recém-ajustada para amambiente. Em suma, este trabalho destaca a importância do controle de pH para alcançar e manter o desempenho digestão anaeróbia ideal.

Palavras-chave: inibição por amônia; digestão anaeróbia; lodo primário.

1. INTRODUCTION

In recent years, the conversion of biomass materials to methane for use as an energy source has prompted interest around the world. This conversion is accomplished by anaerobic digestion, the biological process by which organic materials or feedstocks are degraded in the absence of oxygen to produce a combustible gas consisting of methane (CH₄) and carbon dioxide (CO₂). The gas product is often called biogas and the conversion of waste pollutants to biogas via anaerobic digestion is attracting great interest and has been used successfully for various types of waste effluents, both domestic and industrial.

Compared to aerobic treatment from the standpoint of implementation of sustainable technologies, anaerobic digestion (AD) solves the problem of waste in a more comprehensive manner. AD requires smaller areas to implement, produces useful energy in the form of biogas, recovers fertilizers, has lower energy requirements, produces less sludge, and can be combined with effluent post-treatment methods for recovery of useful products depending on the nature of the treated effluent (Berni and Bajay, 2000). Also, depending on the temperature at which the process is conducted, it may enable higher loading rates than aerobic treatment, and greater destruction of pathogens. However, among the disadvantages of anaerobic digestion, its operational system vulnerability, which is high sensitive to toxicants (Chen, et al., 2014), and its longer retention times are significant limitations compared to aerobic digestion. The anaerobic microorganisms are sensitive to several process parameters such as pH, alkalinity, concentration of free ammonia, hydrogen, volatile fatty acids (VFA), etc. These parameters can be inhibiting factors to some or all bacterial groups, and modern approaches include these inhibition effects in system modelling, during investigation of system behavior, and in controlling the process (Appels et al., 2008). This study will focus on the operational vulnerability of AD processes, specifically on the toxicity caused by free ammonia concentration.

1.1. Anaerobic Digestion Process

The overall AD conversion process is often described as a four subsequent stage process, which may occur simultaneously in an anaerobic digester: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Martin-Ryals, 2012). During hydrolysis, a consortia of bacteria break down complex organic macromolecules (e.g. proteins, cellulose, lignin, and lipids) from the influent into soluble monomers such as amino acids, simple sugars, glycerols, and fatty acids. Extracellular enzymes such as cellulases, proteases, and lipases catalyze the hydrolysis of those complexes, some of which are insoluble. (Batstone and Jensen, 2011). Those products are taken up by various acid forming bacteria (acidogens) in the acidogenesis phase and are converted into volatile fatty acids (VFAs) (e.g. butyric acid, propionic acid, acetate and acetic acid), as well as alcohols, hydrogen, and carbon dioxide. In the third phase, acetogenic bacteria convert VFAs and alcohols into acetate, hydrogen, and carbon dioxide, which are used by the methanogens. Acetogenic bacteria are obligate anaerobes that can tolerate a wide range of environmental conditions (Zaher, Cheong et al. 2007). Potential rate limiting

steps associated with acetogenesis include competition between acetogens and sulfate reducing bacteria (SRB) for acetate, hydrogen, propionate and butyrate (Appels et al., 2008) and insufficient generation of acetate due to low populations of acetogenic bacteria (Martin-Ryals, 2012). Finally, in the methanogenesis step, acetate, hydrogen, and carbon dioxide are converted into methane by methanogenic microorganisms. In this stage, two groups of methanogenic bacteria produce methane: the first group breaks acetate into methane and carbon dioxide and the second group uses hydrogen as electron donor and carbon dioxide as an electron acceptor to produce methane (Appels et al., 2008). Hydrolysis of insoluble polymers is recognized as a major rate-limiting step within this complex succession of degradation; however, if the influent is mostly composed of soluble organics, methanogenesis is the most critical phase (Appels et al., 2008). The subsequent phases of AD are summarized in Figure 1.

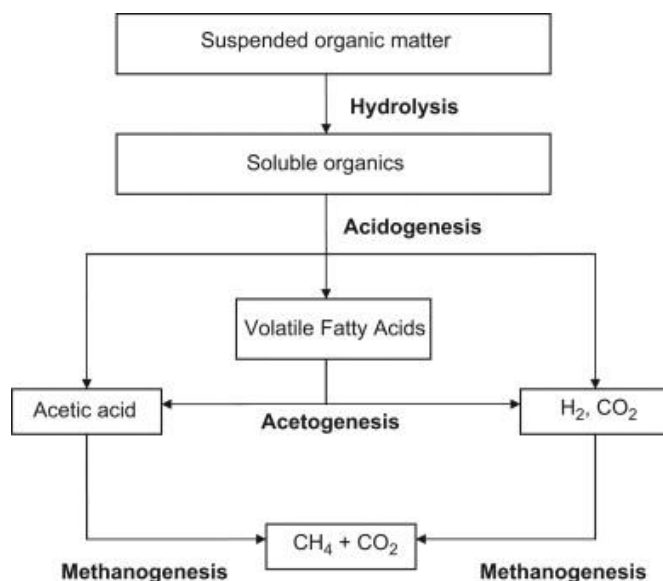


Figure 1. Subsequent steps in the anaerobic digestion process (Appels et al., 2008).

The complete process of AD requires a complex and delicate interaction of several anaerobic bacterial groups that must be in equilibrium in order for the digester to remain stable. Each phase is carried out by a different group of microorganisms, each of them having their own optimum working conditions and differing in terms of their growth kinetics. In this sense, Babel et al (2004) reported that two-stage digesters can be more efficient since it offers conditions to accommodate the microbial groups according to their nutrient needs, growth capacities, and abilities to cope with environmental stress, by physically separating the process into two reactors. In this case, the first digester allows hydrolysis, acidogenesis and acetogenesis to occur while the second optimizes methanogenesis (Raynal et al., 1998; Nguyen et al., 2007).

1.2. Affecting Parameters

Within the anaerobic digester environment, several important parameters affect the rate of each step of the digestion process, and thus affect the efficiency of the overall system and biogas production. These include: pH, alkalinity, temperature, solid and retention times, C/N ratio, organic loading rate, bacterial competition, nutrient content, toxicants, and mixing/agitation (Zaher, Cheong et al. 2007).

1.3 pH and Alkalinity

Each group of microorganisms has a different optimum pH range. Optimal pH for methanogens has been reported from a restrictive range of 6.5 to 7.2 (Boe K., 2006; Turovskiy and



Mathai, 2006) to a little higher range of pH 6.5 to 8.2 (Khalid, Arshad et al. 2011). In contrast, the fermentative microorganisms are somewhat less sensitive and can function in a wider range of pH between 4.0 and 8.5 (Hwang and Jang et al., 2006). Optimal pH for acidogens has been reported in the ranges of pH 5.5 to 6.5 (Khalid, Arshad et al. 2011) and 5.8 to 6.2 (Zoetemeyer, Vandenheuvel et al. 1982). The VFAs produced during AD tend to reduce the pH. In a balanced digester, this reduction is normally countered by the activity of the methanogenic bacteria. This reduction also produces alkalinity in the form of carbon dioxide, ammonia and bicarbonate (Zaher, Cheong et al. 2007). Overall, the pH required in AD for good performance and stability is in the range of 6.5-7.5. Under adverse environmental conditions, the buffering capacity of the system can be upset, eventually stopping the production of methane. The anaerobic treatment system can completely lose its function (process failure) when an accumulation of VFAs drops the pH exceeding a certain level and the methanogenic bacteria are inhibited. On the other hand, prolific methanogenesis may result in a higher concentration of ammonia, increasing the pH above 8.0, where it will impede acidogenesis (Lusk, 1999). A two-phase system can help to mitigate these effects by physically separating the acid and methane phases.

1.4 Temperature

Temperature plays an important role in AD. It affects the physicochemical properties of the components found in the substrate and influences population dynamics in the anaerobic reactor. In general, microorganisms are divided into psychrophilic (10-20 °C), mesophilic (30-40 °C) and thermophilic (50-60 °C) depending on their optimal growth temperature. Most conventional digesters are operated in the mesophilic range, which is more stable and requires less energy inputs compared to operation under thermophilic conditions, and results in a higher degree of digestion compared to operation under psychrophilic conditions (Khalid, Arshad et al. 2011, Chandra, Takeuchi et al. 2012). Thermophilic digestion is faster than mesophilic digestion since the biochemical reaction rates increase with increasing temperature. Other advantages are an increased solids reduction, improved dewatering, and increased death rate of pathogens (Boe K., 2006). The counterpoints, however, are higher energy requirement, a liquid effluent with lower quality and greater amounts of dissolved solids, a higher odor potential, and more careful monitoring due to the decreased stability (Rehm et al., 2000). Moreover, at thermophilic temperatures, there will be an increase of the fraction of free ammonia, which plays an inhibiting role on the microorganisms.

1.5 Solids and Hydraulic Retention Time

The solids retention time (SRT) is the average time the solids spend in the digester, whereas the hydraulic retention time (HRT) is the average time that the liquid fraction is held in the digester. In AD without recycle or supernatant withdrawal, the SRT is equal to the hydraulic retention time. The sequential steps of the digestion process are directly related to the SRT. A decrease in the SRT decreases the time available for the reactions to take place and vice versa. Each time solid material, sludge, is withdrawn, a fraction of the bacterial population is removed thus implying that the cell growth must at least compensate the sludge removal rate to ensure steady-state and avoid process failure (Turovskiy, 2006).

While shorter SRTs are desirable, in order to reduce reactor volumes and thereby reduce capital costs, SRT is limited to some extent by microbial regeneration rates. According to Appels et al. (2008) retention times shorter than 5 days are insufficient for stable digestion because VFA concentrations are increasing due to a washout of methanogenic bacteria, which are relatively slow growers and require an SRT of at least 10-15. Indeed, SRT of 5-8 days is not recommended because the breakdown of some compounds, especially lipids is still incomplete and VFA concentrations are still relatively high. Stable digestion is obtained after 8-10 days, when there is low VFA concentrations and the breakdown of lipids starts. The breakdown curve stabilizes at SRT >10 days,



when all sludge compounds are significantly reduced. Conventional anaerobic digestion processes operate at an HRT in the range of 15-30 days (USDA, 2009).

1.6 Carbon to Nitrogen (C/N) Ratio

The Carbon to Nitrogen (C/N) ratio is a measure of the relative amounts of organic carbon and nitrogen present in the feedstock. The C/N ratio of the collected waste is determined by its composition. If the C/N ratio is very high, the waste used as a substrate will be deficient in nitrogen, which is needed for build-up of bacterial communities. As a result the gas production will be low. If the C/N ratio is very low, nitrogen will be liberated and accumulate in the form of ammonia. This will increase the pH value of the material and a pH value higher than 8.5 will start to show a toxic effect on the methanogenic bacterial communities (Hartmann and Ahring, 2006; Van Opstal, 2006). In general, a C/N ratio of 20-30 is considered optimal for anaerobic digestion (Chandra, Takeuchi et al. 2012; Zaher, Cheong et al. 2007).

1.7 Mixing

Proper mixing during AD is essential for providing optimum performance. Mixing provides intimate contact between the feed substrate and active biomass, yielding uniformity of temperature, substrate concentration, and other chemical, physical and biological aspects throughout the digester, while preventing the formation of surface scum layers and the deposition of sludge on the bottom of the tank. Due to the rise of gas bubbles and the thermal convection currents created by the addition of heated sludge, there is always some degree of natural mixing in the digestion tank. However, this is not sufficient for optimum performance; therefore, auxiliary mixing is needed (Appels et al., 2008).

1.8 Toxicity

One of the main drawbacks to anaerobic digestion is its higher sensitivity to toxicants than aerobic treatment. In an intensive literature review, Chen et al. (2014) summarizes recent work on the effect of nanoparticles and nanotubes on anaerobic digestion and the mechanisms by which they may act. He also shows how a wide range of organic chemicals including halogenated benzene, halogenated phenols, phenol and alkyl phenols, halogenated aliphatics and long chain fatty acids, as well as many inorganic compounds, such as ammonia, sulfide and heavy metals can inhibit anaerobic digestion. Mineral ions and detergents are also some of the toxic materials that inhibit the normal growth of bacteria in the anaerobic digester. In this report, however, only the effect of ammonia toxicity on the methane production is being explored.

1.9 Toxicity of Ammonia to Anaerobic Digestion

Ammonia is an essential nutrient for the growth of microorganisms involved in AD, as well as a potential inhibitor of the AD microbial consortia at certain concentrations (Angelidaki et al., 1993; Koster and Lettinga, 1984). Ammonia is usually formed in anaerobic processes as a result of mineralization of organic nitrogen in wastes rich in protein or urea. The excess ammonia-nitrogen in the fermentation medium could cause an inhibitory effect in three different ways. First, free ammonia, which is more toxic for anaerobic microbial communities than the ammonium ion, due the fact that it can pass through the cell membrane and into the cell causing proton imbalance and potassium deficiency, is formed during the fermentation process. Second, amination of α -ketoglutaric acid by ammonia-nitrogen coupled with rapid disappearance of α -ketoglutaric acid from the metabolic pool of the tricarboxylic acid cycle could cause difficulties in the metabolism of organic compounds. Finally, build-up of ammonia-nitrogen may result in undetected accumulation of volatile fatty acids (VFAs) because ammonia will keep the pH above 8 (Krylova et. al., 1997; Sterling et al., 2001). In terms of a 50% reduction in methane production, a wide range of ammonia concentrations has been documented,



with the inhibitory total ammonia nitrogen concentration ranging from 1.7 to 14 $\text{NH}_3\text{-N}$ g/L (Chen et al., 2008).

Calli et al. (2005) proved that acetogenic bacteria are more sensitive than methanogenic archaea to free ammonia, which has been suggested to be the active component causing ammonia inhibition (Angelidaki and Ahring, 1993; He et al., 2011). Meanwhile, acetate-utilizing bacteria adapted to ammonia were shown to grow within a free ammonia concentration of up to 800 mg-N/L, while many lower free ammonia concentrations (100–150 mg-N/L) have been reported to initially inhibit an unadapted process (Braun et al., 1981; De Baere et al., 1984). Several studies found that the fermentation of high ammonia-containing wastes is more easily inhibited at thermophilic temperatures than at mesophilic temperatures (Angelidaki and Ahring, 1994; Bayr et al., 2012; Braun et al., 1981), which is in agreement with the fact that the ratio of free ammonia to the total ammonium will be much higher at higher temperatures as noted by Garcia and Angenent (2009).

Furthermore, Koster (1986) reported that when the pH value increases, the biogas process becomes more sensitive to ammonia; an increase in pH results in greater free ammonia. In fact, an increase in pH from 7 to 8 will actually lead to an eight-fold increase in free ammonia concentration.

2. MATERIAL AND METHODS

Laboratory-scale Anaerobic Reactor System

The bench-scale two-phase continuous anaerobic system used in this study consisted of a 2.4 L stirred feed tank (raw wastewater – RWW), a 2.0 L stirred acid-phase reactor (referred to as pre-digestion - PD), a 14 L stirred methane-phase anaerobic membrane bioreactor (AnMBR), and a 2 L effluent collection flask. SRT and HRT in the PD phase were the same, at 1.5 days. HRT in the AnMBR was 12 days, with an SRT of 20 days. The reactor was fed daily with primary sludge collected from the local Urbana-Champaign wastewater treatment plant. The biogas production in the AnMBR was monitored by a wet-tip gas-meter (Speece Tip, Nashville, TN), while a water displacement column was used to monitor biogas production in PD phase. A BioFlow 115 Fermentation Unit (Eppendorf) was used for the AnMBR reactor, with automatic mixing, temperature and pH control. pH in the AnMBR was maintained at 7.40 by automatic addition of 10 M NaOH. If the pH went above 7.40, manual adjustment would be made by addition of 12 M HCl. Two continuous-flow pumps were used in the system, one to transfer material from PD to AnMBR, and one to remove effluent from the AnMBR.

At the time of this report, the system had been in operation for more than 800 days, during which changes in various operating parameters were investigated including a change from mesophilic to ambient temperature in the AnMBR. This study reports on data collected during the 629th and 839th days of operation, under the following operating parameters: from the 629th to the 717th days of operation, both, the PD reactor and the AnMBR, were operated at mesophilic temperature (36 ± 2 °C). After the 718th, the PD reactor was still maintained at mesophilic temperature, while the AnMBR was operated at ambient temperature (21 ± 2 °C). The AnMBR was seeded with mesophilic, anaerobic sludge collected from the municipal wastewater treatment facility in Urbana, IL (UCSD, Urbana-Champaign Sanitary District). Bioaugmentation with a proprietary cellulolytic bioculture mixture, provided by Phylein Inc., was added daily to the PD phase in order to enhanced hydrolysis and subsequent acidogenesis of the substrate (Martin-Ryals, 2012). The performance of the reactor was monitored by measuring biogas production, volatile solids (VS), ammonia nitrogen ($\text{NH}_3\text{-N}$) free-ammonia, and pH.

Analytical Methods

Daily samples from the PD, AnMBR and effluent tanks were collected and VS, $\text{NH}_3\text{-N}$, and pH analysis were performed in the Agricultural Waste Management Lab according to Standard Methods for the Examination of Water and Wastewater (APHA, 1989). The biogas composition was



measured using Gas Chromatography (Varian, Model CP-3800), equipped with an Alltech Hayesep D 100/120 column and a thermal conductivity detector (TCD). The carrier gas was helium a flow rate at 30 mL/min. Temperature of both the injector and detector was 120°C. The free ammonia concentration (C_{NH_3}) was calculated according to the Equation (1):

$$C_{NH_3} = \frac{TAN \times K_a}{C_H \times \left(\frac{K_a}{C_H} + 1 \right)} \quad (1)$$

where

TAN is the total ammonia nitrogen concentration, mg/L,

K_a is the temperature dependent dissociation constant (0.564×10^{-9} at 25 °C, 1.097×10^{-9} at 35 °C and 3.77×10^{-9} at 55 °C),

C_H is the concentration of hydrogen ions (Kayhanian, 1999)

3. RESULTS AND DISCUSSION

The performance of the bench-scale two-phase anaerobic digester was investigated in this report based on results obtained from analysis of total and free ammonia concentration, pH, and methane production.

The fermentation of nitrogen-containing materials such as urea and proteins releases ammonia-nitrogen which exists largely as the ionized form (NH_4^+), but this depends strongly on pH as its pKa is 9.3. Hence, the toxic unionized form (free NH_3) increases with increasing pH (Chen et al., 2014). From Equation (1), one can see that the free ammonia concentration (FAN) depends mainly on three parameters: total ammonia concentration (TAN), temperature, and pH. An increased temperature has a positive effect on the microbial growth rate but also results in a higher FAN. An increase in pH obviously can affect the equilibrium of NH_3/NH_4^+ and a high pH is conducive to formation of FAN (Mosquera-Corral et al., 2005). In addition, FAN and its effect is temperature dependent, as temperature influences the dissociation constant of ammonia nitrogen. Base on the Eq. 1, one can conclude that the concentration of free ammonia at a given pH and TAN is two and six times lower for an ambient temperature digestion than a mesophilic and thermophilic digestion respectively.

After measuring total ammonia concentration in the bench-scale reactor system, the concentration of free ammonia was calculated for the period between 4/18/2014 to 11/19/2014. During this period, the system had been operated under both mesophilic and ambient temperature conditions. Overall, FAN was low in the bench-scale reactor, except on two occasion near 8/1/2014 and 10/17/2014, when FAN was 673.4 mg/L (pH=9.28) and 770.7mg/L (pH=10.21), respectively (Figure 2). In both cases, the reason for this observed increase in FAN was due to a sudden, unintended increase in pH due to operation error.

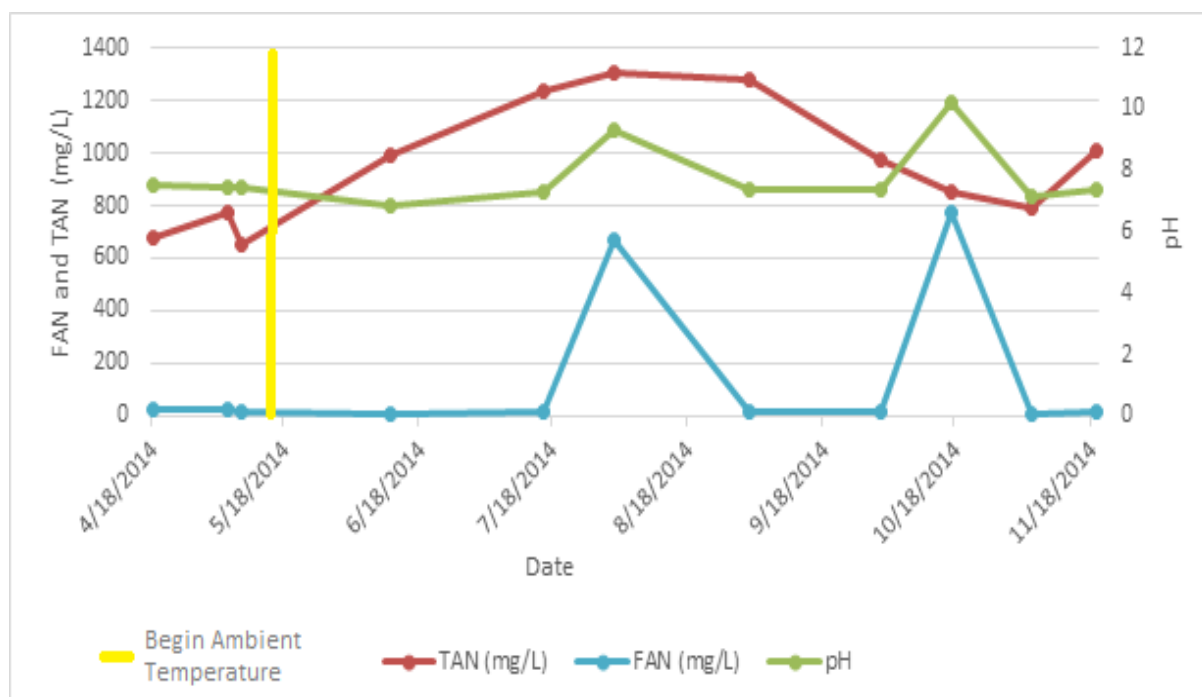


Figure 2. FAN at various TAN and varying pH for the same digester at mesophilic and ambient temperature.

Table 1. TAN, pH and FAN of a digester operating in mesophilic and ambient temperatures.

Date	Day	TAN (mg/L)	pH	FAN (mg/L)	Temperature of operation
4/18/2014	629	680	7.53	24.4	Mesophilic
5/5/2014	646	777	7.48	25.0	Mesophilic
5/8/2014	649	655	7.48	21.0	Mesophilic
6/11/2014	683	995	6.88	4.2	Ambient
7/16/2014	718	1235	7.33	14.7	Ambient
8/1/2014	734	1300	9.28	673.4	Ambient
9/1/2014	765	1275	7.4	17.8	Ambient
10/1/2014	795	970	7.4	13.6	Ambient
10/17/2014	811	855	10.21	770.7	Ambient
11/4/2014	821	795	7.14	6.1	Ambient
11/19/2014	839	1005	7.4	14.0	Ambient

As one can see in Table 1, the increase in 2 units of pH, at ambient temperature and similar TAN, led to an increase in FAN of about 45 times. Angelidak and Ahning (1993) proved that a FAN of approximately 650 mg/l resulted in a 20% reduction in the growth rates of the aceticlastic methanogens in experiments with ammonia toxicity, resulting in a reduction of approximately 25% of methane production.

Daily methane production from the bench-scale system during the period of investigation is shown in Figure 3. It was observed that the system did not yield stable biogas production upon switching to ambient temperature. During the first 15 days after switching to ambient temperature in the AnMBR (day 654 to 669), methane production was observed to decrease substantially from 316.9

to 47.4 mg/g VS added, whereas from day 670 to 740, methane production was observed in the range of 100 to 200 mg CH₄/g VS added. It is suspected that the observed decrease in methane production was due to the previously mentioned fluctuations in pH, which led to an increase in free ammonia nitrogen concentrations which inhibited methanogenesis. It was reported by Chen et al (2008) that high concentrations of ammonia nitrogen is toxic to anaerobes, and will decrease the efficiency of digestion and upset the process. Besides that, the reduction in temperature might have shocked or stressed the micro-ecosystem of the AnMBR, which after time was able to adapt and return biogas production to a higher levels. The lowest level of methane production (17 mg CH₄/g VS added) was measured on day 827, 20 days after an accidental increase of pH from 7.38 to 10.05. This accident caused an increase of free ammonia (FAN) from 13.6 mg/L to 770.7 mg/L. Many lower free ammonia concentration concentrations (27 – 635 mg/L) have been reported to inhibit 50% to 100% of methane producing in un-adapted processes (Siles et al., 2010; He et al, 2011; Bayr et al., 2012 and Garcia and Angenent, 2009). It is clear that fluctuation in pH can directly and indirectly affect methane production. It therefore shows that an effective regulation of pH is mandatory to achieve and maintain optimal biogas production.

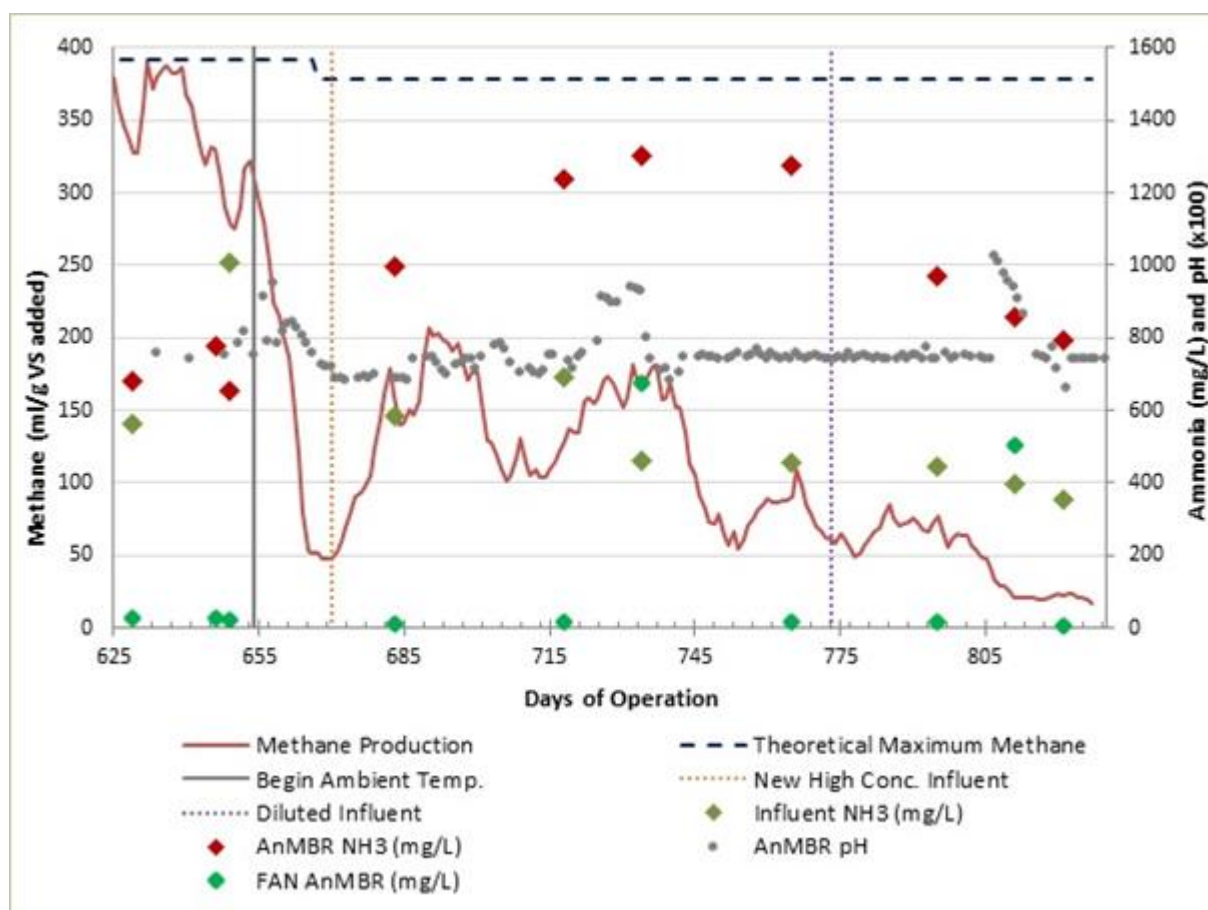


Figure 3. Methane production, pH, total and free ammonia concentrations.

4. CONCLUSION

The literature search revealed that AD is a complex process that depends on the coordinated activity of a complex microbial community to transform organic material into carbon dioxide and methane. Within the anaerobic environment, various important parameters affect the rates of each step within the digestion process, i.e. pH and alkalinity, temperature, and toxicants. Among



other substances that can negatively affect the performance of an anaerobic digester, the concentration of free ammonia is a very relevant one. While ammonia concentrations below 200 mg/L are suggested to be beneficial to anaerobic processes by providing nitrogen, an essential nutrient for anaerobic microorganisms, higher concentrations can result in process failure acting as an inhibitor to methanogen growth. As a result, pH is also a very important parameter in anaerobic digestion systems due, among other factors, to its role in the regulation of free ammonia concentration. When an unadapted digester faces pH fluctuation, the health of the process and hence the biogas production are compromised. In summary, this investigation highlights the importance for proper pH control and monitoring in order to achieve and maintain optimal digestion performance.

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