Two-Stage (Liquid-Solid) Anaerobic Digestion of High Solid/High Ammonia rich Manures at a Low Temperature adopting Recirculation- Percolation Operational Mode

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Abstract

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Prativa Mahato

Globally, livestock and poultry production leads to total emissions of 7.1 Gigatonnes of Carbon-dioxide (CO_2)-equivalent per year, representing 14.5% of all anthropogenic greenhouse gas emissions. Anaerobic digestion (AD) is a sustainable approach to generate methane (CH_4) from manure, but the risk of ammonia inhibition and high-solids can limit the AD process. A two-stage (liquid–solid) batch-mode AD biotechnology at a low-temperature (20±1 °C), using an adapted liquid inoculum, was developed to address the limiting factors. This study deals with chicken manure (CM) and dairy cow manure (DM) as feedstock. Furthermore, liquid inoculum recirculation-percolation mode was adopted to replace mechanical mixing. The findings from physio-chemical and biogas analysis showcased the sustainability of this novel biotechnology technique, yielding impressive results at 20±1 °C. Firstly, CM liquid inoculum was adapted to high-ammonia concentrations. Secondly, mono-digestion of CM (TKN: 23-33g/L; TS: 68-72%) was conducted in the aforementioned AD technology. Then, a start-up study on co-digestion of CM+DM (TKN: 13.6 g/L; TS: 48-51%) was conducted for 190 days using same technology. Moreover, to investigate AD process stability, physio-chemical parameters were monitored. The objective of this study was to demonstrate the operational feasibility of the proposed AD biotechnology. Results showed that, although a better SMY (0.52 ± 0.13 L CH4g−1VSfed) was obtained for mono-digestion of CM, co-digesting CM + DM showed a better methane quality and generated comparatively lower FAN. Finally, techno-economic assessment of the aforesaid AD technology, processing 1tonne-CM/day showed that the AD plant obtained revenue after 14 years from the commencement of the project.

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Contribution of Authors

This thesis would not have been possible without the contribution of the following co-authors:

- Suman Adhikary, initiated the project with primary experimental set-up and has a contribution in writing and editing of Chapter-3 of this thesis.

Chapter-5 has been published with the contribution of co-authors:

- Dr. Rajinikanth Rajagopal has contributed in conceptualization, methodology, investigation, writing, editing and major supervision.
- Dr. Saifur Rahaman have played a role for providing resources and investigating the project.
  Dr. Rajinikanth Rajagopal and Dr. Bernard Goyette provided funding for the project.

Finally, I, Prativa Mahato, have a role in conducting all experiments, preparing original written drafts, reviewing and editing of the entire thesis.

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List of Abbreviations

AD	Anaerobic Digestion
ASBR	Anaerobic Sequencing Batch Reactor
C2	Acetic Acid
C3	Propionic Acid
C4	Butyric Acid
iC4	Iso-Butyric Acid
C5	Valeric Acid
iC5	Iso-Valeric Acid
C6	Caproic Acid
CH_4	Methane
CM	Chicken Manure
CO_2	Carbon-Dioxide
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DM	Dairy Manure
FAN	Free Ammonia Nitrogen
FAO	Food and Agriculture Organization
GHG	Green House Gases
HSAD	High Solid Anaerobic Digestion
NPV	Net Present Value
NO_2	Nitrous Oxide
OLR	Organic Loading Rate
PL	Poultry Litter
R-P	Recirculation-Percolation
SBR	Sequencing Batch Reactor

SCFA	Short Chain Fatty Acids
SCOD	Soluble Chemical Oxygen Demand
SM	Swine Manure
SMY	Specific Methane Yield
TA	Total Alkalinity
TAN	Total Ammonia Nitrogen
TCOD	Total Chemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
TVFA	Total Volatile Fatty Acids
UNFAO	United Nations Food and Agriculture Organization
VFA	Volatile Fatty Acids
VS	Volatile Solids

Chapter 1: Introduction

1.1 Background Information

In the last 50-60 years, there has been a huge shift in the economy, which has directly affected the food consumption pattern worldwide [1]–[4]. Food consumption patterns have mainly shifted from plant-based products to animal-based products and agro-based or livestock industries have overtaken the individual farmers in the urban parts of the world [1], [5]–[7]. One of the major causes of this shift is the growing urbanization and population [8]. United Nations Food and Agriculture Organization (UNFAO) statistics of average meat supply demand per capita per year of the world shows a linear growth in the meat demand [8]. The average meat supply per capita in America, Canada, China and World, as shown in Fig 1.1, illustrates that irrespective of China with its highest population, the average meat supply per capita per year of Canada is approximately double than China's. Figure. 1.1 also indicates Canada having highest average meat supply per capita (100 kg per year); higher than America, China and World. Solely in Canada, poultry (chicken and turkey), pig and cattle are the three major produced and consumed meat products. Among which, chicken rose by about 9 kg per capita between 1999-2018 and was recorded as the most consumed meat in 2018 [2]. This growth was also in accordance with UNFAO livestock counts of the World and Canada between 2000-2014 (Figure 1.2(a) and Figure 1.2(b) which shows a steep rise of chicken counts among other livestock like pigs and cattle. This remarkable difference among chicken counts and other livestock could be due to high protein and energy efficiency in chickens coupled with the low feed demand [7]. Chicken and eggs are cheaper sources of protein and the low feed demand illustrates lower economic investment in chicken or poultry farms [2].

Therefore, chicken production in Canada alone in 2018 was around 1.3 million metric tonnes. Out of which, Ontario(ON) had 34% share among other Canadian provinces (Figure 1.3) followed by Quebec (QC) and British Columbia (BC) with 26.4% and 14.7%, respectively [2]. QC, with its second-highest number of chicken farms, had 597 instalments as of December 2019 [2].

As a result, numerous poultry farms have emerged in the last few decades in all over Canada and worldwide [9]. Therefore, with the proliferation of poultry industry, there has been a huge accumulation of chicken manure (CM) in the world [9]–[11]. According to the 2017 annual report by Egg Farmers Canada, 24.5 million hens were in production in 2017 in Canada, which was a 1.3 million increase over 2016, resulting in a large volume of excreta accumulation [2], [12] . As per the report generated by Food and Agriculture Organization (FAO) in 2012, CM generated worldwide was approximately 42 million tonnes [9]. Additionally, rise in swine manure (SM) and dairy cow manure (DM) also exists in parallel [6], [13], [14].

Thus, this huge amount animal excreta or manure requires a proper management system and channel because improper management of manure can lead to drastic damage to the environment such as climate change, eutrophication, nutrient overloading and degradation of soil and water quality [2], [15], [16].

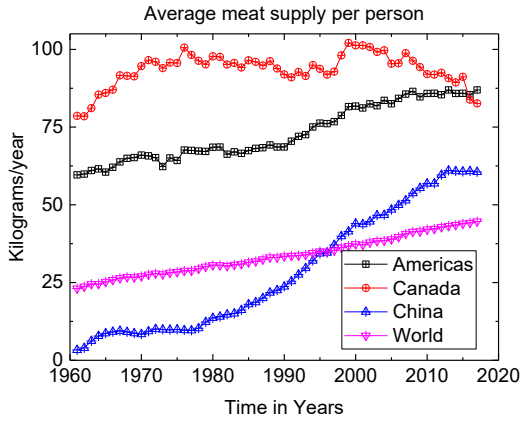


Figure 1.1:Average meat supply [8]

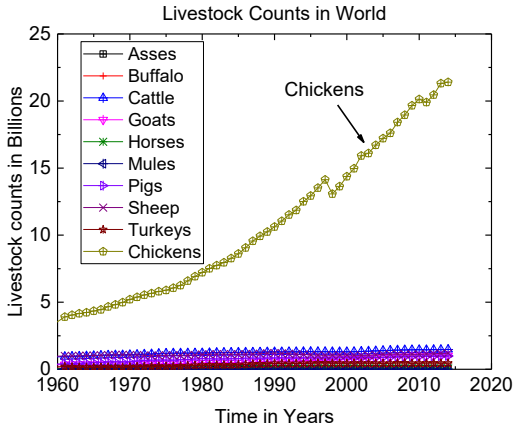


Figure 1.2(a): Livestock counts in World [8]

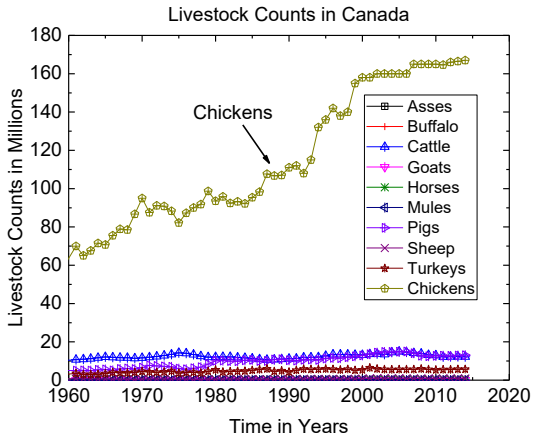


Figure 1.2(b): Livestock counts in Canada [8]

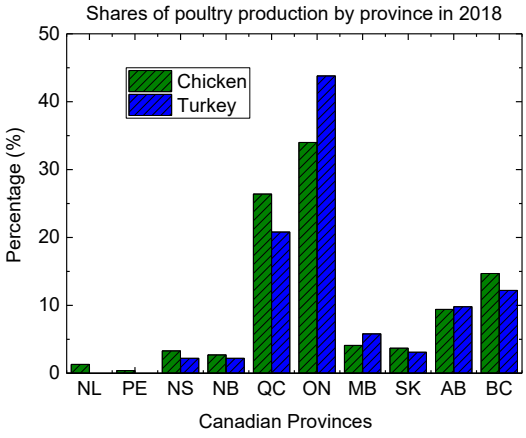


Figure 1.3: Shares of poultry production [2]

Several methods and technologies dealing with the animal manure management have already been studied previously, like land application [17], composting [18], and biochar production [19]. Firstly, land application of manure is widely exercised manure management technique because manure is high in nutrients like nitrogen, phosphorus and trace elements which are beneficial for crop production. Land application also improves the physical and biological fertility of soil. However, the direct application of manure on the crops has been studied to interrupt seed germination and CM being the hottest of all manure can burn the plant roots [20]. Furthermore, manure when applied under soil creates environmental pollution due to leaching of nutrients and tracing its path to the water bodies, ultimately resulting in contamination and eutrophication [18]. Secondly, composting is another simple techniques for manure management as composting reduces waste mass, destroys weed seeds, provides sufficient sanitation, and produces valuable end products for agriculture [21], [22]. However, the high release of soluble nitrates in the soil inhibits the process, thus high carbon crops should be mixed with nitrogen-rich manure (like CM) to maintain the Carbon: Nitrogen(C:N) ratio [23]. On the other hand, biochar has its benefits on

soil amendment because of its positive effects on microbial population, soil nutrients, and growth of plants [24]. Biochar prepared from poultry manure benefits in odour reduction, pathogen reduction and the volume of manure management [25]. Furthermore, hydro char prepared from combustion of CM results in eliminating fossil fuels requirement [26]. However, application of biochar to soil tends to change soil biological community composition, which affects the nutrient cycles and plant growth. In addition, biochar may also emit other GHG from soil such as nitrous oxide (N_2O) or methane (CH_4), thus further accelerating climate change [27], [28].

Moreover, thermal technologies like pyrolysis, combustion, gasification and bacterial methods like AD are also well-known techniques to convert manure into renewable source of energy [26], [29], [30]. Thermal methods reduce waste, odour, and pathogens and ultimately generates electricity, however accumulation of ash and the requirement of preliminary appeal for thermal technologies installments from American Public Health Association (APHA) are some of the major obstacles for this method [28].

Therefore, a bacterial method, like AD, in which microorganisms and bacteria are used to produce energy from organic wastes, can be a sustainable and clean technology to process manure as it proves to be a means to combat climate change and achieve environmental benefits [29]. In recent years, AD has received great attention due to its obvious advantage, i.e., reducing pollution, converting organic waste into high-quality biogas, which is useful in the form of heat and/or electricity [31].

CM, among other livestock manures has the highest organic content (56-62 % Volatile Solids (VS)) and holds a potential capacity to produce methane-rich biogas through AD [29]. Additional benefits of AD of CM are reduction of odour, production of stable end product and renewable energy, however, the success rates of utilization of CM as a feedstock in AD is considerably low due to its high ammonia content especially free ammonia nitrogen (FAN). FAN can rupture the cell walls of the microbes when exceeded above 0.5 g/L and lead to cell lysis and process inhibition [32], [33]. Therefore, to counteract ammonia inhibition, research on methods like ammonia stripping, membranes and struvite precipitation have been conducted [34], [35]. Nonetheless, these methods add up the cost to the AD process and makes economically impractical.

The other major concern with CM as a feedstock is its high solid content (>25% total solids (TS)). In general, wet AD (dilution of organic waste) is the widely practiced AD worldwide for easy operation and maintenance [32], [36]. Dilution facilitates microorganisms to proliferate and speeds up to process high solids and high ammonia manures [36]. However, dilution generates huge quantity of waste and demands larger bio-digesters to operate [37]. The conventional AD system, mainly wet (< 10% TS) and semi-solid (10-15% TS), therefore fails to treat high solid manure like DM and CM having high ammonia concentration (> 6 gTKN/L) as well [11], [36], [38], [39].

Therefore, despite having a great potential as an organic substrate for biogas production, utilizing CM and DM fully still remains a great challenge through conventional AD.

One of the methods to counteract the high ammonia and high solids issues in AD can be a low-temperature high solid anaerobic digestion (HSAD). Low-temperature often declines the AD process but it also facilitates in lowering the FAN concentration in the digester and prevents from AD inhibition [40].

Besides, HSAD requires a smaller reactor volume and less energy input, making it economically feasible [36]. Instead of dilution, HSAD technique can be incorporated with the R-P of adapted liquid inoculum; (i) recirculation of liquid inoculum to HSAD and (ii) percolating liquid inoculum through HSAD. A clear diagram of R-P mode is shown in Figure 1.4. Recirculation of liquid inoculum to HSAD increases the moisture content in HSAD and increases microbial activity and percolation enables flushing of accumulated ammonia and Volatile Fatty Acids (VFA) from HSAD to liquid inoculum reservoir [39]. R-P of adapted liquid inoculum, therefore also eliminates the need of mixing, saves energy input, enhances digestion process and increases production of gas [37], [41]. Furthermore, processing high solid manure in HSAD alone results in poor start-up of the digester, which has been found due to incomplete mixing and accumulation of VFAs [36]. Therefore R-P of adapted liquid inoculum also boosts-up the HSAD process by reducing the start-up phase [37], [39].

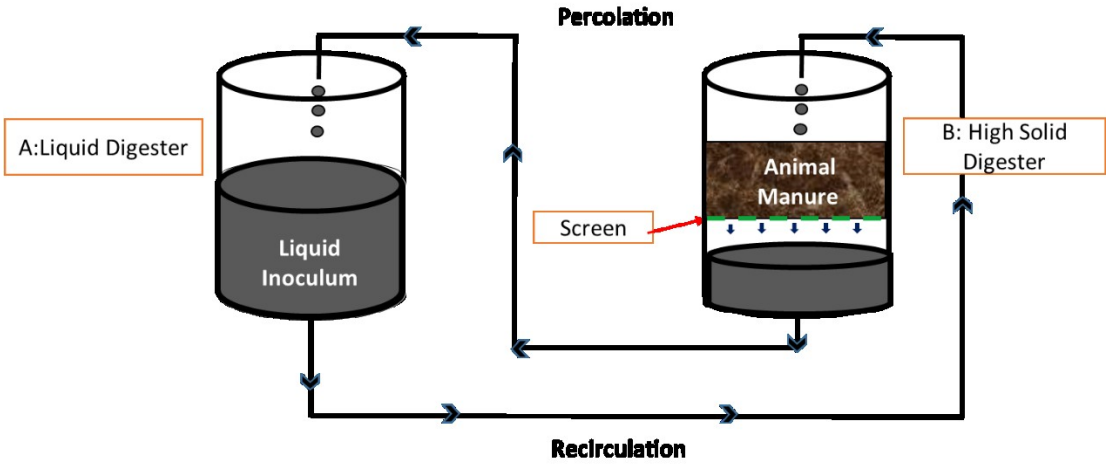


Figure 1.4: Schematic Set-up of two-stage(liquid-solid) HSAD system

Another additional strategy to overcome digester failure can be co-digestion [38]. Co-digestion is one of the sustainable approaches to solid-waste management. It improves the nutrient balance of the digester by the addition of carbon rich and lignocellulosic compounds like crops, DM to the nitrogen rich CM. Co-digestion also increases gas yield [42] and the load of biodegradable organic matter in the digester [43]. Along with this, co-digestion increases bacterial activity, and improves methane production. In addition, co-existence of poultry and cattle are commonly found in the livestock farms. Therefore, co-digestion of manure benefits in many ways like reduction of manpower in the segregation of waste to be processed, facilitates in handling of waste and reduction of toxic compounds [43], [44], [45].

Hence, a low-temperature two-stage (liquid-solid) HSAD can be a suitable and energy efficient biotechnology to process high solid manures like CM and DM, either separately (mono-digestion) or together (co-digestion) in order to avoid ammonia inhibition and maximize biogas production. Low-temperature digesters can be an economically practical solution for the livestock farms located in the cold-region countries like Canada. Therefore, the above-mentioned technology can be boon to the small and medium-sized farmers by providing them a clean, green and sustainable method to process the animal manure waste.

1.2 Objectives

The main objective of this study was to develop a low-temperature (20±1ºC) two-stage HSAD biotechnology to process CM alone and mix of CM+DM, adopting liquid inoculum R-P mode. Another long-term objective of the study was to provide an easy, cost-effective, clean technology for the small to medium size poultry/livestock farms located in cold weather conditions and thus to promote good farming practices. The specific objectives of this thesis are:

i. To investigate the performance of low-temperature anaerobic digestion biotechnology for processing CM rich in high-solids and high-ammonia concentrations.
ii. To develop and acclimate liquid inoculum at high-ammonia concentration to reduce start-up phase of the digester, avoid ammonia inhibition and maximize methane production.
iii. To control short chain fatty acids build-up in the digester processing solid CM.
iv. To demonstrate the operational feasibility of two-stage process for the co-digestion of CM and DM at a low temperature: a start-up strategy.
v. To assess the techno-economic aspect of two-stage HSAD processing CM at a rate of 1 tonne waste/day using Net Present Value (NPV) model.

1.3 Outline of the thesis

The thesis consists of 7 chapters as mentioned below.

Chapter 1 gives a brief background of the growing animal and livestock production (mainly poultry/chicken) and huge quantity of manure generation worldwide and in Canada; this chapter also proposes a solution to tackle the issue of animal manures considered in this study (Figure. 1.5) and states the objectives of this thesis.

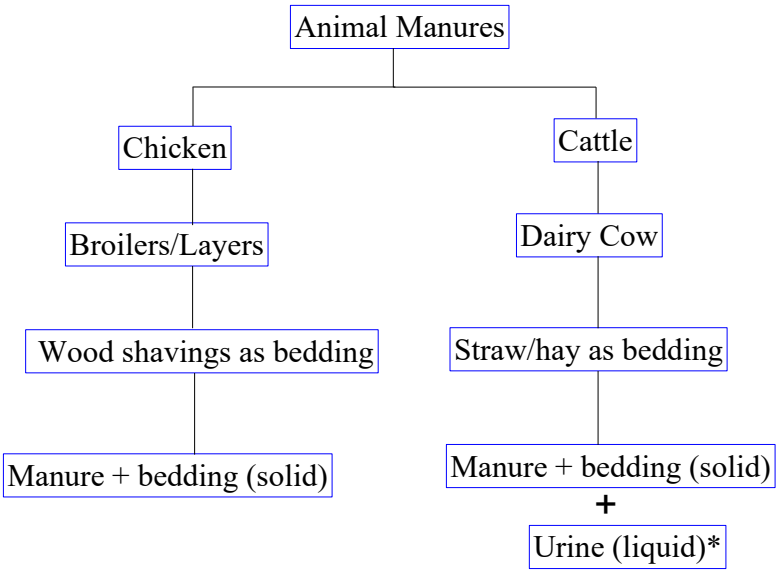
In Chapter 2, literature review on AD, its process and types of AD, influencing factors for biogas production, and previous works conducted AD of high solid and ammonia manures using are mentioned.

Similarly, in Chapter 3, adopting the two-stage HSAD biotechnology, CM is processed for 282 days in 4 different cycles (69-71 days per cycle). The study of AD of CM is followed by the performance assessment and monitoring of the digester in Chapter 4 using critical indicators and ratio limits like TVFA/TA ratio, propionic/acetic acid ratio and (butyric+valeric)/acetic acid ratio.

In chapter 5, A preliminary study (start-up) of co-digestion of CM and DM is conducted adopting the same technology adopted in Chapter 3 and 4.

In chapter 6, a techno-economic assessment of the two-stage HSAD of CM treating 1 tonne CM waste per day is conducted.

Finally, chapter 7 includes the conclusions and future scopes derived from the overall study.



*Urine (liquid) is a wastewater type of waste and was not treated in this study

Figure 1.5: The type and manure used in this study

Chapter 2: Literature Review

2.1 Anaerobic Digestion

Anaerobic Digestion (AD) is a clean and sustainable technology which utilizes organic waste in a series of biochemical conversion processes influenced by different microbial communities and catalyzed by enzymes (intracellular and extracellular) [46]. Organic content is utilized under 5 distinct conversion processes to produce biogas as shown in Figure 2.1. [47], [48]. The organic matter portion of the waste is first disintegrated into biopolymers (carbohydrates, proteins and lipids) from particulate components, then the hydrolysis of the biopolymers takes place which converts biopolymers into monomers (amino acids, sugars and fatty acids) [49]. Further, this is followed by the process of fermentation or acidogenesis in which monomers transform to carboxylic acids like acetic acid, propionic acid and carbonates. After that, carboxylic acids change to hydrogen and acetate in the acetogenesis phase, which finally turns into biogas in the methanogenic phase. Biogas comprises of approximately 60% CH_4 and 40% CO_2, respectively [50]. The percentage biogas composition means the biogas has 60% of the energy in natural gas or approximately 600 BTU per cubic foot (22 MJ/m^3) [51]. However, in order to upgrade biogas to bio-methane (natural gas), methods like CO_2 sorption and separation (membrane) are approved [50]. Therefore, biogas is a renewable energy source and can eliminate the requirement of fossil fuels.

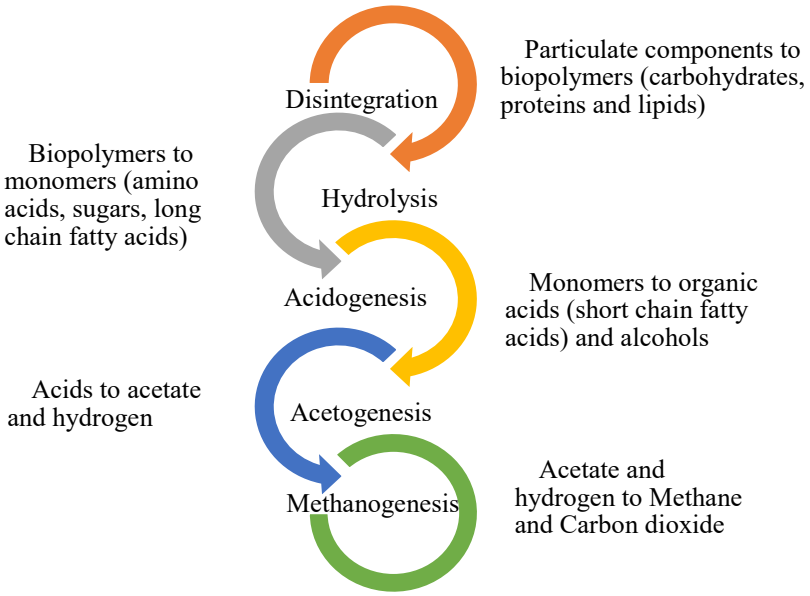


Figure 2.1: Anaerobic Digestion Process [48]

Along with biogas, one of the final end-products obtained from AD process is digestate. Digestate is useful as fertilizer as it contains nutrient-rich elements that are more readily available for crop uptake. Therefore, the digestate can be processed into portable chemical fertilizers by thickening, drying or struvite precipitation [23], [35], [52].

2.2 Anaerobic Digestion of Animal Manure

Animal manure is one of the valuable sources of nutrients and renewable energy [53]. However, improper treatment of animal manure causes emission of air pollutants, soil and water pollution [9], [28]. The pollutants emitted from manure include CH_4, N_2O, ammonia, hydrogen sulfide (H_2S), volatile organic compounds and particulate matter, which can give rise to serious environmental and health concerns [9], [27], [28]. AD is a well-established technology to treat organic wastes like food waste, kitchen waste and animal waste [53]. AD of animal manure has become increasingly attractive in the last decades worldwide as a unique solution to provide a reasonable economic return from energy production and minimize accumulation of waste [39], [45]. In Canada, the principal feedstock used in the farm-based AD is manure. Among the manures, DM is the predominant feedstock ingredient. With the ever increasing poultry farms in Canada, the highest percentage in Ontario (ON) province and Quebec (QC) being the second, the farm-based AD in the poultry farms have become a must to minimize CM waste accumulation [2].

Irrespective of the simple fundamentals of the AD process, the operation and control can be challenging. In addition, manure with its nutrient-rich compounds, can make AD systems more complex [6]. The main difficulty lies in the maintenance of suitable operating conditions for digestion. Furthermore, with one or more feedstock, establishing and maintaining a good recipe becomes equally challenging. These issues can be however tackled with a proper knowledge and understanding of AD dependent operational parameters [36], [43], [54].

2.3 Factors affecting AD Process

AD systems depend upon several factors like materials used (feedstock), mode of operation (mixing, temperature) and type of digesters [48], [49]. Moreover, the main factors that affects biogas production are pH, alkalinity, temperature, ammonia and VFA. The organic content in terms of chemical oxygen demand (COD), solids content (TS and VS), Organic Loading Rate (OLR) and Retention Time or Cycle Length (CL) are some of the additional important factors determining the development and maintenance of a stable digester [36], [37], [48]. These operational parameters are described in detail below.

2.3.1 pH

pH is one of the important parameters of the AD process, it is the concentration of hydrogen ions (H^+) in the system. It is well-known that a neutral pH is an ideal and optimal condition for an AD process. This is because of diverse microbial communities that contribute towards the generation of biogas. The microbial community differs at different AD stages (Figure 2.1), demanding different pH levels to survive and react at an optimum pace [36]. Besides, the stability of the AD process also is highly dependent upon the balance between acidogenic and methanogenic bacteria [32]. For instance, acidogenic bacteria prefers acidic pH (< 6.5), whereas methanogens demand for a neutral pH range (6.8-7.2) [55]. However, acidic pH levels might lead to the accumulation of VFA, followed by decrease in CH_4 gas production [32], [56]. On contrary, a pH between 7-8, under mesophilic conditions (35-40 ^oC) leads to a massive increase in FAN level, which is considered as a major cause of methanogens [32]. Therefore, a neutral and optimum pH is recommended for stable AD in order to minimize VFA and ammonia toxicity and generate a rich biogas yield [57], [58].

2.3.2 Alkalinity

In an AD process, alkalinity has a major role in maintaining pH at a desirable range (6.8-7.2) and buffers VFA generation. Sufficient alkalinity helps to balance the rapid change in pH and enhances the buffering capacity. It also enhances the diversity and richness of microbial populations and favors methane-forming bacteria, facilitating in methane production. For a wet digester, alkalinity is preferable to remain within 1.5 to 5 g/L. In order to maintain desired alkalinity in the digesters, additional sources of alkalinity (calcium or sodium bicarbonates) are generally added to feedstock which are scarce in alkalinity. This, however is not required in nitrogen-rich animal manure. As alkalinity is the outcome of degradation of proteinaceous wastes such amino acids and ammonium ions, CM is generally rich in ammonia compared to other manures. Therefore, an abundant level of alkalinity between 13.5-40 g/L as $CaCO_3$ is observed in high solids CM [37], [59]. However, even higher alkaline conditions are preferred to be avoided in order to prevent the collapse of microbial particles [60]. Besides, increase in alkalinity can serve as an indicator of an adverse operational condition for anaerobic digesters [49]. Therefore, suitable alkalinity facilitates process stability and CH_4 production.

2.3.3 Temperature

Temperature plays an important role in AD process performance as it greatly affects the kinetics and growth of microbial community [13], [36]. Furthermore, temperature can also regulate the intracellular enzymatic activity of microbes in the AD process. The three main temperature ranges in which AD is operated are : (i) mesophilic (20-40^{o}C) (ii) thermophilic (50-60^{o}C) and (iii) psychrophilic (10-20^{o}C) [13], [36], [38]. Among these, the mesophilic condition is the most widely adopted worldwide as it is relatively stable and easy to operate [55]. Thermophilic temperature is an effective condition for pathogen removal and requires less Hydraulic Retention Time (HRT) because of faster particle degradation rate [40]. However, thermophilic temperature consumes more energy for heating and leads to ammonia inhibition. On the other hand, psychrophilic temperature is energy-efficient, stable and easy to manage [13], [61], although it requires longer HRT comparatively to generate equivalent biogas (to other temperatures). In Canada, psychrophilic AD systems are designed and operated in QC province [62].

2.3.4 Ammonia

Ammonia carries a major role in the performance and stability of AD of nitrogen-rich organic wastes. It is the end-product of AD of proteins and nucleic acids [57]. Ammonia also neutralizes organic acids in the acidogenic phase and assists in maintenance of optimum pH. However, studies conducted on effect of ammonia on AD of solid wastes like agro-food and livestock wastes shows that high ammonia concentrations are toxic for AD and causes sudden failure or inhibition [32], [57]. Ammonia inhibition is mainly caused by Total Ammonia Nitrogen (TAN), which comprises (i) ammonium ions [NH_4^+] and (ii) free ammonia nitrogen (FAN) [NH_3]. However, FAN is considered a principal inhibitor [63] as FAN exceeding between 0.60 g/L-1.10 g/L can be a serious problem [32]. However, the threshold limit remains inconsistent and is dependent on the nature of substrates. FAN is also dependent upon pH and temperature, in relation with TAN as mentioned in earlier studies [64], [65], which is shown in the Equation 2.1 below.

$$\text{FAN} = \text{TAN}\left(1 + \frac{10^{-pH}}{10^{-\left(0.09018+\frac{2729.92}{T(K)}\right)}}\right)^{-1} \quad (2.1)$$

AD operated at constant temperature of 35°C and variable pH (7 and 8) illustrated that FAN generated at pH 7 was lower than at pH 8 [66]. Similarly, AD operated at constant pH and variable temperature (35°C and 55°C), demonstrated that FAN was six times higher at thermophilic (55°C) than at mesophilic (35°C) [66]. A comparative study con ducted on FAN generated at different temperature for pH range between 7.2-8.4, clearly stated lowest FAN levels at psychrophilic (20°C) or low-temperature condition [39]. Therefore, low-temperature and a neutral pH level are some of the ways to control high production of FAN.

Other common methods practiced in AD plants to avoid ammonia inhibitions are dilution [67], co-digestion [39], [67] and acclimation of microflora to high-ammonia concentrations [37], [39]. Dilution requires addition of huge amount of water in the high solid substrates like CM having 20-62% TS [68], [69]. Solely, for the dilution of CM having 30% TS to 3% TS, water requirement is around 9 m^3/t CM. However, it increases the consumption of water and decreases the biogas generation per unit of digester volume [70], [71]. Co-digestion, on the other hand can reduce ammonia inhibition and generate higher biogas outputs. For instance, for high nitrogen waste like CM, addition of carbon-rich substrates (DM and straw) with CM can help to avoid ammonia inhibition but co-digestion also increases the complexity of the process and transportation cost [70]. Further, utilization of acclimated-microflora is another technique to combat ammonia-inhibitions. An experiment conducted at thermophilic temperature to determine ammonia inhibitory level revealed that using non-adapted inoculum inhibited at 2.5 gN/L, however adapted inoculum inhibited at 4 gN/L. On contrary, a co-digestion of CM+DM conducted at 20°C using adapted inoculum demonstrated no-ammonia inhibitions [39]. Therefore, pH and low-temperature control, co-digestion (balancing Carbon/Nitrogen ratio), adaptation of microflora to high ammonia concentrations are some of the key ways to significantly reduce ammonia inhibitions.

2.3.5 Volatile Fatty Acids (VFAs)

VFAs are intermediate by-products of AD process and their production is beneficial for efficient biogas production [56]. As shown in Fig 2.1, long chain fatty acids (LCFA) and Short Chain Fatty Acids (SCFA) are generated in the hydrolysis and acidogenic phase of AD process. SCFA are also called Total Volatile Fatty Acids (TVFA) and are vital nutrients for methanogens [58]. SCFA comprises of acetic acid (C2), propionic acid (C3), butyric acid (C4), iso-butyric acid (iC4), valeric acid (C5), iso-valeric acid (iC5), caproic acid (C6). Among which, C3 is an important indicator for AD process stability. It is preferred to be less than C2 because of its toxic nature and lower capability to get oxidized into C2 [72], which might lead to VFA accumulations. In general C2 is the dominant SCFA with 20-75% composition [58].

Along with high ammonia, high VFA level is another inhibitory factor for AD process. Although, sufficient VFA is beneficial for methanogenic activity to obtain methane-rich biogas, however enhanced VFA concentrations production 7.3 g/L-10.5 g/L [73] in a high strength wastewater resulted towards elimination of methanogens in the digester. In addition, exceeding a threshold limit can also lead to acidic pH, VFA accumulations and digester failure. However, the threshold limit is dependent upon the nature of substrate, pH, temperature, HRT and OLR. Among which pH and temperature are the primary factors.

AD operated at variable pH (uncontrolled, 5, 6 and 7) illustrated that VFA generated at pH 6 (39.46 g/L) was higher and suitable for food waste than at pH 7 (37.09 g/L) [74]. Similarly, AD operated at variable temperature (35ºC, 45ºC and 55ºC), demonstrated that VFA was lowest at 55ºC (14.9 g/L), than at 35ºC (41.3 g/L) and 45ºC (47.9 g/L) [74], indicating higher solubilization of food waste at higher temperature. Furthermore, a two-stage (liquid-solid) AD of high-solids CM+DM carried out under pH (7.2-8.4) at 20ºC resulted a maximum VFA concentration of 28 g/L without inhibition, which was supported by high alkalinity (10-16 g/L as $CaCO_3$) and utilization of adapted liquid-inoculum [39]. Taken together, these data highlights significant differences of suitable VFA levels at distinct pH and temperature and depends primarily upon substrates composition [58], [74]. However, in practical application, pH control is the most likely and important factor to determine the VFA yield for a stable operation.

2.3.6 C/N Ratio

C/N ratio in feedstock can play a major role in regulation ammonia content in AD process. Less nitrogen in a substrate often causes incomplete utilization of carbon content. On the other hand, high nitrogen or smaller C/N ratio can also cause ammonia inhibition. Among the other techniques, optimizing C:N ratio is also one of the ammonia inhibitor control strategies. Co-digestion of carbon-rich animal manure (DM) or crops and nitrogen-rich manure (CM) can improve C/N ratio which further increases biogas production and be a cost-effective method for a biogas plant. For the understanding of C/N, TCOD/TKN ratio are also used by some studies [39], [69]. Co-digestion of CM with DM at equal ratio with C/N ratio 30, showed better methane concentration and lower FAN level than CM alone with C/N ratio 26 [39]. Further, co-digestion of 50% CM with 50% organic municipal food waste showed higher methane and biogas yield than mono-digestion of CM alone and stabilized C/N ratio [75]. Similarly, co-digestion of whey with poultry manure also showed better biodegradation rate and was attributed to increase in C/N ratio [67]. However, higher C/N ratio between 59-210 also disturbs the AD process due to the lack of ammonium-nitrogen in the feed which ceases microbial growth [32]. C/N ratio between 20-30 is considered optimal for increase in COD degradation, less FAN generation, high biogas production and improvement in VFA digestion [32], [75].

2.3.7 Organic Loading Rate (OLR)

OLR is the amount of organic content (in terms of VS and soluble COD) per unit volume of a digester for a unit time period [76]. Studies suggest OLR depends upon organic material concentration in a substrate and the operational period (retention time period). It is also one of the key factors for biogas generation and increases with increase in substrate concentration. At an OLR between 2-10 g/L.d, a higher methane biogas was observed. In addition, VFA concentration was observed low and sufficient alkalinity prevented from acidification [76]. Similarly, CM digested at mesophilic and thermophilic at two OLR (1.6 $kgVS/m^3.d$ and 2.5 $kgVS/m^3.d$) revealed lower methane yield at thermophilic condition. Besides, at higher OLR (2.5 $kgVS/m^3.d$), higher FAN and VFA concentrations was observed which affected specific methanogenic activities adversely. In addition, a comparison of OLR at different temperature showed better organic matter utilization rate in psychrophilic condition [77]. However too high OLR can cause AD inhibitions and changes microbial structure. For instance, mono-digestion of poultry litter at psychrophilic condition demonstrated AD inhibitions at higher OLR of 21.6 $gVS/kg_{inoculum}VS/d$ [69].

2.3.8 Hydraulic Retention Time (HRT)

HRT is an important parameter of AD process that affects the conversion of organic matter into biogas [48]. HRT is the time period substrates remain in the anaerobic digester. The term HRT is mainly used for wet AD and digesters treating wastewaters. For solid digesters, a term solid retention time (SRT) is utilized. Some studies also mention cycle length (CL) to mention the operational period [39], [77]. A shorter HRT is mainly desired for an energy-efficient and process efficient AD. However, in order to achieve complete utilization of organic matter, a longer HRT is required. HRT also influences biogas production, methane and VFA concentration. Anaerobic Digestion of wheat straw at different HRT (20, 40 and 60 days) revealed higher biogas yield at HRT 60 days. However, HRT or CL can be reduced by the help of adapted inoculum [39], which boosts the digestion process and reduced start-up phase. Therefore, HRT is an important operational AD parameter that influences the process and stability of AD process.

2.4 Types of AD systems used for manure treatment

Different types of commonly used AD systems for the treatment of manure waste as shown in Table 2.1 are Batch reactors, Continuously stirred tank reactor (CSTR), Plug flow reactor (PFR), Anaerobic sequencing batch reactor (ASBR) [13], [3] and Leach Bed Reactors (LBRs). The unconventional reactors are Up-flow anaerobic sludge blanket (UASB), Anaerobic Fluidized bed reactor (AFB), Oscillatory flow reactors (OFR) [6]. The unconventional reactors are capable of handling biomass with high concentration and have higher reaction rate with better process engineering properties.

Table 2.1: Commonly used reactor for AD systems of animal manure [6]

Types of Reactors	Remarks
Batch Reactors	i. Simplest technique ii. Potentiality to process high-solid waste iii. Re-use of digestate in the next batch
CSTR	i. Mechanical agitation tool ii. Biogas recirculation to mix contents of digester continuously
PFR	i. Unmixed system ii. Waste flows semi-continuously as a plug through the horizontal reactor
Novel Reactors: High Rate of reaction per unit volume of the reactor	
UASB	i. Accumulate high biomass concentration
AFB	ii. Permits long sludge retention time (SRT)
OFR	i. Enhances process engineering properties like mixing and rate of reaction

The installation of the type of anaerobic digesters greatly depends upon the purpose, scale and the location of the biogas plant. As per the scale, centralized, agro-food based and farm-based AD systems are the three main options [62]. Centralized AD system are non-farm based which are on the rise currently in North America. However, at present, centralized systems only process food-processed and municipal wastes. Similarly, agro-food based AD systems are only designed to removed organics from wastewater and only treat their own by-products, but facilitates in reducing

on-site energy costs. On the other hand, farm-based AD systems are designed to process animal manure and crops from local fields [62], [71]. Farm-based AD systems are beneficial for manure management and handling, odour reduction and pathogen removal. They are also capable to handle the digestate locally, generate electricity to use in the form of heat and return a good economic value [71].

In Canada, mostly farm-based plants rely on animal manure, out of which dairy and cattle manure are the main feed because of its stable operation and maintenance compared to chicken and swine manure [62]. CM and SM digestion remains a major challenge because of its high nutrient levels, among which CM becomes more difficult to process. In addition to high nitrogen, CM consists high solids which demands huge dilution and large digester volumes. CSTRs are the widely used anaerobic digesters for high-strength wastewater or diluted CM waste [78], [79]. However for high-solid CM waste, literature suggests batch digestion because of its simple technique and ability to process undiluted raw CM manure [80]. In batch digestion, the digesters are fed only once in the beginning of the operation and nothing is fed during the reaction process. The final digestate, obtained at the end of digestion can also be re-used or sold in the form of fertilizer [39]

2.5 Anaerobic Digestion of High Solids and High Ammonia Manure

The sources of CM are small to large poultry or chicken farms which are laid with different types of bedding like wood shavings, sawdust, peanut hulls, and wheat straw [81]. Generally, a chicken farm is cleaned to ground level after every 4 to 8 flocks, having a growth cycle of 40-65 days [20]. Therefore, CM derived from the farms consists of manure along with bedding, and causes a high solids end product ranging between 60-70% TS. Besides, the excretory system of chickens are unique, the urinary tract in chickens are absent, hence they excrete high ammonia manure. Thus, in addition to high solids, it consists of high ammonia content 23-33 gTKN/L [39]. This category of organic waste is distinctive and is challenging for AD technology.

The types of AD classified based on solid state of a substrate are wet (<10% TS), semi-solid (10-15% TS) and solid (>15% TS) [82]. Widely, Wet AD is used for full-scale operations because of its easy operation, maintenance and higher biogas yield. However, Wet AD are not suitable for organic waste like CM and DM having solid content (>25% TS), which demands 4-6 times dilution than normal operation (DM: 35% TS and CM: 65%TS) [39]. Dilution of high solids manure is uneconomical and requires special collection, processing, and disposal systems. Dilution also makes AD less efficient as it decreases organic matter concentration and increases the cost of post-treatment processes [83]. Similarly, wet ADs are normally operated within 8 TKNg/L, however considering the range of ammonia content of CM, a heavy dilution of 3-4 times would be required to avoid ammonia inhibitions.

Hence, in order to avoid dilution, high solid anaerobic digestion (HSAD) [84] can be an economic option as it eliminates the requirement of large volume digesters and the cost of post-treatment processes. Moreover, HSAD also allows the treatment of substrates with high Organic Loading Rate (OLR). However, poor start-up and requirement of longer retention time are some of drawbacks of HSAD [80]. Due to this, only few works have been conducted on high-solids and high-ammonia manure directly and requires extensive research for a successful farm-based plants.

To address the issue related to poor start-up, liquid inoculum adapted to high ammonia concentrations can be supplied to HSAD for a quick start-up. Acclimated anaerobes have high tolerance of ammonia up to 7000 g/L [37], [85]. HSAD can be paired with a liquid-inoculum

reservoir and liquid inoculum can be recirculated to HSAD and percolated back to liquid-inoculum reservoir for an efficient metabolic activity [39], [69]. In addition, recirculation-percolation also solves ammonia and VFA accumulations problems as the liquid inoculum percolating through HSAD helps not only to provide sufficient moisture content to HSAD but also flushes out high ammonia and VFA concentrations. This further when can be coupled with psychrophilic or low-temperature (< 20^{o}C), which regulates FAN generated from high ammonia manure.

Hence, taken altogether, it is a novel low-temperature two-stage (liquid-solid) AD biotechnology adopting recirculation-percolation of acclimated liquid inoculum. This technology has been developed by Sherbrooke Research and Development Center, Quebec and is proven to be successful to process SM, diluted PL and DM [39], [61], [69]. However, raw CM still remains a challenging substrate and a successful development of AD to treat raw CM can benefit the poultry farms to obtain a clean and sustainable technology.

2.6 AD process stability and performance evaluation indicators

AD process is dependent on several physio-chemical parameters as mentioned above and their ranges indicate the process stability. For instance, neutral pH, high alkalinity, low FAN concentrations and a balanced C/N ratio are some of the monitoring indicators of a stable AD performance. Moreover, literature suggests additional key indicators like propionic acid (C3)/acetic acid (C2) ratio and TVFA/Total Alkalinity (TA) ratio as the representative indicators of digester's failure [86], [87]. Additionally, butyric acid+valeric acid (C4+C5)/acetic acid(C2) ratio is another indicator proposed for digester's performance [88]. The recommended range for C3/C2 ratio is ≤ 1.4, above which represents digester's failure [94]. Similarly, TVFA/TA ratio is suggested below 0.5 for high digester stability [95]. Literature also suggest TVFA/TA ratio below 1 as stable and unstable at ≥ 1. Low TVFA/TA ratio is linked with higher methane yield and higher ratio with lower methane yield [96]. Furthermore, C4+C5/C2 ratio above 1.2 is predicted as the sign of digester failure. Hence, TVFA and their composition and TA are the major indicators to determine AD performance evaluation.

2.7 Techno-economic analysis of AD plant and its feasibility

AD technology is an important renewable energy technology that generates biogas, fertilizer and reduces GHG emissions. Biogas generated from AD plants are beneficial in many ways: (i) Heat, (ii) Electricity, (iii) Natural Gas. Similarly, the end-product digestate is also beneficial in the form of fertilizer which can be sold commercially with further post-treatments. As it solves the fuel shortage and farm-based problems by utilizing organic waste efficiently, hence it becomes crucial to examine the economic benefits of this technology.

Whether the profit gained is high or low, whether it is beneficial for environmental protection and whether the economic benefit is worth to establish a biogas plant. These are some of the basic subjects that needs to be examined prior installing the AD plant. Economic evaluation requires comparison of output gross value of plant construction, operation and maintenance with fuel output and fertilizer value to reach a value. Similarly, the costs of AD system varies widely which often depends factors like labor cost, materials, type and size of digesters, type of feedstock, environmental conditions [89]. The design and cost also depend upon the purpose of the digester as the purpose varies from country to country. In USA, AD plants are installed based on waste management and methane production, however in European countries like Switzerland, primary focus is given on the advanced technologies to process the waste. Often, the plants fail to result

positive economic indicators, hence offsets of earnings can be widened by selling digestate in the form of fertilizers, chemical sources or earning carbon credits [89]. Among the existing methods to assess the techno-economic aspect of AD plant, Net Present Value (NPV) model is a method which uses discounted tax flow based on capital investment. NPV above zero is a sign of beneficial and profitable digester. A techno-economic assessment conducted [90] on anaerobic co-digestion of manure with straw resulted a Net Present Value (NPV) > 0 and a return of the investment in 11 years. However, the assessment of anaerobic mono-digestion of cattle manure showed negative returns and was not able to counterbalance initial investment [90]. Moreover, costs and returns of AD plant treating organic waste of Kawangware, Kenya resulted positive NPV and payback period of 7.9 years [89]. This study further analyzed the possible carbon credits obtained from the plant.

For a potential and profitable AD plants, integrated bio-economy based on digestate requires planning [91]. Similarly, in order to make the biotechnology commercially feasible, an economic feasibility assessment of digestion plant is must. Economic assessment of a low-temperature two-stage AD biotechnology to process high solids and high ammonia manure can give an overview of cash flows to the livestock farmers and facilitate in decision-making process to install an AD plant.

2.8 Summary and Objectives

The literature indicates the role of physio-chemical and operating parameters in AD processes. Besides, due to the AD limiting factors like high-solids and high-ammonia, processing manure, rich in solids and ammonia (CM and DM) becomes challenging. Most of the works conducted on CM were either diluted CM treatment or inhibited due to ammonia accumulation. Adaptation of liquid inoculum to high-ammonia concentration and its supply to nitrogen and solid-rich manure has been proven a promising HSAD technique, resulting high-methane concentration biogas. In addition, operation at low-temperature also reduces FAN generation, hence avoiding ammonia inhibition. Low-temperature also has economic and environmental benefits. Low-temperature HSAD also addresses issues of poultry farms by producing green energy, high value fertilizer, reducing odor and pathogens. Therefore, a low-temperature two-stage (adapted liquid inoculum recirculation to solid and its percolation through solid substrate) HSAD might benefit Canadian farmers and agro-based industries to improve livestock operations and its waste management. Altogether, the HSAD biotechnology would be an economic, sustainable and clean technology for the agri-food industry.

Therefore, efforts are made in this research to develop the aforementioned AD technology processing animal manures (CM and DM in this study) and physio-chemical parameters were carefully monitored and investigated for its operational feasibility. Moreover, to check the economic feasibility and environmental benefits (incentives gained from GHG reduction credits, revenue generated from end products like green energy and organic fertilizer) of the proposed HSAD technique, a techno-economic assessment was also conducted in this research.

Chapter 3: Performance of Low-temperature Anaerobic Digestion Biotechnology for Treatment of Chicken Manure rich in High Solids and High Ammonia Concentrations

3.1. Introduction

CM is one of the drier and bulkier manures, unlike other animal manures that consist of both liquid and solid fractions [81]. CM consists of only solid fractions. The solid fraction of CM mainly consists of excreta, bedding material, feathers and unwanted feed. CM is also high in organic matter and nitrogen, hence is considered as valuable biomass resource. AD technology can generate biogas from CM as a recommended method for manure treatment [78], [92], [93]. Yet, CM as a substrate when digested anaerobically can reduce AD process performance because of its inherent high-solids and high-ammonia content. A customary approach to this is dilution to 0.5-3% TS, resulting in huge volume of waste and making it unpractical and unattractive. Few studies have been conducted at high solids (>25 %TS), resulting in successful methane production at 37°C with the help of acclimated methanogens [38], however ammonia inhibition at higher OLR [69] was observed. Furthermore, high-solids substrate also lead to poor start-up of AD process [32]. To address this issue, utilization of adapted inoculum to high ammonia concentration (TKN > 20g/L) is suggested, as it reduces the start-up phase, minimizes ammonia inhibition and maximizes methane production [57], [69], [93]. In addition, AD operation at low-temperature is also demonstrated as one of the method to avoid ammonia accumulations [61], [77].

Sherbrooke Research and Development Center, Agriculture and Agri-Food Canada (AAFC) has successfully developed the low-temperature AD biotechnology through the years to treat animal manure like poultry litter, swine, and cow manures [39], [61], [69]. However, the capability of digesters to process CM with TS > 50% using adapted liquid inoculum has not been studied. Therefore, the primary objective of this study was to illustrate the performance assessment and operational feasibility of two-stage process (i.e., liquid inoculum reservoir coupled with high-solid anaerobic digestion (HSAD) system), treating CM at 20 ± 1 °C, at its original state without modification. The major objectives of this research were to develop the HSAD, using (i) acclimatized liquid inoculum to quicken the start-up process of high-solid CM manure and avoid ammonia inhibition; (ii) no mixing conditions, as mechanical mixers create complexity in full-scale operations; (iii) to monitor the AD process performance with the assistance of physio-chemical parameters.

3.2. Materials and Methods

3.2.1 Feedstock and Inoculum

Fresh CM was collected at the AAFC laboratory from a small-sized local poultry farm located in Farnham, Quebec, Canada. CM consisted wood shavings and feathers as bedding material and was stored at a room of temperature 4±1°C for 3-4 days to prevent biological activity prior to the experiments. For the characterization of feedstock, CM was diluted with tap water at a dilution ratio of CM: Tap Water (1:5) and ground to prepare homogenized samples, except for TS and VS analysis. The liquid inoculum, on the other hand was prepared in the AAFC laboratory-scale liquid sequencing batch reactor (SBR) in order to adapt to high-ammonia content CM leachate before the HSAD of CM waste.

3.2.2 Experimental Set-Up and Procedure

The experiment was carried out in two phases: (i) Phase I: Liquid inoculum developmental phase, and (ii) Phase II: Two-stage HSAD of CM adopting P-R mode of operation. In Phase-I, the liquid inoculum was developed and adapted to CM with high ammonia concentration to be able to reduce the effect of ammonia inhibition, likely to occur in Phase-II operation.

3.2.2.1 Phase-I: Liquid Inoculum Developmental Phase

The major objective of this experiment was to acclimatize the anaerobes with high-solid and high ammonia content CM leachate since the microflora acclimated to a high concentration of ammonia has been reported as one of the potential strategies to control ammonia inhibition [31]. The experimental set up for the liquid inoculum developmental phase is presented in Figure.3.1 Three sequencing batch reactors (BR1, BR2 and BR3) with 40L working volume were operated in triplicate and operated at a low-temperature of 20 ± 1°C in a batch mode for 14 days. The choice of 14 days was based upon the maximum utilization of organic content and generation of stable methane production. 14 days was taken as one cycle length (CL) and altogether 7 cycles were conducted. In each cycle, approximately 3.5L of CM leachate was fed to each of the BRs as shown in Table 3.1. The process was repeated in 7 multiple cycles to obtain sufficient amount of adapted liquid inoculum required for Phase-II operation.

Table 3.1: Operational Parameters of a single BR for Phase-I

	Cycle 1	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**	**Cycle 6**	**Cycle 7**
Total CM leachate fed in a BR (L)	3.5						
Total liquid Inoculum (L) in a BR	3.5	7 (3.5 L inoculum from Cycle 1 + 3.5L fresh CM leachate)	10.5 (7 L inoculum from Cycle 2 + 3.5L fresh CM leachate)	14 (10.5 L inoculum from Cycle 3 + 3.5L fresh CM leachate)	17.5 (14 L inoculum from Cycle 4 + 3.5L fresh CM leachate)	21 (17.5 L inoculum from Cycle 5 + 3.5L fresh CM leachate)	24.5 (21 L inoculum from Cycle 6 + 3.5L fresh CM leachate)
Cycle length (Days)	14						
Temp (º C)	20 ± 1						
Operation mode	Sequencing batch reactor						
OLR (gVS/L.d)	0.14- 0.41						

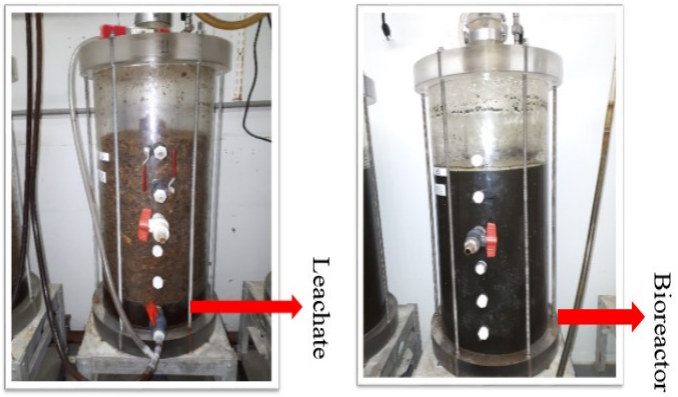


Figure 3.1: Set-Up of Phase I: Liquid Inoculum Adaptation

3.2.2.2 Phase-II: Digester Setup of Two-Stage HSAD of CM adopting P-R mode of operation

The objective of this study was to develop a two-stage HSAD of CM at low-temperature using adapted liquid inoculum prepared in Phase-I operation. The experimental set up for Phase-II operation is presented in Figure.3.2. The two-stage (liquid-solid) HSAD (i.e., liquid inoculum reservoir coupled with HSAD system) to process CM consisted a set of liquid digester (A) and solid digester (B). Three sets of digesters; digester 1 (D1), digester (D2) and digester (D3) with a 60L volumetric capacity were operated in parallel. Each set was a combination of 'A' and 'B', such as (i) D1: 1(A)+1(B), (ii) D2: 2(A) +2(B), and (iii) D3: 3(A)+3(B). Hence, a total of six digesters with a total volume of 60L and a working volume of 20-25L for 'A' and 4.5-10L for 'B' were used for this study. Digester 'A' was fed with adapted liquid inoculum prepared in Phase-I and digester 'B' was fed with CM waste. 'A' and 'B' were placed adjacent to each other and the complete set-up was installed at a controlled low temperature of 20 ± 1°C. The experiment was carried out for 282 days in four cycles, with a cycle length of 69-71 days. The process required that a fraction of the digestate be used as inoculum for the next treatment cycle (Table 3.2). A potential improvement was induced by adopting liquid inoculum recirculation-percolation (R-P) in which (i) liquid inoculum was recirculated from A to B, then (ii) inoculum was allowed to percolated through B and sent back to A [69]. This operation eliminated the need for premixing equipment, which can be costly and difficult to manage.

For R-P, approximately 5L liquid inoculum was pumped from 'A' and sprinkled to the top of 'B' in a way that liquid percolated through the CM substrate. The percolated inoculum from 'B' was further supplied back to 'A', hence liquid inoculum was adapted to a new CM substrate after each R-P. Besides, this also contributed towards the biogas production in digester 'A'. R-P also facilitated in the extraction of accumulated ammonia and VFAs from 'B' to 'A'. A top-down R-P mode was approached in this study and conducted 3 times a week (5 days). For an easy passage of liquid inoculum through CM substrate, diluted liquid inoculum was utilized at the beginning of each cycle operation which is concentrated by the end of operation due to multiple P-R.

Table 3.2: Operational Parameters of HSAD for Phase-II

	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Cycle length (Days)	70	69	69	71
Total manure treated (kg) in solid digester (B)	4.5	4.5 (2.25 kg dry inoculum from Cycle 1 + 2.25 kg fresh CM)	10 (4.6 kg dry inoculum from Cycle 2 + 5.4 kg fresh CM)	10 (4.6 kg dry inoculum from Cycle 3 + 5.4 kg fresh CM)
Total liquid Inoculum (L) in liquid digester (A)	22	22	25	25
Temperature (°C)			20 ± 1	
Operation mode			Batch	
OLR (gVS/Ld.)	8.7	4.4	4.4	4.3

OLR: Organic Loading Rate; CM: Chicken Manure

Figure 3.2: Experimental set-up of Two-Stage HSAD of CM

3.3. Analytical Methods

3.3.1 Sampling

3.3.1.1 Phase I: Liquid Inoculum Developmental Phase

To analyze the process performance, samples were taken immediately after the feeding (day 0) and after 3, 8 and 14 days of each cycle. Altogether, 136 biogas samples and 136 samples for physio-chemical tests were taken from all the bioreactors (BR1, BR2 and BR3) during the entire operation of 98 days.

3.3.1.2 Phase II: Two-Stage HSAD of CM

The experiments for two-stage HSAD were operated in a batch mode, hence the physio-chemical samples from HSAD (1B, 2B and 3B) were only taken twice; once at the beginning and the other, at the end of each cycle. The CM samples were diluted at a dilution ratio of CM: tap water (1:5) and ground for homogenized samples. However, samples from liquid digesters (1A, 2A and 3A) were taken only once a week (5 days) and withdrawn into 100 ml containers. Also, the biogas samples were taken thrice a week from all the six digesters (solid and liquid). Hence, a total of 700 biogas samples and 130 physio-chemical samples were taken during the entire operation of 282 days.

3.3.2 Analysis

3.3.2.1 Physio-chemical Analysis

Physio-chemical samples were analyzed for the tests: pH, alkalinity, total solids (TS), volatile solids (VS), total COD (TCOD), soluble COD (CODs), TKN, ammonia nitrogen, and VFAs. Along with this, TS and VS on a dry weight basis were determined following the guidelines given by the standard methods [94]. pH was determined by using pH Mettler Toledo AG 8603, SevenMulti (Schwerzenbach, Switzerland). Alkalinity was measured using Hach Lagne Sarl, Titralab AT1000 Series (Hach, Switzerland). COD was measured by using a closed reflux colorimetric method [94]. TKN and NH3-N were analyzed using a 2460 Kjeltec Auto-Sampler System (FOSS, Sweden) following the macro-Kjeldahl method [94]. VFA was determined using a Perkin Elmer gas chromatograph, model Clarus 580 (Perkin Elmer, Shelton, CT, USA), mounted with a DB-FFAP high-resolution column, but before the evaluation of VFAs, samples were conditioned according to the procedures mentioned by Masse et al. (2003) [95]. Samples collected from digesters were first centrifuged at 41× g for 15 min and filtered through a 0.22 µm membrane before injected. The injection volume was 0.1 µL.

3.3.2.2 Biogas Analysis

Biogas samples were analyzed thrice a week for the characterization of biogas (methane, carbon dioxide, and nitrogen) immediately after sampling. The characterization was determined with Agilent Technologies 490 Micro GC, Biogas Analyzer (CA, USA) equipped with a thermal conductivity detector (TCD) and Helium gas as the carrier gas at a flow rate of 20 mL/min. The injector and oven temperatures being 110^0C and 180^0C respectively. Furthermore, biogas volume was monitored every day using the wet tip gas meters.

3.4. Results and Discussion

3.4.1 Phase-I: Liquid Inoculum Developmental Phase

3.4.1.1 Characteristics of liquid inoculum and CM leachate

The range of parametric characteristics of the CM leachate and liquid inoculum of 7 cycles are shown in Table 3.3. Liquid inoculum contained a small proportion of TS (1.43-1.48%) and VS (0.60-0.70%). Its pH was in the neutral range (7.71-7.73). The low TVFA/TA ratio (<0.07) indicated the possibility of a stable AD process.

The leachate was characterized by much lower pH (5.84-6.63). The TS and VS were determined up to 4.63% TS and up to 2.9 %VS respectively. Moreover, the organic content in terms of SCOD was in the range of 24.8-69.5 g/L which was significantly high and NH_3-N concentration was also found in the range of 3.7-5.4 g/L indicating the higher limit until which the liquid inoculum would be adapted.

Table 3.3: Characteristics of inoculum and leachate

Parameters	Liquid Inoculum	Leachate
TS (%)	1.43–1.48	2.42–4.63
VS %	0.60–0.70	1.34–2.9
Alkalinity (g/L as $CaCO_3$)	9.2–10.4	11.4–16.3
TCOD (g/L)	16.2–17.6	45.2–75.4
SCOD (g/L)	2.1–2.6	24.8–69.5
TKN (g/L)	2.1–2.2	4.4–6.9
NH_3-N (g/L)	1.7–1.9	3.7–5.4
pH	7.71–7.73	5.84–6.63
TVFA (g/l)	0.5–0.8	21.5–36.7

3.4.1.2 Performance of a liquid inoculum BR of a typical cycle

Figure 3 shows the cumulative methane generation (quantity and content) performance for liquid inoculum in 7 cycles. The methane concentration during the time period of 14 days ranged from 50% (lowest) to a maximum of 80% as is clear from Figure 3.3(a). Similarly, Figure 3.3(b), shows the cumulative methane quantity to be approximately 60L at the end of each 7 cycles indicating the stability of the inoculum developed. The BR also exhibited high alkalinity (up to 18g/L) which provided good buffering capacity and assisted towards the maintenance of a neutral pH (7-8) and provided an ambient condition for the microbes [91]. Towards the end of Phase-I, liquid inoculum was adapted to the ammonia concentration up to 6.9 gTKN/L.

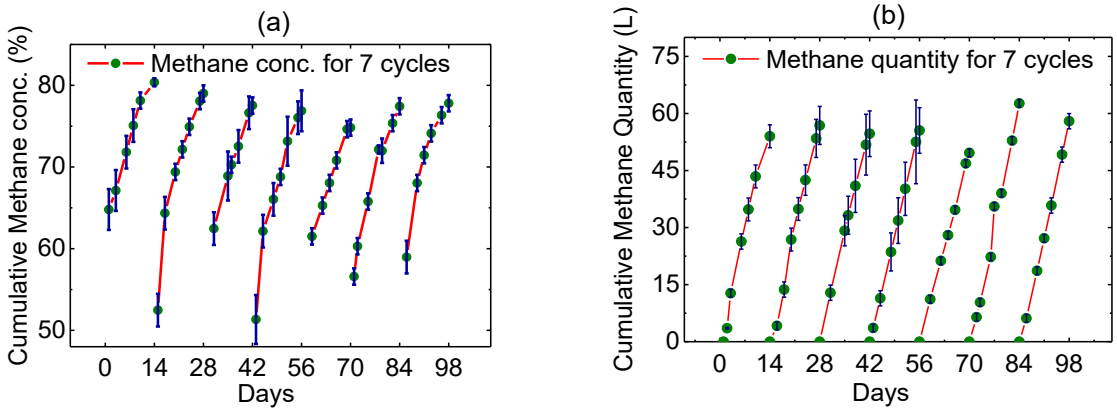


Figure 3.3: Cumulative Methane at different cycles (a) Methane concentration cycles; (b) Methane quantity

From the TVFA and COD profile (Figure 3.4) of a typical cycle, the BR showed a significant reduction of TVFA and COD from liquid inoculum Along with this, a significant reduction in VFA and COD was observed. TVFA reduced up to 93% (26.32 g/L to 2.63 g/L) from 0-day to 14-day,

indicating the maximum utilization of VFA by the growing methanogens by the end of each cycle (Figure 3.4a) [49], [92]. Similarly, COD reduction was found to be in the range of 70-80% (Figure 3.4b). This denotes the acclimation of inoculum to CM leachate with significant number of methanogens.

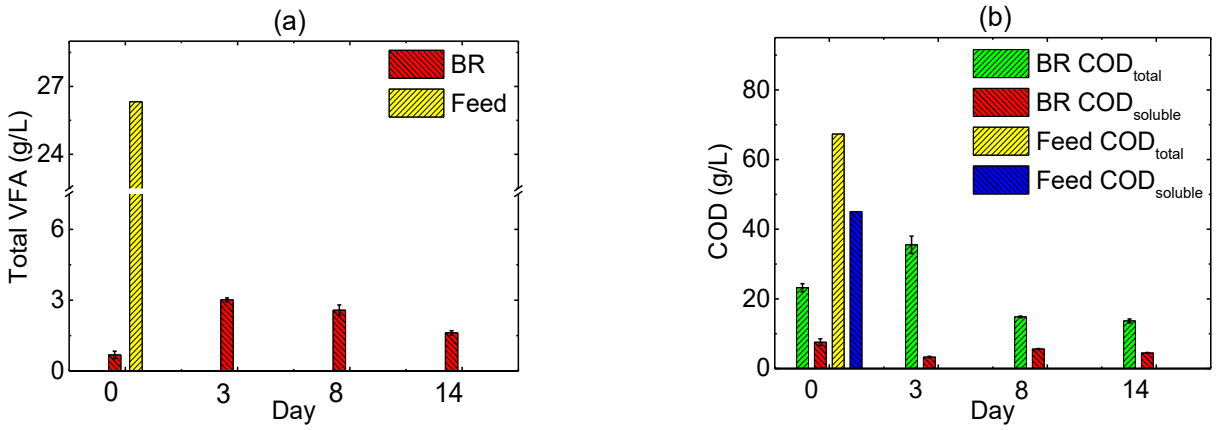


Figure 3.4: (a) VFA reduction during a typical cycle; (b) COD reduction during a typical cycle

3.4.2 Phase II: Two-Stage HSAD of CM using liquid inoculum P-R process

3.4.2.1. Feedstock and Inoculum characteristics

The physio-chemical characteristics of CM and inoculum are shown in Table 3.4. CM contained a high solids content in terms of TS (65-70%) and VS (56-62%), which are close to those found for dry CM [69], [92], [96] . Its pH was an average of 8, which is considered a favourable growth environment for methanogenic bacterial activity and is consistent with the literature values for a poultry litter or CM [70], [93], [97]. The nitrogen content in terms of TKN was 3-4 times higher than the normal dry CM values reported [69], [78], [93], which were conducted at different operating temperatures (20ºC -55ºC). Higher ammonia is often considered as an inhibitory factor for AD, but is expected to be low at lower temperature conditions (≤ 25ºC) [69], [92]. The TCOD/TKN ratio between 26-36 lies in accordance with the optimal C/N ratio (~ 30), as reported to be favourable for microbial activity [32]. However, C/N ratio is studied to be variable and dependent upon characteristics of the feedstock alone [98]. The TVFA/TA ratio of CM was around 10 times lesser than the recommended value of 0.4 for a stable digester [99].

Inoculum was characterized by a pH range between 8-8.4. This is in line with the characteristic values of diluted CM ranging within 8-9 [78], [97], [99] and the low TVFA/TA ratio (<0.1) indicates the aid of inoculum towards a stable AD process [49], [100]. Inoculum also contained a small proportion of TS content (<2%) which is considered as a favourable solid proportion for percolation-recirculation process [39], [69].

Table 3.4: Characteristics of CM and Inoculum

Parameters	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	CM	**Inoculum**	**CM**	**Inoculum**	**CM**	**Inoculum**	**CM**	**Inoculum**
pH	8.39	8.17	8.39	7.87	8.04	8.24	8.04	8.2
Alkalinity (g $CaCO_3$/L)	35	11	35	10.8	33.4	16.6	33.4	11.5
TS %	69.87	1.48	69.87	1.44	65.14	1.5	65.14	1.55
VS %	61.09	0.6098	61.09	0.69	56.24	0.6	56.24	0.608
TCOD(g/L)	864.4	10.3	864.3	12.8	827.8	10.8	827.8	9.7
SCOD(g/L)	291	9.03	291	4.6	303.2	7.3	303.2	5.5
TKN(g/L)	32.8	2.3	32.8	2.9	23.3	4.05	23.3	2.9
NH_3-N(g/L)	8	2	8	2.4	6.1	3.5	6.1	2.4
TVFA(g/L)	1.52	0.85	1.52	2.15	1.7	0.4	1.7	0.18
TCOD/TKN	26.4	4.47	26.4	4.4	35.6	2.6	35.6	3.3
TVFA/TA	0.04	0.07	0.04	0.2	0.05	0.02	0.05	0.01

3.4.2.2 Performance of Low-temperature Two-Stage HSAD

3.4.2.2.1 Biogas and Methane

The aim of this study was to examine the potentiality of biogas and methane production from CM waste in a low-temperature two-stage anaerobic digester under different OLR. Figure 3.5, 3.6 and 3.7 shows cumulative biogas, methane generation and specific methane yield (SMY) performance for CM waste respectively, operated for 282 days in 4 cycles. For performance evaluation, the 4 cycles were divided into 69-71-day periods (1-70 day for Cycle 1, 71-140 day for Cycle 2, 141-210 day for Cycle 3 and 211-282 day for Cycle 4). The results obtained for biogas, methane and SMY at the end of each cycle is also given in Table 3.5.

In Fig 3.5(a) and Table 3.5, the average combined cumulative biogas quantity (D1, D2, D3) is observed to be 578±42 L in Cycle 1 which increased steadily by 26% in cycle 2, abruptly by 91% in cycle 3 and 75% in cycle 4. The increased biogas were likely the results of added proportion of adapted dry CM inoculum with every succeeding cycle (as shown in Table 3.2) which enhanced the microbial activity [92].

The cumulative combined biogas production rate results, presented in Fig 3.5(b) shows a non-uniformity in the production rate in Cycle 1 due to start-up phase of the study to process CM. Apart from that, the trend of production rate was observed to be same for Cycle 2-4. It increased rapidly to 20-25 L/d on the first two days, slowly declining to 6-14L/d between 20-25 days, which further took leap in 45-50 days and finally declining after 50 day. This trend is also consistent with the study conducted on high-solids CM [101]. The highest biogas production rate (~12.5 L/day) was achieved on 70 day of cycle 1 but the operational or cycle time was reduced with the ongoing operation cycles to produce a higher production rate. A maximum biogas production rate (~15.1

L/day) was achieved on 50 day of Cycle 2, ~18.8 L/day on 53 day of Cycle 3 and ~17.6 L/day on 50 days of Cycle 4. The maximum biogas production rate from Cycle 2-4 at a reduced cycle time and constant OLR (4.4 gVS/L.d), likely appears due to the two-stage AD process and addition of liquid inoculum to HSAD (percolation-recirculation) [101], [102].

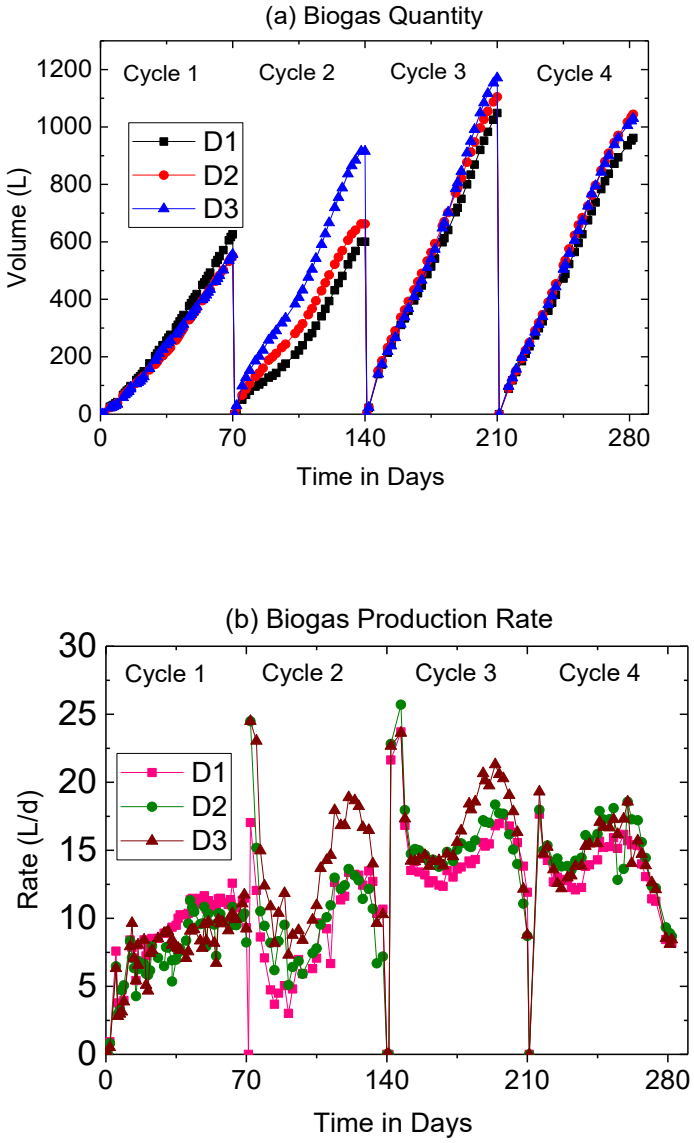


Figure 3.5: Cumulative Biogas production; 5(a) combined average biogas quantity; 5(b) combined average biogas production rates

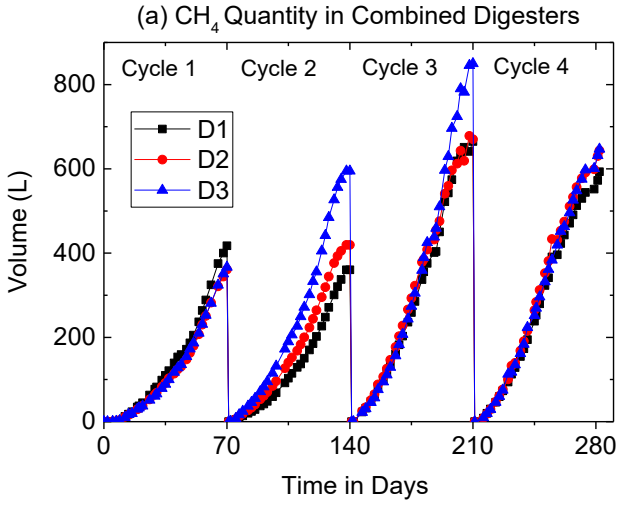
Fig 3.6a shows the methane quantity for the combined digesters D1, D2 and D3 throughout the entire operation time of 4 cycles. A lag phase in cycle 1 was evident which could be due to the adaptation of microbes towards new substrate type, taking longer time in the hydrolysis of the organic matter [49]. The production was observed to be increasing in the later cycles. Although, the methane quantity in cycle 4 was not as high as cycle 3 despite having similar OLR as in cycle 3 (4.3 gVS/L/day) but showed a consistent pattern altogether. Generally, the rise in OLR, boosts

methane production, provided that other parameters are controlled and vice-versa [76]. Regardless, an increase in methane production was noticed with a decrease in OLR (Table 3.5). The reason behind this could be the residual organic matter from previous cycles, contributing to the excess methane production in complete digestion of CM in cycle 1, which was further utilized in cycle 2. As the feed provided in cycle 2 consisted of dry inoculum in addition to CM, hence part of the substrate available for microbes was present in a better digestible form to convert into methane enriched biogas. Therefore, it was evident that acclimatized microbes have played a crucial rule in the conversion of organic matter into methane with better efficiency and rate [49], [101], [103].

Table 3.5: Average values of digesters (D1, D2 and D3) at the end of cycle at different OLR

Cycles	Cumulative Biogas (L)	Cumulative Methane (L)	Methane Content (%)	SMY (L CH_4/ g VS)	OLR(gVS/L.d)
Cycle 1: Day 70	578±42	382±31	70±11	0.46±0.05	8.7
Cycle 2: Day 140	726±167	458±122	70±11	0.56±0.14	4.4
Cycle 3: Day 210	1108±61	728±105	69±7	0.80±0.12	4.4
Cycle 4: Day 282	1012±44	628±30	63±5	0.68±0.04	4.3

As seen from Figure 3.6 (b), cumulative methane concentration was higher in digesters "A" than digesters "B" and the higher moisture content in digesters "A" is presumably the reason behind it which favours the microbial growth environment [77]. Just after 60 days of every cycle, the methane concentration reached ~80% in liquid digesters and ~60% in solid digesters. It was also noticed that cycle 1 and cycle 2 showed some abrupt changes while cycle 2 and 4 were more likely steady.



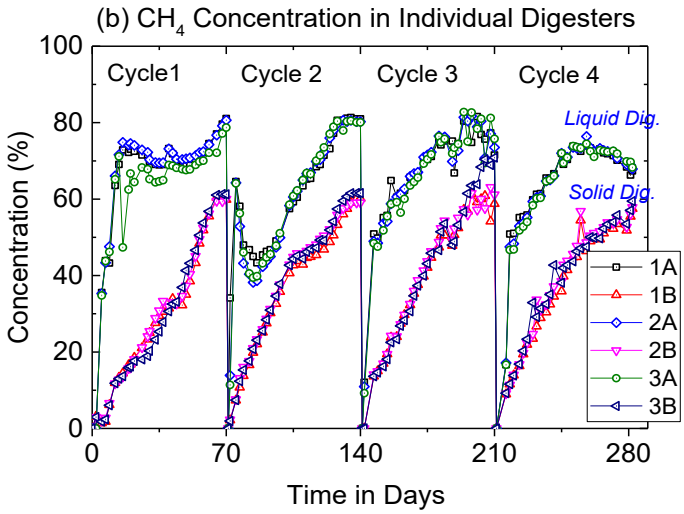


Figure 3.6: Cumulative methane production; 6(a) combined methane quantity; 6(b) combined methane concentration

Figure 3.7 shows the cumulative SMY digesters D1, D2 and D3. SMY indicates the efficiency in terms of the utilization of organic matter in a substrate [36]. SMY was found approaching to 0.5 LCH_4/ g VS in cycle 1 and a slight increase was noticed in cycle 2. Approximately, 40% increment of SMY was gained in the last two cycles with the increment in the feed quantity and well-adapted inoculum. In cycle 3 and 4, SMY resulted to 0.65-0.85 LCH_4/gVS. This was likely attributed to adapted inoculum which consisted robust microbes and utilized maximum organic matter from CM.

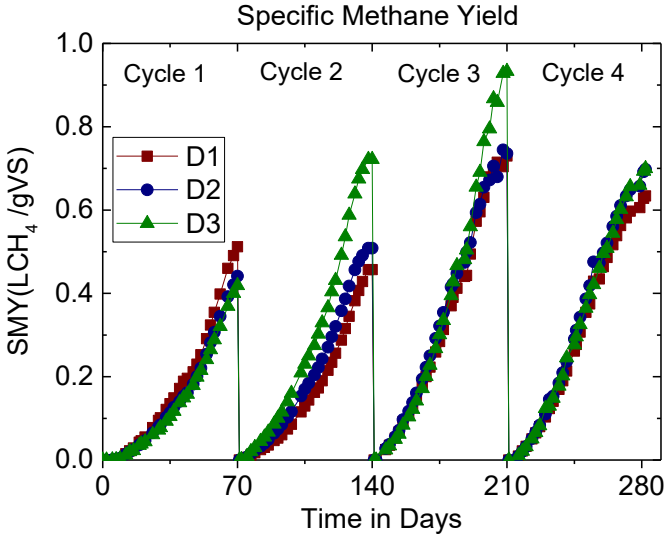


Figure 3.7: Cumulative SMY in combined digesters

3.4.2.2.2 pH, Alkalinity, VFAs

Figure. 3.8 shows the pH, alkalinity and VFA profile of liquid digesters during the entire operation of 282 days. pH, in the digester remaining within a neutral pH zone (7.25-8.5) throughout the entire operation which could be attributed to the ammonia released during hydrolysis, assisting to maintain neutral pH [104]. Besides, the alkalinity in the liquid digesters increased with time and reached to approximately 20g/L as $CaCO_3$ which further helped to buffer pH fluctuations. Figure. 3.8 also shows the VFA profile of 4 cycles. Cycle 1 and cycle 2 generated higher VFA (up to 17.7 g/L) than cycle 3 and 4. A faster consumption of fatty acids was noticed towards the succeeding cycles. This sharp decrease in VFAs corresponded with an increase in methane production during this operating period (see Figure. 3.6a). Hence, cycle 3 and cycle 4 exhibited more stability and balanced conversion of fatty acids to CH_4 and CO_2 which is evident from Figure 3.6. Furthermore, the accumulation of VFAs is considered as an indication of either the high rate of hydrolysis or the inhibition of methanogens in the system. These are important indexes to evaluate digester's efficiency as the VFA build-up induces an imbalance in the metabolism of microorganisms [105], [106] (Figure 3.8). Among the indicators, TVFA/TA is recognized as a reliable indicator to assess digester process stability and the ratio >0.8 results AD inhibition [99]. Results derived from this study showed that TVFA/TA ratio exceeded the limit of 0.8 for cycle 1 (between 42-44 days) only and cycle 2 (between 10-30 days) indicating a potential inhibition. This could be due to the formation of ammonium bicarbonate from ammonium present in CM, contributing towards acidification [103].

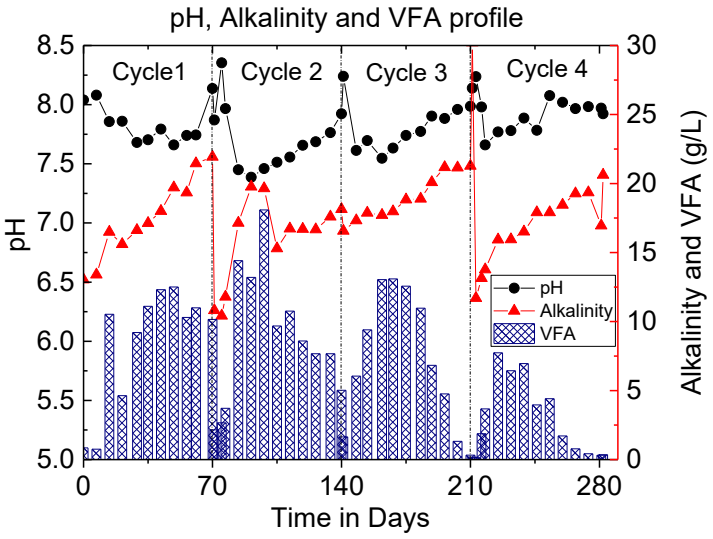


Figure 3.8: VFA, pH and Alkalinity profile of liquid digesters

3.4.2.2.3 Ammonia

CM has high ammonia content and ammonia is one of the most critical parameters causing digester failure. Figure 3.9 shows the evolution ammonia in the form of TAN and FAN. TAN concentration fluctuated between 2.5-5.5 g/L during the operation of 282 days. TAN was 5.5 g/L at maximum in Cycle-1, 5.0 g/L in Cycle-2, 4.5 g/L in Cycle-3 and 4.2 g/L in Cycle-4. The maximum concentration lowered as the cycles succeeded. A similar trend was observed for FAN concentration. Generally, FAN exceeding 1g/L leads to ammonia toxicity in the digester [60]. However, in this study, FAN concentration was shown to be below 300 mg/L throughout the operation. In Cycle-1, FAN was 275 mg/L at maximum, 225 mg/L in Cycle-2, 175 mg/L in Cycle-3 and 172 mg/L in Cycle-3. The concentrations of TAN and FAN was lowered with every cycle superseded, however the methane concentrations and biogas production increased (Figure 3.5 and 3.6). This could be likely due to the utilization of inoculum acclimatized to high ammonia [99], as microorganisms with tolerance to higher ammonia level facilitate towards reducing ammonia inhibition and sustainable operation. Hence, irrespective of a high TAN of 5.5 g/L, significant biogas production was noticed without any sign of inhibitions. The reason behind lower FAN concentrations could also be associated to the high buffering capacity of the system coupled with low-temperature (20±1°C), as FAN is dependent on temperature and pH [66]. Studies showed that temperature plays an important role in the kinetics of microbial population and methane production [33], although lower temperature lowers the metabolic activity. However, inoculum adapted to lower temperature has been proven to be successful [39], [69]. Therefore, the liquid inoculum adapted to 20±1°C, used this study for percolation-recirculation benefitted the AD operation with low FAN (275 mg/L) generation.

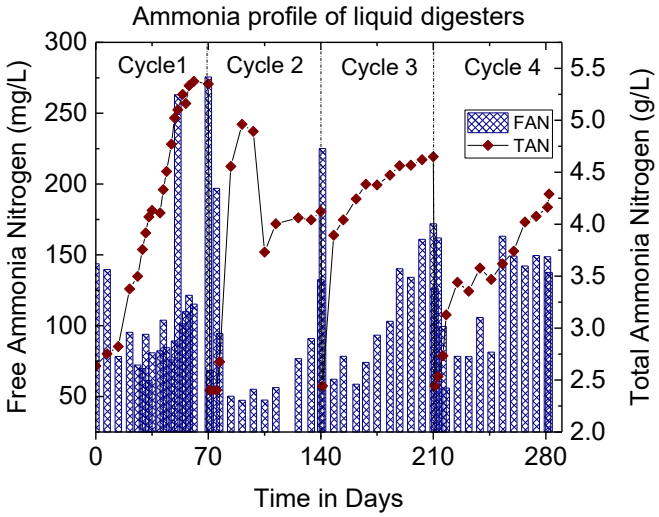


Figure 3.9: Ammonia profile of liquid digesters

3.4.2.2.4 Efficiency analysis of the digester

To assess the performance of the digesters in terms of consumption of organic matter, TCOD, SCOD, TS and VS were analyzed. The analysis is essential to estimate the efficiency of the anaerobic process with regards to the reduction of organic loading, being an important feature to

reduce environmental pollution. In cycle 1, 78% efficiency (Figure 3.10a) was obtained as TCOD is reduced from 864 to 193 g/L. The efficiency of the reduction of SCOD was even higher, i.e. over 92%, as the organic content in the form of COD_s is more digestible for the anaerobes. Furthermore, the proposed two-stage AD treatment can reduce TS and VS over 60% which is clear from figure 3.10b. The slightly higher VS removal efficiency over TS could be due to a faster hydrolysis rate of CM, influenced by operating conditions, and rate of percolation-recirculation [107].

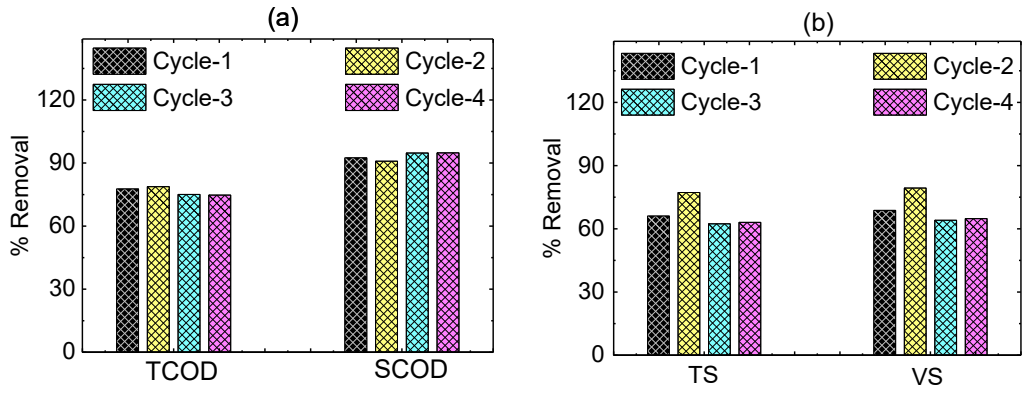


Figure 3.10: (a) TCOD and SCOD removal percentage; (b) Total Solids (TS) and Volatile Solids (VS) removal percentage

3.5 Comparative study of mono digestion of CM

Table 3.6 provides a comparison summary of the mono-digestion of CM from our study with others. The studies conducted so far have dealt with either diluted CM or poultry slurry except with few [38], [69], [70], [92], which have been carried out with raw CM (≥ 25% TS). Whereas, in this study, direct treatment of undiluted raw CM was carried out up to (72% TS). Another distinctive difference is, its operation at low-temperature while all the other studies were performed under mesophilic or thermophilic range. The third major aspect of this study is high OLR which was comparatively higher than reported works, except a work conducted on poultry litter at an OLR of 21.6 gVS/L.d [69]. However, this study revealed excess ammonia inhibition which was evident from low SMY (0.20-0.22 LCH_4/gVS) generation, whereas SMY in our study was observed to be highest (~0.65 LCH_4/g VS) so far. Thus, despite having a 2-4 times higher TAN concentration compared to other studies, the resulting highest SMY and significant methane concentration (>60%) determined the process stability with no signs of inhibition.

Table 3.6: Operating conditions, influent and effluent characteristics of studies conducted on CM

Raw CM	Diluted CM	Dry CM	Raw CM	Fresh CM	Diluted PL	Diluted CM	Raw CM	Diluted PL	Diluted PL	Fresh CM	Diluted CM	Diluted CM	Substrate
Batch	CSTR	Batch	Batch	Bench Scale	Self-mixing	CSTR	Batch	CSTR	CSTR	Semi CSTR	CSTR	CSTR	Reactor Type
4.5-10[d]	5	20	0.13	5	200	10	10	10	10	20	2.3	12.3	Treated Volume (L)
20±1	36±2	20±0.5	37	55±2	37±1	38±1	25	38	40	37	37±2	35±1	Temp (°C)
7-8.5	7.5-8.0	8.3	7.3-9.0	8.5-9.0	7.2-7.4	7.9	8.0	6.8-7.7	7.8-8.0	7.7-8.1	6.5	8.5-9.0	pH
>22.5	20.7-39.8	13.5-15.5	NR	NR	NR	NR	NR	NR	NR	8-30	NR	1.2- 4.4	Alkalinity (g/L as $CaCO_3$)
4.3-8.7	2.75-6	21.6	NR	NR	1.08	6.0	5.3	3.2	3.5	3.00	2.17	1.5-3.5	OLR (gVS/Ld)
70	30	38	NR	NR	80	40	40	40	35	25	12	1.5	HRT/CL
0.65	0.30	0.20-0.22	0.31	0.20	0.14	0.34-0.42	0.39	0.36-0.45	0.43	1.40	0.55	1.2	SMY (L/g VS)
846	NR	888	NR	NR	NR	NR	NR	NR	NR	NR	NR	2-8	TCOD (g/L)
75-93	NR	NR	NR	82	41-44	70-80	40-55	NA	NA	80	60-67	70-80	VS or COD removal (%)
65-72	12	69	25	25	90[c]	14	36	78[b]	75[b]	91[a]	24	NR	TS (%)
87	68	84	NR	NR	80	65	59	70	64	72	60	NR	VS (% of TS)
23.3-33.3	4.8	7	11	2.3-3.0	NR	5.2-6.4	7.0	3.4-4.2	5.9-6.9	3	3.0-3.5	1.25	TAN (g/L)
This Study	[59]	[69]	[38]	[70]	[108]	[101]	[92]	[68]	[53]	[96]	[79]	[78]	Reference

Note: PL: Poultry Litter; CL: Cycle Length; CSTR: Continuous Stirred Tank Reactor; SB: Serum Bottle; NR: Not reported; NA: Not Available; (a) Initial data for raw CM, diluted to <1%TS for operation; (b) Initial data for raw CM, but diluted with tap water for operation (c) Initial raw CM, diluted to 10%TS for operation (d) Unit of treated volume of quantity is in kg.

3.6 Conclusion

The proposed two-stage process (i.e., liquid inoculum reservoir coupled with high-solid anaerobic digestion (HSAD) system), treating CM at 20 ± 1 °C, showed appreciable results despite of its high ammonia content (23-33 g TKN/L) and high solids (69-72% TS). Results showed that the HSAD system could generate a SMY of 0.68±0.04 L CH4g/VS_{fed} at an OLR of 4.4 gVS/L.d. CH_4 concentrations above 50% was also observed with TCOD and SCOD reduction up to 80% and 93%, respectively and VS removal efficiency of 75%. The HSAD was capable to process CM by utilizing the microbial adapted inoculum and the two-stage recirculation-percolation mode of mixing. The positive results obtained, therefore motivates to optimize the system with further experiments so that it could be a sustainable and clean farming option for small to medium sized poultry farms especially in the cold climate weather conditions.

Chapter 4: Acclimation of inoculum and Start-Up strategies to control Short Chain Fatty Acids build-up in High organic and Nitrogen loading Anaerobic Digesters

4.1 Introduction

Anaerobic Digestion (AD) is a series of bacterial processes in which a complex substrate disintegrates into soluble monomers (carbohydrates, proteins and lipids). The soluble monomers gets converted to monosaccharides, amino acids and long chain fatty acids (LCFAs) in the hydrolysis phase [48]. The compounds generated in the hydrolysis phase are then consumed by acidogens to form short chain fatty acids (SCFAs) which are low in molecular weight. The produced SCFAs further gets transformed to acetic acid in the acetogenic phase, which ultimately converts to methane (CH_4) and carbon-dioxide (CO_2) in the methanogenic phase by methanogenic acetoclastic archeas (Figure 4.1) [48], [58], [109]. SCFAs, also referred as total VFA (TVFAs) are the linear short-chain aliphatic mono-carboxylate compounds, ranging from two to six carbon atoms (C2-C6) (Figure 4.2) [56]. These include higher amounts of acetic acid, propionic acid, butyric acid and lower amounts of isobutyric acid, valeric acid, iso-valeric acid and caproic acid. VFA concentration is a health indicator of an AD system, and regular VFA analysis allows the identification of stability state of a digester at an early phase [48]. The level of SCFA is also a dependent factor for high biogas quality since SCFAs are substrates for methanogens [58]. Therefore, the knowledge of mechanism behind SCFA formation and its utilization allows to maintain stability of AD process and its efficiency.

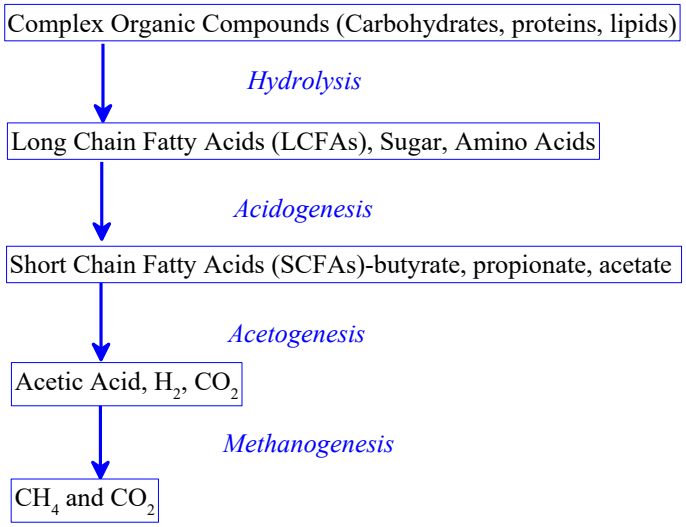


Figure 4.1: Stages of Anaerobic Digestion Process

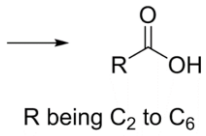


Figure 4.2: Carboxylic group compound (C2 to C6)

The generation of VFA occurs when the soluble substrates are utilized by acidogens and acetogens after hydrolysis and the accumulation takes place due to the variation in the microbial activity between acetogens and methanogens. Acidogenic bacteria have faster growth rate than methanogens and are resilient to high temperature, high organic loading rate (OLR) and high acidity (low pH). VFAs also build up due to the variation in the demand of hydrogen partial pressure among the acetogens and methanogens [58]. Since SCFAs are formed in the acidogenesis stage which is followed by acetogenesis, hence signifies that the accumulation of SCFAs leads to the reduction of pH and acidic failure of the system. Therefore, accumulation of VFAs should be avoided as it ultimately leads to digester failure and hence, the financial loss [110]. Therefore, effective strategies like increasing the rate of hydrolysis (formation of VFAs and hydrogen) and proper consumption of VFAs to yield high methane concentration can be adopted to control VFA accumulation [58].

Build-up of VFAs is highly dependent upon the parameters like pH, total alkalinity (TA), temperature, C/N ratio, moisture content, OLR and the cycle length or hydraulic retention time (HRT) as they have direct influence on the performance of AD system [111], [112]. Besides, VFA yield varies with the operational conditions and the types of substrates. In general, substrates with higher organic content, yield higher VFA concentrations. Therefore, substrates like high solid chicken manure CM) consisting high organic content carries potentiality of generating higher VFAs. Therefore, proper environmental conditions, right selection of parameters and suitable level of stress indicators like TVFA/TA, C3/C2, and (C4+C5)/C2 (details are mentioned in Chapter 2) are essential for a well-balanced operation of the digester to exploit AD to the fullest.

This chapter is a follow up study of Chapter 3 in which a two-stage high solid AD (HSAD) was adopted to process high solid and high ammonia CM. The emphasis on this chapter is mainly given to VFA, its mechanism, formation and effects on the digester's efficiency. Thus, the major objectives of this work was (i) to study the impact of operational parameters on TVFA in the start-up phase of the two stage (liquid-solid) high solid AD process treating CM by adopting P-R mode of operation; (ii) to understand the ratio limits of TVFA/TA, C3/C2, and (C4+C5)/C2 on digester's performance and stability; (iii) to inspect the methane production with respect to the changes in VFA yield, its production and composition in the proposed HSAD biotechnology.

4.2. Materials and Methods

As this study is the detailed analysis of VFA in the digester adopted in Chapter-3, hence the materials and methods are same as that of Chapter-3. The effect of operational and physio-chemical parameters on SCFA build-up in the AD process studied in Chapter-3 is mainly focused in this research, as VFA is one of the health indicators of AD system.

4.2.1. Feedstock and Inoculum

The feedstock used in this study was same as that of Chapter 3.

4.2.2. Experimental Set-up

The description of set-up was same as mentioned in Chapter-3.

4.2.3. Analytical Methods

All the other physio-chemical parameters, biogas and methane analysis were same as mentioned in Chapter-3. However, among the other parameters, only an overview study of VFA was conducted in Chapter-3, but all the VFAs composition (C2-C5) was analyzed in detail in this Chapter.

4.3. Results and Discussion

4.3.1. Impact of operational parameters towards on SCFAs in a two-stage AD processing CM adopting percolation-recirculation mode.

The operational parameters such as pH, temperature, OLR and retention time, and their influence on VFA generation are described in detail in this section [113]. Besides, type of substrates, their composition, the type of digesters and operational mode are also discussed as they are the major driving factors for VFA production [56], [63].

4.3.1.1. pH

pH is one of the major parameters which has direct influence on the production rate of SCFAs [106]. A total of four cycles were operated for 282 days at 20^{o}C in this study. The pH of the digesters was slightly alkaline but mainly neutral during the entire operation, which was between 7.2-8.5. In general, pH level varies at different stages of AD but pH is considered optimum at maximum SCFA concentration [101], [108], [109]. Figure 4.3 shows the variation of VFAs at different pH level in this study. The maximum VFA produced in each cycle were 12.5 g/L (cycle 1), 18.10 g/L (cycle 2), 13.1 g/L (cycle 3) and 7.7 g/L (cycle 4) at pH 7.6, 7.3, 7.5, and 7.8 respectively as it is evident from Figure 4.3. The VFA level was approximately 2.4 times higher at pH 7.3 than at pH 7.8. The results also showed that pH closest to neutrality (pH 7.3 in cycle 1) yielded highest VFA concentration. A similar result was reported, where VFA concentration was 4.1g/L at pH 7 and 3.8 g/L at pH 8 indicating comparatively higher VFA production at pH close to neutral [114]. The reason behind preference of neutral pH lies in the sensitivity of methanogenic bacteria towards pH fluctuation. Their growth rate is reported to be highest around pH 7.0 and reduces significantly below pH 6.6 [86]. Therefore, high VFA production at neutral pH allows methanogens to avail and consume VFAs efficiently in order to yield better quality biogas. Hence, it is necessary to maximize VFA production for methanogens. Under all the pH levels, the VFA concentrations increased quickly at the beginning and then lowered slowly to reach stability. The trend was observed in all the experimental cycles, which is similar to a study conducted on food waste [74]

Furthermore, among the carboxylic acids, acetic acid fraction was higher than propionic and butyric acid in all the cycles except cycle 2. In cycle 2, VFA composition was affected by slightly alkaline pH range (7.7-8.2) when propionic acid raised higher than acetic acid and butyric acid. Nevertheless, on an average acetic acid and propionic acid were the most prevalent VFAs produced, accounting above 70% of TVFAs (Figure 4.4 and 4.5). A similar result on VFA fraction was reported where acetate and propionate accounted up to 70% at pH 5.0 [74]. VFA distribution also varies at different pH level for different types of feedstock. For instant, a study conducted on

food waste reported, at an acidic pH 5.5, acetic acid concentration was higher than other SCFAs, however at a pH range between 5.5-6 and 10-11, butyric acid dominated above all [76]. Similarly, at controlled pH 6.0 and 7.0, butyrate was the main product above acetate and propionate whereas at pH 5.0, acetate was the highest of all [74], [115].

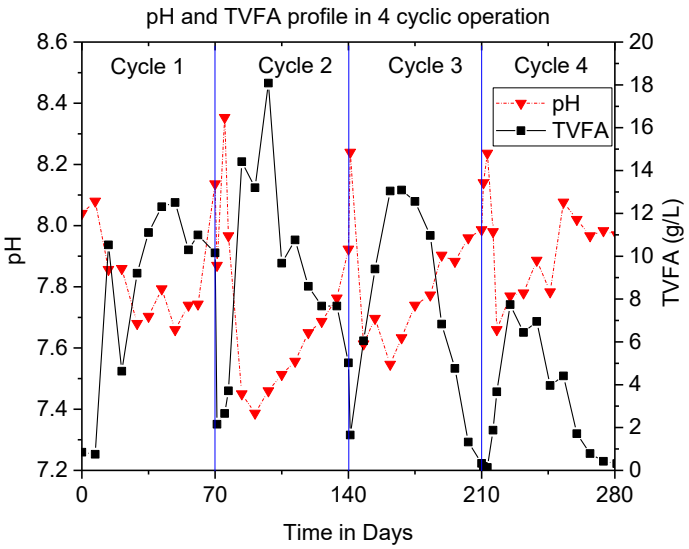


Figure 4.3: pH and TVFA

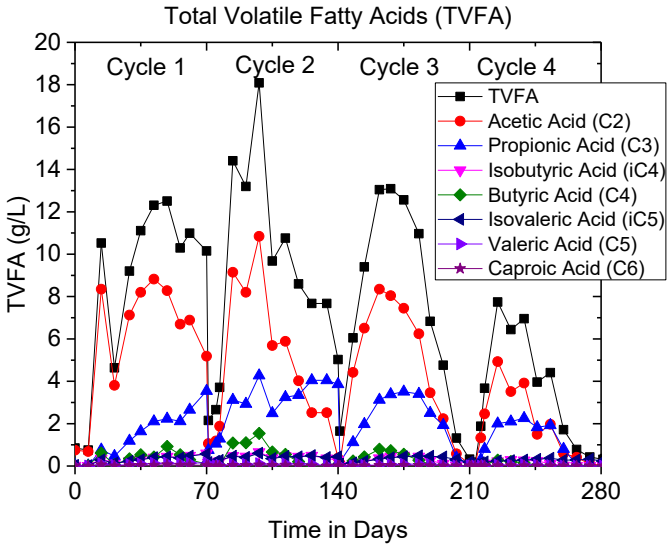


Figure 4.4: Total Volatile Fatty Acids (TVFA)

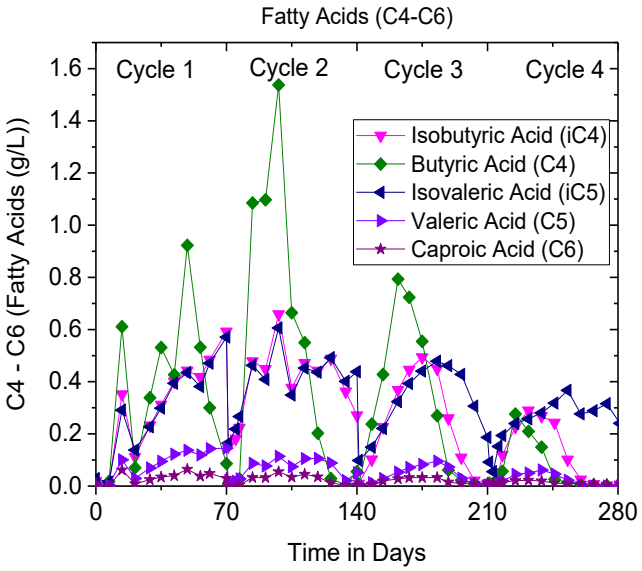


Figure 4.5: Fatty acids (C4-C6)

4.3.1.2. Temperature

Operating temperature in the AD has direct influence on the microbial growth rates and modification of microbial structure [116], [110]. The accumulation of SCFAs is less at lower temperature for example: SCFA 4ºC <SCFA 14ºC<SCFA 24.6ºC [116]. This can be attributed to the faster hydrolysis rate, carbonates, and proteins solubility at higher temperature [117]. However, overheat is proven to inhibit the methanogenic phase lowering the conversion rate from VFAs and H_2 into CH_4 and CO_2. Similarly, low temperature is generally stated to yield low VFA, however the statement is contrary to this work. In this study, at a low temperature of 20 ± 1^0C, VFA production was maximum (up to 18.1 g/L) at an OLR of 8.78 gVS/L/d in cycle 2. This VFA concentration was comparatively higher than the study conducted at a temperature of 35ºC which resulted a maximum VFA of 17 g/L and also the experiment conducted at 55^0C yielded VFA of 11g/L [116]. However, the generation of VFA was lowered in cycle 3 and reduced significantly in cycle 4. Hence, after the proper adaptation to a temperature of 20 ± 1^0C, at cycle 4, a maximum VFA of only 7.5 g/L was generated.

4.3.1.3. Organic Loading Rate (OLR)

The effect of OLR on VFAs was studied at 20ºC for 282 days under 4 different cycle lengths. Figure 4.6. shows variation of VFA at different OLR. The OLR of the digesters were 8.78 gVS/L/d (Cycle 1), 4.4 gVS/L/d (Cycle 2), 4.40 gVS/L/d (Cycle 3) and 4.3 gVS/L/d (Cycle 4). It is clear from cycle 1 that VFA accumulation towards the end of cycle was high (up to 11 g/L) at a high OLR of 8.78 gVS/L/d. Although, high OLR consists of organic content in a substrate to be utilized my microorganisms for biogas production, but high OLR also releases organic acids which reduces pH and accumulates VFA in the system [112]. Hence, after Cycle 1, OLR was reduced to 4.4-4.3

gVS/L/d in Cycle 2-4. Therefore, at lower OLR (4.3-4.4 gVS/L/d), the concentration of VFA towards the end of cycle 2, 3, and 4 was 1g/L, 0.25 g/L and 0.30 g/L respectively which was significantly low, indicating the complete consumption of VFA. This is in accordance with the two-stage experiment conducted by Jiang et al. (2013) [74], in which VFA levels were lower at low OLR because of the death of microorganisms responsible for fermentation in the first stage.

Similarly, the percentage of acetate was also observed to be higher (~72%) at high OLR of 8.78 gVS/L/d in cycle 1 and almost 30% less in cycle 2 at a lower OLR of 4.3 gVS/L/d (Figure 4.4). A similar observation was reported by Jiang et al. (2013) [74]. Although, at high OLR of 8.78 gVS/L/d, high VFA concentration was achieved but high OLR is also responsible for reactor failure. Therefore, in this study an OLR between 4.4-4.3 gVS/L/d was suitable for a stable VFA production.

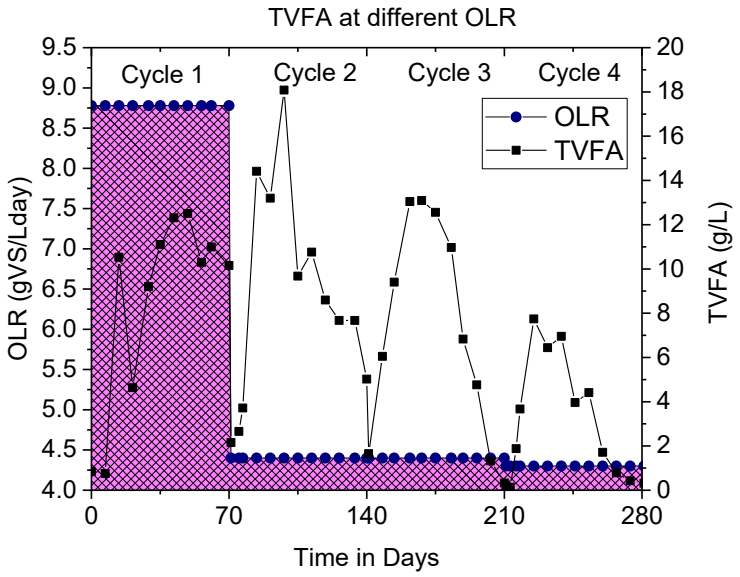


Figure 4.6: SCFA at different OLR

Table 4.1: Short Chain Fatty acids at different OLR in different cycles

Cycles	Digesters' mode of operation	OLR (gVS/L/d)	VS% Day 1	VS% Day 70	SCOD (g/L) Day 1	SCOD (g/L) Day 70	Maximum SCFA produced (g/L)	Corresponding pH level
Cycle 1	Batch	8.78	61.08	12.69	291.15	22.62	12.50	7.65
Cycle 2	Batch	4.40	61.08	12.60	291.15	26.63	18.10	7.30
Cycle 3	Batch	4.40	56.23	20.20	303.16	15.83	13.10	7.50
Cycle 4	Batch	4.30	56.23	19.78	303.16	15.62	7.74	7.80

Average VS degradation = 42%; Average SCOD degradation = 93%

4.3.1.4. Cycle Length (CL)

CL is the time duration or period for which the feedstock remains in the digester. CL influences SCFA generation more than temperature [30]. In this study, CL is the time required for a two-stage batch digester to process high solids CM. An average CL of 69-71 days was taken by digesters for the fullest utilization of the substrate. Studies suggest that cycle length facilitates in the yield of high SCFA however, the cases vary with different substrates and operational conditions [118]. In this study, maximum VFA of 12.5 g/L occurred at 45-d (Cycle 1), 18.1 g/L at 30-d (Cycle 2), 13.1 g/L at 25-d (Cycle 3) and 7.7 g/L at 20-d (Cycle 4) of the total CL. Similarly, the combined and cumulative methane concentration above 55% was observed on 60-d (Cycle1), 35-d (Cycle 2), 35-d (Cycle 3) and 30-d (Cycle 4). This shows the time requirement for maximum VFA generation was lesser than quality methane production. This could be due to faster proliferation rate of acidogenic bacteria (responsible for VFA production) by 30-40 times than the methanogens (responsible for methane production) [48]. Although, longer retention time provides sufficient time for methanogenic activity and enhances biogas production. However, longer retention time of 95-116 days in a study showed low performance of digester [119].

It is also observed from Figure 4.4. that VFA concentrations reduced in Cycle 3 and Cycle 4 compared to Cycle 1 and Cycle 2. In addition, the reduction of VFA generated from its peak (13.2 g/L in Cycle 3 and 7.7 g/L in Cycle 4), required lesser duration than Cycle 1 and Cycle 2, which was 26-d for cycle 3 and 21-d for cycle 4. Therefore, reduced CL duration resulted in lower VFA generation, which is in accordance with the study conducted by Scoma et al (2013) [120].

Similarly, the composition of VFA was also evaluated with respect to CL. Among the SCFAs, the shorter SCFAs (i.e., acetic and propionic acid) were consumed at shorter CL time (Figure 4.4). On contrary, the longer fatty acids (i.e., isovaleric and isobutyric acid) reduced at longer CL, whereas other VFAs (i.e., valeric, caproic and butyric acid) decreased at a shorter period of time (Figure 4.5), which is also in agreement with Scoma et al (2013) [120]. Therefore, CL was found to influence the SCFAs. Furthermore, acetate and propionate always dominated over other VFA components irrespective of different CL duration required for their reduction. However, this result was in contrast to a study where butyrate dominated over other VFAs with reduction of retention time [120]. In all, VFA level decreased in the succeeding cycles (Cycle 3 and 4) in correspondence with the requirement of lesser CL duration.

4.3.2. Understanding the ratio limit of TVFA/TA, C3/C2 and (C4+C5)/C2 on digester's performance and stability.

Different concentrations of TVFAs are considered as quality indicators for the digester's condition [88]. In addition, build-up of SCFAs is considered to be the most stressed indicator for the digester's stability. Moreover, instability in the AD process leading to digester failure can also be clearly observed by the means of C3/C2 ratio. Literature suggests that C3/C2 ratio greater than 1.4 is a representation of digester's failure [86]. Similarly, studies also mentioned that TVFA/TA ratio should be less than 0.5 for high stability of the digester [87]. Study conducted shows the performance of the digester is stable when TVFA/TA ratio is below 1 and is unstable when the

ratio is more or equal to 1. Low TVFA/TA ratio has been attributed to yield higher methane [88] and higher ratio at an OLR of 5 gVS/L/d resulted in decreased methane yield [119]. Similarly, for monitoring methane production in this study, the three ranges of TVFA/TA ratio adopted were : (i) less than 0.4 (verifies stability), (ii) 0.4-0.8 (some chances of instability) and (iii) >0.8 (significant instability) [3].

AD of CM, in this study was processed for 282 days in 4 different cycles with a cycle length of 70 days as shown in Figure 4.7. For a typical cycle (cycle 2) of operation, TVFA/TA ratio reached a maximum value of 0.86-0.97 in between day 13 and day 27, which then further reduced to less than 0.2 towards the end of the cycle. However, in cycle 4, which was noticed to be the acclimatized phase of the digester, TVFA/TA ratio never raised beyond 0.5 indicating promising buffering capacity of the digester with higher stability. Similarly, C3/C2 ratio was always below 1.4 except for the end of cycle 2. Even though higher C3/C2 ratio occurred in the start-up stage of digester, i.e. 1.6, no failure was recognized at later stages of operation. Furthermore, (C4+C5)/C2 ratio in this study remained under the suggested range (always below 0.3), therefore indicating a positive signal for digesters' stability (Figure 4.8).

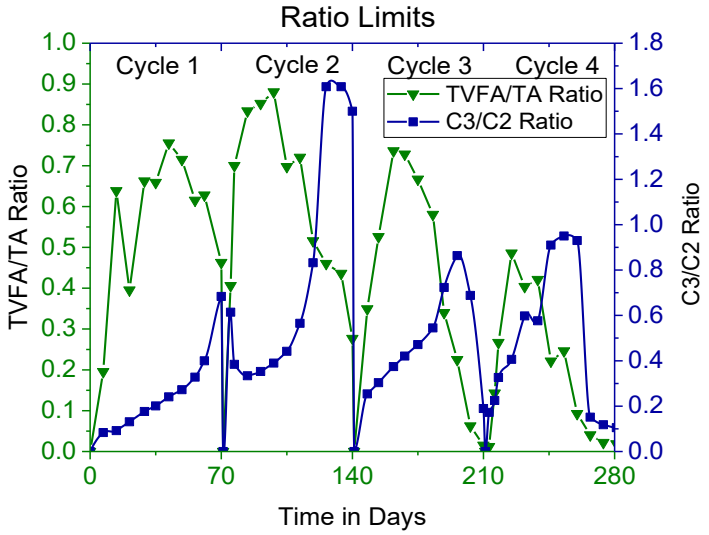


Figure 4.7: Ratio Limit for TVFA/TA and C3/C2

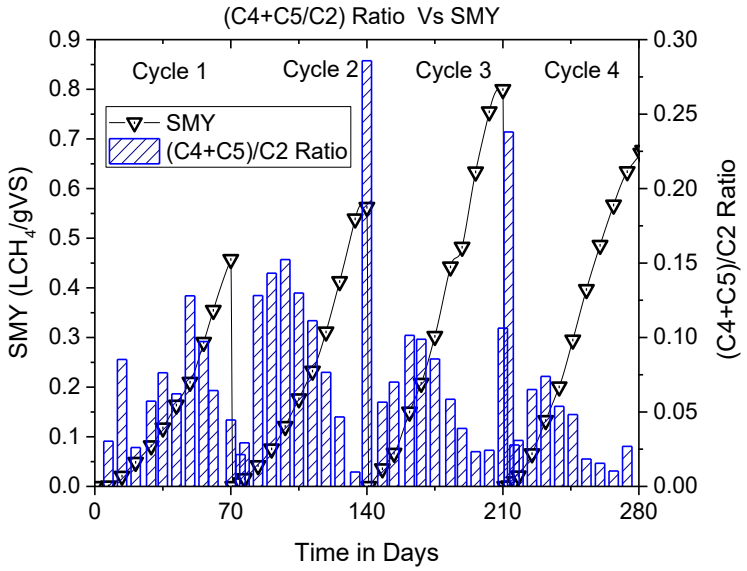


Figure 4.8: (C4+C5)/C2 ratio Vs SMY

4.3.3. Inspection of methane production with respect to the changes in VFA yield, its production and composition in the proposed HSAD biotechnology

Methane production depends on the existence of SCFAs in the digester since SCFAs are the substrate for methanogens and methanogenic activity is higher in the presence of SCFAs. A study suggests higher methane production at higher SCFAs [56], however situation differ when pH becomes inhibitory due to high release of hydrogen through acetic acid [58]. Along with the quantity of SCFAs produced, the knowledge of its composition plays an equally important role to determine the level of hydrolysis and fermentation in the process [113].

4.3.3.1. Effect of SCFAs on methane production

Figure 4.9 and 4.10 depicts the correlation between the evolution of TVFA concentrations and its corresponding methane concentration (%) and SMY, respectively. As it is evident from the Figure 4.9, it took about 50 days to reach a methane concentration of about 50% in cycle 1 compared to that of succeeding cycles (Cycle 2-4). It is to be noted that cycle 1 operation was considered like a start-up phase, as this cycle started with the diluted liquid inoculum as a source of active microbes for the solid CM digestion. Hence, it took time initially to acclimatized to the high solids and high ammonia substrate as shown in Figure 4.7. With time the adaptation was better and hence from cycle 2 onwards, a better TVFA degradation took place resulted in a higher methane concentration (65-70%) towards the end of the cycle.

Similarly, SMY also evolved with every succeeding cycles (from Cycle 1 to Cycle 4) as it is clear from Figure 4.10. On the 70-day of each cycle, SMY was 0.46 LCH_4/gVS (Cycle 1), 0.56 LCH_4/gVS (Cycle 2), 0.80 LCH_4/gVS (Cycle 3) and 0.68 LCH_4/gVS (Cycle 4). Therefore, the enhancement in the VFA utilization is observed with every succeeding cycles which could be due to higher methanogenic activity [63]. In the most advanced cycle of this study (i.e., Cycle 4), it

was observed that SMY raised slowly to 0.1 LCH_4/gVS, when VFA increased up to 8 g/L on 14-d of the cycle. From 15-42 d, VFA reduced from 8 g/L to 4.4 g/L, during which SMY increased up to 0. 3 LCH_4/gVS. Towards the termination of the cycle (70-d), VFA decreased to 0.3 g/L and SMY raised to 0.7 LCH_4/gVS. Compared to Cycle 4, maximum SMY yield in Cycle 1 was only observed to be 0.46 LCH_4/gVS. Hence, it is clear that the microbial adaptation with time played an important role towards faster and higher consumption of VFA and SMY generation at the end of cycle [63].

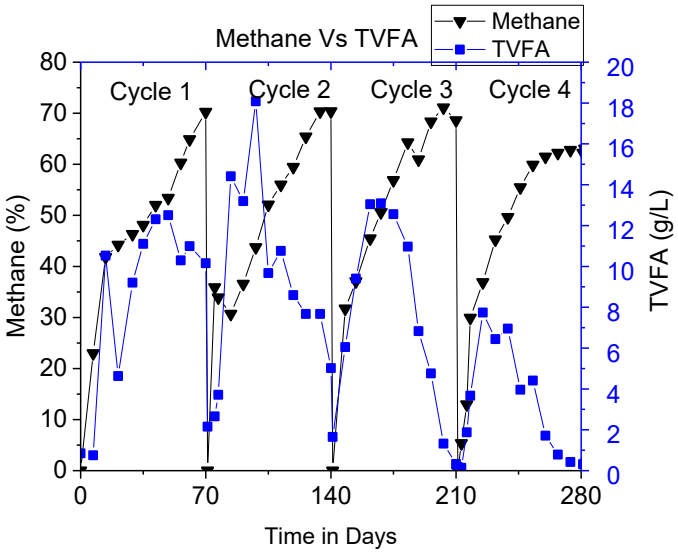


Figure 4.9: Methane Vs TVFA concentration

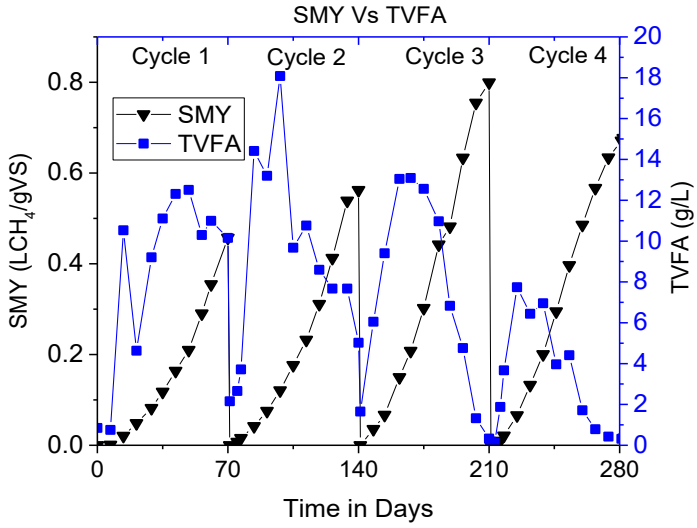


Figure 4.10: SMY Vs TVFA

4.3.3.2. Study of VFA yield, its production and composition

SCFAs consists of carboxylic compounds with carbon atoms between C2-C6 (Figure 4.4 **&** Figure 4.5). The pattern of the concentration of fatty acids among the SCFAs was observed as acetic acid (C2) > propionic acid (C3) > butyric acid (C4) in this study as shown in Figure 4.4. However, among C4, C5, iC5 and C6 in the acidogenic phase shown in Figure 4.5, C4 dominated, followed by iC4 and iC5, which maintained higher concentration comparatively. In a typical cycle (cycle 3) of the experiment, it was observed that VFA concentration was highest on 27-d of the cycle and then decreased with time. The level of VFA increased from 1.6 g/L on the 1st day to 13 g/L on the 27-d and reduced to 0.3 g/L on the 69-d. Similarly, C5 and C6 remained below 0.2 g/L during the entire process and towards the end of the cycle 3 from 48-d to 69-d, iC5 dominated over other fatty acids. Some authors suggested that high butyric and valeric acid content results in digester failure [88], [3], however the concentrations of C4 and C5 where significantly low (<0.2 g/L) in our study.

It was noticed in Cycle 3 and Cycle 4 (Figure 4.4) that almost complete C3 degradation (<20 mg/L) and C2 degradation (<100 mg/L) took place, however in Cycle 1, C3 remained above 3.5 g/L and C2, above 5.1 g/L indicating incomplete degradation. Conversely, in Cycle 2, C3 was higher than C2 which is generally considered inhibitory and the built up of C3 was an indication of poor process performance [121]. However this was balanced by high alkalinity content or buffering capacity of the manure as TVFA/TA was observed below 0.4, which is considered stable [122], [3]. Table 4.2 clearly shows the concentration of acetic acid was higher than propionic acid followed by butyric acid. Figure 4.5 shows significant concentration of propionic acid and was consumed after reaching to a maximum concentration of 3.9 g/L (Cycle 1), 4 g/L (Cycle 2), 3.5 g/L (Cycle 3) and 2.2 g/L (Cycle 4). High concentration of C3 attributes in the consumption of hydrogen ions from acetic acid, which are essential substrates for methanogens to produce methane and carbon-dioxide.

The accumulation of C3 is often a warning sign and toxic, since the methanogens are fragile to C3 above 1 g/L [123]. However, the concentration of C3 was lower than C2 which was a positive sign for a stable digester operation as the conversion of C3 to C2 is a slow kinetic process which holds the possibility of inhibition due to rise in C3 concentration. Therefore, altogether the build-up fatty acids in the acidogenic stage were controlled with its utilization adequately by the microorganisms and enzymatic activity. In addition, washing out of accumulated VFAs was also facilitated with percolation and recirculation of liquid inoculum in the solid digester.

Table 4.2: Percentage composition of SCFAs

	Acetic acid (C2) %	**Propionic acid (C3) %**	**Butyric acid (C4) %**	**Iso-butyric acid (iC4) %**	**Valeric acid (C5) %**	**Iso-valeric acid (iC5) %**	**Caproic acid (C6) %**
Cycle 1	72.98±18.36	15.34±15.50	3.17±2.50	3.98±3.3	3.24±1.80	0.92±0.51	0.46±0.27
Cycle 2	44.76±20.38	39.72±22.07	5.24±1.93	3.47±3.21	5.76±2.66	0.82±0.41	0.19±0.15
Cycle 3	50.97±22.05	24.75±15.74	2.69±1.40	2.82±2.41	10.95±4.21	0.62±0.36	0.54±0.32
Cycle 4	43.17±27.52	20.88±10.46	2.67±2.40	2.01±2.11	29.56±8.11	0.49±0.25	1.21±0.75
Average	52.97±22.07	25.17±15.94	3.44±2.05	3.07±11.03	12.37±4.19	0.71±0.38	0.60±0.37

4.4. Conclusions

The results obtained from this experiment showed step-wise improvement in methane yield from Cycle-1 to Cycle-4, followed by the reduction or consumption of VFAs. VFA concentration was seen to be lower at lower OLR and pH was optimum when closest to neutrality. Similarly, the time duration required for the initiation of methane concentration above 55% shortened from 60-day (Cycle1) to 30-d (Cycle 4). Moreover, the performance of low-temperature digester operating high solids and high ammonia CM was encouraging since the monitoring parameters (TVFA/TA) and C3/C2) ratio limits were within the suggested range. In addition, the ratio of (C4+C5)/C2 also remained under a range of 0.3 throughout the operation. Furthermore, no significant inhibitory effects were observed despite of higher OLRs (8.78 gVS/L/d) and ammonia concentrations (23.3-33.3 g TKN/L). Along with, an appreciable methanogenic activity was also noticed which was justified with methane rich biogas generated (>60%) and an increase in SMY of up to 50% was noticed from Cycle 1 to Cycle 4 during the 282 days operation conducted in four cycles. Hence, the positive results obtained overall motivates to optimize the next operational cycles by varying operating conditions.

Chapter 5: Processing High-Solid and High-Ammonia Rich Manures in a Two-Stage (Liquid-Solid) Low-Temperature Anaerobic Digestion Process: *Start-Up and Operating Strategies*

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5.1. Introduction

In the last few decades, rapid growth in the population has been observed, which is further predicted to increase to 9.6 billion by 2050 [124]. In addition, the accelerated pace of urbanization and growing income is also noticed. Together, these factors pose severe challenges to the food and agriculture sectors. Along with the change in food habits, the elevation in manufactured agriculture products, mostly based on animal sources, the consumption of chicken meat, and egg production, has also increased by 50% and 36.5%, respectively, from 2000 to 2014 [5]. The demand for food is estimated to increase to 73% and 58% for meat and milk, respectively, by 2050. Consequently, this leads to mass production of livestock and, ultimately, a huge generation of manure. Manure causes emissions of greenhouse gases (GHG) [125] if not managed properly. Globally, the poultry-related emissions alone account for about 600 million tons of carbon dioxide (CO_2) equivalent per year [9], contributing to climate change and global warming. In order to manage the manure, one of the widely exercised solutions is the land application as it provides nutrition to the land. However, excessive land application of manure results in nutrition overloading in soil and water bodies, ending up in eutrophication [11]. Open land application of manure also contributes to methane (CH_4) emissions, which carries 23 times more global warming potential than CO_2 alone [126]. Another positive solution towards manure management can be composting as it reduces waste mass and produces valuable end products [22]; however, the huge loss of nitrogen (N) in the form of soluble nitrates is observed in composting, which eventually reduces the fertilizer value. Besides this, composting also causes odor nuisance and environmental side effects like air and water pollution, gases like NH_3, CH_4, and N_2O impacts air quality and, leaching and runoff due to precipitation causes high adverse effect on water pollution [15], [127].

Anaerobic digestion (AD) is a sustainable approach to reduce the ill effects caused by improper processing of manure. In recent years, AD has received great attention due to its obvious advantage, i.e., reducing pollution, converting organic waste into high-quality biogas, which is useful in the form of heat and/or electricity [31]. Moreover, the generation of electricity through AD is a renewable process, thus reduces the cost of fossil fuels and their climatic side effects. Poultry litter is one of the highest biomethane potential organic substrates compared to dairy cow manure (DM). However, one of the major limitations of AD of chicken manure (CM) is the inhibition caused by the production of ammonia [128] due to which its potentiality cannot fully be exploited. CM is also high in solids (63 ± 10% Total Solids (TS)), which makes the process unsuitable in semi-liquid (10–15% TS) or wet (<10%) digesters as the dilution requirement would be 6–7 times than the normal practice to operate in these types of digesters. Similarly, high N in CM (Total Kjeldahl Nitrogen (TKN): 25–35 g/L) also demands huge dilution to avoid inhibitions during the AD process. Unfortunately, dilution by water requires comparatively high energy input, which makes the situation expensive and impractical to process the feedstocks rich in high-solids and high-ammonia. In this scenario, the co-digestion of CM with other crops or C-rich feedstock could be a feasible method.

C/N ratio of CM ranges from 6.3 to 10 [20], [75] and to operate the digester to its utmost condition, high carbon content is essential. On the other hand, the C/N ratio of DM is reported to be between 24 and 40 [75], [129]. Therefore, co-digestion stabilizes the C:N ratio because of the

composition of high lignocellulosic compounds in DM. Co-digestion also minimizes the risk of ammonia inhibitions and, in some cases, improves the methane content in the biogas. Co-digestion of manure also benefits in many ways, like the reduction of manpower in the segregation of waste to be processed. It avoids the separate storage, treatment, and handling of mixed waste [45]. Agriculture and Agri-Food Canada (AAFC) has successfully developed the AD biotechnology over the years to process poultry, swine, and cow manures operating at low temperatures. However, the potential of digesters to process the co-digestion of DM and CM at a TS > 50% using adapted liquid inoculum has not been studied. The positive results obtained from the study of the mono-digestion of CM has encouraged us to explore the possibilities of co-digesting CM + DM mix in an economical way.

This part of research emphasized on the start-up and operating strategies for the development of low-temperature two-stage (liquid–solid) anaerobic co-digestion of CM + DM mixture using adapted inoculum. The primary objective of this study was to demonstrate the operational feasibility of two-stage process (i.e., liquid inoculum reservoir coupled with high-solid anaerobic digestion (HSAD) system), treating CM + DM at 20 ± 1 °C, and to encourage small-scale farmers to adopt this technology at low cost. An effort was made to develop the HSAD start-up protocol, using (i) acclimatized liquid inoculum since obtaining a huge quantity of solid inoculum to treat high-solids waste mix is practically not feasible at many farm locations; (ii) no mixing conditions, as mechanical mixers create complexity in full-scale operations. Besides, the scope of this study was also to assess the comparative performance of digesters co-digesting CM + DM and mono-digesting CM, especially in terms of methane concentration and free ammonia inhibition.

5.2. Materials and Methods

5.2.1. Feedstock and Inoculum

Fresh DM was obtained from the AAFC dairy farm located at our Sherbrooke Research and Development Center, whereas the fresh CM was sourced from a small-sized poultry farm located in Farnham (Quebec province). DM consisted of straw as bedding, whereas the bedding of CM composed of wood shavings. These bedding materials were used for the dairy cow/chicken productions by the farm itself. Hence, the manure used in this study was always contained in the bedding components. The manure was collected and stored in a cold room at 4 °C to prevent biological activity prior to feeding. For the feedstock characterization, manure was diluted and ground primarily to reduce the feed concentration for the analysis purpose and the homogenization of the solid samples, except for the TS and volatile solid (VS) analysis. The liquid inoculum used in the start-up phase was obtained from our ongoing laboratory-scale liquid sequencing batch AD, adapted to high-ammonia content chicken manure leachate. The summary of the feedstock and inoculum materials used is shown in **Table 5.1**.

Table 5.1. Summary of the materials used.

	Cycle 1	Cycle 2
Total weight of feedstock treated	7 kg (CM + DM)	4.7 kg (CM + DM)+ 4.7 kg (Dry inoculum from cycle 1)) = 9.4 kg
Quantity increment (%) per cycle	-	34% w/w
Mix ratio (CM:DM)	1:1	1:1
Volume of liquid inoculum	25 L	25 L
Solid substrate: liquid inoculum digester volumetric ratio	1:3.6	1:2.6
OLR (gVS/L.d)*	3.7	4.7

*OLR calculations were done based on the raw feedstock VS, and the formula used was OLR = VSi * (Q/V), where OLR: organic loading rate (g VS/L.d); VSi: VS of feedstock (CM + DM) in g/L; Q: quantity/flow rate of raw feedstock in kg or L/d; V: volume of the HSAD in L.

5.2.2. Experimental Setup of Two-Stage (Liquid–Solid) Anaerobic Digesters

The experimental arrangement consisted of two-stage (liquid–solid) anaerobic digesters (i.e., liquid inoculum reservoir coupled with HSAD system) for processing CM + DM mixture at 20 ± 1 °C. Two sets of digesters in duplicates with a total volumetric capacity of 40 L were operated in parallel. A set consisted of 2 digesters—one for liquid inoculum reservoir named "digester A", and the other for HSAD named "digester B'. Digesters A and B were kept adjacent to each other, as shown in Figure 1. Therefore, the two sets of digesters were named as digester 1 (1A + 1B) and digester 2 (2A + 2B).

The concept behind this coupled liquid–solid digesters arrangement was to enhance the digestion feasibility of the HSAD content, which was fed without any dry inoculum. Provisions were made in such a way that a known volume of adapted liquid inoculum from 'digester A' was recirculated-percolated through the solid content in the HSAD(digester B), principally (i) to enhance mixing and, thus, waste-microbe interactions in 'digester B' and (ii) also to leach out a significant amount of Volatile Fatty Acids (VFA) and nitrogen from digesters 'B' to 'A'. By doing this, organic and VFA overloading in HSAD were minimized, but, at the same time, methane yield was increased since 'digester A' also contributed to producing biogas as it contained acclimatized microbes. This conception also aimed to increase the buffering capacity of the digesters by maintaining optimum pH and alkalinity in 'digester B'. Similarly, the liquid inoculum 'digester A', which was less in organic matter (Table 5.2), got fed and charged from 'digester B', aiding in additional methane production.

The digesters (A and B) were fit with the biogas pipeline to the tip tank for the release and quantification of the biogas produced. Digester A was connected with 3 additional pipelines; first one was connecting A and B; second, was linked to the pump for mixing. Mixing was done (just in digester A) every day for 5 min, mainly to homogenize the liquid content since it received leachate from digester B and also to release the space for air bubbles trapped in the anaerobic

digesters. Similarly, the third one was connected to B, for recirculating the liquid inoculum from B to A. The first and the third pipe connections were responsible for percolation and recirculation of liquid inoculum. Five liters of inoculum from digester 'A' were recirculated to digester 'B' and then percolated back from digesters 'B' to 'A', thrice a week.

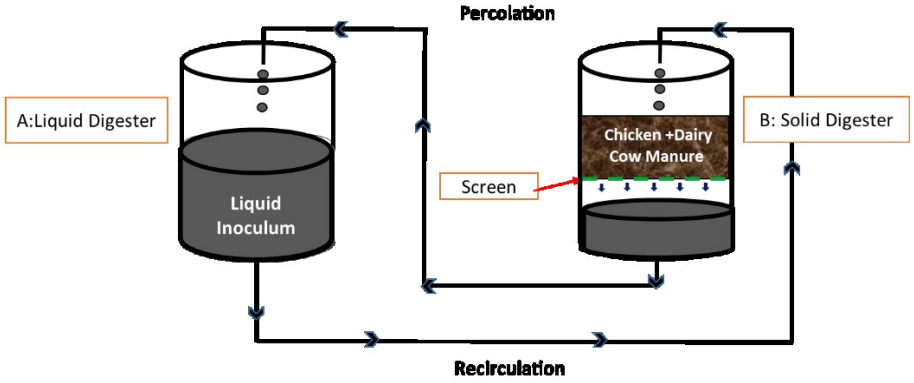


Figure 5.1. Schematic diagram of a single set of two-stage (liquid–solid) digesters.

Embracing this set-up, altogether two batch feeding operations were conducted one after the other immediately; hence, they are named as "cycle 1" and "cycle 2", which represents retention time or treatment duration corresponding to each feeding. Cycle 1 was conducted for 112 days, while cycle 2 was conducted for 78 days only. The operation time or cycle length was mainly dependent upon the desirable methane concentration, methane yield, and VFAs accumulations. CM and DM were mixed in 1:1 (*w*/*w*) ratio for two reasons: (i) to operate the digester with TS of around 50% (instead of about 70% in CM); (ii) to maintain Total Chemical Oxygen Demand (COD_t)/TKN ratio in the range of 30. However, further study is underway in order to optimize several operating parameters, including COD_t/TKN ratio, as our prime aim is to operate at high ammonia levels. As presented in Table 1, a total of 7 kg of mixed manure was fed to the (HSAD) digester (cycle 1 operation). For cycle 2, about 4.7 kg of digested material resulted from cycle 1 was retained in the HSAD as a source of dry inoculum. This was done in order to reduce the start-up period by supplying adopted active microbes for the subsequent (batch) feeding. Our motive was to operate at short cycle length and to maintain a similar volumetric loading rate. Henceforth, about 4.7 kg of mixed (CM + DM) manure (refer Table 1) was mixed to the retained dry inoculum (i.e., 4.7 kg) and fed to the HSAD in order to have the substrate:dry inoculum ratio (*w*/*w*) close to 1:1. Once the stabilization occurred, substrate:dry inoculum ratio would be increased to accommodate more feedstocks for commercial benefits.

It is to be noted that the liquid inoculum used in cycle 1 was adapted to CM leachate with 5500 mg TKN/L. Since the adapted inoculum was not exposed to DM, longer retention time was required for cycle 1 operation to develop an acclimatized inoculum for cycle 2 operation. A volume of 25 L liquid inoculum was fed in the individual liquid digesters in both cycle 1 and cycle 2. The substrate to liquid inoculum digester volumetric ratio was maintained between 1:3.6 and 1:2.6 for cycles 1 and 2, respectively. The solid content of the mix was initiated with approximately 48% TS in cycle 1 and 51% TS in cycle 2.

A similar experimental set-up was used for CM mono-digestion. Two operational cycles (70-d for cycle 1 and 85-d for cycle 2) were conducted in order to have a performance comparison. Mono-digestion of CM was processed with the 65–73% TS, 4.3–4.6 gVS/L.d, and the co-digestion

(CM + DM) was treated with 48–51% TS, 3.7–4.7 gVS/L.d. Two cycles of different cycle lengths, depending upon the consumption of VFAs, methane quality, and digester's stability factors, were carried out. The operating conditions of all the four processes (CM(C1), CM + DM (C1), CM(C2), and CM + DM(C2)) are shown in Table 5.2.

Table 5.2. Operating conditions of mono-digestion (CM) and co-digestion (CM + DM).

	CM (C1)	CM + DM (C1)	CM (C2)	CM + DM (C2)
Cycle length (retention time or treatment period) in days	70	112	85	78
Quantity of raw manure treated (kg)	5.4	7	6.5	4.7
Total volume of HSAD (L)	60	40	60	40
Total amount of solid material treated in HSAD (kg)	10	7	10.8	9.4
Total volume of liquid digester (L)	60	40	60	40
Active volume of liquid digester (L)	25			
Quantity and frequency of liquid inoculum percolated-recirculated	5L-thrice a week			
Mode of operation	Batch			
Temperature (°C)	20 ± 1			
OLR (gVS/L.d)	4.3	3.7	4.6	4.7

OLR = organic loading rate; CM = chicken manure; DM = dairy cow manure; C1 = cycle 1; C2 = cycle 2.

5.2.3. Analytical Methods

The bio-digesters were operated in a batch mode; therefore, the operational physio-chemical parameters were examined only for the liquid digesters on a weekly basis in order to assess the performance of the two-stage digesters. About 100 mL liquid samples were withdrawn from the liquid inoculum reservoir for the physiochemical analysis, whereas samples from the HSAD system was only taken twice *viz.* at the beginning and the end of operation since the HASD was not having weekly sampling provisions. Overall, 290 samples for biogas and 80 samples for physiochemical tests were taken during 190 days of the entire process of CM + DM. For CM alone, 240 gas samples and 50 samples were taken during 155 days of operation.

5.2.3.1. Biogas Analysis

The biogas production and its composition were checked for both A and B digesters on alternative days. The biogas samples were analyzed thrice a week (weekends not included from all the 4 bio-digesters (1A, 1B, 2A, and 2B), and the volume of biogas was monitored every day using the wet tip gas meters. Methane concentration in the biogas was analyzed using a gas chromatograph (Micro GC 490, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) and Helium gas as the carrier gas at a flow rate of 20 mL/min. The injector and oven temperatures were 110 °C and 180 °C, respectively.

5.2.3.2. Physiochemical Analysis

All the other samples were analyzed for the tests like pH, alkalinity, total solids (TS), volatile solids (VS), total COD (TCOD), soluble COD (CODs), TKN, ammonia nitrogen, and volatile fatty acids (VFAs). Along with this, TS and VS on a dry weight basis were determined following the guidelines given by the standard methods [94]. pH was determined by using pH Mettler Toledo

AG 8603, SevenMulti (Schwerzenbach, Switzerland). Alkalinity was measured using Hach Lagne Sarl, Titralab AT1000 Series (Hach, Switzerland). COD was measured by using a closed reflux colorimetric method [94]. TKN and NH_3-N were analyzed using a 2460 Kjeltec Auto-Sampler System (FOSS, Sweden) following the macro-Kjeldahl method[94]. VFA was determined using a Perkin Elmer gas chromatograph, model Clarus 580 (Perkin Elmer, Shelton, CT, USA), mounted with a DB-FFAP high-resolution column, but before the evaluation of VFAs, samples were conditioned according to the procedures mentioned by Masse et al. (2003) [95]. Samples collected from digesters were first centrifuged at 41× *g* for 15 min and filtered through a 0.22 µm membrane before injected. The injection volume was 0.1 µL.

5.3. Results and Discussion

5.3.1. Characteristics of the Feedstock and the Inoculum

The characteristics of the inoculum and feedstocks are shown in Table 5.3. DM had low carbon in terms of CODt (~65% less) and nitrogen content in terms of TKN (~70% less) than CM, which complemented the DM to achieve a desirable nutrient content in the system for AD of CM + DM. The COD_t/TKN ratio of the CM + DM mixture in this study was around 30, which is considered as optimum value, as reported in[130]. However, for inoculum, this ratio was low in the range of 2–3, as it was acclimatized using high ammonia content wastes. The pH of CM, DM, or CM + DM mixture was always above 7.5, although high VFA concentrations of 11.6 g/L were detected for CM, mostly because of the high amount of alkalinity in the respective manures (Table 3). The biodegradability of CM, DM, and CM + DM mixture was generally higher (i.e., VS/TS = 86–89%).

Table 5.3. Characteristics of feedstock and inoculum.

Parameter	Cycle 1				Cycle 2			
	CM	DM	Inoculum	CM + DM	CM	DM	Inoculum	CM + DM
pH	8.68	7.58	7.86	8.2	8.88	8.13	8.37	8.1
COD_t (mg/L)	568,017	208,433	7121	405,534	565,885	188,341	5968	402,921
COD_s (mg/L)	114,768	44,852	4415	94,044	111,545	34,017	3915	96,944
Alkalinity (as mg/L $CaCO_3$)	33,282	13,932	13,313	12,649	30,486	11,126	9575	-
TS (%)	65	23.9	1.28	48	73	21.58	1.02	51
VS (%)	56	21.3	0.54	42	65	19.23	0.40	45
TKN (mg/L)	21,962	6749	3151	13,613	23,072	5194	2359	13,472
NH_3 (mg/L)	6070	1389	2732	3470	7229	1795	2117	-
TVFA (mg/L)	11,588	6973	24	10,582	10,914	6499	116	-
CODt/TKN	25.8	31	2	30	25	36	3	30

5.3.2. Influence of Operational Parameters in the Two-Stage AD Process Treating CM + DM Mixture

Two-sets of two-stage (liquid inoculum reservoir coupled with HSAD) AD digesters, treating CM + DM mixture, were operated for a total period of 190 days, in which cycle 1 was operated for 112 days (i.e., day 0–112), and then cycle 2 was done for 78 days (i.e., from day 113–190). Digester's performance was monitored by a wide range of several physicochemical parameters listed under Section 5.2.2.2, in order to develop a start-up solid-state AD protocol, using adapted liquid inoculum as a microbial source. Operational parameters, such as Organic Loading Rate (OLR), cycle length/treatment period, operating temperatures, recirculation-percolation rate and frequency, and the mode of operation, were controlled as they have a direct influence on the performance of the two-stage AD process. In addition to this, the effect of ammonia concentrations on the digester's performance was also given priority.

5.3.3. Performance of the Two-Stage AD at Different Cycles and OLRs: Biogas and Methane Production and Digester Buffering Indicators

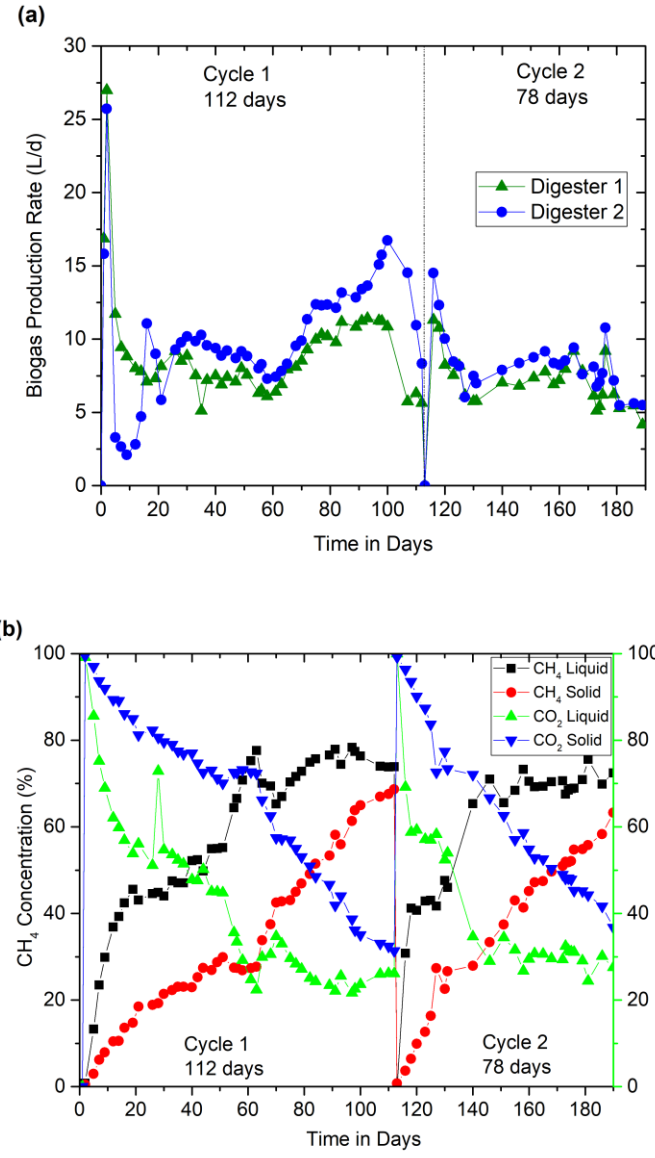
The task of liquid inoculum reservoirs was not just limited to the dilution of solid digesters organic content or to supply active microbes but also played a vital role in providing signals of the ongoing metabolic activity in the HSAD. The indications from liquid digesters assisted in taking the required actions prior to the possible inhibitions that could occur in the system. Liquid digesters also participated in the generation of biogas in addition to the HSAD with a supply of new feed from each time the leachate was recirculated.

Figure 5.2a–c depict the biogas and methane production profiles and their yield along with the digester buffering indicators (pH and alkalinity). For cycle 1 (days 0–112) operation, the OLR was maintained at 3.7 g.VS/L.d, and the corresponding volumetric combined (liquid + HSAD) biogas production recorded was 9.4 ± 3.7 L/d (Figure 2a). Whereas for cycle 2 (days 113–190) operation, OLR was increased to about 4.7 g.VS/L.d, and the corresponding volumetric combined biogas production was observed to be 7.7 ± 1.8 L/d. The cumulative biogas volume was found to be stable in both cases. As far as the methane concentration in the biogas was concerned, during the cycle 1 operation, it took about 82 days, especially for the HSAD to reach 50% CH4, whereas, in cycle 2, it took only 42 days to attain the same value. Interestingly, methane content in the liquid inoculum reservoirs remained always higher for both the cycles (Figure 2b), which demonstrates that the process offered excellent quality of biogas, which remained fairly steady (70–75%) at the end of each cycle. High methane content also suggested that the methanogenic population in the liquid inoculum reservoirs was enhanced for this substrate (CM + DM mix). It is to be noted that a combined (liquid + HSAB) methane concentration at the end of each cycle had reached to about 70%.

Specific methane yield (SMY) is a parameter that quantifies the amount of methane generation per gram of the organic matter, such as VS or COD. The average SMY was reached to about 0.33 LCH_4/gVS at the end of cycle 1 operation (i.e., on day 112), whereas a similar result was obtained within 78 days in cycle 2 (Figure 5.2c). In addition to this, the degradation of organic matter in terms of CODt and VS was monitored. CODt and VS reductions of about 60% and 59%, respectively, were observed at the end of cycle 1. Whereas at the end of cycle 2, CODt and VS removal efficiencies were increased to about 76% and 62%, respectively, even at a shorter cycle length.

From Figure 5.2d, at a pH range of 7.2–8.4, the alkalinity reached up to 18 g/L in cycle 1 operation and 14 g/L in cycle 2, respectively. A slight change in pH generally could affect the

methanogenic activity in AD. However, abrupt changes in pH are balanced by sufficient alkalinity (buffering capacity). Generally, alkalinity generated in the AD system itself controls the system, which is assisted by high protein or nitrogen content in the manure. The levels of VFA remained low (total content below 900 mg/L) at the end of both, indicating high reactor stability, which was confirmed by the presence of more neutralized pH and higher alkalinity values within the digester. There was no sign of inhibition or nutrient deficiency at these operating conditions. The detailed results pertaining to the VFA concentrations are discussed in subsequent sections.



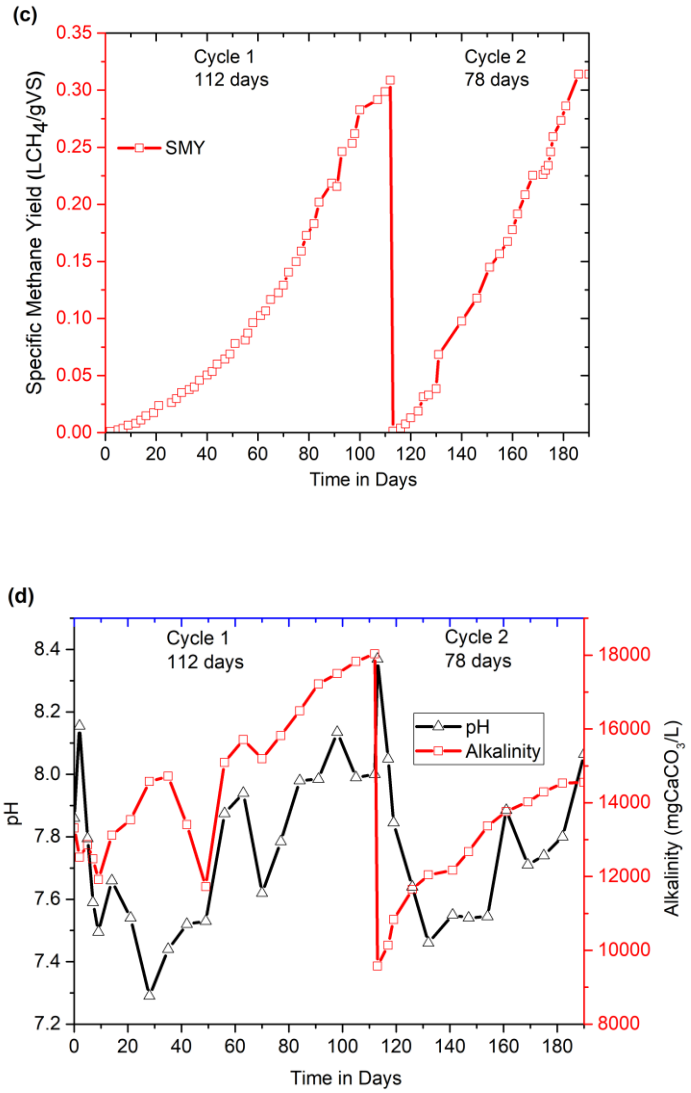


Figure 5.2. Performance of the liquid and solid digesters at different organic loading rates (OLRs) during 190 days of operation. (**a**) Biogas production rate; (**b**) Biogas composition; (**c**) Specific methane yield (SMY); (**d**) pH and alkalinity profiles.

5.3.2.2. Performance Monitoring of Digesters: Correlation between VFAs, pH, and Methane Concentration

Figure 5.3 shows the correlation between pH and TVFA. VFAs are the intermediate products in the AD process, and their accumulation is advised to be avoided. The concentration of VFAs is one of the important parameters for the AD process as the increase in VFA indicates the initiation of the acidogenic phase; however, the rapid increase is a sign of inhibition of microorganisms responsible for methanogenesis. Fluctuations in VFA concentration change the pH with the change in hydrogen (H^+) ions released during the breakdown of organic matter. Maintaining optimal pH is a must for the survival of varieties of microorganisms playing a role in continuing the process without inhibition. The optimal pH range regarded is 6.8–7.2 for both acidogenic and methanogenic bacteria [48]. The pH range in this study was 7.2–8.4, with occasional fall and rise.

The initial decrease in pH means the start of acidification, and a sudden increase indicates the termination of acidification at that point. The growth rate of methanogens is slower than the acidogens; therefore, methanogens require longer retention time than the acidogens, in order to consume the VFAs and produce methane-rich biogas. The extraction of VFAs is also possible by providing longer retention time and can be achieved with a batch mode of operation [1]. Low pH leads to the accumulation of acetic acid and hydrogen, which inhibits the degradation of propionic acid and ultimately accumulating VFAs. In the cycle 1 of this study, TVFA production went highest to 15 g/L (42-d), in which 10 g/L was acetic acid, and propionic acid was below 3 g/L. However, the case differed in the next cycle, which only generated a maximum of 4.5 g/L TVFAs (Figure 3). The reason behind comparatively low VFAs could be due to the amount of fatty acids, which declined rapidly due to an appreciable amount of methanogens generated from the previous cycle.

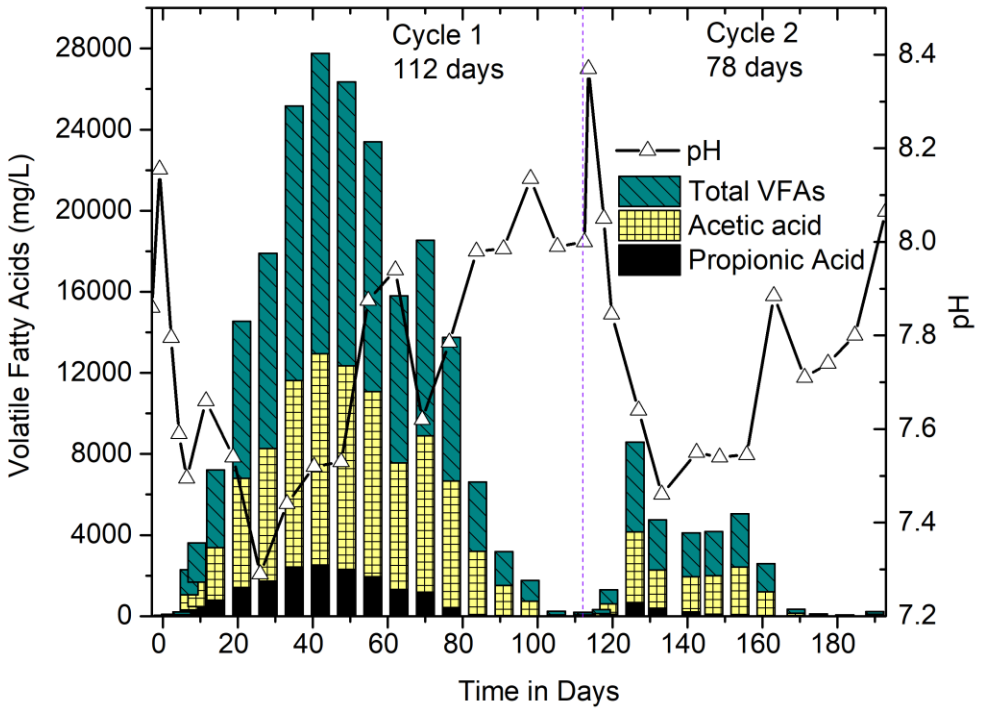


Figure 5.3. Correlation between volatile fatty acids (VFAs), pH, and methane concentration.

As shown in Figures 5.2b and 5.3, between days 51 and 63 of cycle 1, the methane quality was observed to be decreasing (29% on 51-d to 27% on 63-d) in the HSAD digester. Although the decrease was not significant, this could be due to the possibility of scarcity in methanogenic population; therefore, 10 L additional liquid inoculum was recirculated-percolated from liquid inoculum reservoir (digester A) to HSAD (digester B), which then increased the methane concentration due to the increase in their biomass activity. This also facilitated in leaching out the accumulated VFAs from HSAD to the liquid inoculum reservoir for further degradation. Henceforth, after day 65, a significant improvement in methane quality (approximately 45%) in

HSAD and a rapid reduction in VFAs (8500 mg/L (66-d) to <200 mg/L (112-d)) in liquid inoculum reservoir were noticed (Figure 3). As far as the cycle 2 operation was concerned, comparatively less feed material was fed along with 50% (*w*/*w*) of the digested material (considered as dry inoculum) from the 1st cycle, which helped to shorten the cycle length with an enriched methane concentration over 50% with minimal VFA accumulations. However, as far as the OLR was concerned, due to the residual COD or VS accumulations from the digested material from cycle 1 operation, there was a slight increase compared to that of cycle 2. In this scenario, the better performance is mostly linked to the adapted microbial populations within the (liquid–solid) system interactions.

5.3.4. Performance Monitoring of Digesters: Ratio Limits

Monitoring the AD process requires proper selection of operational parameters depending upon its metabolic state. The parameters like pH, total alkalinity (TA), temperature, TVFA, and C/N ratio are important as they have a direct influence on the performance of the AD system. The proper understanding of these fundamental parameters and its implementation can exploit the AD to the fullest and avoid the inhibitions that can occur in certain conditions. One of the major factors, which expresses the stability of AD, is the ratio of TVFA/TA, which is reported to be less than 0.5 for high stability[87] and regarded optimal between 0.4 and 0.6 by [131], beyond which is indicated as overfed. Therefore, the profile of these parameters in the digesters during the operation is shown in Figure 5.4.

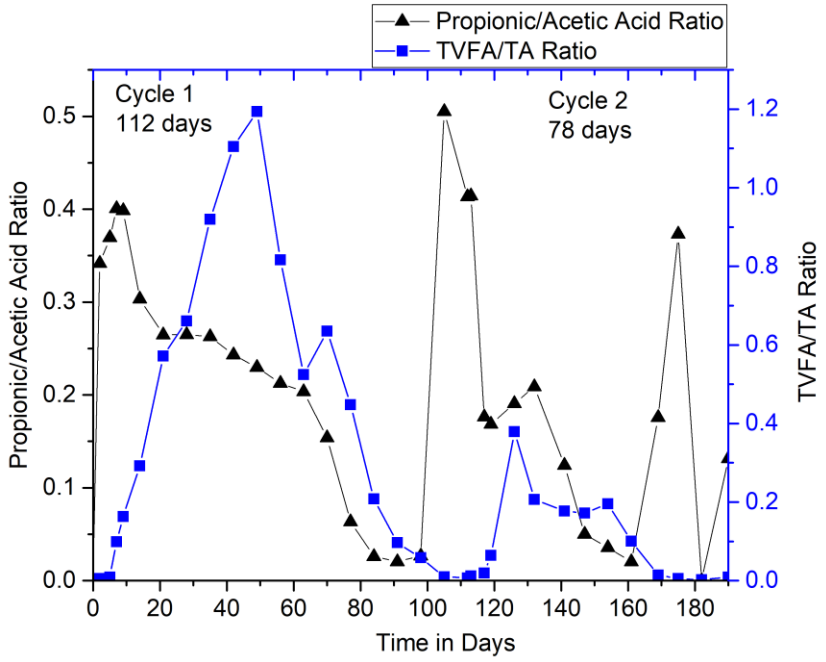


Figure 5.4. Evolution of TVFA/ TA and propionic acid/acetic acid ratios.

Ratio limits like TVFA/TA and propionic acid/acetic acid ratio are the key critical indicators of digester's crash. Studies suggest TVFA/TA to be less than 1 (preferably within a range of 0.1 and 0.6) [18,19] and propionic acid/acetic acid ratio to be less than 1.4[88] for the high stability of the digester. In this study, the TVFA/TA ratio remained below 1 in the entire operation period,

except for days between 40 and 50 of cycle 1 when it reached up to 1.2. This was an indication of inhibitions due to the lack of active microbes in HSAD. These results were in accordance with the methane concentration profiles (HSAD) (Figure 2b), and henceforth, about 10 L of liquid inoculum was recirculated-percolated to HSAD to overcome this situation. However, the propionic acid/acetic acid ratio reached only up to 0.5 throughout the operation (Figure 5.4). These indicators, demonstrating that the digesters were operating favorably without the risk of acid-buildup and better stability, attained with time.

5.3.5. Performance Monitoring of Digesters: Relationship between pH, Ammonia (TAN, FAN), and Temperature

The parameters in the AD process are inter-related; thus, an optimal pH and low temperature coupled with high alkalinity balanced the yield of free ammonia in this system. In Figure 5.5, FAN concentration was shown to be under 200 mg/L in cycle 1 and lower than 180 mg/L in cycle 2. TAN was 3.7 g/L at maximum in the 1st cycle; however, in the 2nd cycle, it was only 3.1 g/L. Generally, exceeding TAN results in the reduction of methane concentration and biogas production. This study, hence, justified the increase in methane-rich biogas with the reduction of TAN.

Around 35–40% of the increase in total ammonia nitrogen (TAN) was observed from the commencement of the operation, and a considerable increment of free ammonia nitrogen (FAN) was also observed.

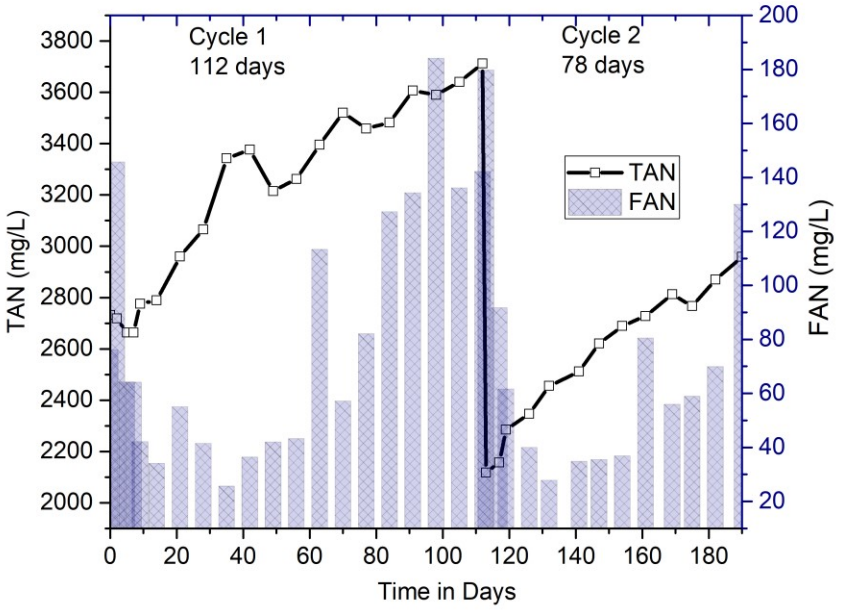


Figure 5.5. Evolution of total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) profiles.

Ammonia has a significant role in supplying nutrients for microbial growth, maintaining buffering capacity (alkalinity) and stability of the digester. Ammonia is dependent on pH, temperature, alkalinity, and substrates. Ammonia exists in two forms: (i) free ammonia (NH_3) and

(ii) ammonium (NH_4). Free ammonia is a gas and toxic, and ammonium is in ionized form, which is non-toxic salt. NH_3 or free ammonia nitrogen (FAN) and NH_4 together make total ammonia nitrogen (TAN). FAN takes part in the inhibitory actions in the AD process since the high concentration of free ammonia in the system ruptures the cell wall of the microbes, leading to cell lysis. Ammonia is mainly dependent upon temperature and pH mentioned by [65] in Equation (5.1).

$$\text{FAN} = \text{TAN}\left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)^{-1} \quad (5.1)$$

where the temperature is in Kelvin (K); total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) are in mg/L.

Microorganisms, which are responsible for the entire AD process, are generally sensitive and survive at certain conditions. Similarly, the temperature is one of the important factors as the growth of the microbes is higher at a higher temperature. However, AD at a temperature >50°C is unstable and generates high FAN, especially while treating ammonia-rich wastes, which is an inhibitor for the process itself. FAN is directly proportional to the temperature; therefore, the generation of FAN is lesser at low-temperature conditions, contributing to the fewer chances of AD inhibition. For proper microbial growth at a lower temperature, substrate acclimation at low-temperature conditions is proven to be advantageous [10]. Therefore, in this study, liquid inoculum adapted to 20 ± 1 °C was utilized for the liquid digester in a two-stage operation, which led to the lower generation of FAN of up to 185 mg/L.

This study also reported the direct proportionality of FAN with different temperatures (for instance, 20 °C, 35 °C, and 55 °C), as shown in Figure 5.6, based on the formula provided in Equation (5.1) by extrapolating the concentration of FAN. This was done to derive a theoretical conclusion based on these calculations. Under an operating pH range of 7.2–8.4, FAN at 20 °C was a maximum of 185 mg/L. Extrapolating the results for FAN at different temperatures based on Equation (5.1) and operating pH range showed that at 35 °C (mesophilic), FAN could have reached up to 500 mg/L, and the same could have ascended to 1300 mg/L at 55 °C (thermophilic). Therefore, a theoretical extrapolation shows the concentration of FAN to be low and balanced at lower temperatures and inhibitory at higher temperatures.

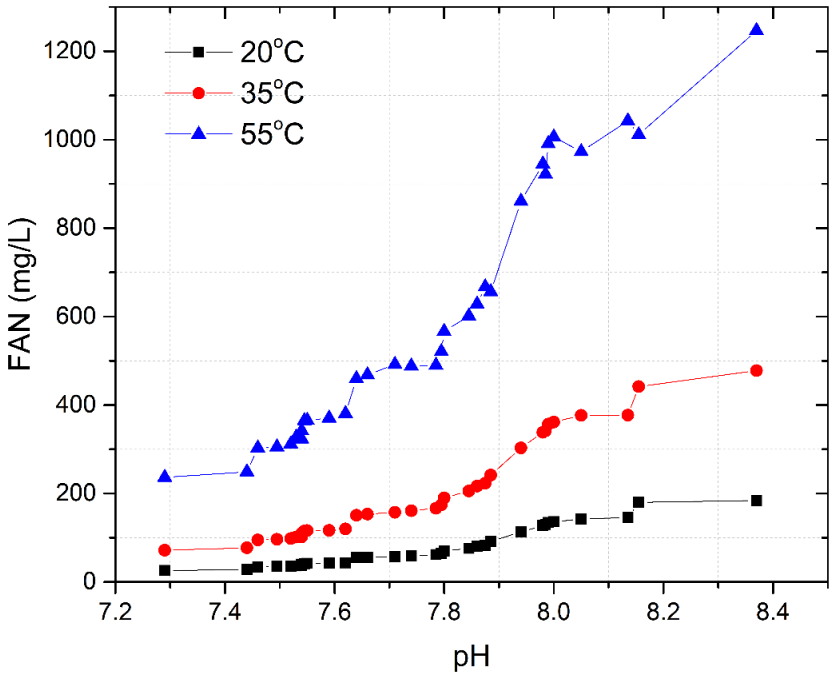


Figure 5.6. FAN at different temperatures under an operating pH range.

5.3.6. Comparative Study of Two-Stage (Liquid–Solid) AD of CM and Co-Digestion of CM + DM

The operating conditions of the two-stage (liquid–solid) AD of CM mono-digestion and CM + DM co-digestion are given in Table 2. The digesters were operated in a similar fashion in order to develop a start-up and operating strategies for these substrates. An attempt was made to compare digesters, treating these two substrates in terms of methane yield and its concentrations, and also the release of FAN during the AD processes in order to determine the inhibitory potential of ammonia in the respective feedstocks (Figure 5.7). The liquid inoculum used to start the digesters for both substrates were adapted to chicken manure leachate. Henceforth, the methane concentrations in cycle 1 of CM mono-digestion showed a quick start-up compared to that of CM + DM co-digestion.

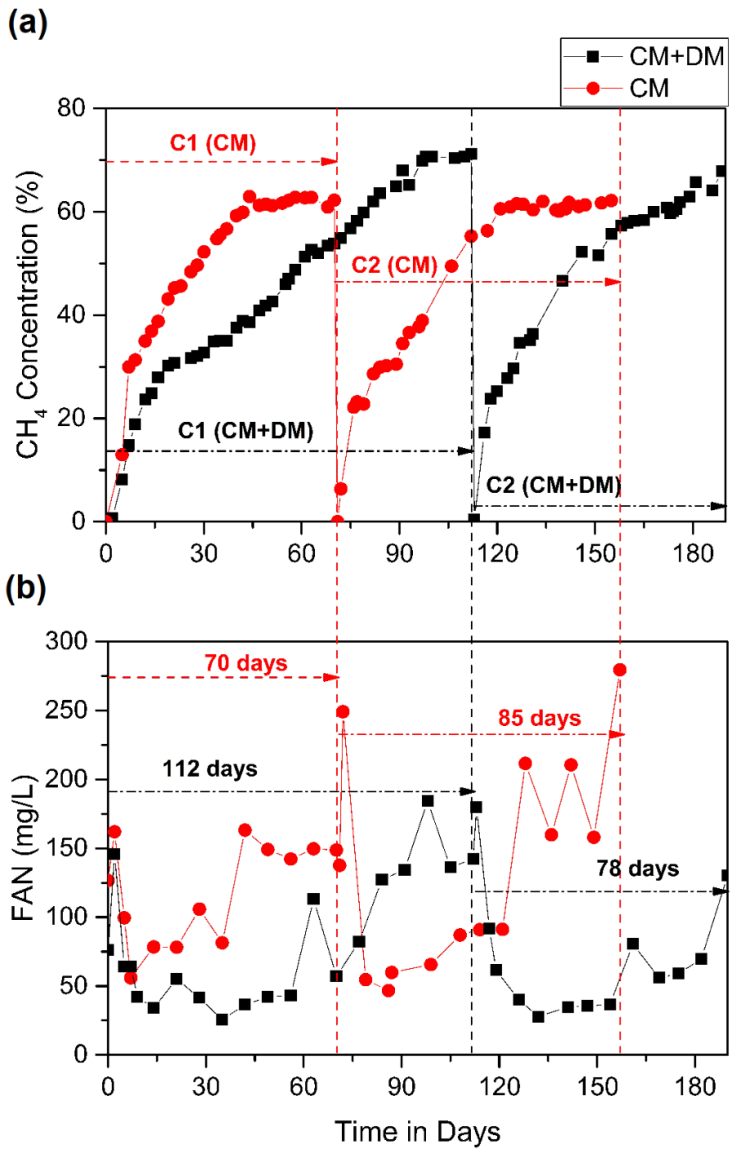


Figure 5.7. Comparative study of chicken manure (CM) mono-digestion and CM + dairy cow manure (DM) co-digestion: (**a**) Methane concentration profile; (**b**) FAN profile.

In cycle 1 of both cases, the concentration of methane was approximately 58%. On the contrary to this, in the cycle 2 of CM + DM, on day 78, the CH_4 concentration was approximately 70%; however, the same for CM was around 60%, making a difference of up to 10% (Figure 5.7a). These results showed that co-digestion using DM had a positive effect in producing a comparatively better methane-rich biogas. However, methane yield or SMY obtained for the CM mono-digestion was 0.52 ± 0.13 L $CH_4 g^{-1} VS_{fed}$, and for CM + DM co-digestion, it was 0.35 ± 0.11 L $CH_4 g^{-1} VS_{fed}$ (detailed data not shown). Similarly, the volumetric biogas production was more in CM (13.6 ± 4 L/d) than CM + DM (7.7 ± 1.8 L/d); however, the quality of methane was observed to be better in the co-digestion process. Since the COD_t:TKN ratio was always higher than 25 for both CM and CM + DM mixture used in this study, better results for CM in terms of methane yield were observed as the CM has a better energy potential than DM.

Furthermore, the release of FAN concentration for both the mono- and co-digestions was monitored to have a better perspective or forecasting of the ammonia inhibition (Figure 5.7b). It is evident from Figure 5.7b that FAN concentrations were comparatively lower in the co-digestion process than mono-digestion due to the dilution of higher ammonia content in CM by DM. Contribution to the generation of ammonia not only lies in the initial concentration of the feedstock but also during the biochemical process in AD. CM is high in nitrogen; hence, the initial concentration had a vital role in a higher concentration of FAN than that in CM + DM. On the 70th day of both cases, FAN was 150 mg/L in CM and 50 mg/L in CM + DM. This can be related to the high nitrogen content in CM mono-digestion than the co-digestion, which helped in the dilution of high ammonia. Although there was an increase in the FAN concentration for the mono-digestion of CM, no apparent inhibitions were reported for both the processes during the start-up phase. The probable reason was that the FAN levels were still in the tolerable range (always below 280 mg/L), and the VFA/alkalinity ratio always remained below 0.5 in both the cases, indicating that the digesters were operating favorably without the risk of acid-buildup. Thus, the presence of ammonia nitrogen did not inhibit the performance of the liquid inoculum reservoir, as well as HSAD, even at high OLRs. Even if the pH was not controlled in the bioreactors, there was no formation of foam or scum observed throughout the study. The mode of operation (process, temperature, percolation-recirculation rate, and frequency) and the appropriate choice of acclimatized inoculum at the start-up phase of the experiment allowed a high stabilization of CM + DM co-digestion, even at higher OLR (4.7 gVS/L.d) studied. Further study is underway to optimize the operating parameters, especially for the co-digestion process.

5.4. Conclusions

The proposed start-up study focused on two-stage (liquid inoculum reservoir coupled with HSAD) anaerobic digestion process using a closed-loop recirculation, and percolation mode operation was found efficient for the treatment of CM + DM at 20 ± 1 °C despite having a waste mix with high TKN (13.5–13.6 gN/L) and solid (TS: 48–51%) concentrations. Results showed that our system could generate a specific methane yield of 0.35 ± 0.11 L CH_4g/VSfed at an OLR of 3.7–4.7 gVS/L.d. We also observed CH_4 concentrations above 60% with CODs and VS reduction by up to 85% and 60%, respectively. A comparative study was done using the same start-up protocol to perform the mono-digestion of CM (TKN: 21–23 g/L; TS: 65–73%). Although a better SMY (0.52 ± 0.13 L $CH_4g^{-1}VS_{fed}$) was obtained for mono-digestion of CM, co-digesting CM + DM showed a better methane quality and also generated comparatively lower FAN. However, no evident inhibitions due to ammonia or VFA accumulations were reported for both the processes during the start-up phase. Compared to the higher-temperature digestion process, more energy is expected to be available for farm uses, especially while treating high solids and ammonia-rich wastes.

Chapter 6: Techno-economic assessment of Two-Stage Anaerobic Digester treating High Solid and High-Ammonia rich Chicken Manures treating at a rate of 1-tonne per day

6.1 Background Information

The poultry industry is one of the most growing agro-food sectors worldwide. Merely, in the year 2018, the total number of broiler chicken production in Canada was 730 million and 192.72 million in the province of Quebec alone, with a total of 597 chicken farms in Quebec [2], [8]. According to Canadian farms, 1 poultry bird excretes approximately 0.2 kg manure/day [2]. Based on this fact, the rate of chicken manure (CM) generation in Quebec is 5280 tonnes per day approximately. CM, being one of the highest bio-methane potential organic substrates, AD technology therefore is a sustainable technology for the treatment of CM in an environmentally friendly way.

This chapter is based on the two-stage high solid anaerobic digester (HSAD) technology treating CM alone as explained in Chapter-3. Chapter-3 depicts the treatment of 10 kg CM for 70 days in a batch-mode in a single digester yielding cumulative biogas of approximately 1000L ($1m^3$) by the end of operation (refer to Table 3.5). Considering the experimental results obtained, an estimate of 1-tonne CM is calculated. The objective of this chapter is to design and analyse the cost for a two-stage HSAD treating CM at a rate of 1-tonne CM per day.

6.2 Methodology

The digester is designed for a small-scale chicken farm generating 1-tonne CM on daily basis. The study conducted at AAFC shows the peak biogas generation at 56^{th}-60^{th} day of operation per cycle (70-d). Hence, for the design of digester, 56 days cycle length or retention time is considered. The size of a single digester for treatment of 56 tonnes using batch mode would be large. Moreover, it will be inefficient as the plant will be non-operational during the loading (feed) and unloading of the CM waste. Nevertheless, the storage of fresh CM will be unmanageable. Hence, to tackle these issues, the CM can be rather stored for 14 days and treated in 4 digesters with equal capacity. This approach would further facilitate to maintain uniform biogas production throughout the AD process and will add the modularity to the system, ultimately leading to the improvement in system reliability.

It is assumed that 1 digester is fed with CM collected in 14 days (2 weeks) which is equivalent to 14 tonnes CM and the digester is operated for 56 days (batch mode) or 8 weeks. Therefore, adopting 4 HSAD (HSAD 1, HSAD 2, HSAD 3, HSAD 4), CM collected after every two weeks is fed. The 4 digesters are then operated for 8 weeks per digester which illustrates that each of the 4 digesters becomes ready for the next feed of CM after 56 days operation period. Hence, 4 digesters operating in parallel with the same capacity assists the farmers to treat the daily manure generated. Along with this, for a two-stage digester, one large liquid inoculum digester is assumed to recirculate all the 4 HSAD equal volume (Figure 6.1). Hence, in this chapter, two digesters: (i) HSAD and (ii) liquid inoculum digester are designed.

One HSAD is designed to treat 14 tonnes CM for 56 days which is equivalent to 250kg CM/day and a Liquid digester is designed correspondingly as per the ratio of liquid inoculum adopted at AAFC laboratory per solid digester.

As per size and number of digesters requirement, an economical assessment of the proposed digester is carried out using net present value (NPV) model.

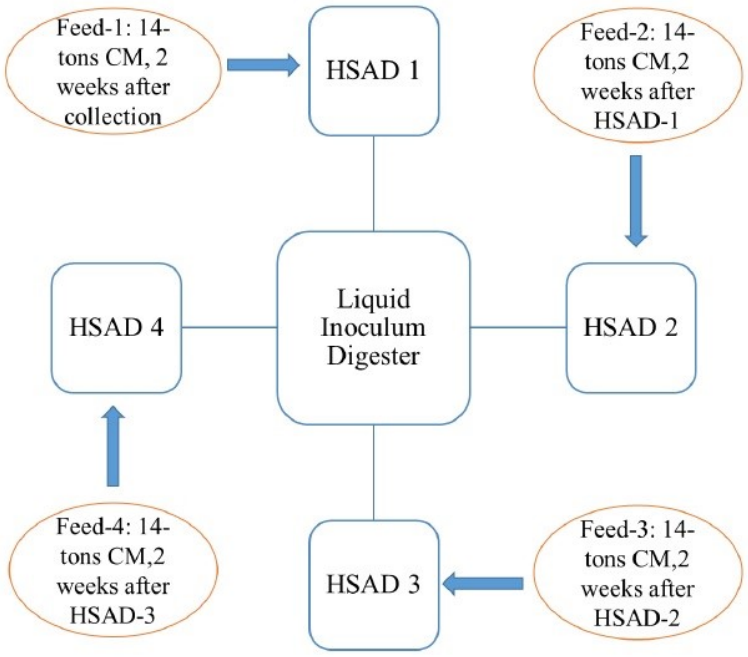


Figure 6.1: Feed pattern of 4 HSAD

6.3 Design of Digesters

As mentioned above, assuming that the farm generates 1-tonne CM/day, one digester would be fed with 250 kg CM/day. Since, solid content of fresh CM is approximately 60%TS. Therefore, TS of CM is 150 kg. A small-scale study for treatment of 10 kg CM conducted at AAFC laboratory required addition of 5L liquid inoculum on the first day and 20 L inoculum was stored in the liquid digester for percolation and recirculation (refer to chapter-3). However, only 25% (5L) liquid inoculum out of 20 L was used for the recirculation-percolation process.

Extrapolation of this study suggests:

1) For 1 HSAD treating 14 tonnes CM, would require 7000 L liquid inoculum on the first day

2) For liquid inoculum storage, 1 HSAD treating 14 tonnes CM would require 28000 L inoculum or a liquid digester of 28 m^3 volume. Assuming that, only 25% (7000L) liquid inoculum out of 28000 L was used for recirculation-percolation process. Hence, only 1 liquid digester of 28 m^3 volume would suffice all the 4 HSAD treating 14 tonnes CM for 56 days.

For the design of HSAD, a cylindrical shaped body is assumed. The geometrical dimensions of the digester is shown in Fig 6.2 and The calculated geometrical dimensions of HSAD is given in Table 6.1. (Refer appendix(a) for calculations)

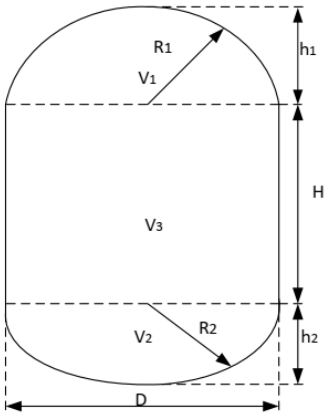


Figure 6.2: Dimensions of a solid digester

Table 6.1 Dimensions of a single solid digester

Dimensions	**Values**
Total Volume (V)	18 m^3
volume of top head (V_1)	3.31 m
volume of bottom head (V_2)	2.04 m
volume of cylindrical part (V_3)	12.57 m
diameter of the cylindrical part (D)	3.42 m
height of cylindrical portion (H)	1.37 m
height of top head (h_1)	0.11 m
height of bottom head (h_2)	0.48 m
characteristic diameter of top head (R_1)	2.48 m
characteristic diameter of bottom head (R_2)	3.60 m

Similarly, the dimensions of one common liquid digester is given in the Table 6.2 (Refer to appendix (a) for calculations)

Table 6.2 Dimensions of a liquid digester

Dimensions	**Values**
Volume of 1 liquid digester (V)	28 m^3
Diameter (D)	3.45 m
Total Height (Ht)	3 m

6.4 Economical Assessment of Proposed Two-Stage Digesters

This section estimates the economical assessment of the proposed digester including cash flows for the initial investment, labour, repairs and maintenance, sale of methane, electricity, organic fertilizer and carbon credits using net present value (NPV) model. NPV is conducted using the depreciation rate of the digester. The objectives of economic analysis are to assess cost-effectiveness of the AD system and maximize the economic benefits. Economic analysis is based on the process design, which includes the cost assessments and investment analysis.

The NPV is the sum of the predicted net cash flows, measured in today's US dollars. Today's dollars indicate the present value (PV) of receipts and expenditures (cash flows). Present value is calculated by multiplying future expenditures and receipts by the appropriate discount rate. The difference between the present value of the receipts and the present value of the expenditures is the NPV. Higher NPV values represent a greater economic benefit. A NPV greater than zero dollars indicates that the digester is profitable than the next best alternative at the same rate of return on investment.

The following formula is used to calculate the NPV [132]. Equation 6.1

$$NPV_N = -C_0 + C_N(1+r)^{-N} + (1-T)\sum_{k=1}^{N}(MR_k + ER_k + FR_k + CC_k)(1+r)^{-k} + T[\sum_{k=1}^{N} D_K\,(1+r)^{-k}] - (1-T)[\sum_{k=1}^{N}(LC_k + RM_k + W_k)(1+r)^{-k}] \quad (6.1)$$

NPV_N = Net Present Value of solid waste digester investment

C_0 = The original investment costs

C_N = The retrieved value of the digester and equipment at the end of Nth year. This is discounted to present value by $(1+r)^{-N}$

r = After tax discount rate

N = Number of years

T = Marginal income tax rate

MR_k = Methane revenue in the kth year

ER_k = Electricity revenue in the kth year

FR_k = Fertilizer revenue in the kth year

CC_k = Carbon credit revenue in the kth year

D_K = Depreciation in kth year. This term is discounted and then multiplied by the

tax rate to arrive at the effective tax deduction for depreciation.

LC_k = Labor cost in the k^{th} year.

RM_k = Repair and maintenance cost in the k^{th} year.

W_k = Water cost in the k^{th} year.

MR_k, ER_k, FR_k, and CC_k, are discounted and multiplied by (1-T) to arrive at the after-tax revenue. LC_k, RM_k, and W_k also are discounted and multiplied by (1 – T) to arrive at the after-tax costs. These costs are deductible expenses; therefore, the effective rate is found by multiplying the costs by (1 – T).

Similarly, the types of cash flows involved in the evaluation are :

(a) Capital investment costs (outflow)

Investment costs adopted in the analysis are the cost of bio-digester, purchase of pumps, tubes and valves, cost of land and electric generator. The entire capital cost will be borrowed from agencies promoting small businesses and encouraging renewable energy production for the reduction of GHG emissions. Investment costs are based on a project life of 20 years for them to depreciate to zero salvage value.

i. **Cost of bio-digester:** It is based on the volume and the number of bio-digesters required. The cost also includes the construction and engineering of the proper set-up of the digesters. In this study 1 liquid and 4 solid digesters is required.
ii. **Cost of Land**: It is based on the cost of land required for the installation of AD plant. As per the 2017, depreciation rates for non-residential buildings is 6%. Residual production through anaerobic digestion of waste is normally 90% including solid and liquid content. For two-stage digesters treating high solid waste, it is estimated to produce 70% residual waste after each operation considering 30% waste used as solid inoculum for the next batch operation and lost during transfer or accumulated in the pipes. This estimate is based on the vertical cylindrical batch anaerobic digester used at AAFC laboratory.
iii. **Electric generator:** In order to produce electricity, an 80KW electric generator is assumed.
iv. **Miscellaneous costs**: It includes the cost of pumps, tubes and valves. Pumps are required for feeding and extracting the digesters. They are also essential for mixing and recirculation of inoculum. Tubes are connected for gas collection, mixing and connections between liquid and solid digesters. Similarly, the cost of valves depends on the types and capacity requirement. Butterfly valves are considered to be useful for heavy load feed.

Table 6.3 Capital Investment Costs

Item	Description	USD	Project Life in Years
Biodigester	100 cubic meter	178,800[(a)]	20
Land	½ acre	14,500[(a)]	20
Electric Generator	80 Kw	180,000[(a)]	20
Backhoes loader with bucket and fork	80 hp	75,000[(a)]	20
Miscellaneous (Pumps, tubes, valves)		5000[(b)]	20
Total		453,300	

Note: (a): Data taken are based on the USD costs [89]; (b) Assumed

(b) Operating Costs (outflow)

Operating costs consist of labour costs, repair and maintenance cost, and water costs (Table 6.4).

i. **Labour Costs:** One part-time employee will be required to operate and monitor the digester on daily basis and will earn 20-22$/hour and will work for 10 hrs/week. A part-time employee will be involved in the sales and marketing of the energy and fertilizer 15-17$/hour and will work on interval basis for 2-3 hrs/week. One administrator will be paid 20-22$/hour who will work for 4-5hrs/week. The hourly rate is based on an informal employment.

ii. **Repair and maintenance costs:** Annual operation and maintenance cost of 1% on the initial total capital investment cost excluding land or non-residential will be considered for this project. The 1% allocation to cover recurring operation and maintenance of the installation and equipment is the average allocation for most investments of this kind.

iii. **Water costs:** In this study, water is only used once to develop inoculum and is stored in the liquid digester for percolation-recirculation purpose. The solid digester is not diluted, hence the requirement of water for dilution is eliminated which ultimately reduces the water cost. However, a nominal annual water cost is assumed.

Table 6.4. Labour, repair and maintenance and water costs

Item	Description	Annual Cost (USD)
Labour	One Administrator	4,500
	One full-time employee (operate and monitor)	10,000
	One part-time (sales and marketing)	2,000
	Total labour cost	16,500
Repair and Maintenance	1% of capital cost	4,533
Water		600

(c) Revenue or Income (inflow): The money earned or received from the sales of methane as natural gas, electricity, fertilizer and carbon credits are accounted as the cash inflow. The potential revenue sources are (1) methane sales, (2) electricity sales, (3) fertilizer sales, and (4) carbon credits.

Based on the quantity of biogas obtained from laboratory-scale experiments, 0.066 m^3 biogas is generated from 1 kg CM, hence the extrapolated biogas quantity for 250 kg is approximately 16.5 m^3 Biogas. Therefore, the annual biogas, methane, fertilizer and electricity generated from treating 250 kg CM per day is calculated below. In addition, carbon credits are also measured for the same.

i. Annual Biogas Production

For 250 kg/day = 0.066m^3/kg waste x 250 kg waste/day × 365 days/year
= 6022.5 m^3

ii. Annual Methane

Biogas is approximately 70% methane (CH_4) and 30% carbon dioxide (CO_2). Therefore, this yields an approximate of 0.0462 m^3 CH_4/kg CM and 0.0198 m^3 CO_2/kg CM based on 0.066 m^3 biogas generated from per kg CM.

Hence, CH_4 and CO_2 production per day for 250kg CM are:

CH_4 volume = 70% = 250 *0.0462 = 11.55 m^3

And CO_2 Volume= 30% = 250*0.0198 = 4.95 m^3

Therefore, annual CH_4 generation is 11.55 m^3× 365 days = 4215.75m^3
Assuming 50% biogas generated is utilized for methane gas as natural gas.
Therefore, annual CH_4 generation from 1 HSAD = 2107.86 m^3

iii. Annual Fertilizer

Assuming that 80% of the treated waste is used as fertilizer [89], therefore the annual fertilizer becomes = 0.8 kg/kg waste × 250 kg waste/day × 365 days/year = 73000 kg

iv. Annual Electricity

An electric generator produces about 1.7kwh of electricity from each cubic meter of biogas [14].
Hence, the annual electricity generated from 250 kg CM per day is = 1.7kWh x 6022.5 m^3 biogas = 10,238.25 kWh
Assuming 50% of biogas generated is utilized for electricity, therefore the annual electricity generated from 250 kg CM per day is 5,119.12 kWh

v. Greenhouse gas reduction

Anaerobic digestion produces biogas which contains CH_4 and CO_2. 250 kg CM per day, when digested produces 11.55 m^3 of CH_4 and 4.95 m^3 of CO_2 per day.

Since, 1 volume of methane when combusted yields 1 volume of carbon dioxide

$CH_4 + 2O_2 = CO_2 + 2H_2O$

Thus, the combustion of 11.55 m^3 CH_4, generates 11.55 m^3 CO_2. On the other hand, natural CO_2 generated is 4.95 m^3. Hence, total CO_2 generation is 16.5 m^3/day.

If the organic waste is left to decompose in a pile or in a landfill it will produce 30% carbon dioxide and 70% methane gas. Assuming the annual waste, i.e. 91,250 kg CM is not digested, annually biogas production will be as follows:

i. CO_2 production = 4.95 $m^3 CO_2$/day x 365 = 1806.75 m^3
ii. CH_4 production = 11.55$m^3 CH_4$/day x 365 = 4215.75 m^3

Both gases are responsible for global warming, among which CH_4 contributes 23 times than CO_2 in terms of global warming potential (GWP) [133].

Therefore, a volume of 4215.75 m^3 CH_4 would result an emission of 96962,25 m^3 CO_2 equivalent. Further, an addition of actual produced 1806.75 m^3 of CO_2 would effectively contribute to the emission of 98769 m^3 CO_2 equivalent annually.

The anaerobic digester reduces CO_2 emissions which is the difference between 98769 m^3 CO_2 and 16.5 m^3 x 365 of CO_2 which is 92746.5 m^3 CO_2. This is also known as carbon credits. Hence, AD reduces CO_2 emission of 93.9 % in total.

The outputs and market prices used to estimate revenue are listed in Tables 6.5, 6.6 and 6.7

Table 6.5. Output of the Project

Output	**Annual Capacity from 1 HSAD**
Methane gas	2107.86 m^3
Fertilizer	73,000 kg = 73 tonnes
Electricity	5119.12 kwh
GHG abatement (CO_2 equivalent)	92746.5 m^3 of CO_2 equivalent 183.64 tonnes

Table 6.6. Annual Revenue

Output	**Annual Revenue from 4 HSAD (USD)**
Methane gas	4× 2107.86 m^3 x 0.0519 \$/m^3 = 437.59
Electricity	4× 5119.12 kwh x \$0.04/kwh = 819.06
Fertilizer	4 × 73000 kg × \$0.20175/kg = 58911
Carbon Credits	4× 183.64 tonnes x \$5.6/ t/CO_2-e = 4113.5
Total Revenue	= 64281

Note: 4 HSAD = 4× 250 Kg CM/day = 1 tonne CM/day

Table 6.7. Depreciation Expenses deductible (US $)

Years	Digester	Generator	Backhoes	Pumps	Total
2020	178800	180000	75000	5000	453300
2021	160920	162000	67500	4500	407970
2022	144828	145800	60750	4050	367173
2023	130345	131220	54675	3645	330455
2024	117310	118098	49208	3280	297410
2025	105579	106288	44287	2952	267669
2026	95021	95659	39858	2657	240902
2027	86081	86659	35872	2391	216811
2028	77141	77659	32122	2141	195130
2029	68201	68659	28372	1891	172465
2030	59261	59659	24622	1641	149800
2031	50321	50659	20872	1391	127135
2032	41381	41659	17122	1141	104470
2033	32441	32659	13372	891	81805
2034	23501	23659	9622	641	59140
2035	14561	14659	5872	391	36475
2036	5621	5659	2122	141	13810
2037	0	0	0	0	0
2038	0	0	0	0	0
2039	0	0	0	0	0
2040	0	0	0	0	0

Hence, from equation, 6.1, NPV is calculated (Appendix (b)) excluding governmental taxes. It is clear that NPV gets positive after 14 years (Figure 6.3).

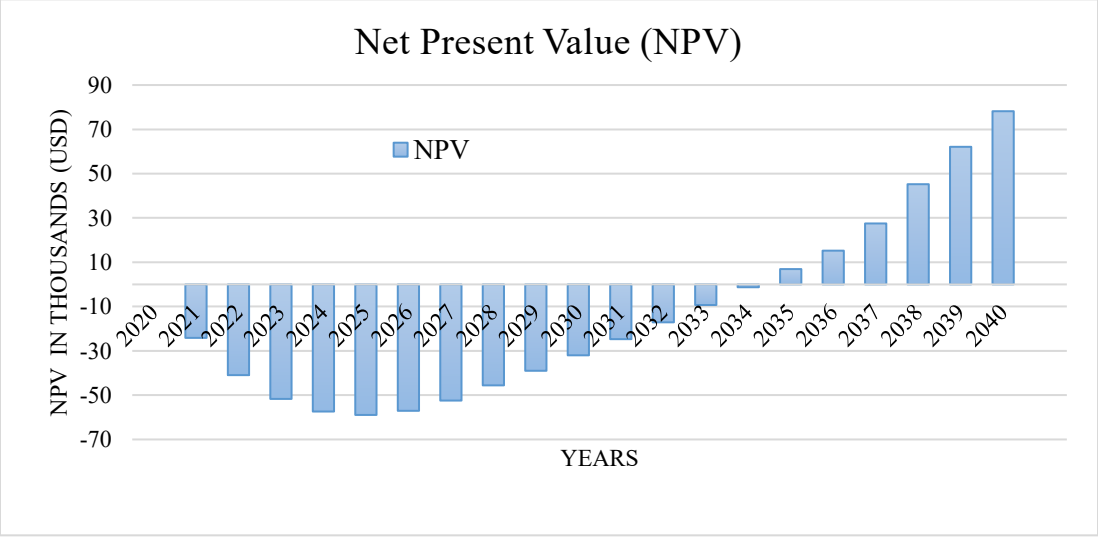


Figure 6.3. NPV profile in 20 years

6.4. Results, Conclusions and Recommendations

The designed digester and estimated cash flows shows the AD plant obtaining revenue after 14 years from the commencement of the project. The NPV analysis resulted positive returns; NPV>0, and payback period after 14 years and few months. For a sustainable AD plants, animal manure as a feedstock is highly recommended and the heat energy obtained from the plants is recommended to recover the heat usage to reduce GHG emissions. Digestate needs to be managed well and used as fertilizer. In addition to methane being used as natural gas, CO_2 can be used as a chemical source to extend the economy of AD plants. In addition, the funding programs available for Quebec province of Canada are Canadian Agricultural Partnership, Government of Canada Agricultural Clean Technology Program, Partnership Streams of the Low Carbon Economy Challenge, Quebec's Biomethanation and Composting Organic Matter Treatment Program (PTMOBC). These agencies promote the sectors that improve air, water, and land and reduce greenhouse gas emissions. These organizations support sector growth by supporting diversity and a dynamic sector, focusing on agriculture and/or bio-products.

Chapter 7: Conclusions and Future Perspectives

In this study, a low-temperature (20±1 °C) two-stage (liquid-solid) HSAD biotechnology using recirculation-percolation of adapted liquid inoculum was used to treat animal manure, specifically CM and DM along with their bedding. The main findings showcased the sustainability characteristics of this novel biotechnology technique, yielding impressive results at 20±1 °C. Throughout the research, the operation, monitoring and performance evaluation of the aforementioned HSAD was carried out using all the AD dependent physio-chemical parameters.

Firstly, the mono-digestion of CM was operated for 282 days. The operation was found efficient irrespective of high TKN (23–33 gN/L) and solid (TS: 68–72%) concentrations. Results showed that the designed system could generate a specific methane yield of 0.68±0.04 L CH4g/VS fed at an OLR of 4.4 gVS/L.d. CH_4 concentrations above 50% was also observed with TCOD and SCOD reduction up to 80% and 93%, respectively and VS removal efficiency of 75%.

Moreover, the performance HSAD treating CM was encouraging since the monitoring parameters (TVFA/TA and C3/C2) ratio limits were within the suggested range. In addition, the ratio of (C4+C5)/C2 also remained under a range of 0.3 throughout the operation. Furthermore, no significant inhibitory effects were observed despite of higher OLRs (8.78 gVS/L/d) and high ammonia concentrations. Altogether, the biotechnology was proven to be robust and efficient to process CM. In addition, no major signs of digester failure were noticed.

Secondly, a start-up study for the co-digestion of CM+DM was carried out. The operation was found efficient despite having a waste mix with high TKN (13.5–13.6 gN/L) and solid (TS: 48–51%) concentrations. Results showed that our system could generate a specific methane yield of 0.35 ± 0.11 L CH_4g/VS_{fed} at an OLR of 3.7–4.7 gVS/L.d. We also observed CH4 concentrations above 60% with CODs and VS reduction by up to 85% and 60%, respectively. A comparative study was done using the same start-up protocol to perform the mono-digestion of CM (TKN: 21–23 g/L; TS: 65–73%). Although a better SMY (0.52 ± 0.13 L $CH4g{-}1VS_{fed}$) was obtained for mono-digestion of CM, co-digesting CM + DM showed a better methane quality and also generated comparatively lower FAN. However, no evident inhibitions due to ammonia or VFA accumulations were reported for both the processes during the start-up phase. Compared to the higher-temperature digestion process, more energy is expected to be available for farm uses, especially while treating high solids and ammonia-rich wastes.

Finally, in order to assess the cost-effectiveness of the proposed AD biotechnology and maximize the economic benefits, a techno-economic analysis of the aforementioned technology treating CM alone was conducted. Adopting NPV model, the estimated cash flows showed the AD plant could obtain the revenue after 14 years from the commencement of the project. The NPV analysis resulted positive returns; NPV>0, and payback period after 14 years and few months.

Therefore, the proposed study focused on two-stage (liquid inoculum reservoir coupled with HSAD) anaerobic digestion process using a closed-loop recirculation, and percolation mode operation was found efficient for the treatment of CM alone and CM + DM at 20 ± 1 °C. Hence, the positive results obtained overall motivates to optimize the next operational cycles by varying operating conditions as it can be a sustainable and clean farming option for small-medium sized livestock farm, especially in the cold-climate weather conditions.

Based on this research, following recommendations can be made for future studies

- A comprehensive study of the process can be investigated to build an Anaerobic Digestion Model (ADM) to optimize and operate AD system efficiently. ADM model will also help to describe the digester behavior, mainly in transient operating conditions.
- Efforts towards the reduction of Cycle length or HRT should be taken place, provided that adapted inoculum quickens the start-up phase for solid wastes.
- Different mixing ratio of CM and DM can be experimented to optimize the C/N ratio of co-digested substrates for a “best recipe” towards higher biogas production.
- AD of CM and mix of CM+DM can be conducted at higher OLR with step-wise increase to push the digester’s limits.
- Study on microbial activity, mainly methanogenic activity during the AD process should be conducted to understand the microbial behaviour towards the substrates.
- A larger scale or full-scale study should be performed to get the real scenario for the farms.
- Analysis of energy and nutrients recovery from the digestate should be studied using membranes or struvite precipitation to make the system more sustainable.

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Appendix

Appendix (A): Design of Digesters

(i) Design of solid digester

Considering the capacity of single digester, the dimension details given in the **Figure 6.1 and Figure below** are evaluated as follows:

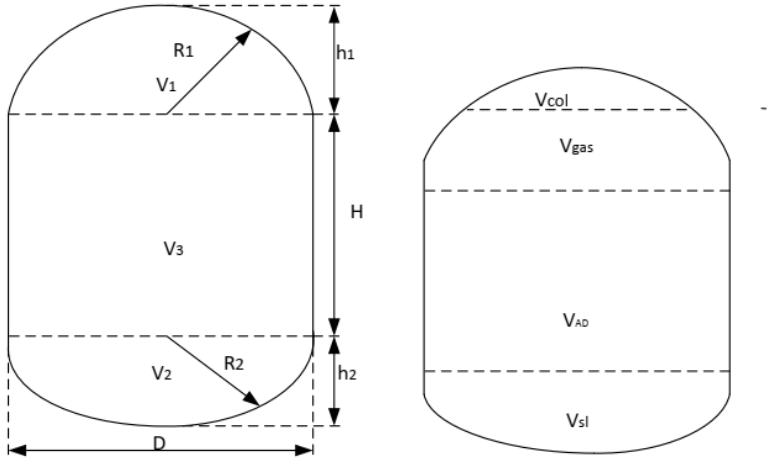

Figure : Geometry and dimension of a digester

Under the assumption of 1000 kg = $1m^3$,

The active volume of the solid digester is Vgs + Vad = 14 m^3.

Since, Vgs + Vad = Q. SRT

Since, Vgs + Vad = 0.8 V, which gives the total digester volume (V) is equal to $18m^3$.

The equation to get the diameter of the digester (D) is below:

$D = 1.3078 * V_{1/3} = 3.42$ m

Further use of D determines other dimensional details as follow,

$V1 = 0.0827\ D^3$

$V2 = 0.05011\ D^3$

$V3 = 0.3142 * D^3$

h1 = D/5

h1 = D/5

R1=0.725*D

R2=1.0625*D

$V3= \pi (D^2 H)/4$

The obtained dimensional details are V1 = 3.308 m^3, V2= 2.004 m^3, V3 = 12.568 m^3, and h1 = 0.684 m,

h2=0.4275 m, R1=2.4795 m, R2=3.63375 m and H = 1.368 m.

The operating digester is divided` into four sectional volumes as shown in Figure 6.1. The sectional volumes are based on the occupancy of different components in the digester.

The volume of sludge is ≤ 15% of total volume(V), i.e. Vsl is 2.7 m^3.

While, volume of the gas collection section (Vgc) is≤ 5% of V, i.e. Vgc is 0.9 m^3. Another major section is the gas storage section that is just above actual digestion section.

The volume of this gas storage section (Vgs) is given by

Vgs=0.5*(V- Vgc)*K

where, K=0.4. Therefore, Vgs is found to be 3.42 m^3

While, the active digestion volume (Vad) =10.98 m^3, which is calculated using the relation

Vad =V-Vgc-Vgs-Vsl.

With help of equation $\pi(D^2 H1)/4 = (Vgs+Vgc)-V1$, the height of the cylindrical portion left empty for gas storage i.e. H1 is determined to be 0.11 m. All of these digester's details are given in the Table 6.1.

(ii) Design of liquid digester

From experimental basis,

10 kg CM processed for 70 days required 5L liquid inoculum on 1st day + 20 L on storage for percolation-recirculation

Hence, for 14-ton CM to be processed for 56 days would require 7000 L liquid inoculum on 1st day and 28000L on storage

For 4 identical digesters, total liquid inoculum required on 1st day will be (7000x4) 28,000L.

However, a common storage would be sufficient for supplying all 4-solid digesters, hence only one 28000 L storage digester needs to be designed, which is 28 m^3.

Appendix(B) : NPV calculation

Assume,

C= capital Investment costs

M= Maintenance +labour+ water costs

R = Total Revenue

r = depreciation rate

D = Depreciation deductible expenses

Therefore, MATLAB code for NPV calculation is given below:

```
%%%%%%%%%%%%%%%%%%%%
clc
C=453300
M=21633
R=64281
r=0.05
D=[407970
367173
330455.7
297410.13
267669.117
240902.2053
216811.9848
195130.7863
172465.7863
149800.7863
127135.7863
104470.7863
81805.78629
59140.78629
36475.78629
13810.78629
0
0
0
0 ]
mc=0
re=0
for (n=1:1:20)
    mc=M/(1+r)^n+mc
    re=R/(1+r)^n+re
```

```
    npv(n)=-C-mc+re+D(n)/(1+r)^n%
end
n=1:1:20
plot(n,npv/1000)
xlabel('years')
ylabel('Net Present Value in thousand')
```

Appendix(C): Pictures

Figure (a): (From left) Chicken manure, cow manure, digestate and liquid inoculum.

Figure (b): Feeding of CM+DM

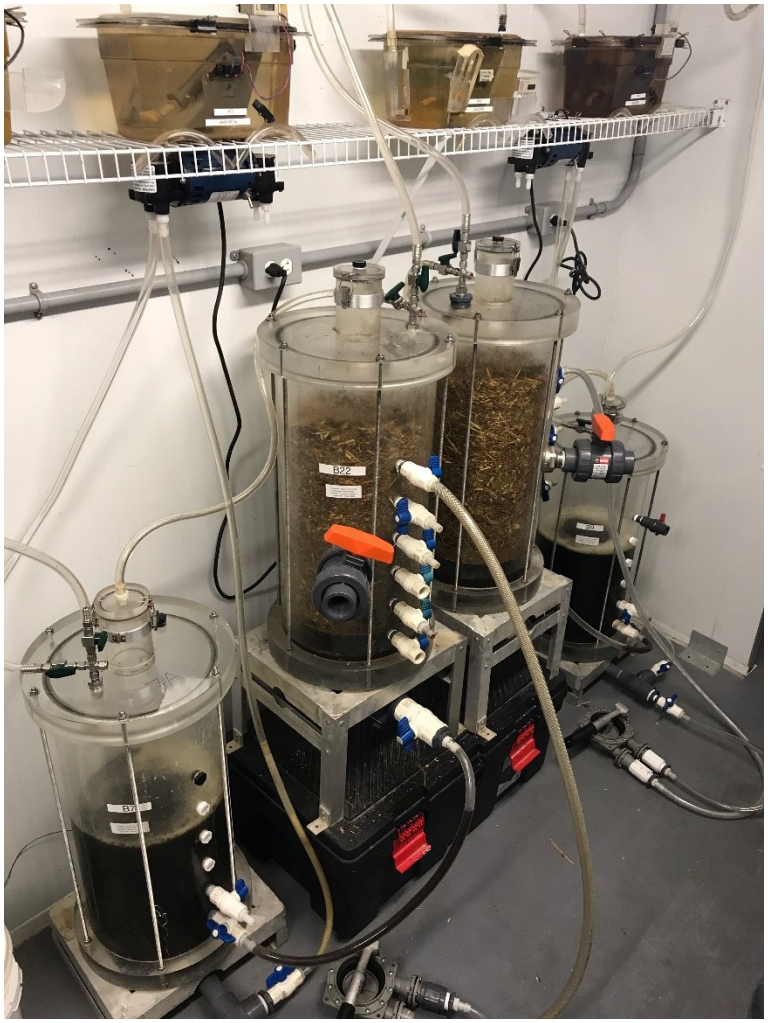

Figure (c): Set-up of CM+DM co-digestion