Biogas Production from Small Farm Cow Manure by High Solid Three-Stage Anaerobic Digester

Burachat Sripitak

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Energy Technology

Prince of Songkla University

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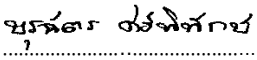

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์ การผลิตแก๊สชีวภาพจากมูลวัวของฟาร์มขนาดเล็กด้วยการย่อยสลายไร้อากาศสามขั้นตอนในสภาวะปริมาณของแข็งสูง

ผู้เขียน นายบุรฉัตร ศรีพิทักษ์

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ปีการศึกษา 2565

บทคัดย่อ

การผลิตก๊าซชีวภาพด้วยการย่อยสลายไร้อากาศในสภาวะปริมาณของแข็งสูงเป็นทางเลือกสำหรับการย่อยสลายแบบใช้น้ำในกระบวนการน้อยโดยจะมีสัดส่วนของของแข็ง 10-20 เปอร์เซ็นต์ งานวิจัยมีวัตถุประสงค์ศึกษาระบบการผลิตก๊าซชีวภาพด้วยการย่อยสลายไร้อากาศสามขั้นตอนในสภาวะปริมาณของแข็งสูงที่อุณหภูมิตามสภาพแวดล้อมปกติ จากการศึกษาศักยภาพในการผลิตก๊าซชีวภาพแบบแบชระหว่างซับสเตรทมูลวัวกับหัวเชื้อจากมูลวัวผสมกันในอัตราส่วน 2:1 4:1 และ 6:1 (VS Basis) โดยแปรที่ 15, 20 และ 30 เปอร์เซนต์ของแข็งทั้งหมดพบว่าสูตรของซับสเตรทมูลวัวกับหัวเชื้อจากมูลวัวอัตราส่วน 2:1 (VS Basis) ที่ 15 เปอร์เซนต์ของแข็งทั้งหมดสามารถผลิตแก๊สมีเทนได้สูงสุดคือ 343.97 mL-CH4·gVS-1 สัมประสิทธิ์จลนพลศาสตร์ไฮโดรไลซิสลดลงเมื่อสัดส่วนของของแข็งและซับสเตรทเพิ่มขึ้นส่งผลให้การผลิตก๊าซมีเทนลดลง เมื่อนำข้อมูลก๊าซที่ได้ไปแทนค่าในสมการ Modified Gompertz Model พบว่าประสิทธิภาพการผลิตก๊าซมีเทนลดลงและระยะเวลาปรับในการผลิตมีเทนเพิ่มขึ้นเมื่อสัดส่วนของของแข็งและซับสเตรทเพิ่มขึ้น เมื่อนำอัตราผสมระหว่างซับสเตรทมูลวัวกับหัวเชื้อจากมูลวัวดังกล่าวไปใช้ทดสอบผลิตแก๊สชีวภาพด้วยระบบการผลิตแก๊สชีวภาพด้วยการย่อยสลายไร้อากาศสามขั้นตอนในสภาวะปริมาณของแข็งสูง ระบบดังกล่าวสามารถผลิตแก๊สมีเทนได้ 316.09 mL-CH_4·gVS^{-1} ปริมาณก๊าซมีเทนที่ผลิตด้วยระบบนี้จะเพิ่มขึ้นที่ความเข้มข้นของซับสเตรทเริ่มต้นที่ 5 g-TS·L^{-1}-R1 ถึง 20 g-TS·L^{-1}-R1 แต่จะลดลงเมื่อความเข้มข้นของซับสเตรทเริ่มต้นที่ 25 g-TS·L^{-1}-R1 และ 30 g-TS·L^{-1}-R1 จากข้อมูลการวิเคราะห์กลุ่มเชื้อด้วยวิธี PCR-DGGE และทดสอบศักยภาพในการย่อยของจุลินทรีย์แสดงให้เห็นว่าระบบการผลิตแก๊สชีวภาพด้วยการย่อยสลายไร้อากาศสามขั้นตอนมีส่วนช่วยในการเพิ่มประสิทธิภาพในการทำงานของกลุ่มจุลินทรีย์แต่ละขั้นตอนและยังลดตัวยับยั้งกระบวนการผลิตแก๊สมีเทนในช่วงเมทาโนเจเนซิสอีกด้วย นอกจากนี้ยังพบว่าการสะสมของกรดไขมันที่ระเหยง่าย ปริมาณและความถี่ในการป้อนวัตถุดิบสำหรับกระบวนการผลิตแก๊สชีวภาพมีอิทธิพลอย่างมากในการเสริมสร้างจุลินทรีย์ที่ไม่ใช้ออกซิเจนที่เกี่ยวข้องกับระบบก๊าซชีวภาพ เมื่อนำข้อมูลต้นทุนและรายได้จากกระบวยการผลิตแก๊สชีวภาพไปศึกษาอัตราผลตอบแทนภายใน มูลค่าปัจจุบันสุทธิและระยะเวลาคืนทุนพบว่าการผลิตแก๊สชีวภาพ

ด้วยระบบการผลิตแก๊สชีวภาพด้วยการย่อยสลายไร้อากาศสามขั้นตอนในสภาวะปริมาณของแข็งสูงมีความน่าสนใจในการลงทุน ด้วยข้อมูลที่ได้จากงานวิจัยข้างต้นนี้สามารถสรุปได้ว่างานวิจัยนี้เป็นประโยชน์ต่อภาคอุตสาหกรรมการเกษตร โดยสามารถนำข้อมูลสภาวะการดำเนินงานผลิตแก๊สชีวภาพไปใช้ประกอบการออกแบบติดตั้งระบบถังปฏิกรณ์แบบแบทช์และ/หรือแบบป้อนกึ่งต่อเนื่องแบบสามขั้นตอนในระดับอุตสาหกรรมขนาดเล็กได้ รวมทั้งยังช่วยในการปรับขยายกำลังการผลิตก๊าซชีวภาพด้วยการย่อยสลายไร้อากาศสามขั้นตอนในสภาวะปริมาณของแข็งสูงอีกด้วย

คำสำคัญ: การผลิตก๊าซชีวภาพด้วยการย่อยสลายไร้อากาศในสภาวะปริมาณของแข็งสูง, ก๊าซชีวภาพ, มูลวัว

Thesis Title Biogas Production from Small Farm Cow Manure by High Solid Three-Stage Anaerobic Digester

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Major Program Energy Technology

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ABSTRACT

High solid anaerobic digestion (HS-AD), a so-called semi-dry or dry anaerobic system is an alternatively potential option for substrate degradation under less water content. The solid-state anaerobic process is categorized into the semi-solid or high solid-state for having total solids between 10% and 20% in a system and solid-state for a system having total solids higher than 20%. This thesis aimed to study the three-stage high-solid anaerobic digestion (TSHS-AD) at ambient temperature with cow manure. Biomethane Potential was conducted at the various substrate to inoculum (S: I) ratios of 2:1, 4:1, and 6:1 on a volatile solid (VS) basis, each of which was conducted at different total solid concentrations (15%, 20%, and 30%). The highest cumulative methane of 343.97 mL-CH4·gVS-1 was achieved at 2:1-S: I ratio and 15 %TS. The first-order hydrolysis constant (kh) decreased, when the initial %TS and S: I ratio was increased. Consequently, methane production was correspondingly reduced. According to the modified Gompertz parameters, the maximum biogas production rate (Rmax), the time period for 90% of total biogas yield (T90), and the effective biogas production period (Tef) were increased, when the initial %TS and S: I ratio was decreased. Additionally, it was found that the lag phase (λ) became shorter by using less initial % TS and S: I ratio. Meanwhile, TSHS-AD of three tanks in a row operated at S: I 2:1 and 15 %TS (20g-TS·L-1 reactor1(R1)) provide a maximum methane yield of 316.09 mL-CH4·gVS-1. The methane yield was increased by stepwise increasing initial manure concentrations from 5 g-TS·L-1-R1 up to 20 g-TS·L-1-R1. However, continuously increasing initial manure concentration to 25 g-TS·L-1-R1 and 30 g-TS·L-1-R1 could cause a decrease in methane production rate. Methane production from compact TSHS-AD of cow manure produces 309 mL-CH4.gVS-1, which is rather similar to methane yield obtained from 1-L TSHS-AD. The stage separation of TSHS-AD reduces inhibitors in the methanogenesis step. Moreover, it could create a full microbial function in each stage. Accumulation of VFA and semi-continuous feeding have considerable influence in enhancing anaerobic microbes involved in the biogas system. The annual income from biogas has the greatest impact on the investment attractiveness of TSHS-AD, according to the computation of the internal rate of return (IRR), Net present value (NPV), and the payback time (PB). The results obtained in this research could be potentially helpful to scale up the TSHS-AD system

Keywords : high solid anaerobic digestion, biogas, cow manure

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List of Publication and Proceeding

- The Three-stage High Solid Anaerobic Digestion (TSHS-AD) under Ambient Temperature for Enhanced Biogas Production from Cow Manure, Chiang Mai Journal of Science, Vol.49 No.5 (September 2022), https://doi.org/10.12982/CMJS.2022.078
- Strategies of biogas production supplement in three stages high solid anaerobic digestion (TSHS-AD) with digestate reusing, the Proceeding of ASEAN Bioenergy and Bioeconomy Conference October 15th, 2021 THAILAND, No.1 Vol.4 October 2021 - September 2022, ISSN: 2586-9280

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Introduction

Recently, with Thailand's population that has already approached 70 million peaks, meat at dairy foods, as good sources for the high quality of proteins are increasingly required, resulting in more livestock farms. The number of cattle raised in the 14-provinces of southern region had increased from 685,669 in 1999 to 914,668 in 2019. Generally, an adult native cow produces manure approx. 10 kg/day hence, generating a large amount of manure for further processing. Without proper management, cow manure could negatively impact the environment. Local farmers utilized cow manure for soil enrichment and bio-fertilizers. An alternative option, cow manure with a high amount of organic matter could be suitably used for producing low-cost renewable energy carriers in biogas form by anaerobic digestion. In addition, digestate discharged from anaerobic digestion of cow manure is defined as a good bio-fertilizer, having low pathogen bacteria and weed seed (Eckford et al., 2012).

Biogas production is normally carried out by using low solid anaerobic digestion (LSAD) known as liquid state anaerobic digestion (He., 2010), where total solids (TS) in the system is less than 10% (Bolzonella et al., 2003). The LS-AD system, regularly used in conjunction with high-strength wastewater treatment has major limitations of poor substrate loading rate, high heating required due to larger volume, and not being suitable for hydrophobic lignocellulose base substrate (Van et al., 2020). Nonetheless, digestate generated from LSAD is highly required for dewatering and has less stable quality (Fagbohungbe et al., 2015).

High solid anaerobic digestion (HS-AD), a so-called semi-dry or dry anaerobic system is an alternatively potential option for substrate degradation under less water content. According to (Guendouz et al., 2012), the solid-state anaerobic process is categorized into the semi-solid or high solid-state for having total solids between 10% and 20% in a system and solid-state for a system having total solids higher than 20%. As HS-AD has considerably less water than LS-AD, less heating for the HS-AD process is therefore required (Xu F et al., 2014). Nutrient-rich digestate discharged from HS-AD is indeed convenient to be transported for land applications as fertilizer or to be alternatively pelletized as solid bio-fuel (Li et al., 2011). Moreover, HS-AD is capable of being operated at a much higher organic loading rate (OLR) than LS-AD (Nagao et al., 2012). However, in a single-stage HS-AD system, there is insufficient mass transfer due to more organic matter filling, resulting in decreasing methane production but increasing inhibitors of ammonia and volatile fatty acids (VFAs). Consequently, methane production yield using single-stage HS-AD is generally much lower than that using single-stage LS-AD (Xu et al., 2016).

Various approaches to enhancing methane production through a single-stage HS-AD include mixing the main substrate with other organic wastes that could potentially

improve methane production by 80-91% (Brown and Li, 2013) comparison of methane production from different substrates (Wijaya et al., 2020), pretreating substrate and improving inoculum efficiency (Yang et al., 2015). Furthermore, operational approaches of such partial premixing (Zhou and Wen., 2019), adjusting solid loading rates (Zulkifli et al., 2020), leachate recirculation (Ilarri et al., 2020), total solids variation, and temperature adjustment (Ge et al., 2016), and division of the anaerobic digestive process into several stages (Van et al., 20 20) have been deployed to stimulate biogas production in a single-stage HS-AD.

Operating in a separate stage, one of the potential means to enhance biogas productivity over the single-stage HS-AD is a very attractive technique, due to having less inhibition caused by pH, ammonia (NH_3), and volatile fatty acids (VFAs) from separation which enhances methane-producing (Willquist et al., 2012). Over the years, several investigations on two stages of HS-AD have been reported. (Wu et al., 2017) investigated microbial communities in a pilot enhanced two-stage high solid anaerobic digestion system. They found that methanothermobacter sp. hydrogenotrophic methanogen dominated in such a two-stage process system with relative abundance up to about 100% in both thermophilic and mesophilic anaerobic digestion. Inoculum pre-treatments, including heat shock, alkaline treatment, aeration, and a novel pretreatment using waste frying oil (WFO) were comparatively investigated in the two-stage anaerobic digestion of food waste for hydrogen and methane production. The inoculum prepared by WFO provided the highest H2 and CH4 yields of 76.1 and 598.2 mL-CH4·gVS-1, respectively (Rafieenia et al., 2018). (Lavagnolo et al., 2018) studied the organic component of municipal solid as a major substrate fed to batch two-stage anaerobic digestion (AD). The effect of varied combinations of initial pH (5.5, 7, and 9) and food to microbe (F/M) ratio (from 0.5 to 6 g-VS/g-VS) on hydrogen and methane generation were examined. Methane production rate of 37.3 mL-CH4·gVS-1 obtained from the single-phase AD was increased up to 68.5 mL-CH4·gVS-1 by using the two-stage AD. Lag time and cultivating time to attain maximum methane production in two-stage AD was 2 times less than those in the single-phase AD. (Wu C et al., 2018) investigated comparatively the effects of digestate recirculation between the single-stage and two-stage anaerobic digestion. Recirculation of digestate had a positive effect on methane yield and organic loading rate (OLR). A systematic hydrolysis degree of greater than 75% was required to complete the conversion of metabolite for methane production from FW. Digestate recirculation could also considerably improve the alkalinity system, allowing methanogens growth at their optimal pH. Those investigations demonstrate the efforts to increase methane production capacity by applying the two-stage anaerobic system.

Additionally, stage separation also has a positive effect on high solid-state AD. However, the two-stage process also has major limitations on the pH and alkalinity regulation, which could possibly occur in the first stage, in which pH and alkalinity imbalance potentially result in a reduction of methane production in the methanogens

stage (Wu C et al., 2018). Recently, a three-stage AD process having three separate phases of hydrolysis, acidogenesis/acetogenesis, and methanogenesis connected in series has emerged to optimize biogas production to overcome previously mentioned problems associated with one-stage and two-stage AD systems (Chatterjee and Mazumder., 2020). The three-stage AD process shows technologically advanced methods combining the advantages of high solids AD and wet AD. In the three-stage reactor, the optimal pH ranges for hydrolysis (pH 4–5), acidogenesis/acetogenesis (pH 5–7), and methanogenesis are possibly regulated. Consequently, hydrolyzing bacteria, fermenting bacteria and methanogenic archaea are capable of having optimal growth in each separate stage.

The most advantages of three-stage AD over the traditional anaerobic digestion are about having a compact digester, 25-54% methane yield increasing, improvement of hydrolysis and acidogenesis efficiency, higher treatment capacity, small reactor volume due to high solid reduction rate (Zhang et al., 2019). Although a compact digester of the three-stage anaerobic digestion using food waste has been developed and subsequently evaluated in term of its performance, insight batch high solid anaerobic digester consisting of three major phases separately operated has yet to be reported. Nevertheless, three-stage anaerobic digestion is difficult to handle slurry or liquid effluent. Therefore, it would be better to solidify all three phases in order to easy manage effluent and save handling and energy cost to control the temperature yet still able to produce biogas efficiently.

Thus, this thesis aimed at investigating the possibility of initiating and maintaining a three-stage high-solid anaerobic digestion TSHS-AD by using cow manure as a substrate at ambient temperatures. Specifically, the objective of this study was to determine the effect of organic loading on key parameters of pH, alkalinity, and VFAs profiles in the TSHS-AD system. Identification of in-depth correlations between anaerobic process parameters, bacterial, archaeal communities, and their dynamics at different stages affecting methane production are of utmost importance. In addition, the experimental methane yield obtained was projected for economic assessment. Information obtained would be further helpful to develop a compact three-stage digester suitable for manure generated from a small farm scale.

Objective

- To find suitable enhanced inoculum and AD parameters with cow manure for run ambient temperature HS-AD.
- To study methane production potential (BMP) of ambient temperature HS-AD.
- To develop a reactor prototype and operating conditions for TSHS-AD.
- To analyze the economic assessment of the TSHS-AD biogas system.

Chapter 2
Literature review

2.1 cow manure

The mostly cow manure is solid, consisting of a fraction of the plant and animal that is ingested and cannot be digested or used. These food fragments are partially digested in the digestive tract. So, the dung is so rich in nutrients for producing biogas by anaerobic digestion. Each cow species will have more or fewer nutrients, depending on the type of food that the cow feeds, digestive system, and other. The mostly cow manure is solid, consisting of a fraction of the plant and animal that is ingested and cannot be digested or used. These food fragments are partially digested in the digestive tract. So, the dung is so rich in nutrients for produce biogas by anaerobic digested. Each cow species will have more or fewer nutrients, depending on the type of food that the cow feeds, digestive system, keep management and other (Kasetsart University, Kamphaeng Saen Campus, 2006).

Table 1 The characteristics of some digestible feedstock types (AL SEADI, 2001)

Type of feedstock	**Organic content**	**C: N ratio**	**DM %**	**VS % of DM**	**Biogas yield m^3*kg-1 VS**	**Unwanted impurities**	**unwanted matters**
Pig slurry	Carbohydrates, proteins, lipids	3-10	3-8	70-80	0,25-0,50	Wood shavings, bristles, water, sand, cords, straw	Antibiotics, disinfectants
Cattle slurry	Carbohydrates, proteins, lipids	6-20	5-12	80	0,20-0,30	Bristles, soil, water, straw, wood	Antibiotics, disinfectants, NH_4^+
Poultry slurry	Carbohydrates, proteins, lipids	3-10	10-30	80	0,35-0,60	grit, sand, feathers	Antibiotics, Disinfectants, NH_4^+

Stomach/intestine content	Carbohydrates, proteins, lipids	3-5	15	80	0,40-0,68	Animal tissues	Antibiotics, disinfectants
Whey	75-80% lactose 20-25% protein	-	8-12	90	0,35-0,80	Transportation impurities	
Concentrated whey	75-80% lactose 20-25% protein	-	20-25	90	0,80-0,95	Transportation impurities	
Flotation sludge	65-70% proteins 30-35%lipids	-				Animal tissues	Heavy metals, disinfectants, organic pollutants
Ferment. slops	Carbohydrates	4-10	1-5	80-95	0,35-0,78	Non-degradable fruit remains	
Straw	Carbohydrates, lipids	80-100	70-90	80-90	0,15-0,35	Sand, grit	
Garden wastes		100-150	60-70	90	0,20-0,50	Soil, cellulosic components	Pesticides
Grass		12-25	20-25	90	0,55	Grit	Pesticides
Fruit wastes		35	15-20	75	0,25-0,50		
Food remains			10	80	0,50-0,60	Bones, plastic	Disinfectants

Girija et al., (2013.) have studied the excrement of cows consumed from former times in husbandry as it has a key part in crop outgrowth advancement and plant protection. It is also being used in various religious practices as a purifier. Thence mere a small scrap of the total animalcule promiscuity can be recovered by culturable methods, a culture-independent 16S rDNA approach was taken up for a more detailed

analysis of cow dung the microorganisms of a particular site. Total community DNA was extracted from fresh cow dung and bacterial 16S rRNA genes were afterward amplified, cloned, sequenced, and deposited in GenBank. Bacteria belonging to the phyla Bacteroidetes (37.3%), Firmicutes (28.8%), Proteobacteria (21.4%), and Verrucomicrobia (2.1%) were identified. A genus of gram-negative produced asexually from one ancestor or stock included the genera Bacteroides, Alistipes, and Paludibacter; while Clostridium, Ruminococcus, Anaerovorax, and Bacillus were distinctive in Firmicutes. α- and γ-proteobacterial genera included Acinetobacter, Pseudomonas, Rheinheimera, Stenotrophomonas, and Rhodobacter. The Verrucomicrobial clone showed up parity to Akkermansia. Unculturable bacterium performed 83.4% in the phylum Bacteroidetes and 87.6% in Firmicutes. whole clones underneath genre Proteobacteria were culturable bacteria. The clone library of 8% be regarded as hitherto uncharacterized and unidentified bacteria following table 2 and figure 1.

Table 2 Distribution of cow dung microbiome (Girija et al., 2013)

Phylum	**No. of clones (% of total)**	**Bacteria**	**No. of clones**	
			Culturable	**Non-culturable**
Bacteroidetes	18 (38.3)	Phylum: Bacteroidetes	-	1
		Order: Bacteroidales	-	5
		Family: Rikenellaceae		1
		Bacteroides sp.	1	-
		Alistipes sp.	2	5
		Paludibacter sp.	-	3
Firmicutes	14 (29.8)	Phylum: Firmicutes	-	2
		Order: Clostridiales	-	1
		Family: Ruminococcaceae	-	3
		Ruminococcus sp.	-	1
		Clostridium sp.	1	4
		Anaerovorax sp.	-	1
		Bacillus sp.	1	
Proteobacteria	10 (21.3)	Acinetobacter sp.	2	
		Pseudomonas sp.	4	
		Rheinheimera sp.	1	
		Stenotrophomonas sp.	1	
		Rhodobacter sp.	2	
Verrucomicrobia	1 (2.1)	Akkermansia sp.	-	1
Unknown	4 (8.5)	Uncultured bacteria	-	4
Total	47		15	32

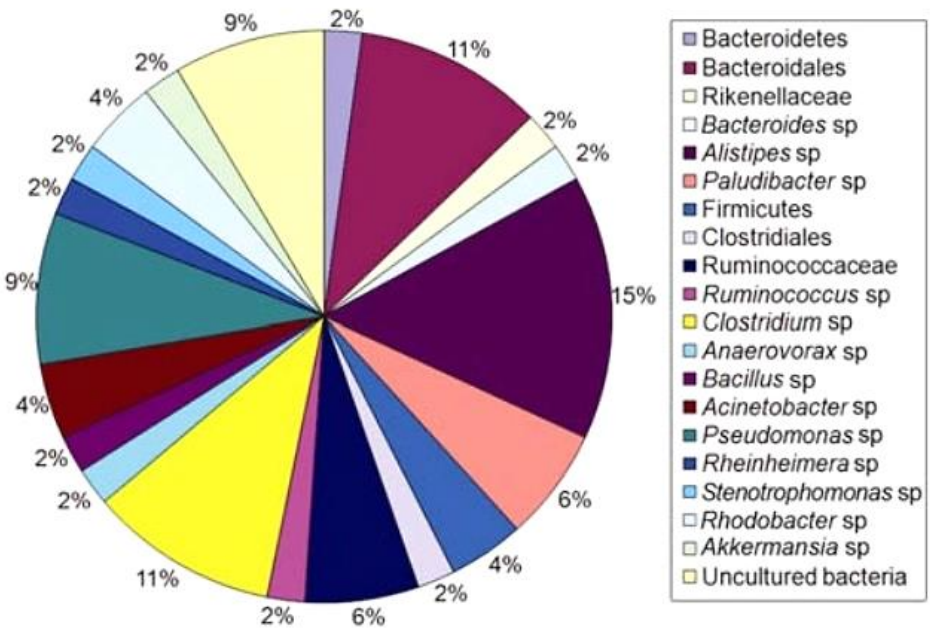

Figure 1 Diagrammatic representation of cow dung microbiome (Girija et al., 2013).

2.2 The biochemical process of Anaerobic Digestion (AD)

While hitherto specified, the AD is a microbiological operation of putrefaction of biological matter in nonappearance oxygen. The principal products of this operation are biogas and digestate. Biogas is a combustible gas, consisting primarily of methane and carbon dioxide. Digestate is the decomposed substrate resulted from the production of biogas. During the AD, very little heat is generated in contrast to aerobic decomposition (in presence of oxygen), like it is the case of composting. The energy, which is chemically bounded in the substrate, remains mainly in the produced biogas, in form of methane. The operation of biogas alignment is a consequence of linked process storeys, in which the commencing material is ceaselessly dilapidated into diminutive units. Different consortia of microorganisms with different function in the anaerobic digestion process are needed. Essential cluster of microorganisms have been analysed with other purpose in the gross decomposition process and what not the hydrolyzing and undergo fermentation microorganisms, the obligate hydrogen-producing acetogenic bacteria and two kind of methanogenic Archaea (fig.2). These organisms successively decompose the products of the previous steps.

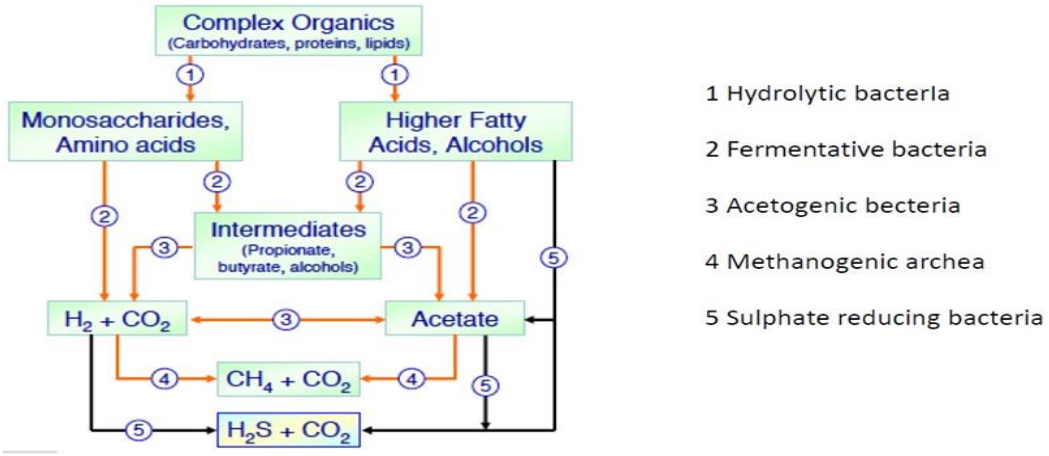

Figure 2 Major groups of microorganisms have been identified with different functions in the overall degradation process (Cristina, 2011).

Enzymes in anaerobic digestion is divided into 3 types shown in Figure 3. For hydrolysis stage exoenzymes officiate solubilization of particulate and colloidal wastes. In stage of acid forming endoenzymes act conversion of soluble organic acids and alcohols to acetate, carbon dioxide and hydrogen. And methanogenesis stage endoenzymes produce methane and carbon dioxide.

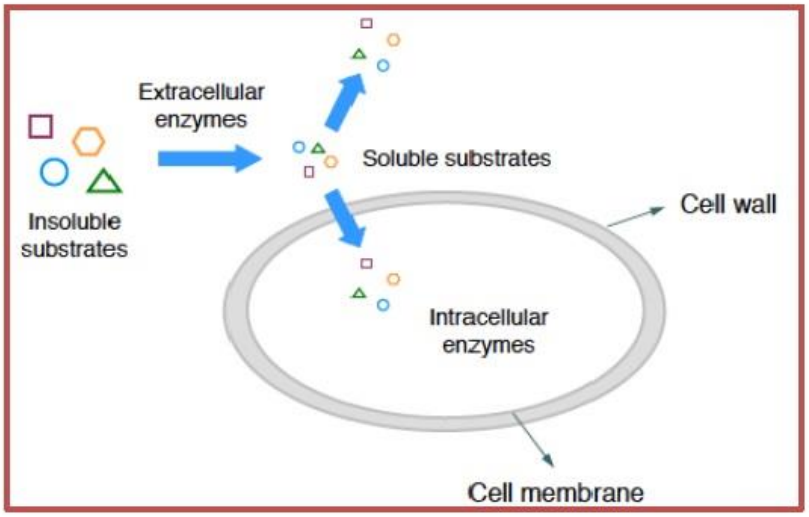

Figure 3 Enzymes in anaerobic digestion (Cristina, 2011).

The clarify representation of the AD operation, shown in Figure 4, pinnacles the four essential operation steps: hydrolysis, acidogenesis, acetogenesis together with methanogenesis. The process steps quoted in Figure 4 run parallel in time and space, in the digester tank. The speed of the total decomposition process is determined by the slowest reaction of the chain. In the case of biogas plants, processing substrates

containing cellulose, hemi-cellulose and lignin, hydrolysis is the speed determining process. During hydrolysis, relatively small amounts of biogas are produced. Biogas production reaches its peak during methanogenesis.

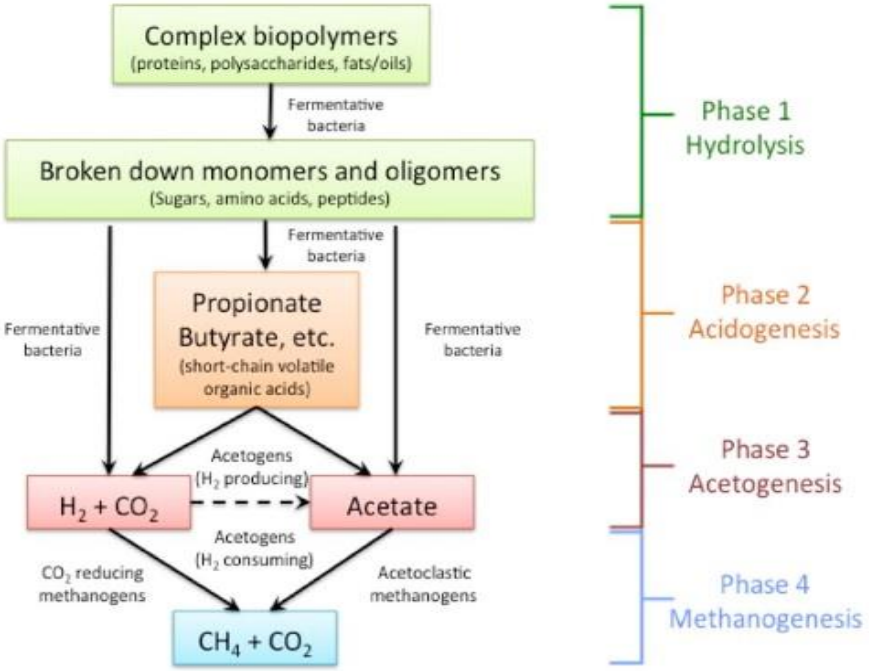

Figure 4 Schematic of pathways of anaerobic digestion processes for organic substrate (Michael Olalekan Fagbohungbe, 2015)

2.3 AD parameters

The productivity of AD is prejudiced by certain serious factors, consequently it is essential that suitable situations for anaerobic bacteria are provided. The development and movement of anaerobic microbes is meaningfully prejudiced by circumstances for example prohibiting of oxygen, constant temperature, pH-value, nutrient source, stirring intensity in addition to attendance besides quantity of inhibitors (e.g. ammonia). The methane microbes are fastidious anaerobes, with the intention of the occurrence of oxygen into the digestion procedure must be severely circumvented.

2.3.1 Temperature

The AD procedure be able to take place at dissimilar temperatures, alienated into three temperature ranges: psychrophilic (below 25^{o}C), mesophilic (25^{o}C-45^{o}C) also thermophilic (45^{o}C -70^{o}C). There is a straight relative flanked by the procedure temperature also the HRT (Table 3).

Table 3 Thermal stage and characteristic retention times (Schlundt, 1984 and I. Placha, 2001).

Thermal stage	Process temperatures	Minimum retention time
psychrophilic	< 20 °C	70 to 80 days
mesophilic	30 to 42 °C	30 to 40 days
thermophilic	43 to 55 °C	15 to 20 days

Table 4 The decimation time (T-90)* of some pathogenic bacteria - comparison among animal slurry preserved by AD and the unprocessed slurry (Bendizen, 1995)

Bacteria	preserved by AD		unprocessed slurry	
	53°C (thermophilic temperature) hours	35°C (mesophilic temperature) days	18-21°C weeks	6-15°C weeks
Salmonella typhi murium	0.7	2.4	2.0	5.9
Salmonella dublin	0.6	2.1	-	-
Escherichia coli	0.4	1.8	2.0	8.8
Staphilococcus aureus	0.5	0.9	0.9	7.1
Mycobacterium paratuberculosis	0.7	6.0	-	-
Coliform bacteria	-	3.1	2.1	9.3
Group of D-Streptococi	-	7.1	5.7	21.4
Streptococcus faecalis	1.0	2.0	-	-

* Decimation time T-90 is the time of survival of the observed micro-organisms. The decimation time T-90, is demarcated as the time taken for feasible totals of a inhabitants to reduction by one logarithmic unit (log10), which is corresponding to a 90% lessening (Schlundt, 1984 and I. Placha, 2001).

The temperature steadiness is conclusive for AD. Actually, the process temperature is selected with deliberation to the feedstock used and the essential route temperature is typically on condition that through ground or partition heating schemes,

inside the digester. Figure 5 demonstrations the degrees of comparative biogas yields contingent on temperature besides retention time.

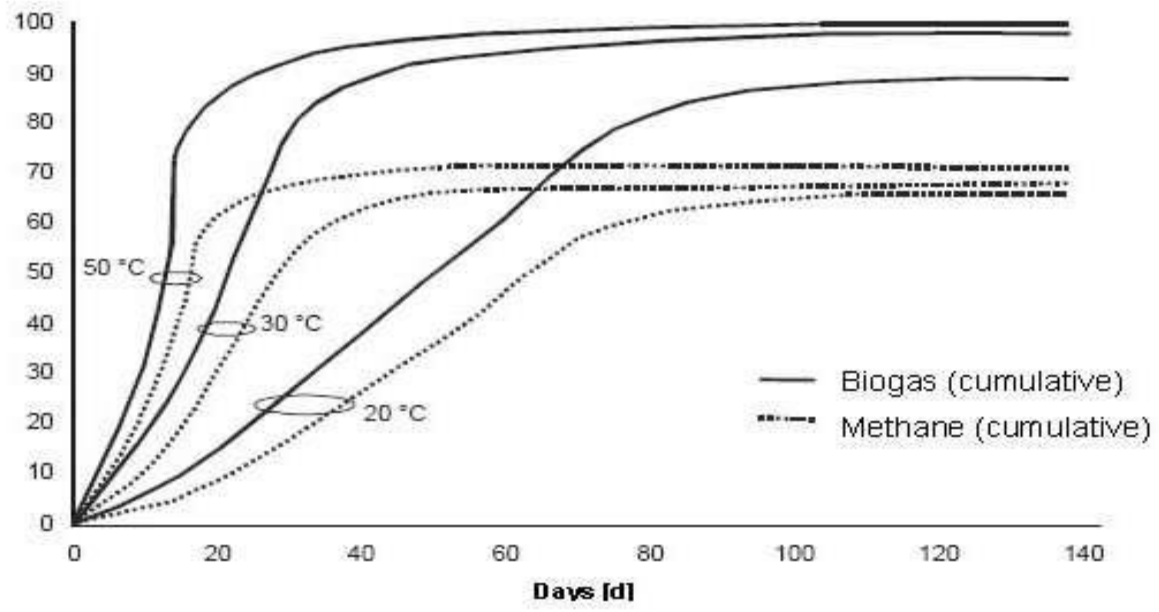

Figure 5 Relative biogas yields, depending on temperature and retention time (LfU, 2007)

Several contemporary biogas vegetation function at thermophilic procedure temperatures as the thermophilic procedure make available numerous compensations, related to mesophilic and psychrophilic procedures:

· in effect obliteration of pathogens.
· higher cultivate degree of methanogenic microorganisms at upper temperature.
· abridged retention time, manufacture the progression more rapidly and more well-organized.
· better-quality digestibility and obtainability of substrates.
· improved dreadful conditions of solid substrates and improved substrate utilization.
· enhanced opportunity for straightening out liquid also solid portions.

The thermophilic procedure has likewise particular disadvantages:

· superior degree of inequity
· higher energy demand in arrears to high temperature
· advanced risk of ammonia inhibition

Procedure temperature impacts the intoxication of ammonia. Ammonia intoxication upsurges with upsurges temperature also be able to be reassured by lessening the procedure temperature. Nevertheless, as soon as declining the temperature to 50°C or underneath, the progress degree of the thermophilic bacteria will crash considerably, in

addition to a risk of disappointment of the bacteriological inhabitants can transpire, as a result of a progression degree inferior than the definite HRT (Angelidaki, 2004). This means that a acceptable operational thermophilic digester can be encumbered to a upper grade or activated at a lesser HRT than an e.g. mesophilic one for the reason that of the evolution degrees of thermophilic organisms (Figure 6). Knowledge demonstrations that at high loading or at low HRT, a thermophilic functioned digester has advanced gas produce and higher transfiguration degrees than a mesophilic digester.

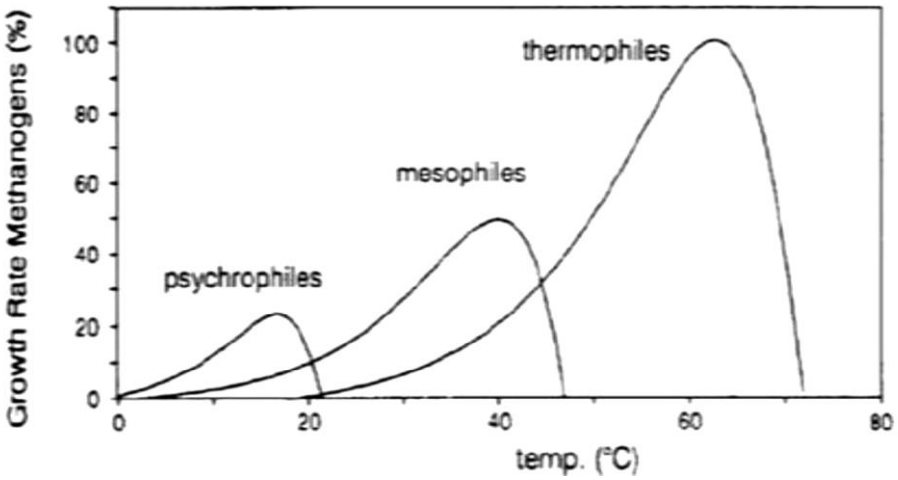

Figure 6 Comparative growth proportions of methanogens (ANGELIDAKI, 2004)

The solubility of several compounds (NH_3, H_2, CH_4, H_2S and VFA) likewise be contingent on the temperature (Table 5). This can be of great meaning for resources which have an constraining outcome on the procedure.

Table 5 The relative in the middle of temperature and the solubility in water of specific gases (Angelidaki, 2004)

Gas	Temperature (°C)	Solubility mmol/l water	Changed solubility 50-35°C
H_2	35	0.749	3.3 %
	50	0.725	
CO_2	35	26.6	36 %
	50	19.6	
H_2S	35	82.2	31 %
	50	62.8	
CH_4	35	1.14	19 %
	50	0.962	

The tackiness of the AD substrate is contrariwise relational to temperature. This means that the substrate is supplementary liquid in elevation temperatures also the distribution of liquefied material is accordingly simplified. Thermophilic procedure temperature consequences in more rapidly chemical reaction proportions, as a result improved productivity of methane manufacture, advanced solubility and inferior viscosity. The advanced demand for energy in the thermophilic procedure is vindicated by the advanced biogas return. It is significant to preserve a constant temperature throughout the digestion procedure, as temperature changeability or fluctuations will have emotional impact the biogas manufacture destructively. Thermophilic microorganisms are additional delicate to temperature instability of ±1°C and necessitate extended time to become accustomed to a new-fangled temperature, with the intention of reach the extreme methane manufacture. Mesophilic microorganisms are a smaller amount delicate. Temperature instabilities of ±3°C are endured, starved of noteworthy decreases in methane manufacture.

2.3.2 pH-values and optimum intervals

The pH-rate is admeasure of acidity/alkalinity of a solution (correspondingly of substrate combination, in the circumstance of AD) also is articulated in parts per million (ppm). The pH rate of the AD substrate guidance the development of methanogenic microbes and have sensitive impact the detachment of particular compounds of significance for the AD procedure (ammonia, sulphide, organic acids). Involvement demonstrations that methane materialization takes place in the interior a comparatively constricted pH interval, on or after about 5.5 to 8.5, through the most favorable interval in the middle of 7.0-8.0 for furthermost methanogens. Acidogenic bacteria typically have inferior charge of optimal pH. The optimal pH interval for mesophilic digestion is among 6.5 and 8.0 also the procedure is relentlessly repressed if the pH-value declines underneath 6.0 or increases directly above 8.3 (C. Liu et al., 2008 and Noxolo T Sibiya et al., 2014). The solubility of carbon dioxide in water reductions at increasing temperature. The pH-value in thermophilic digesters is consequently advanced than in mesophilic ones, as liquefied carbon dioxide forms carbonic acid by reaction with water. The value of pH know how to be enlarged by ammonia, manufactured throughout poverty of proteins or through the attendance of ammonia in the feed stream, even though the accretion of VFA diminutions the pH-value. The rate of pH in anaerobic reactors is primarily measured by the bicarbonate buffer scheme. For that reason, the pH value inside digesters be contingent on the fractional pressure of CO_2 and on the concentration of alkaline and acid constituents in the liquid stage. If accretion of pedestal or acid ensues, the buffer volume neutralizes these vicissitudes in pH, up to a convinced level. As soon as the buffer measurements of the scheme is surpassed, sweeping vicissitudes in pH-values arise, wholly constraining the AD procedure. Consequently, the pH-value is not not compulsory as a stand-alone procedure

verification parameter. The buffer measurements of the AD substrate know how to be different. Understanding on or after Denmark demonstrations that the buffer volume of livestock dung differs with the time of year, perchance prejudiced by the arrangement of the livestock feedstuff. The pH-value of national animal manure is for that reason a adjustable which is problematic to use for proof of identity of procedure disproportion, by means of it vicissitudes very slight and very leisurely. It is, on the other hand, significant to communication that the pH-value can be a rapid, comparatively dependable and inexpensive approach of recordkeeping scheme unevenness in more inadequately buffered schemes, for instance AD of a number of wastewater categories.

2.3.3 Volatile fatty acids (VFA)

The constancy of the AD procedure is reproduced by the concentration of transitional produces comparable the VFA. The VFA are in-between compounds (acetate, propionate, butyrate, lactate), manufactured throughout acidogenesis, through a carbon chain of unequal to six atoms. In furthermost circumstances, AD procedure unpredictability will principal to build up of VFA inside the digester, which can central in addition to a moving toward a lower position of pH-value. Acidic pH levels have destructive influence on these reactions. Methanogens are respond to slight changes to pH external the range 6 to 8, from this time, a diminution in pH underneath 6 decreases the activity of the methanogens more than that of the acidogens/acetogens. This reasons a collection of organic acids, supplementary dropping pH. On the other hand, the accretion of VFA will not continuously be articulated by decreases of pH value, attributable to the buffer volume of the digester, over and done with the biomass kinds controlled in it. Animal droppings e.g. has a surplus of alkalinity, which means that the VFA accretion would go beyond a assured level, previously this can be noticed attributable to noteworthy reduction of pH value. By the side of such fact, the VFA concentration in the digester would be so high, that the AD procedure will be already relentlessly repressed (Ahring et al. 1995, Vaccari et al. 2005 and ZhiyangXu et al., 2014). Besides Andreas OttoWagner et al. (2014) revision considered the metabolism of dissimilar acetate:propionate ratios (0.25, 0.33, 0.5, 1.0, 2.0, 3.0, 4.0) in equimolar carbon concentration throughout an anaerobic putrefaction procedure further down well-defined laboratory circumstances and appraised the betrothed methanogenic communal. Momentous changes on a metabolic level (gas manufacture, gas configuration, volatile fatty acid (VFA) concentration) were experimental in the middle of acetate:propionate ratios ≤ 1 and ≥ 2. In the main ratios ≥ 2 occasioned in a more rapidly methane manufacture and VFA putrefaction related to ratios ≤ 1. Applied understanding demonstrations that two dissimilar digesters can perform completely poles apart in admiration to the identical VFA concentration, subsequently that one and the similar concentration of VFA can be optimum for one digester, on the other hand inhibitory for the other one. One and only of the conceivable clarifications can be the

detail that the arrangement of bacteria inhabitants diverges from digester to digester. Consequently, as well as like in the circumstance of pH, the VFA concentration can not be not compulsory as an unrelated process observing parameter.

2.3.4 Ammonia

Ammonia is created by the biological dilapidation of the nitrogenous matter, regularly in the custom of proteins and urea. It is commonly supposed that ammonia concentrations underneath 200 mg/L are advantageous to anaerobic procedure from the time when nitrogen is an indispensable nutrient for anaerobic bacteria (Chen et al. 2008). Ammonia (NH_3) is a significant compound, with a noteworthy purpose for the AD method. NH_3 is an imperative nutrient, allocation as a predecessor to foodstuffs and manures and is customarily stumble upon by means of a gas, by way of the distinguishing pungent odor. Proteins are the foremost basis of ammonia for the AD procedure. In addition high ammonia concentration inside the digester, particularly free ammonia (the unionized form of ammonia), is well thought-out to be in authority for procedure embarrassment. This is mutual to AD of animal slurries, as a result of their high ammonia concentration, instigating on or after urine. Intended for its inhibitory influence, ammonia concentration ought to be held in reserve underneath 80 mg/l. Methanogenic microorganisms are particularly respond to slight changes to ammonia inhibition. The concentration of free ammonia is straight comparative to temperature, consequently there is a greater than before risk of ammonia reserve of AD procedures functioned at thermophilic temperatures, compared to mesophilic ones. The free-ammonia concentration is calculated from the equation 2.1

$$[NH_3] = \frac{[T - NH_3]}{(1 + \frac{H^+}{ka})} \tag{2.1}$$

Where $[NH_3]$ and $[T\text{-}NH_3]$ are the free and correspondingly the overall ammonia concentrations, besides *ka* is the disconnection parameter, with values cumulative with temperature. This means that cumulative pH and increasing temperature will principal to enlarged reserve, as these reasons will rise the portion of free ammonia. As soon as a procedure is reserved by ammonia, an upsurge in the concentration of VFA will main to a reduction in pH. This will moderately respond the influence of ammonia attributable to a diminution in the free ammonia concentration. High whole ammonia nitrogen levels (TAN, i.e. $NH_4^+ + NH_3$; > 3000 mg/L) have been exposed to be a significant cause modifiable the swing from aceticlastic methanogenesis to syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis in mesophilic biogas procedures (Schnürer & Nordberg 2008). The swing is almost certainly an importance of reserve of the goings-on of the aceticlastic methanogens. Free ammonia nitrogen

(FAN, i.e. NH_3) has been well thought-out for example the foremost reason of ammonia reserve as it possibly will without restrictions pass over and done with the cell membrane. Numerous appliances for ammonia reserve have been planned, for instance a modification in the intracellular pH, upsurge of preservation energy prerequisite, and reserve of a specific enzyme reaction (Chen et al., 2008). It is supposed that aceticlastic methanogens are further respond to slight changes to high ammonia levels than hydrogenotrophic methanogens and that methanogens in general are additional sensitive than SAOBs (Fotidis et al., 2013). Conversely, there are suggestions that certain hydrogenotrophic methanogens are similarly or all the more lasting to high TAN than SAOBs (Wang et al. 2015). This creates it rigid to assume simplifications on ammonia reserve in AD. Reserve of the AD procedure is customarily designated by a reduction in the stable state methane manufacture degrees and upsurge in the intermediate digestion produces like VFA concentrations, predominantly acetate and propionate. A moral select of temperature, controller of pH and C/N ratio, and application of familiarized bacteria and microscopic algae and fungi, especially those living in a particular site or habitat to higher ammonia concentrations might confirm a steady and uninterrupted digestion (Rajagopal et al. 2013). Subsequently FAN has been not compulsory to be the definite toxic agent, a growth in pH would consequence in improved poisonousness as designated in equation (2.2).

$$FAN = \frac{TAN}{1+\frac{10^{-pH}}{K_a}} \tag{2.2}$$

Procedure unpredictability attributable to ammonia frequently consequences in VFA buildup, which all over again indications to a reduction in pH and in this manner deteriorating concentration of FAN. Together bacteriological evolution proportions and FAN concentration are exaggerated by temperature variation meanwhile Ka is at the mercy of on temperature. A greater than before procedure temperature in common has a optimistic consequence on the metabolic rate of the microbes nonetheless likewise consequences in a higher concentration of FAN. Cutting-edge anaerobic digesters functioned at pH 7 at 35^{o}C, FAN characterizes less than 1% from the whole ammonia, even though, at the similar temperature, nevertheless pH 8 the FAN upsurges to 10% (Rajagopal et al. 2013). In other words, an upsurge in pH as of 7 to 8 will principal to an eightfold upsurge of the FAN levels in mesophilic circumstances and all the more at thermophilic temperatures. More than a few approaches have been recommended to counteract ammonia inhibition (Chen et al. 2008, Rajagopal et al. 2013). Convinced ions for instance Na^{+}, Ca^{2+}, and Mg^{2+} have antagonistic effects on ammonia embarrassment, a singularity in which the poisonousness of one ion is reduced by the

attendance of additional ion(s). Air stripping and chemical precipitation might eliminate ammonia after the substrate. Dilution decrease the concentration of ammonia, nonetheless it likewise upsurges the waste capacity. The use of struvite precipitation, anammox, zeolite and carbon fiber fabrics has also been planned (Rajagopal et al., 2013). Additional communal technique is to rise the retention time of the biomass either by improved sedimentation or by restriction. Acclimation of methanogenic groups is an established valuable and inexpensive scheme on the other hand it is not yet unblemished whether the adaptation is a significance of metabolic evolution of already current bacteriological inhabitants or from fluctuations in the communal arrangement owing to dissimilar ammonia concentrations. TAN concentrations of nearby 1700–1800 mg/L may be totally inhibitory with unacclimated inoculum, even though with acclimation, inhibitory TAN levels could exceed 5000 mg/L (Yenigun & Demirel 2013).

2.3.5 Decrease of sulfate

There are three conceivable sulfur bases in anaerobic digestion (Vaccari et al. 2006). Sulfides may be presented to anaerobic digesters through the sludge streams as i) constitutive cellular elements (e.g. sulfur-bearing amino acids) unconstrained unswervingly from lysed bacteriological cells originate in the subordinate sludge (ii) abridged metallic-sulfide (e.g. FeS) precipitates found in the interior abridged sludges (iii) received soluble sulfates being rehabilitated straight to sulfides attributable to the extremely dropping circumstances surrounded by the digester. Etcetera their foundation, sulfides contained by anaerobic digesters possibly will then go through a variability of significant revolutions. Sulfate dropping microorganisms (SRB) might decrease sulfate to sulfide. SRB are very miscellaneous in positions of their metabolic pathways and over may totally or incompletely cut down branched-chain also long chain fatty acids, ethanol and further alcohols, organic acids, and aromatic compounds (Chen et al. 2008). SRB may contend with methanogens, acetogens, or fermentative microbes for obtainable acetate, H_2, propionate, and butyrate in anaerobic coordination. The consequence of the rivalry governs the concentration of sulfide in the reactor method. Sulfide is toxic to methanogens as well as to the SRB themselves. Sulfide-grounded precipitation may transpire to a degree that could decrease the disposable solubility of numerous metal classes (e.g. iron, copper, nickel and zinc), conceivably unfluctuating to the grade that would decrease their obtainability as metabolically essential trace elements. Hydrolysis of the sulfide possibly will correspondingly take place assumed the to some extent acidic circumstances set up in several anaerobic digesters (i.e. due to the low acid ionization constant intended for sulfide hydrolysis, H_2S $HS^- + H^+$). This would principal to gas altercation by the use of stripping into the headspace and impurity of the methane item for consumption to a degree that could

require elimination in advance of by the methane as an energy-rich feed gas (Vaccari et al. 2006).

2.3.6 Macro- and micronutrients (trace elements) and toxic compounds

Microelements (trace elements) similar to iron, nickel, cobalt, selenium, molybdenum or tungsten are correspondingly significant for the development and continued existence of the AD microbes as the macronutrients carbon, nitrogen, phosphor, and sulphur. The optimum proportion of the macronutrients carbon, nitrogen, phosphor, and sulphur (C:N:P:S) is measured 600:15:5:1. Inadequate establishment of nutrients and trace elements, along with excessively high digestibility of the substrate can reason reserve and instabilities in the AD procedure. Additional issue, influencing the activity of anaerobic microbes, is the occurrence of toxic compounds. They can be transported interested in the AD scheme composed with the feedstock or are engendered throughout the procedure. The submission of starting point principles for toxic compounds is problematic, on one hand for the reason that these category of materials are frequently bound by chemical procedures and conversely for the reason that of the volume of anaerobic microbes to become accustomed, surrounded by particular bounds, to environmental circumstances, together with this to the being there of toxic compounds.

2.4 Operational parameters

2.4.1 Organic load

The manufacture also set-up of a biogas plant is an amalgamation of reasonable in addition methodological contemplations. Procurement the extreme biogas produce, by dint of absolute digestion of the substrate, would necessitate an elongated retention time of the substrate inner recesses the digester and a consistently great digester magnitude. In rehearsal, the select of method purpose (digester size and category) otherwise of appropriate retention time is permanently established on a finding the middle ground in the middle of in receipt of the uppermost conceivable biogas yield besides having defensible plant economy. In this high opinion, the organic load is a significant in operation factor, which point toward by what method plentiful organic dry matter know how to be nourished obsessed by the digester, per volume and time unit, on the word of the equation 2.3 A lot of features have emotional impact the presentation of anaerobic digestion procedures. Particular of them are connected to feedstock physical appearance, reactor draw up plans and in operation circumstances (Hawkes et al., 1980). The organic loading rate (OLR) is a significant factor for the reason that it designates the quantity of volatile solids to be fed into the digester every day (Mattocks R et al., 1984). Volatile solids characterize that percentage of the

organic-material solids that can be assimilated, despite the fact that the remains of the solids is immovable. The 'absolute' solids and a percentage of the volatile solids are non-biodegradable. The definite loading rate be determined by on the categories of wastes nourished interested in the digester (Mattocks R et al., 1984), for the reason that the forms of waste control the level of biochemical activity that will take place in the digester.

$$BR = m \cdot c / VR \quad (2.3)$$

BR organic load [kg/d.m³]
m mass of substrate fed per time unit [kg/d]
c concentration of organic matter [%]
VR digester volume [m³]

Satoto (2009) recommended at advanced OLR might discovery establishment of massive foam occasioned in enlarged quantity of pressure water in the upcoming consequently the COD elimination effectiveness capable of OLR control. For instance the organic loading rate was greater than before, the VS dilapidation and biogas yield reduced (Azadeh and Jalal, 2011).

2.4.2 Hydraulic retention time (HRT)

Anaerobic digestion is a multifaceted biochemical procedure, whereupon microbes decompose organic matter and products biogas. Amount of factors could effect the implementation and biogas manufacture for semicontinuous or incessant anaerobic digestion (R. Chandra et al., 2012), together with substrate physical characteristics, organic loading rate (OLR), hydraulic retention time (HRT), temperature, and pH. HRT is a significant in operation factor for the anaerobic reactors which can encouragement the transformation of volatile solids (VS) obsessed by biogas (M. A. Dareioti and M. Kornaros, 2014 and D. Ho et al., 2014). In the main, comparatively extended HRT is desirable in anaerobic digestion of lignocellulosic wastes for this category of substrates is tenacious to anaerobic microbes (S. Yadvika et al., 2004). Rivard et al. (C. J. Rivard et al., 1988) recommended that 60–90 days is obligatory in order to accomplish extreme digestion of polymeric substrates, despite the fact Banks (C. Banks, 2004) correspondingly described HRT of 20 days in anaerobic digestion of maize. Undersized HRT is necessary as it is in a straight line interrelated to the decrease of resources cost and the upsurge of procedure competence. An imperative parameter for dimensioning the biogas digester is the hydraulic retention time (HRT). The HRT is the mean time intermission as soon as the substrate is reserved inner parts the digester reservoir. HRT is interrelated to the digester capacity and the capacity of substrate fed per time unit, as stated by the following equation 2.4

$$HRT = VR / V \quad (2.4)$$

HRT hydraulic retention time [days]
VR digester volume [m^3]
V volume of substrate fed per time unit [m^3/d]

In relation to the directly above equation, cumulative the organic load diminishes the HRT. The retention time necessity be satisfactorily extended to make sure that the quantity of bacteria uninvolved with the discharge (digestate) is not advanced than the quantity of replicated microbes. The reduplication rate of anaerobic microbes is customarily 10 days or more. A small HRT be responsible for an improvement substrate flow rate, but a lower gas yield. It is therefore important to adapt the HRT to the specific decomposition rate of the used substrates. Knowing the targeted HRT, the daily feedstock input and the decomposition rate of the substrate, it is possible to calculate the necessary digester volume.

2.4.3 Solids Retention Time (SRT)

The Solids Retention Time (SRT) is the furthermost significant factor regulatory the transformation of solids to gas. It is correspondingly the furthermost key factor in preserving digester constancy. Even though the calculation of the solids retention time is every so often indecorously stated, it is the amount of solids preserved in the digester divided by the quantity of solids wasted each day follow 2.5 equation.

$$SRT = \frac{(V)(C_d)}{(Q_w)(C_w)} \quad (2.5)$$

Where V is the digester volume; C_d is the solids concentration in the digester; Q_w is the volume wasted each day and C_w is the solids concentration of the waste.

In a predictable absolutely miscellaneous, or plug flow digester, the HRT equivalents the SRT. On the other hand, cutting-edge a variability of engaged biomass reactors the SRT go beyond the HRT. Consequently, the reserved biomass digesters know how to be abundant less important even though accomplishing the similar solids conversion to gas.

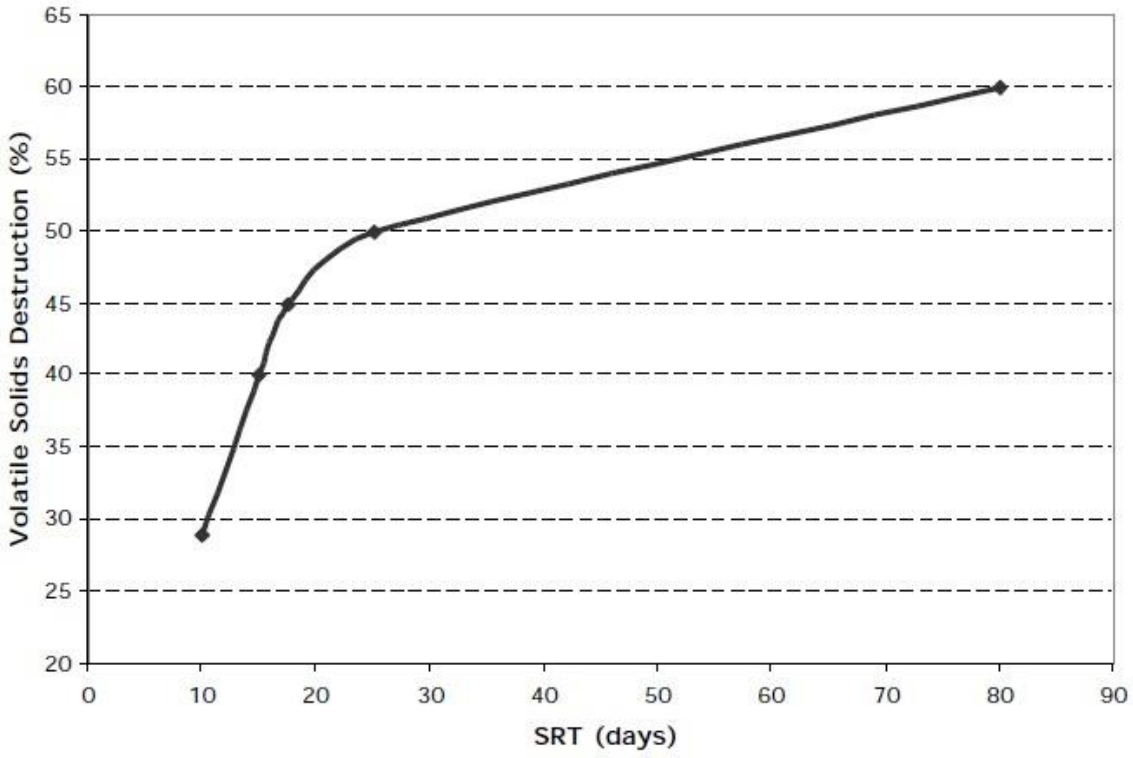

Figure 7 Dairy Waste Volatile Solids Destruction

The volatile solids transformation to gas is a purpose of SRT (Solids Retention Time) rather than HRT (Fig. 7). By the side of a low SRT appropriate time is not obtainable for the microorganisms to raise and substitute the microbes misplaced in the discharge. If the rate of bacteriological harm surpasses the rate of microorganisms evolution, "wash-out" ensues. The SRT at which “wash-out” instigates to ensue is the "critical SRT". Jewell, W. J., R. M. Kabrick, et al. (1981) recognized that an extreme of 65 percent of dairy manure's volatile solids possibly will be transformed to gas with long solids retention times. Burke, D. A. (2000) recognized that 65 to 67 percent of dairy manure COD possibly will be transformed to gas. Extended retention times are essential for the transformation of cellulose to gas. Inka et al. (2015) determine that shortening the SRT and intensifying the temperature are valuable approaches for dynamic bacteriological populations in the direction of manipulated manufacture of in height levels of particular volatile fatty acids. The objective of procedure engineers in excess of the previous twenty years has been to progress anaerobic developments that preserve biomass in a diversity of procedures such that the SRT can be enlarged even though the HRT is lessened. The objective has been to preserve, slightly than waste the biocatalyst (bacterial consortia) in control for the anaerobic procedure. For instance a consequence of this determination, gas yields have enlarged and digester volumes reduced. A measure of the achievement of biomass retention is the SRT/HRT proportion. In predictable digesters, the proportion is 1.0. In effect retention schemes

will have SRT/HRT proportions more than 3.0. At an SRT/HRT ratio of 3.0 the digester will be 1/3rd the scope of a predictable digester.

2.4.4 Food to Microorganism Ratio

The nourishment to microbe proportion is the important issue monitoring anaerobic digestion. By the side of a given temperature, the bacteriological consortia can individual put away an inadequate quantity of nourishment every day. With the intention of munch through the essential number of pounds of waste one necessity resource the appropriate amount of pounds of microorganisms (Dennis A. and Burke P.E., 2001). The percentage of the pounds of waste provided to the pounds of microorganisms obtainable to put away the waste is the food to microbe ratio (F/M). This relation is the adjusting feature in altogether biological handling procedures. Lopes et al., (2004) originate that as soon as cramming the anaerobic digestion of municipal organic waste with the cow stomach for instance its inoculums, it was extraordinarily significant to choice the suitable feature relation of substrate and inoculums (F/M) for sustaining the immovability of the scheme whose inoculums was cow stomach and municipal organic waste was raw material. Agus et al. (2015) originate that the proportion F/M concern the quantity of biogas formed. In the intervening time, the retention time (HRT) is merely have an effect on by the ratio F/M. An inferior the F/M ratio determination consequence in a better percentage of the waste existence transformed to gas. Inappropriately, the bacteriological mass is problematic to estimate subsequently it is challenging to discriminate the microbial mass from the influent waste. 0% Plagiarism The task would be unsophisticated if altogether of the influent waste were transformed to biomass or gas. Cutting-edge that circumstance, the F/M ratio would basically be the digester loading divided by the concentration of volatile solids (biomass) in the digester (L/Cd). Designed for whichever given loading, the productivity can be enhanced by lowering the F/M ratio by rising the concentration of biomass in the digester. Moreover for whichever given biomass concentration in the interior the digester, the competence can be enhanced by lessening the loading. Inappropriately, a fraction of the influent waste is not treated or transformed to biomass or gas by the microorganisms. In that situation the F/M ratio is equal to the VS loading divided by the digester VS controlled (VS_D) minus the unprocessed Volatile Solids (VS_{UP}). The untreated volatile solids might include refractory or non-degradable organic products created by the microorganisms.

$$\frac{F}{M} = \frac{L_{VS}}{VS_D - VS_{UP}} \tag{2.6}$$

2.5 Biochemical Methane Potential, BMP (Angelidaki et al., 2009)

Anaerobic digestion of solid organic waste for instance biowaste, sludge, cattle manure, energy harvests and additional biomasses, for bio-energy production is an extensively utilized knowhow. For the reason that the growing appeal for renewable energy manufacture it is enlarging more and more demand and thoughtfulness also amid result manufacturers. An importance of the incremental application of this expertise is the requirement to define the fundamental biogas potential for numerous solid substrates. In actual fact, this is an important parameter for measuring conceive, economic and handling matters for the full scale application of anaerobic digestion procedures. An amount of data having been produced, relationship of biodegradability data in the works is very problematic. This is not only attributable to the diversity of apparatus used, nevertheless likewise to the several dissimilar environmental circumstances and protocols that are used. Maybe, the inoculum-nutrients mix, liquid and headspace volumes, pH, headspace pressure and the recognition method can all fluctuate from on experiment to one more. In addition, the consequences are often obtainable in mutable units creation relationship very problematic. Owens & Chynoweth (1993), Angelidaki & Sanders (2004) and Hansen et al. (2004) were devoted to these features, planned full procedures for the strength of character of the biomethane potential of organic wastes, despite the fact (Fernandez et al., 2001, Neves et al., 2004, Raposo et al., 2006, Lin et al., 1999 and Raposo et al., 2006) concentrated on specific challenges like the substrate-inoculum relation or very specific substrates. Nonetheless, the meaning of a typical protocol is an encounter as the procedure of anaerobic dilapidation is an extremely complex and energetic system where bacteriological, biochemical and physico-chemical features are strictly related. The procedure encompasses the hydrolysis of multifaceted high molecular weight carbohydrates, fats and proteins or both obsessed by soluble polymers by way of the enzymatic accomplishment of hydrolytic fermentative microorganisms and the change of these polymers into organic acids, alcohols, H_2 and CO_2. Volatile fatty acids (VFAs) and alcohols are then transformed to acetic acid by the H_2 creating acetogenic microorganisms and lastly methanogenic bacteria change acetic acid besides H_2 gas into CO_2 and CH_4. The constancy of the procedure is reliant on the serious equilibrium that occurs in the middle of the symbiotic development of the main metabolic crowds of microorganisms i.e., acid creating microbes, obligate hydrogen producing acetogens and methanogens. In relation to this development, the explanation of an ordinary procedure for the meaning of BMP is powerfully demanded by both the expert and investigation world. By means of that essential, a task group (TG) on Anaerobic Biodegradation, Movement and Inhibition (ABAI) was planned to be formed by Rozzi and Remigi (2004). A quantity of dissimilar examines designated as ISO standards, have been expressed for the last 20 years (Mulleret al.2004). In the beginning, these approaches can be distributed in two principal groups, one group arrangements with the

meaning of anaerobic biodegradability of chemical compounds or plastic (ISO 14853-1999; ASTM D 5511-1994; ASTM 5210-1992; ASTM E 2170-2001; ISO 15473-2002) even though additional group compacts with the eventual biodegradability of multifaceted organic substrates and biogas manufacture (ISO 11734-1995; ISO/DIS 14853-1999). Essentially, they fluctuate for the investigational arrangement. This produced dissimilar consequences, in the main not equivalent. Furthermore, altogether these approaches hitherto described in official documents still encompass certain significant discrepancies or errors. Nonetheless, these approaches are used otherwise and frequently adapted by investigators to describe the anaerobic biodegradability of organic compounds. Consequently, it is estimation of the ABAI-TG that a normal protocol is desirable to amalgamate and standardize examines with the intention of gain similar consequences.

The consequence of a BMP test is the relationship of the methane (or biogas) formed from a given weight of an assured substrate. Gas can be restrained through different methods: volumetric approaches (characteristically acidic water dislodgment), manometric (purpose of pressure variation by transducers), gas-chromatographic methods with flame ionization (FID) or thermal conductivity (TCD) detectors. In this guiding principle the methane accrued in the headspace of the sealed vessel is restrained by gas chromatography (GC). Designed for that, an example volume of e.g., 100 mL ought to be composed with a gas-tight syringe and inserted interested in the GC. The attained peak area should be related to that attained by injecting the similar volume of a standard gas mixture of recognized composition. The standard gas mixture have a duty to be injected at the atmospheric pressure is 1 atm or 760 mmHg or 101,325 Pa, N/m^2 for the reason that question mark the gas sample is taken with a gas tight over pressurized syringe, and related with a gas standard injected underneath atmospheric pressure the methane (%) will be more than 100%. The volume of methane created is attained by multiplying the headspace volume by the percent of CH_4 in the headspace as established by GC investigation. Designed for journal and relationship with additional studies, the values should be considered to standard temperature and pressure (STP) (1.013×10^5 N/m^2, 273 K) conditions, i.e., rehabilitated to 0^oC and 1 atm. Information clarification and journalism. The BMP consequences must always be escorted by an unblemished explanation of inoculum supply, activity and VS or VSS satisfied, medium composition, substrate explanation, and dilutions used. The methane production profiles with respect to time together with the profiles for the blank and control assays should be presented. In the final report, the following items should be considered:

- date, time of start and end of the test
- tested substrate, amount or quantity and physical chemical characteristics
- inoculum, origin and activity, amount or quantity and chemical-physical characteristics
- test conditions: temperature, substrate/inoculums (S/I) ratio, volume of the vessel, number of replicates

- results of blank and controls methane production (report graphics)
- methane production in the triplicate and relative average and standard deviations for a complete statistical, (report graphics)
analysis of data obtained;
- specific methane production: this can be reported as volume of CH_4 per gram VS, or CH_4 per gram COD, or CH_4 per gram of sample.

2.6 The constant kh for a first order hydrolysis model

Results from BMP tests, if properly obtained and of good quality, can be used to obtain further information on the substrate studied like the hydrolysis rate provided that hydrolysis is limiting the anaerobic conversion process. In fact, using the first part of the experimental curve build for the determination of the ultimate methane production of a given substrate (e.g., the first five days of the example given in Figure 8)

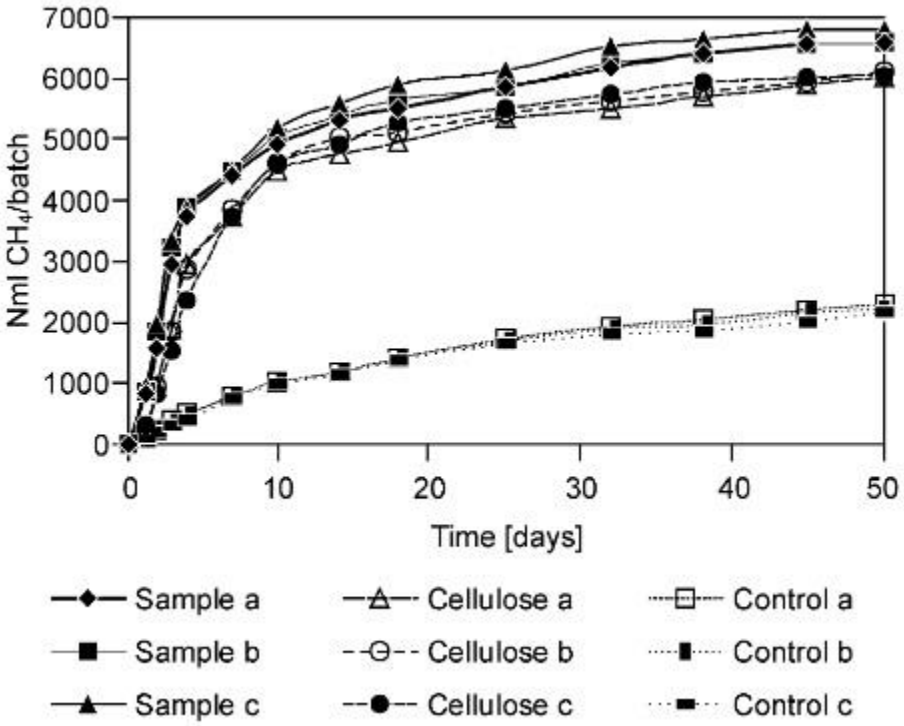

Figure 8 Methane production curves for triplicate samples of solid organic substrate (household), cellulose and inoculum (control). From Hansen et al. 2004.

It is possible to define the constant k_h (day 21) for afirst-orderr hydrolysis model (2.7).

$$\frac{dS}{dt} = -k_h S \tag{2.7}$$

Where, S is the biodegradable substrate, t is the time and kh is the first order hydrolysis constant. Once the variable are separated and integrated and the existing relation between the biodegradable substrate and the methane generated is taken into account, it is then possible to write is the equation (2.8)

$$\ln \frac{B_\infty - B}{B_\infty} = -k_h t \tag{2.8}$$

Where B_∞ is the value of the ultimate methane production and where B is the methane produced at a given time, t. Now, the value of the first order hydrolysis constant, kh, can be determined as the slope of the linear curve obtained. This value is characteristic of a given substrate and gives information about the time required to generate a given ratio of the ultimate methane potential (Mace et al. 2003).

2.7 Gompertz equation

Gompertz equation, particularly its modified form is widely used to describe growth and product formation data for various types of dynamically biological systems. In anaerobic digestion, it becomes an empirical representation of biogas/methane/hydrogen accumulation data (Chairat et al., 2016). Their analysis suggested that much more insightful mechanistic understanding of anaerobic digestion could be achieved by combined and systematic analysis of those experimental data using the best model from extended Gompertz models with the best one from substrate limiting type. The modified Gompertz model could be better in predicting biogas and methane production from swine manure. The difference in the measured and predicted gas yield was in the range of 1.2-1.5% when using first order kinetic model and 0.1% when using modified Gompertz model (Kafle and Kim, 2012). Apart from the specific methane yield and the cumulative methane yield, the duration of the lag phase (λ) is also an important factor in determining the efficiency of anaerobic digestion. The lag phase can be calculated with the modified Gompertz model (2.9) (Kafle and Kim, 2013).

$$G(t) = G_m \times \exp\left\{-\exp\left[\frac{R_{max} \times \mathrm{e}}{G_m}(\lambda - t) + 1\right]\right\} \tag{2.9}$$

where

G(t) = Cumulative biogas production at digestion time t hours (mL/g VS).

G_m = Biogas production potential of the substrate (mL/g VS).

R_{max} = Maximum biogas production rate (mL/g VS day).

λ = Lag phase (day).

t = Time (day).

e = exp (1) = 2.7183.

A nonlinear least-square regression analysis was performed using SPSS (IBM SPSS Statistics 19, 2010) to determine R_{max} and λ.

2.8 Solid-state anaerobic digestion ; SS-AD / high solid anaerobic digestion ; HSAD (M.O. Fagbohungbe et al., 2015)

In the 19th century, non-oxygen dissolution (AD) was discovered in the basic, using low solid anaerobic digestion (LSAD) methods (He, 2010; Mccarty, 2001). AD technology is increasingly accepted for the treatment of biodegradable organic waste (De Baere, 2000). Over the past 23 years, hundreds of large plants have been established across Europe. As a result, the biomass capacity will increase by several million tons per year. Subsequently, a high solids digestion (HSAD) was developed (De Baere et al., 2010). HSAD is a high-solid, oxygen-free microbial digestion system with low water content. May be called semi dry or dry system. In Europe, several sub-tank technologies such as the Dranco silo and the cylindrical Valcera (Li et al., 2011) have been developed to support less water degradation. (Garcia-Bernet et al., 2011). In addition to these technologies, water use is reduced (OLR). And reduce the need for heating. However, the recovery of methane is reduced and the evaporation of solids is less than 50% (Dong et al., 2010; Nagao et al., 2012). However, HSAD has difficulty in pumping water and equipment. Difficulties in pumping raw materials are influenced by total solids (TS). In the case of high solids, such as 30% -40% (TS) Pumping and mixing requires equipment complexity (Vandevivere et al., 2003). The residual organic material from AD process is called digestate and it contains nutrients, which are beneficial to agriculture as a nutrient source and/or soil conditioner. The HSAD system provides a better option for a cost effective digestate handling operation and a nutrient-rich digestate material. The amount of nutrient obtainability per gram of digestate is frequently cooperated contingent on the TS content of the organic waste. It is predictable that the accumulation of water will dilute the accessible nutrient content and successive dewatering possibly will decrease the concentration of the remaining nutrients in the solid portion. The HSAD digestate is additional compact on condition that external zone for nutrient adsorption and steady release of nutrients into the soil. With the intention of reduce the movement of nutrients, wastewater corporations regularly thicken digestates by adding polymers and additional thickening agent (Mangwandi et al., 2013; Watanabe and Tanaka, 1999). These chemical alterations are often managed previously dewatering to upsurge the solid content of the digestate to in the region of 15%-25%. Nutrient administration is indispensable for make the most of digestate consumption on land. HSAD make available an improved possibility for nutrient retention in arrears to the dryness of the digestate. This is for the reason that farming material, for the most part fiber can also work for as an adsorbent (Achak et al., 2009). Supplementary line of attack such as application of adsorbents have been

described to advance nutrient uptake and retention from wet digestates, on the other hand this methodology often increases the cost (Estevez et al., 2014).

Table 6 Comparison of LSAD and HSAD processes (HSADM.O. Fagbohungbe et al., 2015).

Parameter	LSAD	HSAD
Total solid	<10%	10%–40%
Operational mode	Single and multi-stage AD	Single and multi-stage AD
Feeding regime	Semi and continuous	Batch, sequential batch, semi and continuous
Biogas production	High moisture, High biogas production	Low, moisture, low biogas production
Volatile solid reduction	50%–70%	< 40%
Substrate loading rate	<7 gVS m^3/day	7–15 gVS m^3/day
Inhibition	More dispersion and diffusion	Less dispersion and high adsorption into organic material
Mixing device	Internal mixing device, liquor and biogas recirculation	Leachate and biogas recirculation, biogas and partial mixing
Heating requirement	High heating is required due to larger volume	low heating is required due to smaller volume
Operational problem	Pumping equipment is less sophisticated due to high moisture	Sophisticated pumping equipment is required
Substrate	Not suitable for hydrophobic substrates like the lignocellulosic materials	Most suitable for hydrophobic substrates
Digestate handling	Dewatering is required	Dewatering is minimal
Digestate quality	Less stable but nutrient content is high	More stable with low nutrient continent

Mixing in the LSAD system will reduce sedimentation. Karim et al., 2005a, b), but the HSAD system uses leachate flow to assist in mixing instead of stirring (Nkemka and Murto, 2013). Sponza and Agdag, 2004). However, HSAD has a challenging

proposition to lead to more research. In the field of high-quality HSAD digestion. The rate of lysine content on the size of the fermentation tank. Low water consumption and reduce the cost of HSAD degradation.

2.9 High solid anaerobic digesters

The design of the HSAD methane production system depends on the nature of the subtraction and the pattern of the production process. Non-oxygenated digestion can be divided into total solids, called total solids, called HSAD. According to Abbassi-Guendouz et al (2012), the HSAD process can be grouped into semi-HSAD (10% 20% solids) and HSAD (treatment of> 20% total solids). Currently, HSAD reduces the cost of raw materials and process production. (Et al., 2011). According to Vandevivere et al. (2003), the HSAD system divides the HSAD system into two types of digestion steps. The system differs in cost and rate. Organic Trucks (OLR) single stage shredders are inexpensive, but OLRs are limited when compared to multi-stage operations. Multi-stage reactors will incorporate two or more reactors to optimize the process (Vandevivere et al., 2003). Methanogenesis causes higher OLR resulting in more methane production.

2.9.1 Single-stage HSAD systems

Single-stage degradation is widespread in Europe, reaching 90 percent in 90 percent, using a 50 percent reduction in non-oxygenation (De Baere et al., 2010). The Valorga, Dranco and Kompogas digesters are examples of continuous oxygen systems, while the German rectangle is a batch system. Total solids are 20% to 40%

The valorga system

Valorga systems have been implemented at 25% and 35% of TS. The centrifuge of the reactor divides the two-thirds of the diameter of the cylindrical reactor (Figure 9). The wall supports the flow of organic matter. The area of the digestive tract is wider (Li et al., 2011). At the base of the digesters, there is an inlet and outlet valve for the inlet and outlet of the system. Valorga has an internal nozzle at the base of the digesters. This will allow the produced gas to flow through the high viscosity liner. The biogas circulation increases the distribution of the lysine to the microbial cells. However, this system has to put pressure on the biogas, resulting in high power consumption resulting in high management costs. Injections may also be clogged with organic materials (Li et al., 2011).

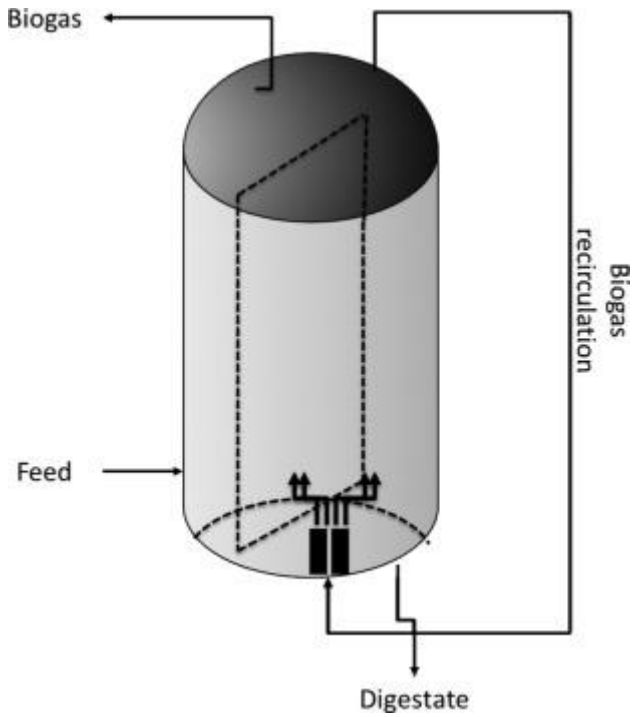

Figure 9 Valorga high solid anaerobic digester (Li et al., 2011).

The Dranco system

The Dranco system has no internal mixing mechanism. The system is vertical, with a silo-shaped base (Figure 10). Similar to the Valorga system, this technology works at 40% TS. The Dranco system mixes the substrate before the raw material into the AD system. Valorga system with nozzles to mix. The mixture is a mixture of new subset and recycled 1: 6 ratio before being transported to the next batch (Martin et al., 2003). This method reduces the mixing energy consumption. This may cause the conveyor to mix and then to discontinue. The amount of raw material added to the Valorga system (De Baere, 2008)

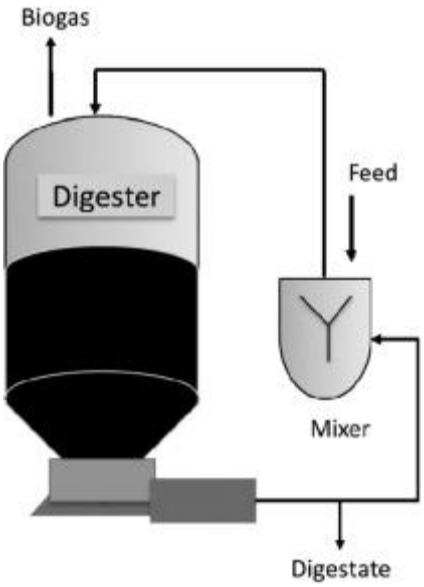

Figure. 10 Dranco high solid anaerobic digester (Martin et al., 2003).

The Kompogas system

In 1980, Switzerland tested the Kompogas system by adjusting the horizontal flow of liquid with a vertical mixer (Figure 11), resulting in a dense solid in the bottom. Increased contact between microorganisms and organic matter and decomposition time (Li et al., 2011). The system utilizes a new mix ratio with a DRANCO-like recycled sub-state. As a raw material, it helps maintain the amount of active microorganisms within the reactor. The digesters work between 23% and 28% TS.

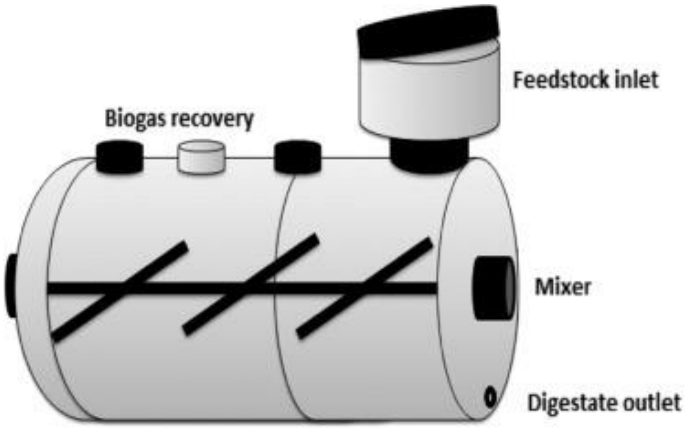

Figure 11 Kompogas reactor digester (Li et al., 2011).

The rectangular batch digester

The rectangular batch digester is similar to the bioreactor system, similar to organic landfill leachate systems (Berge et al., 2009). The system releases leachate to the new lining and recycling before it enters. This AD solution will not solve the problem of mixing the sub-strands with water. And the spread of microorganisms is not uniform. The organic degradation of the rectangular batch digester requires drainage and outflow, which may result in the methane production halting. The system is the cheapest process in the market. But commercially, it requires more research and development. This system works at TS% 40 (Li et al., 2011).

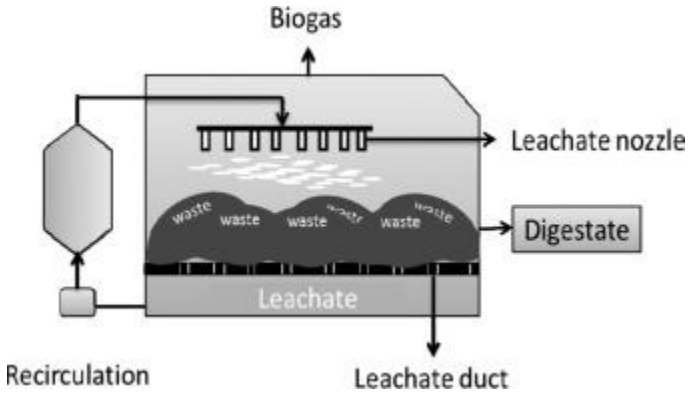

Figure 12 The German rectangular batch digester (Berge et al., 2009).

2.9.2 Multi-stage HSAD systems

Multi-stage HSAD research has evolved steadily. The system has been successful in many countries, such as in Europe, Asia and Canada. The popularity of commercial multi-stage degradation systems is limited. There are many types of HSAD, such as Biotechnische Abfallverwertung (BTA), Linde-KCA, Super Blue Box Recycling (SUBBOR), as well as systems. Batch anaerobic composting (SEBAC)

Biotechnische Abfallverwertung (BTA) system

The BTA multi-stage system was created for the purpose of community solid waste (MSW). The system separates solids and liquids using sprayers and hydrocyclones. The treated leachate is mixed with solids and pumped into the tank for hydrolysis (Figure 13). The remaining liquid from the hydrocyclone and the hydrolysis system are transported to another tank. However, the VS in this system decreased between 70% and 75%, indicating that the degradation in such systems is still low in the use of solid waste. (Chavez-Vazquez and Bagley, 2002; Williams andDavis, 2005)

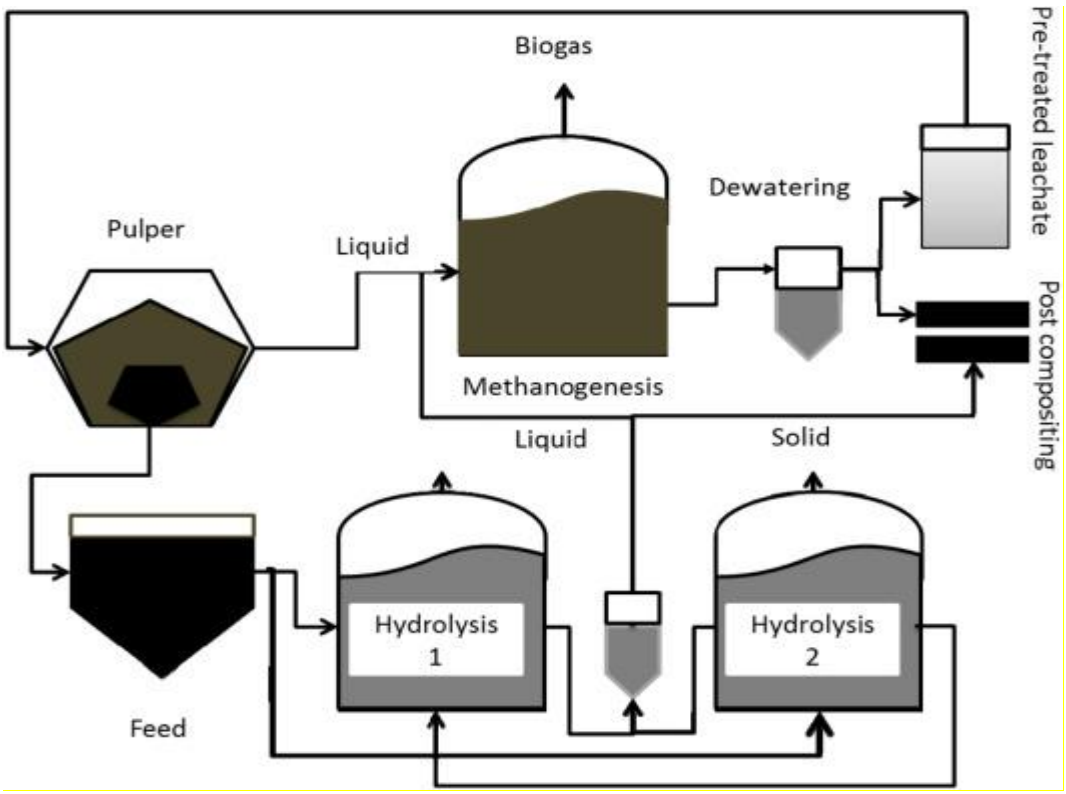

Figure 13 Process scheme of BTA multi-digestion (Williams and Davis, 2005).

Linde-KCA system

Linde-KCA is a two-stage digestion system, air and aeration (Williams and Davis, 2005) (Figure 14). A single plug flow system requires sludge preparation. AD is expensive to operate (Curtis, 2010). As a result, aerobic therapy can reduce the energy cost of raw materials, depending on the duration of the air. This system can operate in the range of 15% to 40% of TS.

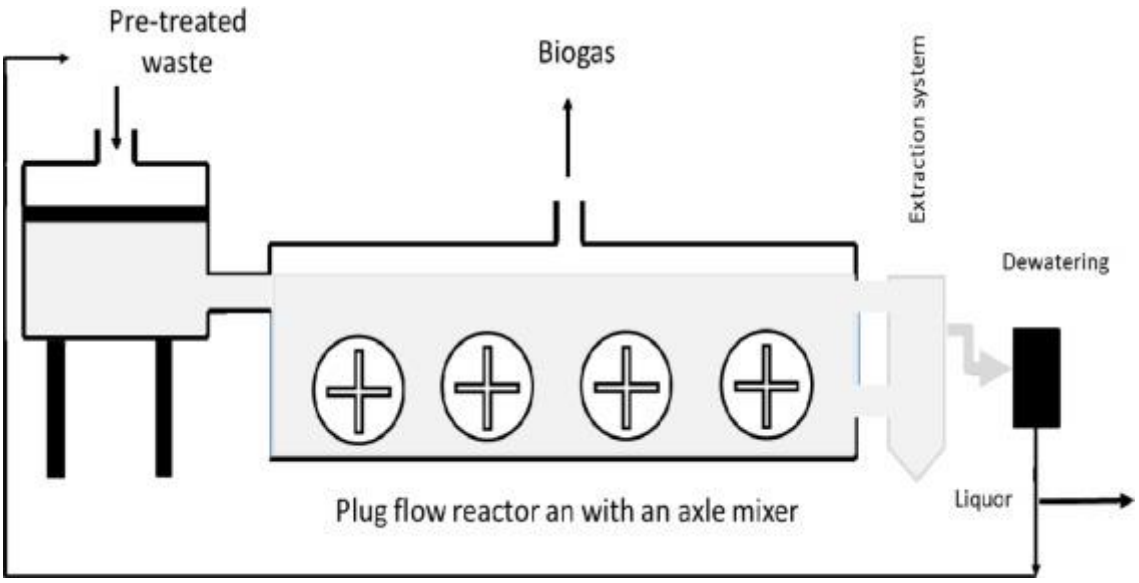

Figure 14 Linde-KCA two-stage dry digester (Curtis, 2010).

Super blue box recycling (SUBBOR) system

The SUBBOR digestion system uses 55-63 bar steam to produce microbial fermentation prior to production (Figure 15). This method is an improvement from the Linde-KCA system. Stays in a small bucket. And want to reduce the energy mix of raw materials. Broths et al., 2011). However, spraying with steam requires more energy, which may make the process less sustainable for small operators (Vogt et al., 2002).

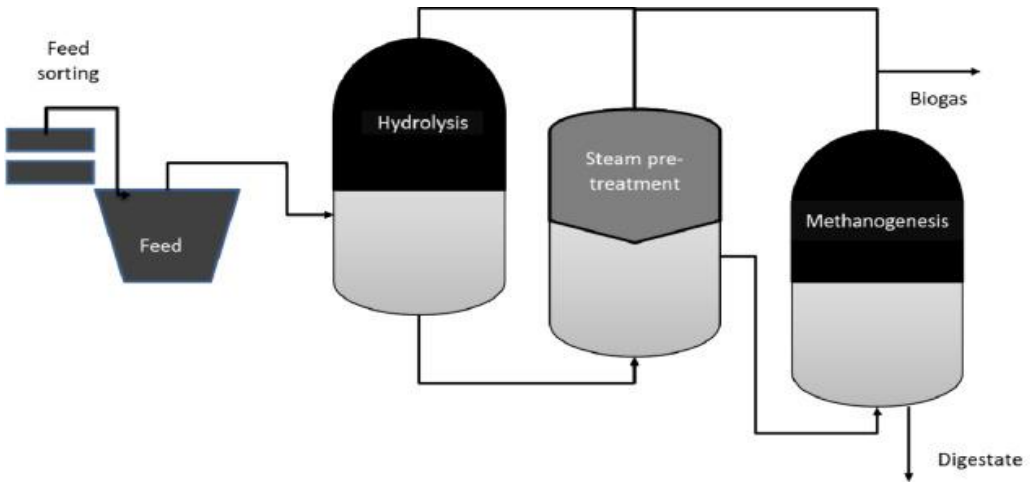

Figure 15 A two-stage SUBBOR anaerobic digestion process (Brodeur et al., 2011).

Biopercolat system

Biopercolat is the first step in aerobic digestion. At this stage it improves the efficiency of digestion and reduces the cost of energy. The second step is to use an anaerobic digestion system. Unlike the Linde-KCA system, before entering the second stage, AD is sprayed with leachate and sub- The liquid part is then conveyed to the methane production stage (Figure 16).

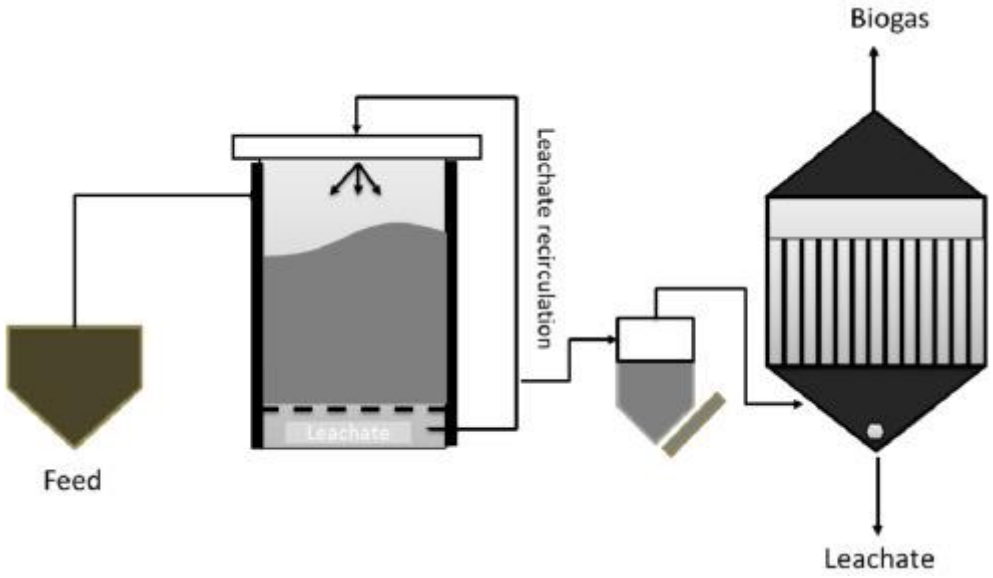

Figure 16 A schematic of two-stage Biopercolat process (HSADM.O. Fagbohungbe et al., 2015).

Sequential batch anaerobic composting (SEBAC) system

This system is intended to reduce the handling of high-volume solids (Chynoweth et al., 1991). Substrates are transported into the first reactor together with leachate injection derived from previous digestive tracts in the first and second tanks in the air-degraded system. In the next step, the lining of the first two tanks will be transported into the third tank for further methane production. In this tank, the leachate is sprayed only from the process of this tank (Figure 17). This technology will leachate until the efficiency of the production is reduced. This system takes a long time to produce methane. Research has reduced the process time from 200 to 100 days. However, the production time is still long for commercial production. SEBAC technology is still under development.

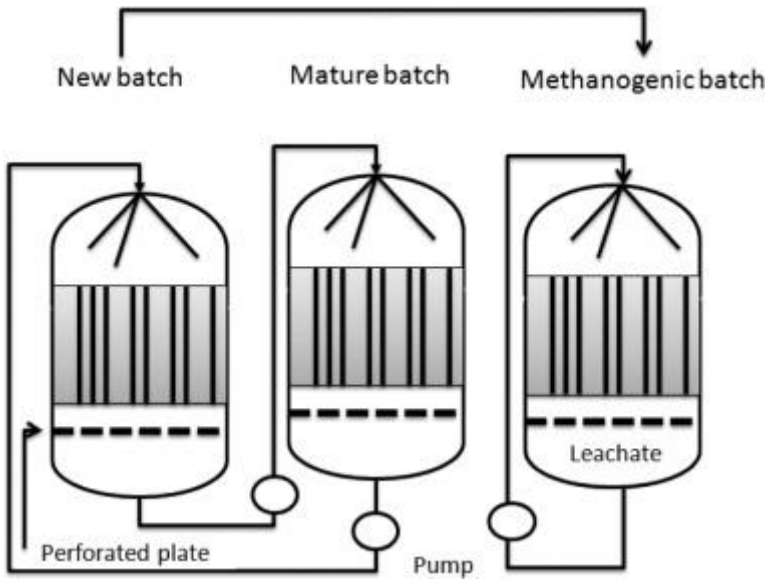

Figure 17 SEBAC process diagram (Fdéz-Guëlfo et al., 2010).

2.10 Factors affecting methane production within HSAD

Highly digestible solids degradation systems are limited in the distribution of sub-samples. Relevant microorganisms and volatile fatty acids (Nagao et al., 2012) due to the spread of VFAs to the microorganisms. Especially acetic acid to microbial cells, which results in the production of methane. The water is taken. Low dissemination has the potential to significantly influence methane production during the HSAD. High toxicity levels, such as ammonia, fatty acids, D-limonene and furfurals, are responsible for the inhibition of methanogenic bacteria.

2.10.1 Fatty acids

Short chain fatty acids, otherwise known as volatile fatty acids (VFAs), and long chain fatty acids (LCFAs) are produced during the acidogenesis and acetogenesis stage in the AD of organic substrates (Madsen et al., 2011).

Volatile fatty acids (VFAs)

Organic compounds are small soluble molecules that are formed by hydrolysis processes. Facultative bacteria are used as food and energy sources by early stage of digestion. The result is a volatile fatty acid (Buyukkamaci and Filibeli., 2004), which contains up to five carbon atoms, such as acetic acid (CH_3COOH), propionic acid (C_2H_5COOH), butyric acid (C_3H_7COOH), etc. And other substances such as ethanol (C_2H_5OH), hydrogen (H_2), carbon dioxide (CO_2). Acid forming bacteria, the type of bacteria that are called differently depending on the type of organic substances

(Zahedi et al., 2013b). Later on, volatile organic acids are formed by acetic bacteria (Acetogenic bacteria) converted into acetate forms, hydrogen and carbon dioxide. This is an important compound in methane production. This reaction is an important step in avoiding the accumulation of volatile organic acids and hydrogen in quantities high enough to inhibit methane production. HSAD schemes are recognized to products lower quantities of methane level at upper organic loading rates (OLR) among 8 and 14 gVS m^3/day (Dong et al., 2010; Nagao et al., 2012). These intermediates may stick to the solid part of the organic material. Due to the deterioration of the decline in the proportion of lower substrates (Dong et al., 2010; Nagao et al., 2012). The HSAD system may be more stable than the LSAD system, although the system has a high VFA concentration, which is high in acid, thus inhibiting microorganisms (Dong et al., 2010; Nagao et al., 2012). Sponza and Ağdağ (2004). The leachate turnover was evaluated and this turnover was used to reduce the VFA inhibition in HSAD system.

Long chain fatty acid (LCFA)

LCFAs are formed throughout the biological breakdown of lipid-containing substrates. Lipid is transformed to LCFAs and glycerol throughout anaerobic hydrolysis even though LCFAs (oleate, stearate, and palmitate) are transformed into hydrogen and acetate over and done with the oxidation pathway (Weng and Jeris, 1976). On the other hand, LCFAs have been described to obstruct methanogenesis by misrepresenting the electron transport scheme in the cellular membranes of the microbes (Hanaki et al., 1981; Rinzema et al., 1994). Sousa et al. (2013) described an extreme tolerance concentration of 1 mM of LCFAs for methanogens.

2.10.2 Temperature

Because the operation of the HSAD system under the mesophilic temperature has a low startup. The HSAD operation at thermophilic temperature (55 °C) was subsequently developed and accepted as suitable for the HSAD process at turbulent temperatures. Underneath anaerobic circumstances, the collaborations between microbes and organic substrates are in need of on temperature. Bacteria can be separated into two core groups: mesophilic and thermophilic microflora (Fernández-Rodríguez et al., 2013). Thermophilic microbes are known to be active at temperatures of 50–60 °C and AD at these raised up temperatures has been described to rise degree of methane manufacture and reduce hydraulic retention time (HRT) (Fernández-Rodríguez et al., 2013; Hidaka et al., 2013). Thermophilic temperature can be accelerated metabolic activities of anaerobic digestion. It also benefits the reduction of pathogen contamination during anaerobic digestion. Increased investment budgets to increase operating heat HSAD system can compensate for increased gas output. The

operation of the thermophilic system for community organic waste treatment has been proven to be reliable and acceptable. The yield of biogas from the anaerobic degradation of OFMSW at thermophilic temperatures was significantly higher compared to the operation at the mesophilic temperature. The influence of thermophilic functional temperatures is not only limited to increasing the metabolic actions of microbes, on the other hand furthermore develops the solubilization of organic substrates. For example, Battistoni (1997) described that upper temperatures rise the solubility and viscosity of organic substrates throughout AD. This can be clarified by the frequent evaporation and condensation of water within the AD system. Water is known to evaporate from 20 °C and, as it condenses, the water molecules flow through the wall of the digester into the scheme (Vieira da Silva et al., 2013). Moreover, owing to the low water content in HSAD scheme, upper temperatures have been described to rise the viscosity among the substrate constituent part, in that way increasing diffusion of organic substrates to bacteriological cells (Battistoni, 1997; Bollon et al., 2013). The disadvantage of operating AD under thermophilic conditions is the high energy demand as well as the lower diversity of robust methanogens desirable for dependable methane manufacture (Biey et al., 2003). Even though, there are information of AD disappointment underneath thermophilic temperature, this suitcases are not in a straight line related with the in service temperatures, on the other hand rather the high OLR and imbalances in the carbon to nitrogen proportion (Hidaka et al., 2013; Lianhua et al., 2010). For example, Fernández-Rodríguez et al. (2013) established that HRT was reduced by 52% underneath thermophilic HSAD for MSW with 20% TS. Conversely, the presentation of mesophilic temperature is more extensive as soon as compared to thermophilic AD systems. Because mesophilic temperatures enrich the multiplicity of methanogenic microorganisms, nevertheless, the rate of methane production is not as fast as thermophilic AD. The mixture of thermophilic and mesophilic temperature in a binary phase AD procedure could be responsible for a improved selection for enhancing HSAD, meanwhile the methanogens are more robust at mesophilic temperature (Biey et al., 2003).

2.10.3 Inhibition

Many researches are concerned. Likewise, LSAD may be more likely to occur in HSAD systems due to lower OLR and moisture content in the HSAD system (Vandevivere et al., 2003). The processes that lead to the production process. Methane between different groups of microorganisms, such as biochemical reactions and the results of various parameters. It may affect the stability and efficiency of AD and methane production. In the methane production process. The restraint is about. The increased concentration of intermediate products in the fermentation process is exaggerated (Chen et al., 2008). For example, compounds are formed in the intermediate stage during fermentation, such as inorganic nitrogen VFAs. And long

chain fatty acids (LCFAs). These substances are produced by the interaction between microorganisms and organic matter. Inorganic nitrogen and LCFAs are protein and fat-rich nutrients. These compounds may be highly toxic (Koster and Lettinga, 1988; Rinzema et al., 1994). In contrast, compounds such as D-limonene, furfural, and phenolic compounds, which are organic compounds but not from the interaction of microorganisms. It inhibits the growth of microorganisms (Mizuki et al., 1990). Nevertheless, the researchers found that the low HSAD liquid composition may result in decreased propagation and reduced mobility, resulting in reduced efficacy of the toxin or inhibitor of the microorganism. Achak et al. (2009) Reported that banana peels adsorb phenol from water from olive plant. It shows that the surface of some agricultural products may have some toxic properties. Also found the inhibitory substances can not be distributed uniformly in the absence of agitation (Martin et al., 2003; Vavilin et al., 2003).

Ammonia

Proteins are essential for the growth of microorganisms. We can divide the two main types of inorganic nitrogen into ammonia (NH_3) and ammoniam (NH_4^+). The Nitrogen inorganic becomes an inhibitor of microbial growth (Koster and Lettinga, 1988). Formation (NH_3) and (NH_4^+) Research has shown that (NH_3) is more toxic (NH_4^+) because (NH_3) can pass through the cell membrane, which limits the cell entry. Cause imbalance of protons It can also interfere with the enzymes used in the microbial metabolism process. Sung and Liu, 2003). Many studies have used more than one subtype to solve the problem of toxicity. (NH_3), a digestive enzyme that interacts between two subunits. Organic compounds with high carbon content are often digested with high levels of protein. For example, Yangin-Gomec and Ozturk (2013) reported a methane production increase of 1.2 times when corn was digested with animal manure. Both subtracts can balance C / N, which reduces toxicity (NH_3).

D-limonene and furanic compounds

Some compounds can inhibit methanol production. D-Limonene is a colorless and liquid substance. The substance is in the Terpene cycle (Wilkins et al., 2007). These compounds are considered to be disinfectants (Mizuki et al., 1990) and have been studied to degrade orange peels. Methane production at OLR is higher than 2.0 g per liter per day (Martín et al., 2010). Microbial cell degradation mechanisms are similar to other hydrocarbons as D-limonene is a liquid hydrocarbon (Ruiz and Flotats, 2014). D-limonene solubility enhances Spreading it into microbial cells makes D-limonene permeable to the membrane more easily. Leakage and cell degradation (Burt, 2004). Removal of D-limonene can be done in a number of ways, including eliminating the precipitate before the AD process (Martín et al., 2013; Srilatha et al., 1995). Or

solvent extraction. It requires investment in energy supply for disposal. Another method is to submerge the fungus into the digestive tract. Martini et al., 2013; Srilatha et al., 1995). Other compounds that inhibit methane production are furanic substances such as furfurals and 5-hydroxyl methyl furfural (5). -HMF). This substance is a dehydration product of hemicellulose (Ramos, 2003). Hendriks and Zeeman (2009) found that hemicellulosic monomers these can inhibit the bacteria that do not use oxygen. These vaccines cause cellular secretion by destroying DNA and inhibiting glycoprotein-related enzymes (Palmqvist and Hahn-Hagerdal, 2000). According to Barakat et al. (2012) when the concentration of furans is ≤1 gram / liter, AD will be stable. For furfurals and 5-HMF, respectively, the methane content decreased (Badshah, 2012).

2.11 Optimizing HSAD through technological integration

AD technology has evolved over the past 20 years, for example. Preparation of raw materials for glucagon cellulose before ethanol production (Hendriks and Zeeman, 2009) has recently become a useful method for the dissolution of lignocellulosic raw materials before entering the AD process or digestion technology. Substrate in combination with reactor mix or thermal digestion to promote large AD operations for technology to optimize the HSAD system. Subtraction is used more when it is found. Mono-digestion The HSAD system is also capable of maintaining the stability of the system. It also adjusts the temperature to suit the microorganisms in each system.

2.11.1 Co-digestion

The common decomposition of the substrate is an example of the development of AD technology. It allows the system to mix and decompose more than one type of organic material. The advantage is that it reduces the defect of only one organic substance. Increase the nutrient supply of other microorganisms. Adjust the balance of C/N derived from the substrate. For example, Kim and Oh (2011) reported a decrease in VSC content in organic material by more than 80% on paper and scrap. Diets are degraded at a rate of 40%. TS is a source of protein to generate NH_3 and high-carbon paper fractions. The combination of these wastes can make the proportion of carbon to nitrogen equivalent (Zhang et al., 2011). Co-digestion is beneficial for all forms of AD, as reported by (Navaneethan et al., 2011). Zhang et al., (2012) Classification of organic substances by nutrient and energy values in three categories: (1) energy (2) nutrient content and (3) methanogens, such as degradation of waste water from pig farms and food waste. Nutrient-rich diets and high energy, respectively (Zhang et al., 2011), as well as manure degradation Food waste and sludge from solid waste including energy nutrients. Angelidaki and Ellegaard, 2003; Navaneethan et al., 2011). The above research has contributed to the improvement of the HSAD system. Principles to consider when using digestion. HSAD is the proportion of organic synthesis

(Sosnowski et al., 2003), as it affects the balance of the microbial populations. Nutrition and organic carbon. Maranon et al. (2012) reported that degradation of dairy cattle waste and sludge using 70:10:20 and 70:20:10 ratio, it was found that the ratio of 70:20:10 was able to produce methane by 22%. To reduce the inhibition of C / N imbalance which results in potential toxins. And improve the HSAD system.

2.11.2 Mixing technologies

Karim et al. (2005a) found that mixing was significant when the TS of the raw material was higher than 5%. OLR Methane production and solid retention time are described as three main factors in the design of AD systems that require continuous methane production (Karthikeyan and Visvanathan, 2013). Advantages of the interaction between microorganisms and materials. The combination of substrate and microbial cells in the effective AD range increases the exposure and can be achieved by Install the tank mixer. Recirculation of leachate from tank subsystem and the biogas pump produced through the system (Karim et al., 2005a). Research has shown that fluid turnover is significantly higher than 15% TS of gas in HSAD (Karim et al., 2005b).

Mechanical mixing

Non-oxygenated decomposition Continuous stirring tanks (CSTR) are often built with internal mixers to enhance the interaction between microbial and organic molecules. CSTR systems are homogenizations. In Europe, the use of humus as a ligand is most commonly used in this system (De Baere et al., 2010). In addition, this combination improves the interaction between microorganisms and the subtypes. This also reduces bubble formation and fragmentation within the AD system. However, there are indications that this method is not suitable for high protein content because high concentrations of ammonia can increase toxicity. Pommier et al., 2007). Although internal mixers are not widely used in HSAD, Kompogas has built a slow-spinning inner core mixer. In addition, the Dranco and Valorgas systems have an external mixing device to homogenize before being fed to the digesters (Fruteau de Laclos et al., 1997).

Fluid mixing through recirculation

There are several ways to optimize the mix in the AD system. Like fluid circulation in the system. Fruteau de Laclos et al., 1997; Shahriari et al., 2012), such as the Valorgas digester, are injected at the base of the reactor to release biogas. Fruteau de Laclos et al., 1997). This design improves the mixing of materials in the digesters (Koster and Lettinga, 1984). Another way is to use leachate from renewable systems to increase density microorganisms (Bolzonella et al., 2003). Due to the high water content of leachate, organic degradation was faster (Bolzonella et al., 2003). In the case

of HSAD, the rectangular German design is a good example (Li et al., 2011). The leachate from the system flows from the bottom of the system to the top of the system again (Pommier et al., 2007 Sponza and Ağdağ, 2004). Recirculation of leachate reduces the mixing load from the mixer in the system (Bollon et al., 2013) and helps to maintain the moisture balance in the HSAD system. The potential for leachate leaching in the HSAD system depends on the properties. Sponza and Ağdağ, 2004; Bollon et al., 2013). In the research, higher solids content increased the viscosity of the sub-Battistoni, 1997; Bollon et al., 2013). Increased viscosity reduces the gaps in raw materials. The leaching of water slowly leaked, found that lignocellulose Tends to hydrophilic due to the polymerized outer surface, which may increase the space between the particles, regardless of the amount of TS, will increase the permeability of the leachate. However, fluid turnover in HSAD may be harmful to methane production, especially when the inhibitor compounds such as Ammonium and Chloride dispersed from leachate aid (Sponza and Ağdağ, 2004; Chen et al., 2008). In some studies, for example, Shahriari et al. (2012) found that leachate flow Similarly, Sponza and Ağdağ (2004) reported ammonia abundance during municipal sewage effluent discharge, which could have reduced the incidence of methanogens, especially when using only one food source. Some researchers have solved this problem by circulating old lithotripsy and leachate with fresh ingredients, for example Dranco and Kompogas (Li et al., 2011). This system does not mention the digestion or mixing process before the AD process, but it is expected to use the fermentation method to produce fresh manure (Bustamante et al., 2012). J. Mumme et al., (2010) reported that an anaerobic solid-state (UASS) upflow reactor acts to degrade solid biomass by using a stream of water flowing from the bottom up and mixing it with a high-purity subassembly. This process reduces bubbles from leaching motion. The combined function of the UASS reactor is divided into three parts: the liquid zone below the liquid top. The high solids content of the lining is in the middle of the liquid part. Solids are limited by the sieve in the center of the UASS reactor. The sieve acts as a 3-phase separator and allows the solid to undergo organic leaching. Leaching reduces the concentration of VFA, thereby reducing the growth of microorganisms.

Gas mixing

Gas mixing can be unconfined or confined. In unconfined mixing, biogas is collected from the top of the digester and pumped at the bottom through nozzles. The bubbles rise in columns via buoyancy and transfer momentum to the surrounding sludge. This momentum transfer takes place due to the push force that the bubbles exert to the surrounding liquid, and the riptide effect arising from the low-pressure region created by the motion of the bubbles. In confined mixing, biogas is collected and injected in the same way, but the discharge takes place inside confined

tubes within the digester. This generates a forced sludge flux throughout the tubes, which in turn creates convective currents out of them (Tchobanoglous et al., 2010).

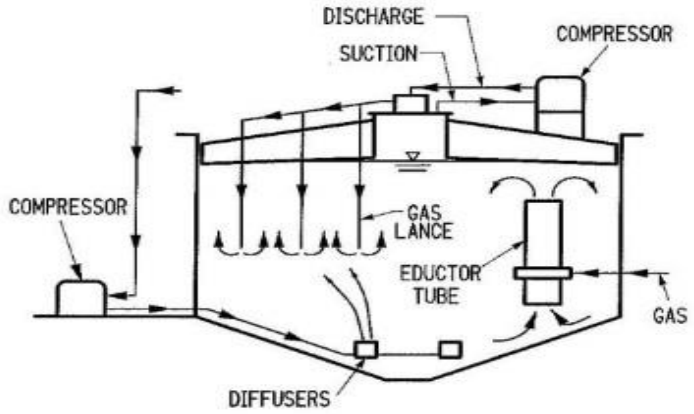

Figure 18 Gas Injection Systems (http://www.walker-process.com/pdf/Anaerobic_Digester_Mixing_Systems.pdf.)

Gas Injection Lance type is an example of an unconfined gas injection system that marketed initially as the Perth System and later copied by a number of competitors. Shear Box Diffusers is an example of an old technology gas injection system. Eductor Tube Systems is an example of a confined gas injection system that creates a pumping action to produce mixing through continuous or intermittent gas injection. Gas mixing involves recirculating a fraction of the digester gas through the digesting sludge via a compressor and a series of lances and nozzles. Sequencing the gas flow to various points causes mixing action to be distributed throughout the tank. Gas mixing – Bottom diffusers, all diffusers receive and discharge equal amounts of compressed gas, creating a rising gas column. Gas flow must be checked periodically, because diffusers are prone to plugging. Plugging can be cleared by directing entire gas flow through affected diffuser or by flushing it with high pressure water. Bottom diffuser mixing systems include diffusers on the floor. Gas mixing-Bubble gun system, Re-circulated gas is continuously fed to bubble generator and intermittently discharged into stack pipe as a large piston bubble. Piston bubble fills the entire cross section of pipe, driving out liquid as it rises and creating a siphon. As one bubble leaves stack pipe at the top, another enters from generator for both continuous mixing and prevention of solids settling. Large bubbles burst as they leave liquid surface, creating substantial turbulence that prevents scum buildup. Gas lifter, Gas is injected into a vertical tube via lances typically below the midpoint of draft tube. As the gas is released, it carries solids upward through draft tube, drawing in more solids at the base of tube. Solids that leave the top of tube flow away radially. Large tanks are equipped with multiple draft tubes; smaller vessels typically contain a single tube located in the center. Advantages of gas mixer are the compressors are mounted externally as is most of the pipework, easing

the maintenance requirements. With some systems, a separate mixing chamber is provided which is accessible from the outside of the tank for maintenance. And disadvantages are initial purchase cost is slightly more expensive than other systems but whole life costs are usually the lowest.

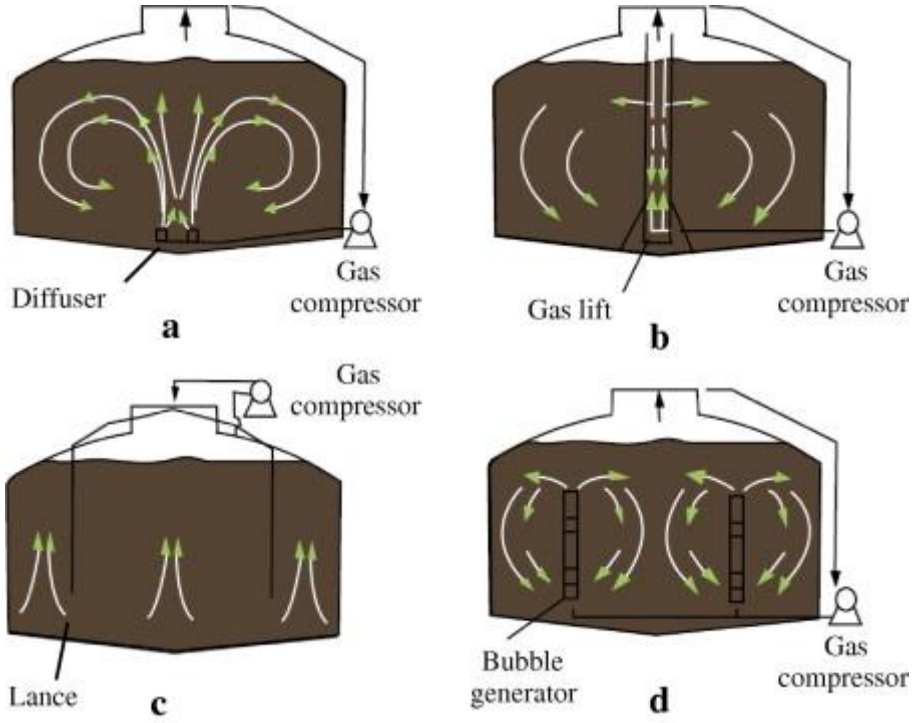

Figure 19 Schematic diagram of four gas mixing designs: (a) bottom diffusers, (b) gas lift, (c) cover mounted lances, and (d) bubble guns (Binxin Wu., 2014)

2.11.3 Attached microbial growth

Attached microbial growth processes have recently become increasingly important in water and wastewater treatment. Research has significantly advanced biofilm technology over the past decade. Despite this progress, the basic conceptual assumption of a biofilm is still in question and therefore needs more investigation. The general concepts applied to biofilms and the associated kinetic models will be reviewed in the following sections.

Biofilm

ZoBell (1943) first suggested that nutrients in very dilute nutrient solutions may be concentrated on solid surfaces by adsorption, thus enhancing the bacterial activities. It was also pointea out by ZoBell that solid surfaces retard the diffusion of exoenzymes away from the cell thereby promoting the assimilation of those

nutrients which may have to be hydrolyzed extracellularly. Marshall et al. (1971) confirmed ZeBell's suggestion that bacterial sorption occurs in stages. The bacteria are first weakly attached to a surface (reversible sorption), and after several hours became firmly attached (irreversible sorption). Daniels (1972) concluded that the adsorption of cells onto surfaces is dependent upon the microorganism, the adsorbent, and the environment. Environmental factors responsible for this process include: hydrogen ion concentration, salt concentration, agitation, time of contact and temperature. Characklis (1973) postulated that initial deposition of organisms is related to the characteristics of the attachment surface and the shear force at the surface. Sutherland (1983) demonstrated that the secretion of polysaccharides or other carbohydrate-containing polymers by many adherent microorganisms play an important role in the attachment process. The structure, function, genetics, and morphologic aspects of known proteinaceous adhesive materials of bacteria were discussed by Jones and Isaacson (1983). Audic et al. (1984) reported that the specific activity of bacteria increases due to attachment. Switzenbaum and Eimstad (1987) noted that 40-50% of the biofilm is due to inorganic material, with increasing amounts found at increased loading rates. This ash content is most likely due to chemical precipitation resulting from nutrient salts used in the experiments.

Biofilm Models

Atkinson and Davies (1974) principal industrialized a microbial biofilm classical integrating together dispersion and Monod-type substrate operation equations which was consequently adapted by Williamson and McCarty (1976a, b). Harremoes (1976) simulated thebiofilm kinetics by a pore diffusion model and found that zero order heterogeneous reactions in a pore will lead to a bulk half-order reaction and that first-order heterogeneous reactions in a pore will lead to a bulk first-order reaction. Rittmann and McCarty (1981) divided the biofilm model into three categories according to the substrate concentration profiles within the biofilms. A completely entered biofilm remains unique with a constant substrate concentration which is the same to the bulk solution concentration. A deep biofilm is one in which the substrate concentration decreases asymptotically to zero within the biofilm. The flux into a deep biofilm is the maximum possible. The shallow biofilm is an intermediate case in which the substrate concentration does not decrease to zero at the wall. The diffusion of substrate into the biofilm may be rate limiting and substrate conversion efficiency will be significantly reduced if the bacteria inside the biofilm cannot be reached by the substrate. On the other hand, the out-diffusion of the products is of equal importance. Riemer (1977), Riemer and Harremoes (1978) and Arvin and Kristensen (1982) reported a pH increase in the biofilm due to alkalinity production during the denitrification process. Similarly, nitrification 0% Plagiarism products acidity in addition let down the pH interior the biofilm. This build-up of alkalinity or acidity,

which may inhibit microbial activity in the biofilm cannot be detected from monitoring the alkalinity or acidity of the bulk liquid. The gaseous end products of anaerobic fermentation, CH_4 and $C0_2$, represent two completely different situations due to their difference in solubilities and their chemical reactivity. $C0_2$ is very soluble and its buildup will decrease the pH, whereas CH_4 is very insoluble and may form gas bubbles within the biofilm. Harremoes et al. (1980) suggested that a gaseous product of low solubility may cause bubble formation and increase the sloughing of the biomass. Methane, which is much more insoluble than $C0_2$, can be expected to form bubbles when supersaturation in the bulk solution is reached (Henze and Harremoes, 1983). Switzenbaum and Eimstad (1987) reported channels and holes which probably occurred from gas bubbles ripping through the film. The "outgassing" of product gas from the films, therefore, deserves more recognition as one of the rate-luniting steps. How the formation of bubbles might affect the reactor performance is still unknown, but the

effect may be more significant at higher organic loading rates. In fully penetrated biofilms the effect is not clear. In deep (thick) biofilms with substantial diffusional resistance, the outdiffusion of bubbles may break up the diffusional pattern. Microcurrents generated by bubbles movements may increase the apparent diffusion rate and make the diffusional resistance less significant. However, the sloughing of biofilms caused by release of the biogas bubbles may be more damaging in the deep biofilms.

2.11.4 Single and multi-stage AD systems

Over 95% of commercial AD plants are operated as a single stage system, principally because two stage systems are more expensive to run (Lissens et al., 2001). The two-stage systems have been reported to be more efficient because it allows the separation of the acid and methane producers, thereby reducing the impacts of pH fluctuations and potential fermentative inhibitors (Demirel and Yenigün, 2002). According to Llabrés-Luengo and Mata-Alvarez (1988), two-stage stabilization of feedstock would be the most suitable configuration for HSAD. Unlike a single stage system, the two-stage AD system can incorporate two different operating temperatures. In a study involving the performance of five different reactor configurations for AD of substrates, the report showed that the two-stage system out-performed single-stage digestion with higher COD removal (Azbar et al., 2001). With regard to HSAD, the choice of digester in a two-stage system must incorporate the necessary solutions needed to enhance methane production. In recent years, several studies have been carried out on multi-stage AD with various digesters including two or more CSTRs, CSTR and high-rate digesters (HRD), particularly anaerobic filter and up-flow anaerobic sludge blanket (UASB) system (Table 6.1). Unlike other high-rate digesters, the UASB system has been extensively integrated with other digesters owing to higher efficiency, flexibility and simplicity of operation (Chong et al., 2012). For example, the

integration of leachate bed and the UASB system for the HSAD of blue mussel and reed was investigated (Nkemka and Murto, 2013). The leachate bed enhances accumulation of leachate to the base of the digester, which invariably will be pre-treated by pumping it through the USAB digester before reintroducing it into the leachate bed system. The leachate bed is similar to the German garbage type rectangular batch digesters in which the solid and liquid phases are demarcated by perforated layer (Sponza and Ağdağ, 2004; Pohl et al., 2012). This perforated surface allows moisture to trickle to the base of the reactor for easy collection and recirculation, particularly within the HSAD system (Macias-Corral et al., 2008). Similarly, the combination of an up-flow solid state and anaerobic filtration has been reported to optimize the AD of wheat straw, thereby increasing the methane output by 36% (Pohl et al., 2012). Reactor integration, particularly solid phase and high-rate reactors enhance leachate pre-treatment prior to recirculation, but do not necessarily provide an outright solution for substrate induced inhibition. However, the adaptive potential of agglomerated microbial cells in all high-rate reactors may survive better and continue to metabolize under unfavourable conditions (Chen et al., 2008; Francois et al., 2007). Despite the major advances in improving HSAD through multi-stage systems, most operators would prefer a single-stage AD system because of the additional operation and maintenance costs (Lissens et al., 2001). In 2010 Jan Mummea et al designed and operation of the upflow anaerobic solid-state with two reactor anaerobic filter (UASS-AF) test system. The UASS reactor digests solid biomass even though the the particulate organic matter (POM) rises in the procedure of a solid-state bed (SSB). The vertical movement of POM takes place in self-separated liquor and is induced by the adherence of self-produced microgas bubbles. The total working volume of the UASS reactor is separated into three sections: a lower liquid zone, an upper liquid zone and the SSB in between. The upper end of the SSB is defined by a sieve in the head of the UASS reactor. The sieve serves as a 3-phase separator and keeps the SSB below the liquor surface. The withdrawal of solid residues is arranged at the highest point below the sieve. By means of liquor recirculation, microbial biomass is transported back to the bottom of the reactor. In order to prevent an accumulation of volatile fatty acids, the process liquor was continuously recirculated through anaerobic filters (figure 20).

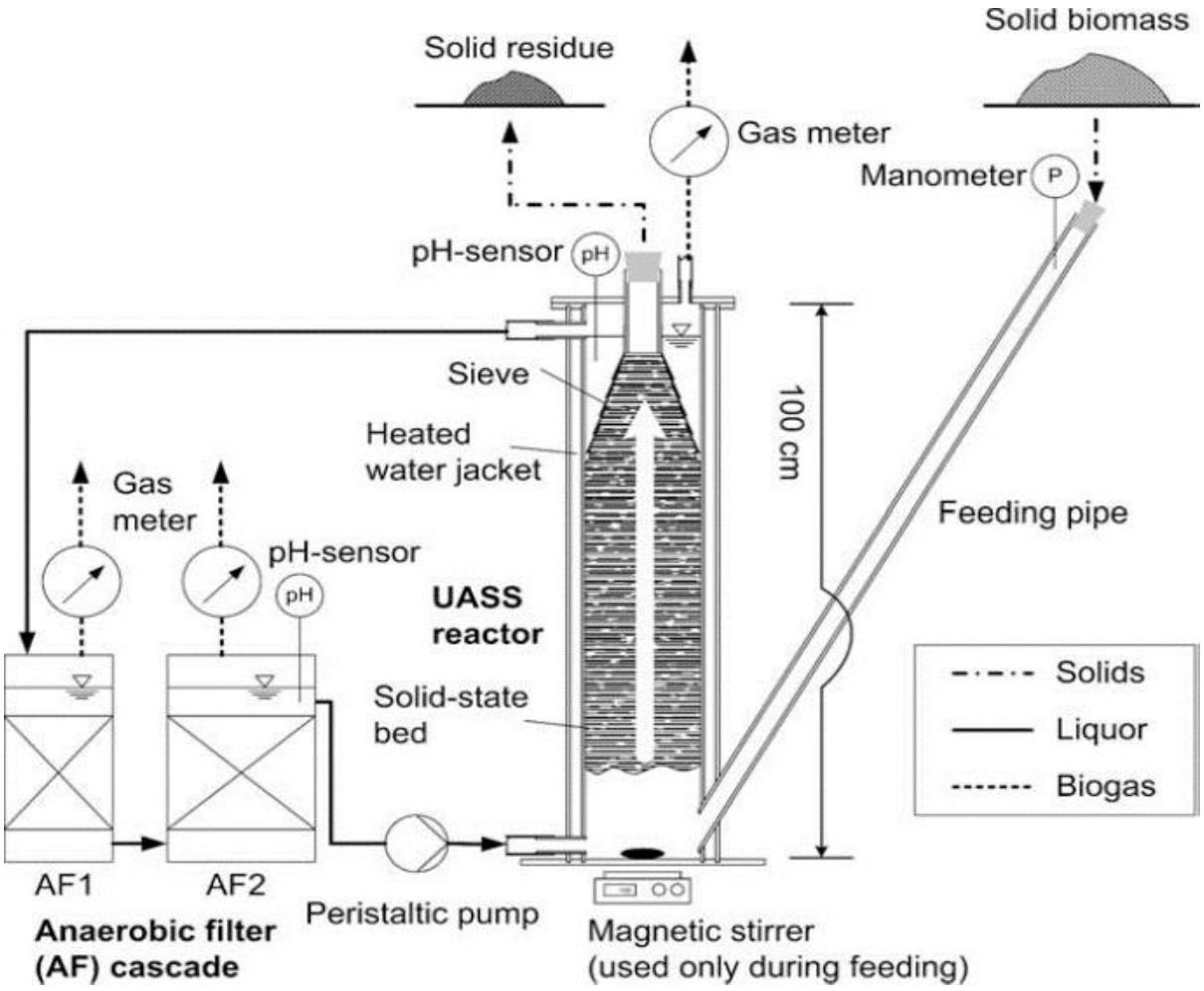

Figure 20 Schematic diagram of the experimental set-up and the laboratory scale UASS-AF Reactor (Jan Mummea et al., 2010)

Throughout stable state thermophilic digestion was brought into being to have advanced methane yield than mesophilic although the hydrolysis degree constant greater than before (Marcel Pohl et al., 2012). Moreover the oxygen tolerance capacity of up flow anaerobic solid-state (UASS) with anaerobic filter (AF) system showed good oxygen tolerance capacity, aeration pretreatment obviously enhanced anaerobic digestion in hydrolysis process and an aeration optimum intensity increased the methane yield (Yao Meng et al.,2016).

Table 7 The performance of different HSAD processes.

Substrate	Configuration	Reactor	Mixing device	Temperature	CH_4 yield	References
OFMSW	Single	Batch	Mechanical stirring	Thermophilic (55 °C)	0.4–0.49 L/L.d	Forster-Carneiro et al. (2008)
OFMSW	Single	CSTR	Mechanical stirring	Mesophilic (35 °C)	1.324 L/L.d	Fdéz-Guëlfo et al. (2010)
Food waste and paper waste	Single	CSTR	Mechanical stirring	Mesophilic (35 °C)	0.25 m^3 gCOD added	Kim and Oh (2011)
Meat and bone meal	Single	Co-digestion	Mechanical stirring	Mesophilic (35 °C)	351–381 ml g TVS	Wu et al. (2009)
OFMSW	Double	CSTR	Mechanical stirring	Thermophilic (55 °C)	5.4 ± 0.3 lCH4/l/d	Zahedi et al. (2013b)
Blue mussel and reed	Double	Leach bed UASB	Leachate recirculation	Mesophilic (35 °C)	0.33 m^3/kgVS	Nkemka and Murto (2013)
Food waste	Single	Leachate bed	Leachate recirculation	Mesophilic	–	Sponza and Ağdağ (2004)
Wheat straw	Double	UASS AF	Leachate recirculation	Thermophilic (55 °C) and mesophilic (35 °C)	–	Pohl et al. (2012)
Food waste and Livestock waste	Single	CSTR	Mechanical stirring	Mesophilic (35 °C)	0.26 m^3 gCOD added	Kim and Oh (2011)
Thin silage and poultry litter	Single	CSTR	Mechanical stirring	Thermophilic (55 °C)	–	Sharma et al. (2013)
Sewage sludge	Double	CSTR	Mechanical stirring	Thermo–mesophilic (TPAD)	424–467 ml gVS	Song et al. (2004)

Substrate	Configuration	Reactor	Mixing device	Temperature	CH_4 yield	References
Foodwaste	Single	CSTR tubular reactor	Mechanical stirring	Mesophilic (35 °C)	2.51± 0.17 $m^3/m^3/d$	Cho et al. (2013a,b)

Temperature-phased anaerobic digestion (TPAD) combines more than one operating temperature for the anaerobic digestion of organic substrate. The term thermo–mesophilic digestion is otherwise grouped under TPAD. The technology simply incorporates the advantages of thermophilic and mesophilic conditions into an AD process (Song et al., 2004). However, the combination of thermo–mesophilic conditions in AD may be a better option for HSAD, but this approach can only be successfully carried out in a multi-stage AD system (Song et al., 2004). The combination of thermophilic and mesophilic conditions has also been reported to operate at high organic loading rates, particularly with shock-loading of substrates. Ge et al. (2011) reported that when two-stage digesters were used, higher volatile solid reduction (34%-48%) was observed for thermos-mesophilic TPAD, 11%-30% higher than meso-mesophilic TPAD. According to Ge et al. (2011), the thermophilic stage of hydrolysis was 27% more effective than the mesophilic hydrolysis stage. This is similar to the results obtained by Roberts et al. (1999), where higher amount of methane was recovered from a two-stage thermo–mesophilic AD system. The application of thermo–mesophilic TPAD is not only limited to optimization of methane output; there are also reports that this can lead to great reductions in pathogenic organisms. Currently, the application of mesophilic, thermophilic and TPAD have been able to achieve pathogen inactivation (Fu et al., 2014). However, recent reports have shown that pathogen reduction is higher in thermophilic AD systems. In a report by Astals et al. (2012), the thermophilic AD of sewage sludge recorded a greater pathogen reduction than the mesophilic AD. Similarly, Riau et al. (2012) recorded greater reductions in pathogens when thermo–mesophilic TPAD was operated at sewage sludge to inoculum ratio of 0.25. With regards to HSAD, owing to the high OLR and low moisture content, the abundance of pathogens in the digestate could be relatively higher if the AD process is limited to mesophilic temperatures. However, there are indications that thermophilic AD only alters the culturable state of the pathogenic microorganisms rather than killing them, thereby increasing the potential for cell reactivation under favorable conditions (Fu et al., 2014). The proliferation of pathogens is a major problem with organic substrates; however, this challenge could be minimized if a pre-treatment or post-treatment stage is included in the operational processes.

Three stage high solid anaerobic digestion

The two types of AD plants now in operation are high-solids (15% total solids) Both of these processes experience certain operational or technical obstacles, whether it be dry AD (TS) 40%) or wet AD (TS 15%) (Kothari et al., 2014). For the example, challenges connected to high-solids AD include mass transfer restriction, accumulation of VFAs, and lengthy retention times, whereas concerns connected to wet AD include high water consumption, demand for a bigger digester volume, and expensive downstream processing of the digestate (Zhang et al., 2017). A three-stage digester has been created to combine the advantages of high-solids AD and wet AD in order to enhance digestion performance and methane generation. The three-stage anaerobic digester might combine wet methanogenesis (stage 3), acidification, and high-solids hydrolysis (stage 1) into a single digester.

Three optimal pH ranges for hydrolysis (pH 4-5), acidogenesis (pH 5-7) and methanogenesis (pH 7-8) could be maintained in the three-stage digester through functional segregation. This enhanced the simultaneous growth of various functional microorganisms, such as hydrolyzing bacteria, fermenting bacteria, and methanogenic archaea, was achieved. The three-stage digester has only been tested so far with FW (Zhang et al., 2017) or FW and horse dung (Zhang et al., 2017) as the feedstock, increasing methane outputs by 11-54%. The goal of the current study is to use the well-known three-stage AD digester to co-digest FW and WAS. The appropriate bacterial and methanogen populations were identified, and a three-stage anaerobic digester system was assessed for improved methane production during co-digestion of waste activated sludge and food waste. According to the findings, the three-stage digester's average methane yield (0.496 L/gVS) was 13-52% greater than that of the one- and two-stage digesters. The three-stage digester (69.3 6.7%) increased the volatile solids removal by 12 to 47% when compared to controls. Proteobacteria, Firmicutes, and Bacteroidetes were the most prevalent bacterial phyla in one-, two-, and three-stage digesters, respectively. However, due to functional segregation, Pseudomonas, Tissierella, and Petrimonas were selectively enriched in the three-stage digester. Eight prominent methanogen genera were found by taxonomic analysis, with Methanosarcina, Methanosaeta, Methanobacterium, and Methanolinea collectively accounting for 80% of those genera. The dominating methanogenic pathway changed from a hydrogenotrophic pattern to an acetoclastic pattern and eventually reached a final synergy of these two with rising OLR and digester stage numbers. For the treatment of food waste, a portable three-stage anaerobic digester (TSAD) was created. The benefits of Wet AD and High Solids AD were integrated in TSAD. Compared to conventional anaerobic digesters, methane production in TSAD rose by 24-54%. (Zhang et al., 2019). Efficiency of hydrolysis and acidogenesis was dramatically increased by functionalized partitioning in TASD. With smaller reactor volume needed, TASD has a better treatment capacity and solid reduction rate.

2.11.6 High solid anaerobic digestate

As stated earlier, recent reports have suggested that HSAD containing >20% TS will produce lower methane (Dong et al., 2010; Nagao et al., 2012). Consequently, many studies are being conducted to optimize methane production from HSAD (Benbelkacem et al., 2013; Cho et al., 2013a,b; Fernández-Rodríguez et al., 2013; Li et al., 2014; Liang et al., 2014a,b; Zahedi et al., 2013a,b; Zhu and Jha, 2013). However, the HSAD system provides a better option for a cost effective digestate handling operation and a nutrient-rich digestate material. This section will be focusing on how HSAD can improve digestate handling and increase its nutrient content.

Nutrient content

The residual organic material from AD process is called digestate and it contains nutrients, which are beneficial to agriculture as a nutrient source and/or soil conditioner. 0% Plagiarism According to Alburquerque et al. (2012), the addition of digestate to the soil will increase the immediate availability of nutrients for microbial and plant uptake. Digestate application to land is currently considered to be the most effective route for maintaining nutrient recycling, particularly in developing countries (Tambone et al., 2010). However, the amount of nutrient availability per gram of digestate is often compromised depending on the TS content of the organic waste. It is expected that the addition of water will dilute the available nutrient content and subsequent dewatering may reduce the concentration of the residual nutrients in the solid fraction (Table 6. 2). A report by Vaneeckhaute et al. (2013) shows that more nutrients are contained in a digestate liquid fraction and in the event of dewatering most of this nutrient could be lost. Table 8 shows that more nutrients can be retained in the digestate if it is not dewatered.

Table 8 Physiochemical characterization of pig slurry, mixture of solid and liquid fraction of digestate (A) and liquid fraction of digestate (B) Adapted from Vaneeckhaute et al. (2013).

Parameter	A	B
Dry matter (%)	6.2 ± 0.1	2.5 ± 0.1
Organic carbon (%)	38 ± 0.1	25 ± 0.1
Total nitrogen (g kg^{-1})	4.7 ± 0.0	3.6 ± 0.0
–N (g kg^{-1})	3.1 ± 0.1	2.8 ± 0.0
Mineral nitrogen (%)	66 ± 0.0	77 ± 0.0
Total phosphorus (g kg^{-1})	0.9 ± 0.1	0.27 ± 0.0
K_2O (g kg^{-1})	2.6 ± 0.1	3.5 ± 0.0
Ca (g kg^{-1})	1.3 ± 0.3	0.11 ± 0.0
Mg (g kg^{-1})	0.34 ± 0.04	0.016 ± 0.00
S (g kg^{-1})	0.4 ± 0.0	0.11 ± 0.0
Na (g kg^{-1})	2.0 ± 0.0	3.1 ± 0.0
Cl (mg kg^{-1})	2.7 ± 0.0	2.9 ± 0.0

Apart from dilution of nutrients in digestate, applications of digestate to water-logged farmland have been reported to contribute to leaching, runoff and eutrophication of watercourses (Mangwandi et al., 2013). On the other hand, the HSAD digestate is more compact providing surface area for nutrient adsorption and gradual release of nutrients into the soil. In order to reduce the mobility of nutrients, wastewater companies usually thicken digestates by adding polymers and other thickening agent (Mangwandi et al., 2013; Watanabe and Tanaka, 1999). These chemical amendments are often administered before dewatering to increase the solid content of the digestate to approximately 15%-25%. Nutrient management is essential for maximizing digestate utilization on land. HSAD provides a better option for nutrient retention owing to the dryness of the digestate. This is because agricultural material, particularly fiber can also serve as an adsorbent (Achak et al., 2009). Other approaches such as application of adsorbents have been reported to improve nutrient uptake and retention from wet digestates, but this approach often increases the cost (Estevez et al., 2014).

The operation of HSAD offers a better option for reducing water usage and enhancing digestate handling. This approach to AD will be most suitable in regions with shortage of freshwater and high demand for organic fertilizer. In addition, the application of decentralized small-scale anaerobic digestion in homes, small and medium scale business could be further achieved using HSAD because it reduces or avoids dewatering and effluent handling. However, the technology is faced with challenges of limited methane production when compared with LSAD. More research is required to explore the potential in thermos-mesophilic digestion, co-digestion, multi

stage digestion, particularly combination of high-rate reactors, and high solid digesters for higher methane production.

- Why do it.
- Man
    - Low man power for operation
- Machine
    - compact reactor
    - easy for maintenance
    -
- method
    - easy handling
    - Low water inputs
    - non complicated
    - Serve small farm-scale biogas plants
    - Leachate recirculation increase amount of MO. and surface between MO. with MO. food (substrate)
    - AF part increase concentration of MO.
- material
    - Save cost
    - less transportation,
    - local substrate
    - main manure has lignocellulose composition that help to C/N balance
    - Destruction of weed seeds
- environment
    - reduce GHG
    - Renewable energy source
    - Reduced dependency on imported fossil fuels
    - Waste reduction
- social
    - Job creation
    - Reduced odors
- economic
    - value added for manure, Additional income for the farmers involved
    - Digestate is an excellent fertilizer
    - Operational cost, Maintenance costs
    - Using/selling the produced heat

CHAPTER 3
Materials and Methodology

3.1 Inoculum and substrate

The substrate is raw cow manure collected from Satun province in the south of Thailand. The enriched inoculum was prepared by mixing liquid fraction of cow manure (as substrate) and anaerobic sludge (as original inoculum) collected from a small farm-scale biogas plant fed with cow manure with the following on the weight basis proportions: 0:1, 1:1, 1:2, 1:3, and 1:4. The liquid fraction of the mixture containing suspension solid including microorganism was separated by filtering with 0.71 mm sieve (24 mesh) in order to remove large inert particulate (Rico et al., 2015) and subsequently added to triplicate 500 mL serum bottles around 350 mL for each bottle. The filled bottles closed with butyl rubber and sealed with the aluminum cap were purged with nitrogen gas for 3 minutes to create the anaerobic condition. All bottles were subsequently incubated at ambient temperature for 20 days. Biogas volume and compositions were daily measured. The enriched inoculum was selected from an assay providing the highest methane production for further single-stage HS-AD and TSHS-AD experiments. For the TSHS-AD, enriched inoculum consisting of three micro-organisms groups for hydrolysis, acidogenesis/acetogenesis, and methanogenesis was inoculated in three separate reactors of the TSHS-AD to have individual optimum conditions, as suggested by Zhang et al. (2019).

3.2 Biochemical methane potential (BMP) of single-stage HS-AD

The substrate and inoculum (S:I) ratio was initiated at 2:1 as previous used by Tepsour et al. (2019) for the single-stage HS-AD of oil palm fruit bunches and palm oil decanter cake. Then substrate portion of S:I ratio was double increased to 4:1, and 6:1 (VS basis), respectively. Complete mixing between the substrate and inoculum for each S/I ratio was added to have 350 mL with total solid (TS) concentrations of 15%, 20%, and 30% in a 500-mL serum bottle. All bottles were later closed with butyl rubber and sealed with aluminum cap. Purging with N_2 gas for 3 minutes was applied to create anaerobic conditions for all closed serum bottles, which were subsequently incubated for 60 days at ambient temperature. The quantity and quality of biogas were measured daily. BMP could be reported in the term of mL-CH_4·gVS^{-1}, which is a ratio between cumulative methane generated and the amount of VS-based substrate added.

The empirical formula ($C_nH_aO_bN_eS_s$) of cow manure was used to calculate the theoretical biochemical methane potential (BMP_{th}) at STP conditions in mL-CH_4 g-VS^{-1} by using Eq. 3.1 (Raposo et al., 2019). The BMP_{th} at STP conditions was then converted to the BMP_{th} at ambient temperature (30 °C) (BMP_{thABT}) by using the ideal gas law.

$$\mathrm{BMP_{th}(mL-CH_4\ g{-}VS^{-1})} = \frac{22.4\ \mathrm{x}\ (\frac{n}{2}+\frac{a}{8}-\frac{b}{4})}{12n+a+16b}\ \mathrm{x}\ 1000 \quad (3.1)$$

3.3 Methane production from manure by 1-L TSHS-AD

Three-stage high solid anaerobic digestion (TSHS-AD) was set up by connecting three 1-L containers having 0.7-L working volume each in series denoted as R1, R2, and R3 respectively responsible for hydrolysis, acidogenesis/acetogenesis, and methanogenesis with the reactor. Polyethylene tube (ID 1 cm) was connected from three reactors to one water replacement gas meter (Fig 21). Initial TS concentration and S: I ratio used for the first high-solids hydrolytic reactor (R1) were selected from the previous batch experiment of single-stage HS-AD. All three reactors were operated at an ambient temperature and stirred for mixing purposes by using a stirrer at 60 rpm for 2 minutes per day. The performance of batch TSHS-AD was tested at various initial manure loading of (5, 10, 15, 20, 25, and 30 g-TS/L-R1). On day 1, the feedstock was filled to 0.7- L working volume of R1 and later hydrolyzed for two days. Then all hydrolyzed substrate from R1 was transferred to R2 and carried out for 3 days. On day 4, the substrate was filled according to the given initial manure loading, e.g., 5 g-TS/L-R1 in R1.

On day 6 all hydrolyzed substrate from R1 was transferred to R2 and carried out for three days. Meanwhile, all acidified substrate from R2 was transferred to R3 for further methanogenesis. Then, three digesters were operated in a semi-continuous mode by feeding 5 g-TS/L-R once for every 2 days at ambient temperature. The corresponding hydraulic retention time (SRT) for all digesters was 60 days. The different feeding times in R1, R2, and R3 was due to different solid retention time (SRT) assigned for each reactor. Each SRT was determined by evaluating pH and methane content. In addition, leachate in the TSHS-AD system of each step is normally generated from digesting activity of microorganisms with small and uncertainly amount. It was re-circulated only in the individual reactor of the TSHS-AD. The process was similarly repeated for the changing the initial manure loading to 10, 15, 20, 25, and 30 g-TS/L-R1. In the case of leachate coming out from R1, R2 and R3, they were only recirculated in each reactor. Daily biogas production volume was recorded by the water displacement gas meter. Biogas compositions were measured by gas chromatography. The steady state was justified when variation of methane yield is less than 10% (Kongjan et al. 2014). Liquid samples were daily taken from R1, R2, and R3 and were subsequently analyzed for pH, total solid (TS), volatile solid (VS), volatile fatty acid (VFA) concentration, and total alkalinity. The microbial activity and microbial community samples were collected on day 2 and day 5 for R1 and R2, respectively. While R3 was collected on day 10, 20, 40, and 60.

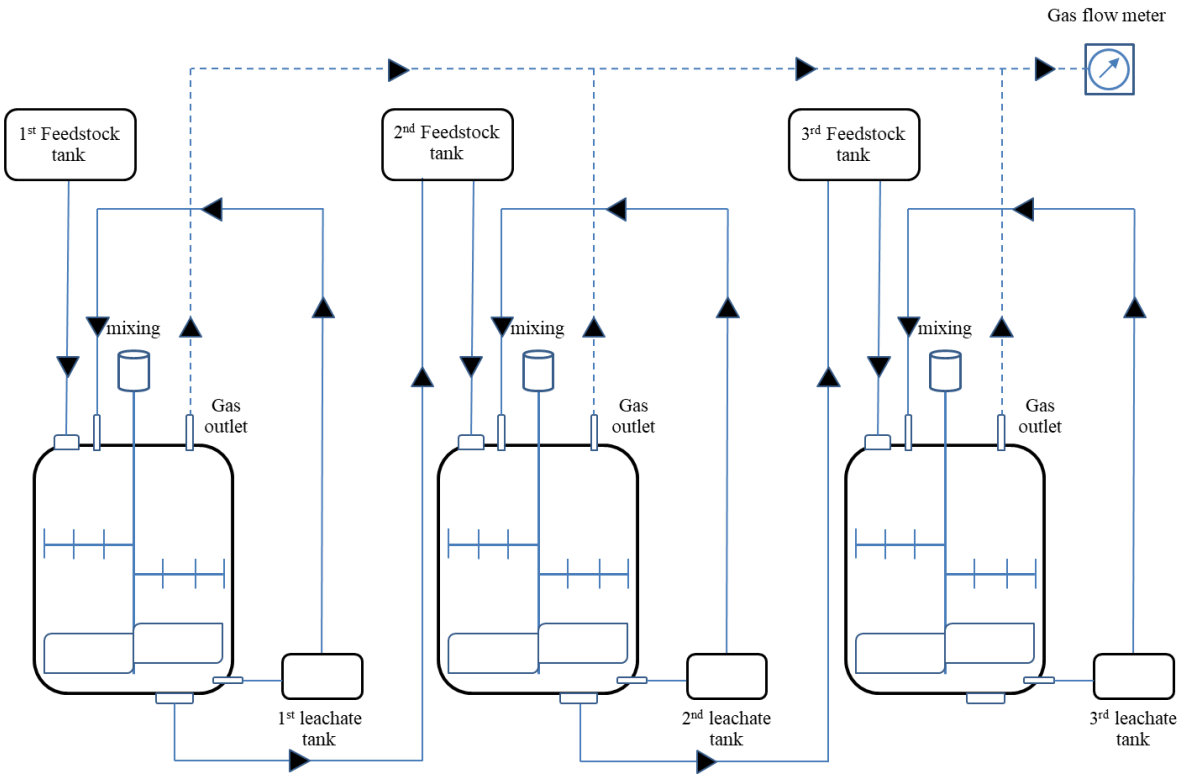

Figure 21 configuration of 1L anaerobic digestion reactors (TSHS-AD)

3.4 Analytical methods

Major parameters (pH, VS, TS, and alkalinity) were measured by using APHA standard methods (APHA, 2012). Elemental composition was analyzed by CHNOS Analyzer. VFA concentration was analyzed by using GC-17 A (Shimadzu, Kyoto, Japan) equipped with stabilwax®-DA fused silica column (30 m of length, 0.53mm of diameter, 85 °C) and flame ionization detector (FID) at 240 °C. Helium was used as a carrier gas, controlled at a flow rate of 30 mL min^{-1}. The biogas production volume was measured through water displacement. Methane contents in biogas were measured using gas chromatography (Shimadzu GC 14A equipped with a thermal conductivity detector) fitted with a 2.5 m Porapak S column with Hayesep Q (80/100). Helium was used as a carrier gas at 30 mL/min -flow rate. The injection port, oven, and detector temperatures (°C) were set at 100, 60, and 110, respectively. The 0.5 mL- gas sample was injected in triplicate.

3.5 Kinetic analysis

Cumulative methane yield obtained from single-stage HS-AD could be further used for first-order hydrolysis constant (k_h) as demonstrated in Eq. 3.2

$$\ln \frac{B_\infty - B}{B_\infty} = -k_h t \quad (3.2)$$

Where, B_∞ is the ultimately cumulative methane production obtained from batch experiment and B is the methane produced at any cultivating time (t). By plotting $\ln((B_\infty\text{-}B)/ B_\infty)$ against t, k_h (1/t) is a slope of that linear equation and is a characteristic of a given substrate to provide information about the time required to generate a given ratio of the ultimate methane potential (Mace et al., 2003).

Modified Gompertz equation (Mace et al., 2003) is demonstrated in Eq. 3.3

$$G(t) = G_m \times exp\left\{-exp\left[\frac{R_{\max} \times e}{G_m}(\lambda - t) + 1\right]\right\} \quad (3.3)$$

Where, G(t) is cumulative biogas production at certain cultivating time (t); G_m is maximum biogas production; R_{max} is maximum biogas production rate (mL/g VS day); λ is lag phase time, and e = exp (1) is equal to 2.7183. T_{90} = time period for 90% of total biogas yield; Tef = T_{90}-λ.

3.6 Specific activity test for hydrolytic activity (SHA), acidogenic activity (SAA), and methanogenic activity (SMA)

SHA, SAA, and SMA tests were described by Regueiro et al. (2012) The assays for SHA and SAA were performed in triplicate 250 mL- serum bottles, each having 175 mL working volume along with biomass concentration of 1.5 g volatile suspended solids (VSS) L^{-1}. A blank bottle was added distilled water instead of inoculum for measuring the abiotic disappearance of the substrates (starch and avicel). The substrate was used with a concentration of 1.5 g COD L^{-1}, in order to maintain an inoculum-to-substrate ratio of 1:1. Samples of the supernatant (1.5-3 mL) were taken every 2-3 h to determine the remaining starch/glycogen concentrations in the flasks. The evolution of the concentration of substrate (expressed in g COD L^{-1}) versus time was plotted and the SHA was calculated as the ratio between the maximum slope of the disappearance of the substrate (g COD L^{-1} d^{-1}) and the concentration of biomass used (g VSS L^{-1}).

The specific acetoclastic methanogenic activity (SAMA) was determined by using a mixture of VFA (1.5 g COD L^{-1}, 50% acetic acid, 25% propionic acid, and 25% butyric acid), and acetate (1.5 g COD L^{-1}). Meanwhile, the specific hydrogenotrophic methanogenic activity (SHMA) was estimated by using H_2:CO_2 (80:20, v/v). All the

activity tests were performed in triplicate. A blank with inoculum, but without substrate, was also included. To pack 350 mL sample into 500 ml serum bottle, the air in the bottles was replaced with nitrogen gas for 3-5 minutes. Methane production over time was observed. The methanogenic activity was computed as the ratio amid the extreme slope of the cumulative methane produced over time (g COD L^{-1} d^{-1}) and the intensity of biomass used (g VSS L^{-1}). SHA SAA and SMA test for inoculum prepared, hence studying the behavior of the microorganisms from changing the time of anaerobic digestion.

3.7 Microbial analysis

3.7.1 DNA Extraction

By using a tad changed standard bacterial genomic DNA separation technique (Blackall et al., 1998), complete genomic DNA was extracted from the sludge sample. The bottom pellet cells were extracted by centrifugation at 10,000 rpm for 10 min. The pellet cells were suspended by using 500- mL TENS buffer having pH 8.0 and containing: 100 mM Tris-HCl, 100 mM EDTA, 100 mM NaCl, and 100 mM Na3PO4. Subsequently, 40 mL- lysozyme (closing concentration 3.7 mg/mL) was added and incubated at 37 °C for 1.5 h with mild hand-operated merging every 10 minutes. Meanwhile, 200 mL of 10 percent sodium dodecyl sulfate (SDS) and 50 mL of proteinase K (1.2 mg/mL) were applied and combined with mild hand-operated merging every 10 minutes by inverting the tubes by hand after incubation at 60 °C for 1.5 h. The DNA was then retrieved from the tube by phenol/chloroform extraction. The combinations were given an equal quantity of phenol/chloroform/isoamyl alcohol (25:24:1) and were mildly assorted. The aqueous coat was centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was later transferred to a new sterile tube.

The extraction of chloroform/isoamyl alcohol (24:1) was then repeated. The crude DNA extract was obtained after centrifugation and subsequently precipitated with absolute ethanol for 2h or overnight at -20 °C. It was then followed by centrifugation at 12,000 rpm for 10 min. The genomic DNA pellet was re-suspended in TE buffer around 30-50 mL and preserved in a -20°C freezer before being used. The genomic DNA was envisaged on 1.0% agarose gel by electrophoresis.

3.7.2 PCR-DGGE Analysis

The microbial community structure inside the three-stage HS-AD was characterized by using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) (O-Thong et al., 2008). Two steps of PCR amplification were employed in this study. After DNA extraction, the bacterial 16S rDNA (~1400 base pair) with universal primers 16Sr and 16Sf was amplified by PCR. PCR Master

Mix II(5X) (GeneDirex) containing 25 pmol of a piece primer around 25 mL was used for mixtures amplification under preliminary denaturation at 95.5 °C for 5 min and subsequently followed by 30 denaturation sequences at 95°C for 1 min, fusing at 54 °C for 40 s, extension at 72 °C for 1 min, and last extension at 72 °C for 10 min. The reaction was then cooled down to 4°C. By using electrophoresis with 1.0% of agarose gels, the PCR product was evaluated. In the second PCR, the CG clamp primers 517r and 340f was used to intensify the V3 area fragment of the 16S rDNA product obtained from the primary PCR through the sequencer under the initial denaturation at 95 °C for 3 min, then followed by 30 cycles of three steps, consecutively at 95 °C for 1 min, 55°C for 30 s, and 72°C for 1 min. The final extension was later carried out at 72°C for 10 min. PCR products were kept at 4°C prior to further use for DGGE analysis by deploying electrophoresis with 1.5% of agarose gel. The amplification of archaea 16S rDNA sequences were performed by using archaea unique primers. Arch 958r and Arch 21f primers were used to amplify the bulk of the Archaea 16S rDNA fragment.

The amplification mixture was carried out using the same mixture for bacteria. PCR was firstly started with denaturation at 94°C for 2 min and followed by 35 denaturation sequences at 94°C for 1 min, fusing at 51°C for 1 min, with an extension at 72°C for 1 min. The last extension was finally carried out at 72°C for 10 min. To be the prototype for the next PCR analysis, a 16S rDNA PCR product was used. The V3 region fragment was amplified with the PARCH 519r and PARCH 340f-GC primers and carried out with the same mixture as stated above. The state of amplification begun by initial denaturation at 94°C for 3 min. and followed by 34 three-step cycles, consecutively at 94°C for 1 min, 53°C for 1 min, and 72°C for 2 min. The final extension was then performed for 10 min. at 72°C. PCR products were kept at 4°C prior to further use for DGGE analysis by deploying 1.5% agarose gel electrophoresis. The DGGE analysis of PCR products obtained from the second PCR was performed using the DGGE unit, V20-HCDC with 8% (v/v) polyacrylamide gels and a denaturant gradient of 40-60%.

The DGGE gels were stained for 60 minutes with Sybr Gold and photographed on the Gel Doc XR device. The gel bands were then separated. DNA was incubated in 20 mL-distilled waters at 4°C for 24 h in the excise gel slices and re-amplified by PCR with the second PCR primers. PCR products were purified and sequenced by the Macrogen sequencing facility after re-amplification, via primer 518r for bacteria and PARCH 519r for archaea. Raw sequenced DNA data were analyzed until associated strains were performed with Chromas and BioEdit tools. The ribosomal database was projected with SeqMatch software. Simple local alignment search tool (BLAST) with nucleotide database in the National Center for Biotechnology Information was applied to identify the closest matches for partial 16S rRNA gene sequences.

3.8 To study methane production of compact three-stage high solid anaerobic digestion (TSHS-AD)

The compact TSHS-AD reactor has a volume of 42 liters. The topmost chamber, middle chamber, and bottommost chamber symbolize the high-solids hydrolysis step (T1), acidification step (T2), and methane-production stage (T3), correspondingly. The appropriate initial manure loading, SRT and operating parameter from 3.3 (1-L TSHS-AD reactor) will launch by the 42-L compact TSHS-AD reactor shown in Figure 22. The baffle at the bottommost of each chamber can be unlocked by a linking rod from the external of the digester. Thus, feedstock was gravity transported from the topmost chamber to another. The reactor operated at an ambient temperature and stirred at 60 rpm for 2 minutes per day. The performance of 42-L compact TSHS-AD was tested at 20g-TS/L for 120 days. Three digesters were operated in a semi-continuous type by 20 g-TS/L-R1 feedings once every two days at ambient temperature. Daily biogas production volume was recorded and its compositions were measured by gas chromatography. Samples were taken from R1, R2, and R3 and were subsequently analyzed for pH, total volatile solid (VS), volatile fatty acid (VFA) concentration and total alkalinity.

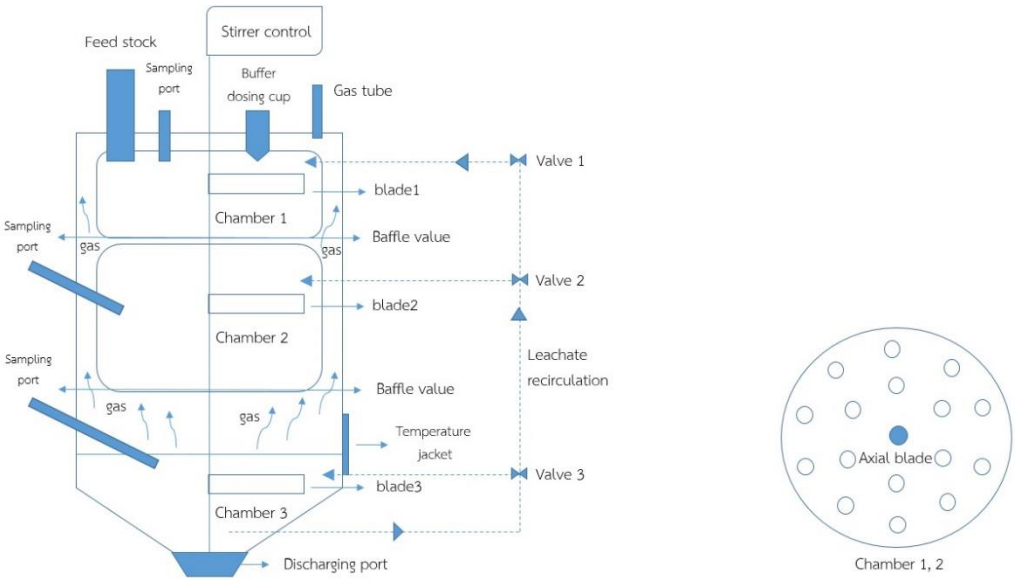

Figure 22 The 42-L compact TSHS-AD reactor schemes

3.9 Method of Economic Assessment

Net present value, the internal rate of return and the payback time as suggested by Li et al. (2018) were applied in the analysis of this research. Net present value (NPV) was deployed to determine the economic viability of biogas production processes. The profitability of the investment is generally indicated by an NPV > 0. The NPV was calculated, according to Eq. 3.4.

$$\mathrm{NPV} = \sum_{t=0}^{n} \frac{\mathrm{GMt}}{(1+r)^t} - CF_0 \tag{3.4}$$

Where n is the lifespan of the plant (5 years); r is the discount rate (4 percent); CF0 is the initial investment, equivalent to the overall investment of the facility. GMt as demonstrated in Eq. 3.5 is the system's gross margin gain for a year.

$$\mathrm{GMt} = \mathrm{Rg} + \mathrm{Rd} - (\mathrm{Cfs} + \mathrm{Com} + \mathrm{U} + \mathrm{L}) \tag{3.5}$$

Where, Rg and Rd are annual gas and solid digestate revenues, respectively; Cfs is the cost of feedstock, which is believed to be zero since they are presented without commercial value; Com is the cost of service and maintenance; U is utility and other prices; L is labor wage (gross).

The internal rate of return (IRR) is estimated, according to Eq. 3.6.

$$0 = \sum_{t=0}^{n} \frac{\mathrm{CFt}}{(1+IRR)^t} \tag{3.6}$$

Where, CFt is the net cash flow in the t-th year.

Lastly, the payback time (PB) is used for process analysis. The PB could be determined by using Eq. 3.7 beyond constant gross margin.

$$\mathrm{GM} = \mathrm{CF}_0 \{r(1+r)^{PB} / [(1+r)^{PB} - 1]\} \tag{3.7}$$

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Characteristics of cow manure and inoculum

The characteristics of anaerobically digested sludge, inoculum, and cow manure were demonstrated in Table 9. Cow manure used in this investigation had TS and VS content approximately 19.98% and 85.74%, respectively. Since having high TS content, cow manure is then considered to be suitable to be used for the dry fermentation process, which could potentially have high anaerobic bio-degradation, due to being rich in VS content. The pH of cow manure was 7.27 that are in an optimal range between 6.5 and 8.0 for mesophilic digestion. The anaerobic process is relentlessly repressed if the pH is less than 6.0 or greater than 8.3. The C/N ratio was 20.99, which is suitable for the anaerobic digestion process. It is a significant and useful parameter for evaluating the performance and stability of the AD process. The ideal C/N ratio for anaerobic digestion is generally agreed to be 20-35 (Mao et al., 2015). The characteristics of pH, TS, TVFA, alkalinity of both anaerobically digested sludge and enriched inoculum were not much different. Interestingly, the enriched inoculum had VS content greater than the anaerobically digested sludge, indicating more possibility to convert organic matter to biogas in the anaerobic system. Since manure and enriched inoculum have an alkalinity to TVFA ratio higher than 2, the anaerobic digestion of cow manure in this investigation is defined as a well-buffering system (Arelli et al., 2021).

Fig. 23 shows methane yield achieved during the enriching inoculum process. Using a liquid fraction of weight-based mixture between cow manure and anaerobically digested sludge at various ratios (0:1, 1:1, 1:2, 1:3, and 1:4), cumulative methane yields of 280, 390, 435, 395, and 380 mL-$CH_4 \cdot gVS^{-1}$ were achieved, respectively. Thus, it could be clarified that the liquid fraction of mixed cow manure and anaerobically digested sludge could provide sufficient microorganisms to generate methane. Microorganisms naturally produce less methane when they have too little enriching inoculum. This has resulted in limited microbial dispersal, less interaction between microorganisms and substrates, and less methane production. However, if there is too much enriching inoculum it will turn to depletes the substrate for microbial life and result in less methane being produced. Therefore, the anaerobically enriched sludge 1:2-mixing ratio between cow manure and anaerobically digested sludge was selected as inoculum, due to the ability in providing the highest methane yield for further investigation in single-stage HS-AD and three-stage HS-AD.

Table 9 Characteristics of sludge, inoculum and cow manure

Parameters	Anaerobic digested sludge	Enriched inoculum	Cow manure
pH	5.82±0.03	5.54±0.06	7.27±0.09
TS (%w/w)[a]	2.95±0.02	3.25±0.03	19.98±0.20
VS (% of TS)[b]	55.32±0.06	64.48±0.60	85.74±0.86
(TVFA) (g/kg)[a]	0.10±0.06	0.16±0.00	0.17±0.00
(TKN) (g/kg)[a]	1.95±0.06	2.30±0.02	2.65±0.03
(NH_4^+-N) (g/kg)[a]	1.13±0.06	1.40±0.01	1.88±0.02
Alkalinity (g/kg)[a]	2.33±0.06	2.70±0.03	5.20±0.05
C/N ratio[b]	2.72±0.06	2.53±0.03	20.99±0.20
Carbon (%wt)[b]	0.30±0.06	0.38±0.00	23.93±0.24
Hydrogen (%wt)[b]	10.12±0.06	9.98±0.02	4.54±0.06
Oxygen (%wt)[b]	71.68±0.06	77.86±0.20	29.20±0.31
Nitrogen (%wt)[b]	0.11±0.06	0.15±0.00	1.14±0.01
Sulfur (%wt)[b]	0.10±0.06	ND	0.22±0.00

[a] Based on wet weight.

[b] Based on dry weight.

ND = Not determined.

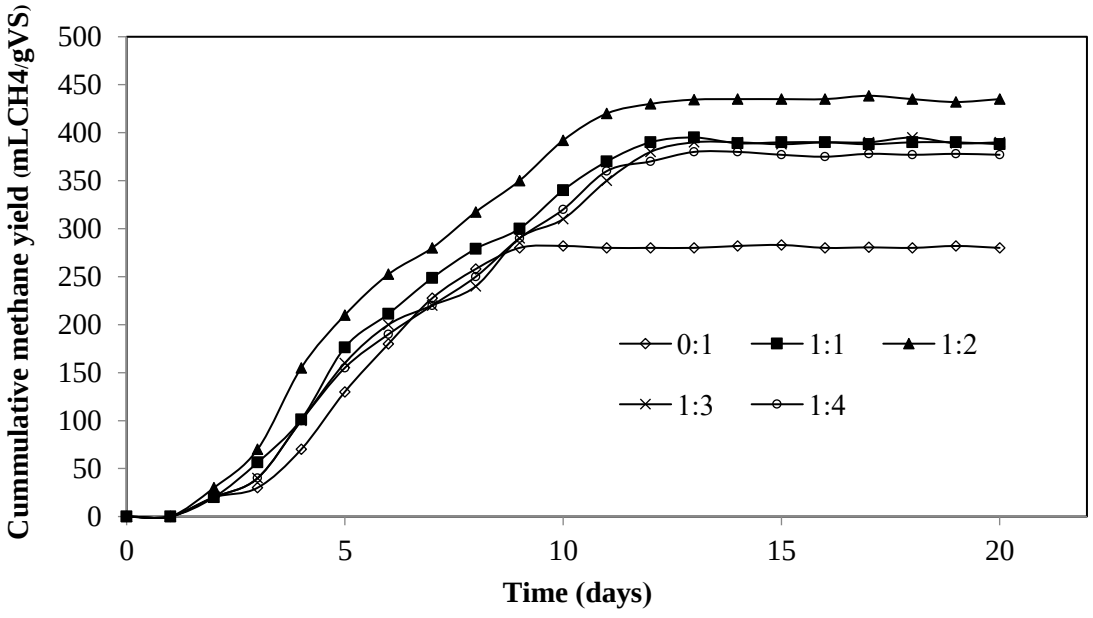

Figure 23 Enhanced inoculum efficiency of cow manure and anaerobic digested sludge at different ratios based on wet weight

4.2 BMP of cow manure using single-stage HS-AD

In this investigation, BMP has differently indicated methane production from given organic matters contained in cow manure at S/I ratios (2:1, 4:1, and 6:1) on VS basis, each of which had initial TS concentrations of 15%, 20%, and 30% (fig. 24 and table 10). The highest methane yield of 344.0 mL-CH_4·gVS^{-1} was obtained by using S/I ratio of 2:1 at initial loading of 15% TS. Table 10 exposed that when S: I and TS increased, the methane yield decreased. As the total solid increase, it means the liquid part in the anaerobic digestion system decrease. The decreased liquid part had caused the spread of microbes in the substrate to be uneven, resulting in reduced activity of the microbes in the production of methane. In addition, the excessive increase in the content of the solid substrate has resulted in difficult substrate movement. Microbial activity values of inoculum and effluent findings from single high solid anaerobic digestion were shown in Table 11. The microbial activity of the inoculum is similar to a single HS-AD end effluent. The SMHA is highest in both inoculum and effluent values. Due to the attainment of inoculum from the steady-state of anaerobic digestion, inoculum has higher microbial activity than effluent, since effluent is the end product of anaerobic digestion. Most bacteria had reached the dead phase. Overall, low accessibility of nitrogen, limited trace elements, and low mass transfer could contribute to low specific microbial activity in HS-AD sludge. However, SMA and SHMA are somewhat more predominant than SHA and SAA since both inoculum and effluent are part of residual that has undergone methanogenesis, resulting in the active group being the methanogen group.

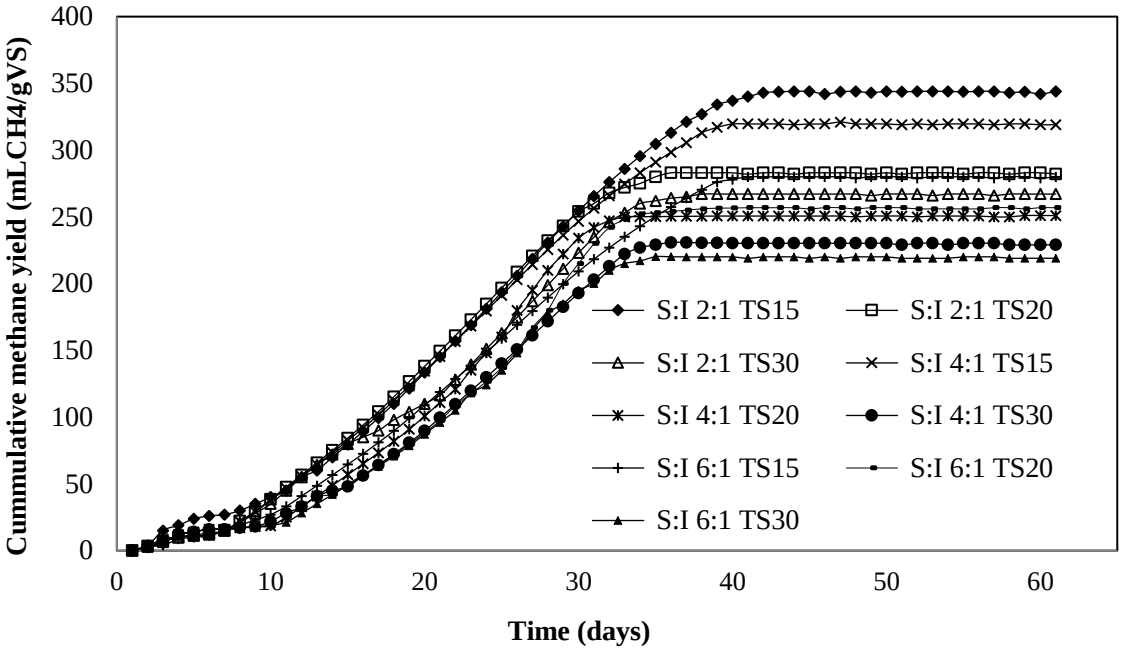

Figure 2 4 The methane production at various substrate to inoculum (S:I) ratios and %TS (15, 20 and 30) on single HS-AD

Table 10 Comparison of the performance of substrate and inoculum at mixing ratios of 2:1, 4:1 and 6:1 on %TS (15, 20 and 30) on single HS-AD.

Parameters	%TS 15			%TS 20			%TS 30		
	S:I			S:I			S:I		
	2:1	4:1	6:1	2:1	4:1	6:1	2:1	4:1	6:1
Cumulative methane yield ($mLCH_4$/gVS)	344± 6.88	319± 3.19	278± 5.56	283± 5.66	251± 5.02	257± 2.57	267± 5.34	230± 6.90	220± 4.40
NH_4^+–N (g/kg)	1.76± 0.04	1.79± 0.04	1.82± 0.04	1.83± 0.05	1.85± 0.04	1.86± 0.04	1.89± 0.04	1.92± 0.04	1.95± 0.04
pH	7.13± 0.14	7.15± 0.14	7.19± 0.07	7.22± 0.14	7.23± 0.14	7.25± 0.22	7.25± 0.15	7.25± 0.15	7.26± 0.07
VS removal rate (%)	33.6± 0.67	31.3± 0.63	29.7± 0.30	30.5± 0.61	18.2± 0.36	18.5± 0.37	19.3± 0.39	17.0± 0.51	15.2± 0.30

Table 11 Microbial activity results of inoculum and effluent from BMP determination at S:I 2:1 on %TS 15 (assays performed in triplicate).

Microbial activity (g COD g^{-1} VSS d^{-1})	**Inoculum**	**effluent**
SHA-Avicel	0.010±0.0025	nd
SHA-strach	0.013±0.0021	0.006±0.00002
SAA-	0.027±0.0014	0.006±0.00012
SMA-	0.030±0.0003	0.017±0.00021
SHMA	0.049±0.0011	0.023±0.00023
SAMA	0.003±0.0001	0.002±0.0001

n.d., not detected.

4.3 Kinetics analysis of single stage HS-AD

The first-order kinetics, depending on solid substrate concentration and the first-order kinetic constant rate (k_h) could be deployed to describe enzymatic hydrolysis by observing solid conversion rate. Hydrolysis is generally a limiting step for the anaerobic digestion of organic solid waste or manure (Palmowski et al., 2000). The modified Gompertz model is widely used to describe growth and product formation data for various types of dynamically biological systems. In anaerobic digestion, it becomes an

empirical representation of biogas/methane/hydrogen accumulation data. Moreover, predicted biogas yield from both models suggests that the modified Gompertz model is more suitable than the first-order kinetic model.

The single HS-AD results shown in Table 12 indicated that the k_h decreased, when the initial %TS and S: I ratio were increased. At this juncture, it could be implied that when increasing solid fraction in the feedstock, it could result to have decreased hydrolysis rate due to cellulose conversion yields, so-called high-solids effect (Weiss et al., 2019). Consequently, methane production was correspondingly reduced. According to the modified Gompertz parameters, the R_{max}, T90, and T_{ef} were increased, when the initial %TS and S: I ratio were decreased. Additionally, it was found that the lag phase (λ) became shorter by using less initial % TS and S: I ratio. The four parameters show that the efficiency of methane production would be decreased as the proportion of solid content was increased.

Table 12 The first-order kinetic model and the modify Gompertz model in single HS-AD.

Parameters	%TS, S:I								
	15, 2:1	**20, 2:1**	**30, 2:1**	**15, 4:1**	**20, 4:1**	**30, 4:1**	**15, 6:1**	**20, 6:1**	**30, 6:1**
First order kinetic model									
K (1/day)	0.093±0.002	0.087±0.003	0.080±0.003	0.088±0.002	0.081±0.002	0.076±0.002	0.083±0.002	0.079±0.003	0.071±0.001
Predicted biogas(mL.gVS^{-1})	336.1±0.2	299.2±0.1	260.0±0.2	312.0±0.1	256.0±0.2	263.0±0.1	267.0±0.2	263.0±0.1	235.1±0.2
%Difference biogas yield	2.3±0.07	5.4±0.16	3.0±0.03	2.4±0.05	2.4±0.07	12.5±0.13	4.7±0.14	2.4±0.10	6.5±0.20
Modified Gompertz model									
Rmax (mL.gVS^{-1}-d)	8.96±0.18	8.75±0.18	7.94±0.24	8.58±0.17	8.07±0.32	6.94±0.14	7.36±0.07	7.8±0.16	6.77±0.20
λ (d)	5.4±0.11	6.23±0.12	6.23±0.19	6.8±0.14	6.87±0.21	6.48±0.13	6.85±0.14	6.99±0.07	6.25±0.13
T_{90} (d)	35±0.7	29±0.9	31±0.6	34±0.7	29±0.3	31±0.6	35±0.9	30±0.6	30±0.6
T_{ef} (d)	29.6±0.59	22.7±0.42	24.7±0.49	27.2±0.54	22.1±0.40	24.5±0.49	28.2±0.52	23.0±0.46	23.8±0.49
Predicted biogas yield (mL.gVS^{-1})	340.1±0.3	282.3±0.5	270.6±0.3	318.4±0.2	249.8±0.2	229.7±0.1	279.7±0.4	257.0±0.3	218.0±0.2
%Difference biogas yield	1.1±0.033	0.25±0.008	0.2±0.009	0.11±0.003	0.12±0.008	0.13±0.006	0.12±0.004	0.1±0.003	0.9±0.022

T_{90} = time period for 90% of total biogas yield; T_{ef} = T_{90}

4.4 The 1-L TSHS-AD of cow manure at different initial loading

The TSHS-AD was started-up by using S: I ratio 2:1, initial TS 15%, according to highest methane yield of 344.0 mL-CH_4·gVS^{-1} obtained from the previous single stage HS-AD experiment. According to methane production from different cow manure loading (g-TSL^{-1}-R1) of 5, 10, 15, 20, 25, and 30 as demonstrated in Fig. 25, it can be summarized as an overview of each initial manure concentration, indicating that the lag phase of TSHS-AD is slightly lower than that of the single HS-AD. The methane gas production of TSHS-AD increased dramatically over the first 20 day- operation. it later became narrow oscillated stationary. The methane yield was increased by stepwise increasing initial manure concentrations from 5 g-TSL^{-1}-R1 up to 20 g-TSL^{-1}-R1. However, continuously increasing initial manure concentration to 25 g-TSL^{-1}-R1 and 30 g-TSL^{-1}-R1 could cause a decrease in methane production rate, corresponding to remained VS concentration as shown in Fig. 26. As generally well known in the anaerobic digestion system, organic matters represented in VS concentration is disappeared by transforming to methane. The initial manure concentration of 20 g-TSL^{-1}-R1 provides maximum methane yield of 316.1 mL-CH_4·gVS^{-1}. Methane production from food waste with a yield of 307 mL-CH_4.gVS^{-1}, which is rather similar to methane yield obtained from this investigation was reported by Zhang et al. (Zhang et al., 2017) for the compact TSHS-AD reactor configured with the third wet methane-production stage at controlled mesophilic temperature (35 °C). However, considerable methane yield of 265 mL-CH_4 gVS^{-1} was achieved from single stage HS-AD of solid fraction of cow manure (Rico et al., 2015).

Fig. 27 show an effect of different initial manure concentrations (g-TSL^{-1}-R1) on pH and alkalinity detected in R1, R2, and R3 at the end of operating time in each reactor. Detected pH could evidently reflect to main function in each reactor of the TSHS-AD as previously reported by Zhang et al. (2019). pH is one of the monitored parameters in the anaerobic digestion having fastest response to increasing organic load, especially for carbohydrates rich substrate, thus it is often used to monitor reactor performance. However, for a well-buffered system of indicated by rather high alkalinity as shown in Fig. 27 for cow manure digestion, pH change in the anaerobic digestion system may not reflect imbalance process caused by the VFA accumulation (Boe et al., 2010). Therefore, individual VFA of acetic acid, butyric acid, and propionic acid, as the main pre-methanogenic intermediate was previously suggested to be a combined parameter to early diagnosis the anaerobic process imbalance. Organic overload in the anaerobic digestion could be defined by accumulation of propionic acid, due to it is the most thermodynamically unfavorable (Li et al., 2014). As demonstrated in Fig. 28, considerable higher propionic concentration at 25 and 30 g-TSL^{-1}-R1-initial loading than other loading at initial loading less than 20 g-TSL^{-1}-R1, corresponding to methane production obtained in Fig. 25. Acetic and butyric acid in R1 and R2 gradually increase and decrease in R3. But propionic acid increased in R3, indicating that acetic and

butyric more acid is used in the process of acetate formation than propionic. When considering free energy of reaction, it was found that propionic acetate generation The acid uses more energy than the other two acids, indicating that it is more difficult to react. As a result, propionic acid is formed in R3. Fig. 29, and Fig. 30 show profiles of pH, VFA, total alkalinity, and VFA/alkalinity ratio in the TSHS-AD with initial manure concentration of 20 g-TSL^{-1}-R1. TVFA rises quickly from 0.05 g kg^{-1} to 0.35 g kg^{-1}), resulting in a lower pH range of 6.5-7 as demonstrated in Fig. 27. Alkalinity ranging between 3.5 g $CaCO_3$ kg^{-1} and 7.41 g $CaCO_3$ kg^{-1} within the first six days could be suitable for methanogenic archaea. Subsequently, TVFA was reduced and methane was eventually generated. The suitable pH for acidogenesis/acetogenesis in the TSHS-AD is between pH 5 and pH 7. Thus, fermenting bacteria can be developed optimally in each stage of the three-stage process (Zhang et al., 2019). During the first week, the VFA/Alkalinity ratio steadily rose to 0.03-0.08 and stabilized at 0.02-0.03 after the first week. The VFA/alkalinity ratio of less than 0.4 is usually considered to be ideal for the anaerobic digestion process in high solid stages and the VFA/alkalinity ratio of more than 0.6 is considered to be indicative of substrate overload (Brown and Li., 2013). Therefore, having a VFA / alkalinity ratio of less than 0.4 at initial loading 20 g-TSL^{-1}-R1 indicates the optimum activity in the TSHS-AD process. Furthermore, TVFA range between 0.12 g kg^{-1}and 0.23 g kg^{-1} and alkalinity range between 6.2 g $CaCO_3$ kg^{-1} and 7.2 g $CaCO_3$ kg^{-1} during the first 10 day-operation could play an important role in maintaining proper pH balance for methanogenesis. Anaerobic microorganisms could produce methane in a comparatively narrow pH range, approximately 7.1 to 7.5. In fact, a suitable pH range for methanogenesis stage is between 7 and 8 (Zhang et al., 2019).

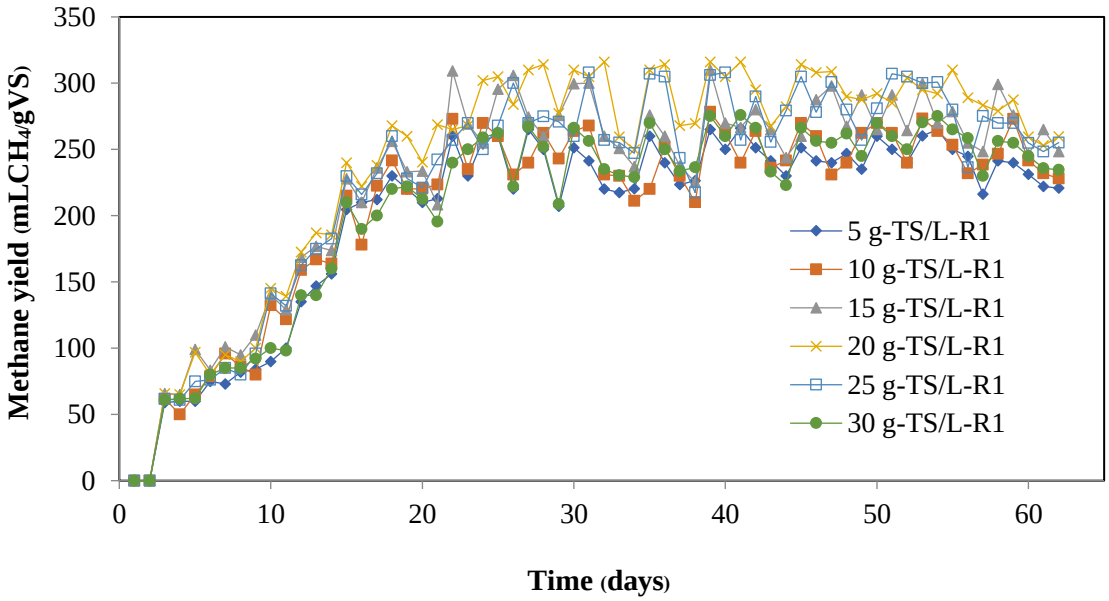

Fig. 25 The methane production of cow manure at (S:I) 2:1 on %TS 15 under ambient temperature in each 5, 10, 15, 20, 25, and 30 g-TS/L-R1 on TSHS-AD.

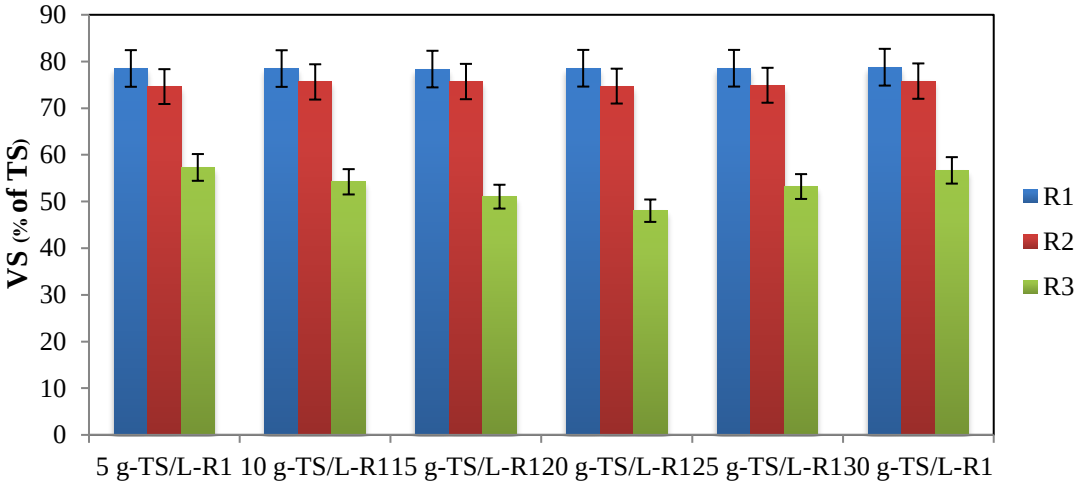

Fig. 26 VS average of R1, R2, R3 respectively in each g-TS/L-R1 on TSHS-AD.

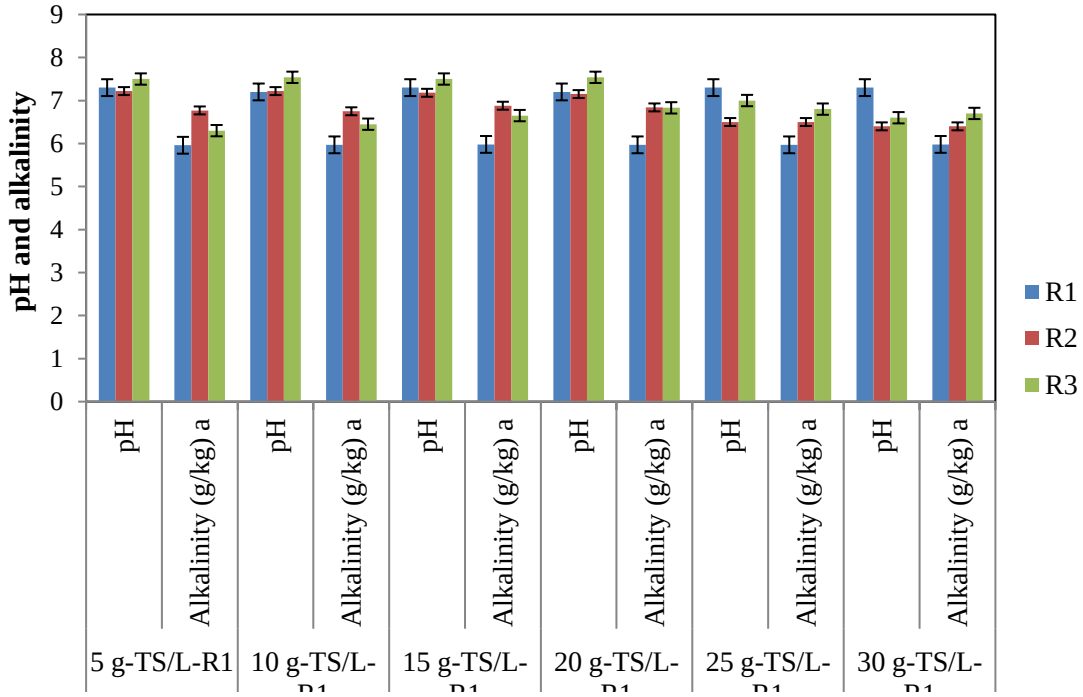

Fig. 27 pH and alkalinity average of R1, R2, and R3 respectively in each g-TS/L-R1 on TSHS-AD.
(a = based on wet weight)

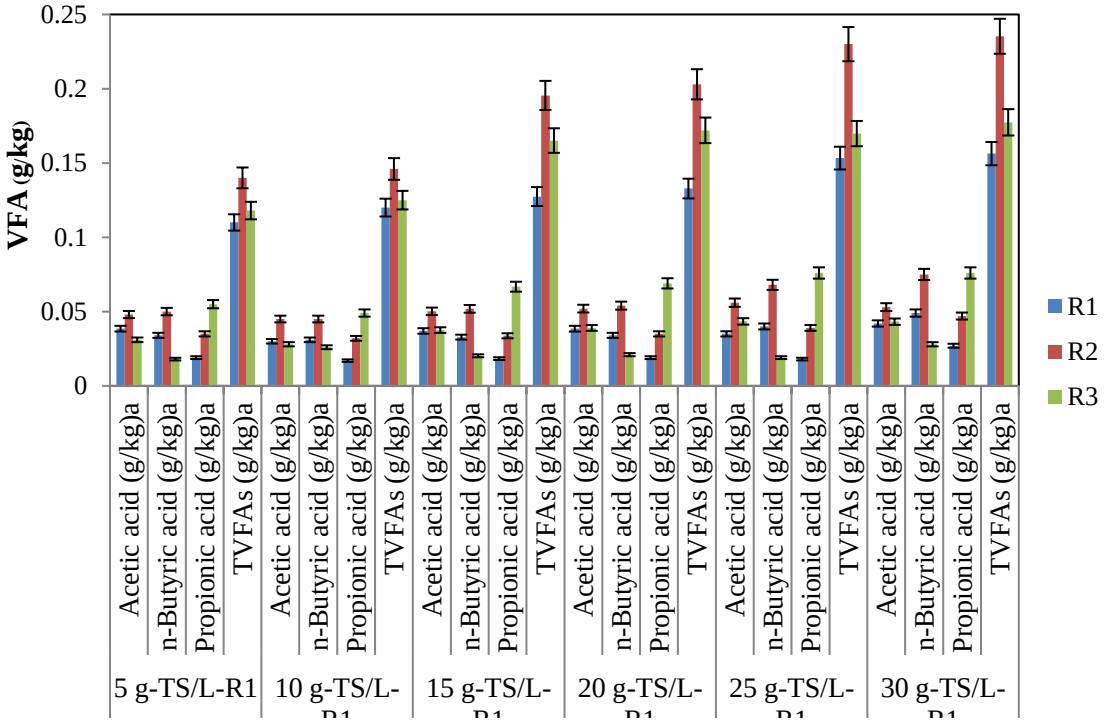

Fig. 28 VFA average of R1, R2, and R3 respectively in each g-TS/L-R1 on TSHS-AD. (a = based on wet weight)

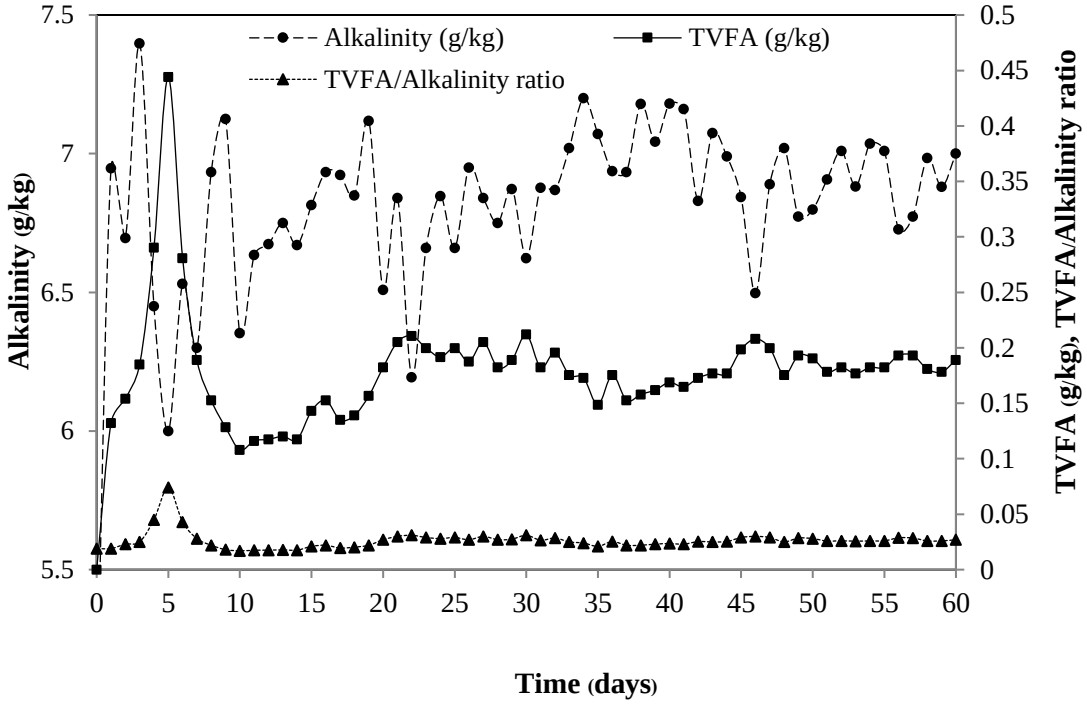

Fig. 29 Profiles of alkalinity, TVFA, and TVFA/Alkalinity ratio in the TSHS-AD of cow manure at initial load 20 g-TS/L-R1

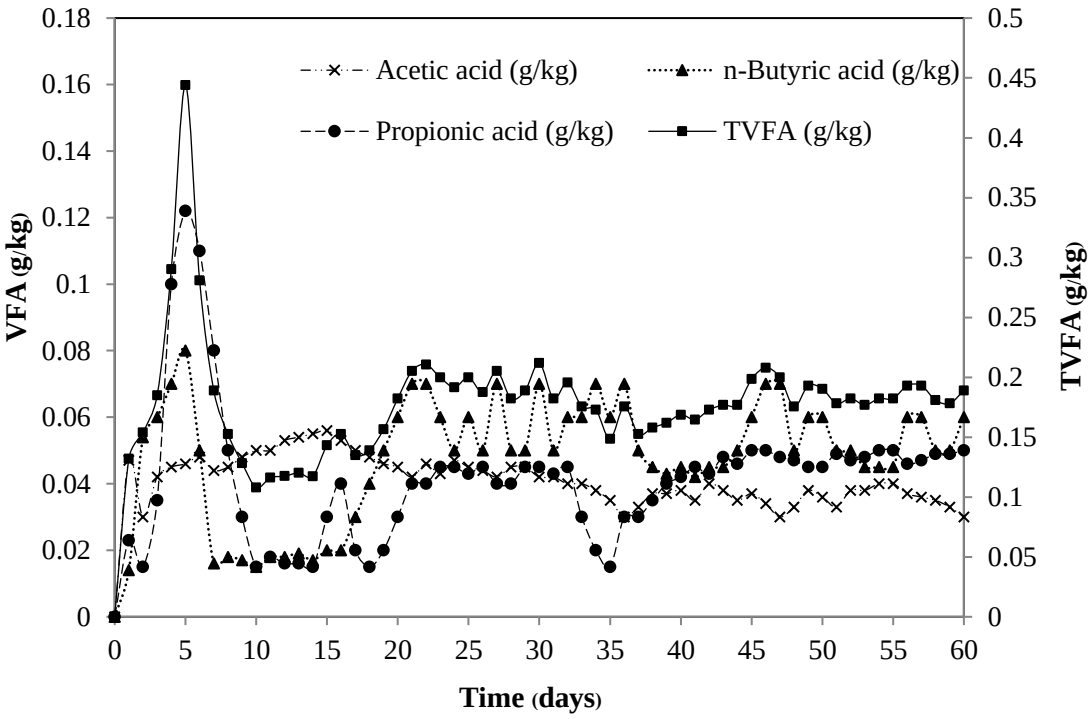

Fig. 30 VFA profile in the TSHS-AD of cow manure at initial load 20 g-TSL^{-1}-R1

4.5 Dynamics of the bacterial and archaeal community

Table 13 shows that SHA, SAA, SMA, SHMA, and SAMA depend on the order of the anaerobic digestion process in each reactor (R1, R2, and R3), highlighting that operational circumstances in each tank affect the growth of particular microbial populations. On the other hand, there were no discernible variations between the SHA in R1 and R2. SHA and SAA decreased in R3. SMA in R3 was higher than in R1 and R2.

The bacterial and archaeal population was identified by denaturing the gradient gel electrophoresis (DGGE) method for a better understanding of the behavior in each stage of the TSHS-AD process for cow manure. Fig. 31 demonstrates bacteria and archaea community in the TSHS-AD fed with 20 g-TS/L-R1 initial manure concentration gathering with a mixing ratio of 2:1 on VS basis and 15 %TS initial loading under ambient temperature on an operating day 2, 5, 10, 20, 40, and 60. Although quantification was not applied, the different intensities would imply relative differences in amounts. As the same loading quantity of PCR products for each lane was applied, different intensities of each band at different lanes revealed by DGGE should therefore indicate the relative difference of dominances of the microbial community. Abundance clustering heat maps from the strains based on their abundance information in each sample. Different color means the different relative abundance of the strains in all four treatments (Muyzer et al., 1993). Archeae, *Methanoregula* sp.,

Methanosaeta sp., and *Methanothrix* sp. containing enriched inoculum were detected substantially. *Cellulosilyticum sp., Hydrogenophaga sp., Lactobacillus sp., Clostridium sp., Methanothermobacter sp., and Methanoplanus sp,* which are essentially bacteria involved in the anaerobic digestion system were also dominantly found Bacteria community in R1. *Cellulosilyticum* sp., *Marvinbryantia* sp., *and Clostridium* sp. are considered capable of excreting extracellular hydrolytic enzymes for instance lipase, xylanase, cellulase, and protease to subsequently degrade lignocellulose (Yue et al., 2013) and methylcellulose. Fig. 25 shows low methane output is produced following the most bacterial hydrolysis group during the hydrolysis process which does not produce methane. *Lactobacillus* sp., *and Clostridium* sp. found mainly in R2 are bacteria responsible for the degradation of polysaccharides and monosaccharides (Francisci et al., 2015). The polysaccharide and monosaccharide hydrolysis results in acidification, which corresponds to a higher TVFA in Fig. 29. Moreover, *Dehalogenimonas* sp. also found in R2 is an alkane-degrading microorganism and is classified as one of the acetogenic bacteria (Johnson et al., 2015). Meanwhile, *Hydrogenophaga* sp., *Alkalitalea* sp., *and Dehalogenimonas* sp., as dominant bacteria in R3 can produce propionate and acetate (Contzen et al., 2000), and sugars (arabinose, xylose, mannose, galactose, xylan, and cellobiose) as carbon and energy sources (Li et al., 2018).

In addition to bacteria majorly detected in R3, *Selenomonas sp.* was the one that could convert lactose to major metabolites of ethanol, methanol, acetic acid, and butyric acid (Belkacemi et al., 2018). The methanogenic archaea detected in the TSHS-AD were *Methanoregula* sp., *Methanothermobacter* sp., *Methanothrix* sp., *Methanosaeta* sp., *and Methanomicrobium* sp. as previously observed in the enriched inoculums. *Methanothrix* sp. and *Methanosaeta* sp. are acetoclastic methanogen. *Methanoregula* sp., *Methanothermobacter* sp. and Methanomicrobium sp. are hydrogenotrophic methanogen. Among these archea, *Methanoregula* sp., *and Methanosaeta* sp. were the two dominant genera found in this anaerobic process. *Methanosaeta* sp. is an obligatory acetate consumer that dominates at low acetate concentration. This acetoclastic methanogenesis is considered to be a major pathway for methane in anaerobic co-digestion of fats, oil, and grease (Kurade et al., 2019). Meanwhile, (Fig. 31) *Methanoregula* sp., *Methanothermobacter* sp. and Methanomicrobium sp. are hydrogenotrophic methanogen that produces methane hydrogenotrophic from H_2 and CO_2. It is also an important co-worker with syntrophic acetate oxidation. Syntrophic acetate oxidation (SAO) is an anaerobic process where two microorganisms are responsible for the degradation of acetate. In this process, syntrophic acetate oxidizing bacteria (SAOB) oxidize acetate and produce H_2 and CO_2 or formate. Hydrogen and formate can serve as interspecies electron carriers (IECs) that are utilized by syntrophic partners, which in most cases are hydrogenotrophic methanogens or sulfate-reducing bacteria (SRB) (Timmers et al., 2018). However, finding hydrogenotrophic methanogen than acetoclastic methanogen, indicating that

the anaerobic process digestion needs reduce H_2 partial pressure, thus making the hydrogenotrophic methanogenesis predominantly from day 40 onwards.

Table 13 Microbial activity results of leachate after R1, R2, and R3 on TSHS-AD in 20 g-TS/L-R1 (assays performed in triplicate).

Microbial activity (g COD g^{-1} VSS d^{-1})	**Leachate after R1**	**Leachate after R2**	**Leachate after R3**
SHA-Avicel	0.014±0.09	0.013±0.002	nd
SHA-starch	0.015±0.02	0.014±0.04	0.001±0.0004
SAA	0.025±0.01	0.038±0.02	0.006±0.0003
SMA	0.020±0.0005	0.022±0.0005	0.027±0.0009
SHMA	0.032±0.004	0.035±0.0005	0.024±0.002
SAMA	nd	0.001±0.0004	0.008±0.0005

n.d., not detected.

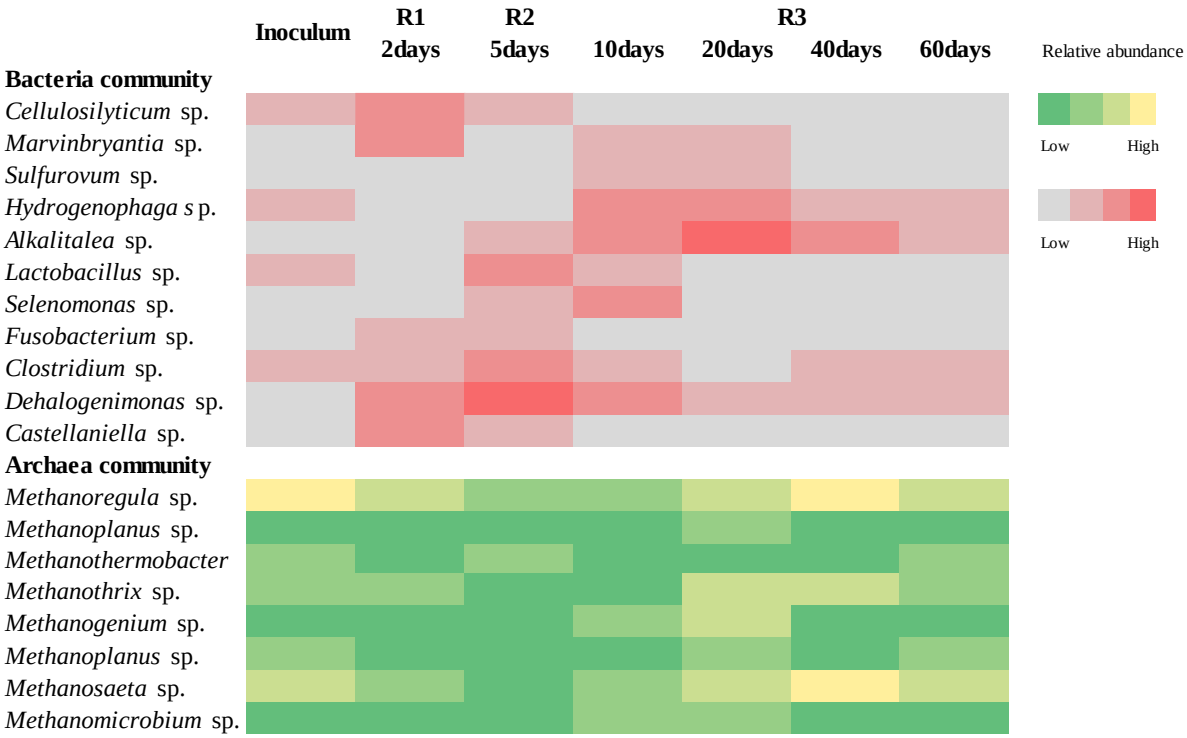

Fig. 31 Bacterial and archaeal community in the TSHS-AD

4.6 Methane production of the 42-L compact TSHS-AD

The 42-L compact TSHS-AD was using S: I ratio 2:1, initial TS 15% under ambient temperature in 20 g-TS/L and SRT value is 11. It produce methane yield was 309 mL-CH_4.gVS^{-1}, which is rather similar to methane yield obtained from 1-L TSHS-AD. Methane increases rapidly during the first 20 days which is also consistent with a sharp drop in VS values (Fig. 32). After that, the methane yield will fluctuate between 235-309 mL-CH4.gVS^{-1}. Subsequently, methane values will fluctuate in a narrower range up to day 120 (Fig. 32). The maximum production rate of 42-L compact TSHS-AD was 1.08 L_{CH_4} L $reactor^{-1}$ d^{-1} after day 20^{th}. TSHS-AD provides production rate higher than 1stage 32% (Suksong et al., 2019) and 17% higher than two stage (Akinshina and Azizov., 2019). This data shows that TSHS-AD increases the potential for methane production rate. In the 42-L compact TSHS-AD with an initial manure concentration of 20 g-TSL^{-1}, profiles of VFA, total alkalinity, VFA/alkalinity ratio and pH are shown in Fig. 34. Within the first six days, TVFA swiftly increases from 0.05 g kg^{-1} to 0.42 g kg^{-1}, with alkalinity varying between 5.5 g CaCO3 kg^{-1} and 7.19 g CaCO3 kg^{-1}. TVFA was subsequently decreased, and eventually, methane was produced. The pH range of TSHS-AD were 6.8-7.5. As a result, each stage of the three-part process can be developed with fermenting bacteria to their full potential. The VFA/Alkalinity ratio rapidly increased to 0.023-0.074 during the first week and then steadied at 0.021-0.031.

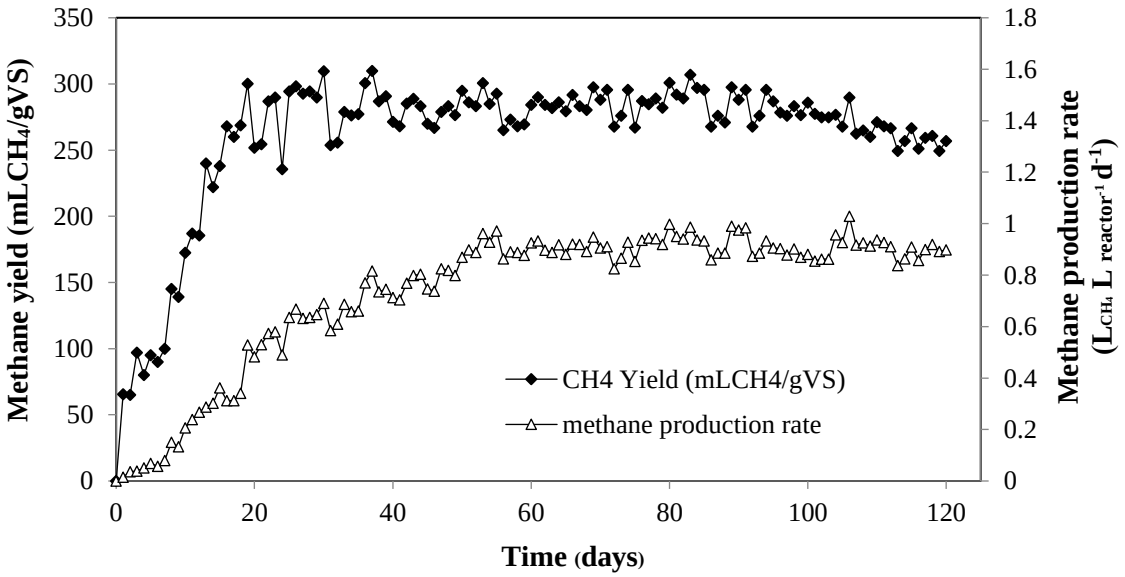

Fig. 32 The methane production of cow manure at (S:I) 2:1 on %TS 15 under ambient temperature in 20g-TS/L on 42-L compact TSHS-AD.

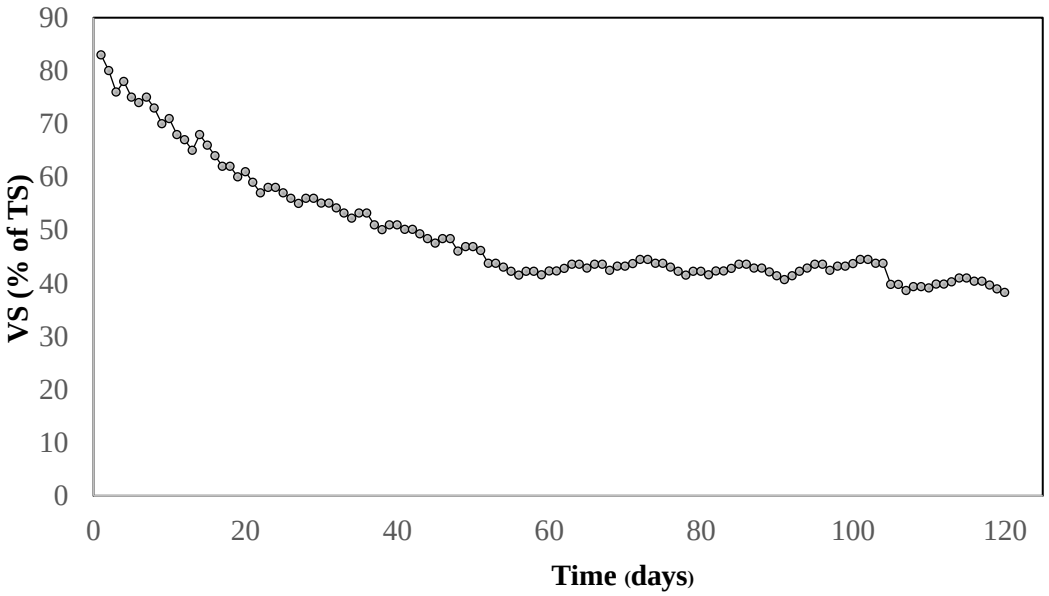

Fig. 33 VS (% of TS) profile in 42-L compact TSHS-AD of cow manure at initial load 20 g-TSL^{-1}

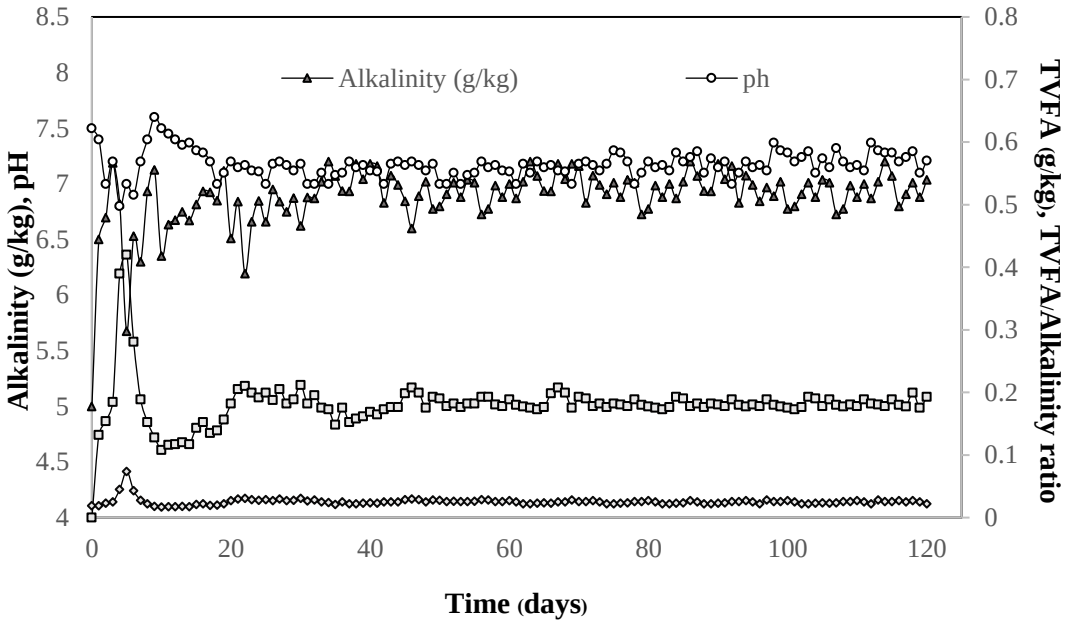

Fig. 34 Profiles of alkalinity, TVFA, and TVFA/Alkalinity ratio in 42-L compact TSHS-AD of cow manure at initial load 20 g-TSL^{-1}

4.7 TSHS-AD anaerobic digestate

A more advantageous choice for a digestate processing operation that is both affordable and produces a substance that is rich in nutrients is the TSHS-AD system. Digestate, the leftover organic material from the TSHS-AD process, includes nutrients that are useful to agriculture as a source of nutrients and a soil conditioner. Digestate

will boost the immediate availability of nutrients for plant and microbial uptake in the soil. The TSHS-AD digestate has a greater surface area for nutrient adsorption and a more progressive release of nutrients into the soil because it is more compact. Maximizing digestate use on land requires effective nutrient management. Because the digestate is dry, TSHS-AD presents a better option for nutrient retention. Comparison of physiochemical characterization of TSHS-AD digestate (A) with standards for organic fertilizers in the case of non-liquid organic fertilizers (B) by announcement of the Department of Agriculture of Thailand 2014 shown that TSHS-AD digestate can be used as a fertilizer. Potassium is also higher than the norm, which plays an important role in the transport of nutrients or photosynthetic products in plants. Potassium will help strengthen cell walls, increase leaf area and chlorophyll content, slow down leaf drop, increase number of seeds and number of good seeds per ear, increase seed weight. For this reason, digestate from TSHS-AD production is another source of income for farmers.

Table 14 Comparison of physiochemical characterization of TSHS-AD digestate (A) with standards for organic fertilizers in the case of non-liquid organic fertilizers (B) by announcement of the Department of Agriculture of Thailand 2014.

Parameter	A	B
Moisture content (%dry weight)	11.22±0.18	≤30
Total nitrogen ($g.kg^{-1}$)	1.10±0.04	≥1.00
Total phosphate ($g.kg^{-1}$)	0.47±0.02	≥0.5
Total potassium ($g.kg^{-1}$)	2.37±0.19	≥0.5
C/N Ratio	18.2	≤30

4.8 Economic assessment

The contributions of the financial investigation were comprised of those charges related to raw materials, conveyance, digester, equipment correlated parts, employment, operational, preservation, and utility is shown in Table 15. An NPV greater than zero indicates that the net benefit project is the present value of net cash inflows greater than the present value of the investments paid, meaning it can be invested in the project. An NPV of zero indicates that the net benefit project is the present value of net cash inflows equal to the present value of the investment paid. But if the NPV is less than zero, then the net benefit plan illustrated that the present value of net cash inflows is less than the present value of the investments paid implied that the project should not be invested. IRR is a calculation of the rate of return that will be received from the investment in the project. This rate of return will be the rate at which the net present value equals zero or the return earned is equal to the initial investment. If the rate of return on investment is higher than the desired rate of return or greater than the cost of capital, it means that the project should be invested in it. The amount

produced from the economic analysis was positive in terms of sale prices of biogas and solid digestate. The NPV prices for the HS-AD single-stage and TSHS-AD at ambient temperature biogas schemes were appropriate to investment, but TSHS-AD is more attractive.

IRRs obtained from both single-stage HS-AD and TSHS-AD were higher than the benchmark discount rate (4%), indicating a viable economic achievability in these two processes. Even though the preliminary investment of the TSHS-AD process is higher than the single-stage HS-AD, the annual income of the TSHS-AD process is however surpassingly higher than that of the single-stage HS-AD. The IRR of the TSHS-AD is more favorable for investments than the single-stage HS-AD. The key reason for the better viability comes from different revenues of gas from both processes (Table 15). The project sets a target payback period equal to 10 years. By observing the payback period, this research found that the time required to regain the funds expended in an investment or to reach the break-even point of the TSHS-AD is faster than the single-stage HS-AD. In addition, when comparing NPV IRR and PB of the TSHS-AD in this investigation of solid-state anaerobic digestion reported at initial %TS of 15 and 20 (Li et al., 2018) found that the NPV, IRR, and PB of TSHS-AD fall into the appropriate range for the project initiative. According to IRR NPV and PB, it could be concluded that annual income from biogas has the most impact on the investment attractiveness of both HS-AD processes. Therefore, if farmers can produce biogas and digestate by deploying the TSHS-AD, they could potentially obtain a more promising profit. In addition, Table 15 presents the NPV, IRR and PB data of manure production and biofertilizer from cow dung. From this data it is shown that single-stage HS-AD and TSHS-AD are more cost-effective than cow dung manure production and biofertilizer from cow dung both in terms of investment and payback.

Table 15 Economic results of the biogas with single HS-AD and TSHS-AD.

Category	**single HS-AD** (assume Volume 60L) TS 15%	**TSHS-AD** (assume Volume 60L) TS 15%	**farmyard manure and bio extract** (assume Volume 60L)	**Li et al., 2018 [35]**	
				Volume 5L TS 15%	Volume 5L TS 20%
Fixed cost	**490.38**	**2428.38**	**56.25**	**5559.63**	**5559.63**
Mixing machine [b] ($)	93.39	93.39			
Digester [b] ($)	311.33	2023.66			
Total facility investment ($)	85.66	311.33			
variable cost ($/year)	**61.87**	**233.72**	**1.6**	**29160**	**40389.30**
feedstock cost ($)	0	0			
Labour wage (gross)[d] ($/year)	18.68	85.23			
Operating and maintenance costs ($/year)	24.51	121.41			
Utility and other prices [c] ($/year)	18.68	27.08			
Revenues ($/year)	**203.87**	**1411.69**	**10.19**	**58251**	**65909**
revenues of gas [e] ($/year)	43.82	1243.60			
revenues of solid digestate [a] ($/year)	160.05	168.09			
NPV ($)	**3642.45**	**12734.32**	**147.45**	**196589**	**263986**
IRR (%)	**6.66**	**7.18**	**4.12**	**8.10**	**11.70**
PB (year)	**6.12**	**2.06**	**8.05**	**17.60**	**10.90**

Note: single stage was under S/I ratio 2:1 and 15%TS; three stage was under S:I 2:1, 15%TS and 20 g-TS/L-R1 under ambient temperature.

[a] the average market prices.

[b] the total cost includes facility involve machine.

[c] the data is calculated according to Provincial Electricity Authority and Provincial Waterworks Authority of Thailand.

[d] the data is calculated according to Hourly minimum labor according to the Ministry of Labor of Thailand.

[e] the data is calculated according to gas price as announced by the Ministry of Energy of Thailand.

4.9 The practical feasibility of the TSHS-AD of cow manure

The TSHS-AD of cow manure in this current research is successfully demonstrated at ambient temperature with satisfactory methane production yield and rate. Furthermore, the third methanogenic stage assigned for methane generation is not wet process. Additionally, main parameters effecting on process stability such as pH VFA concentration, and alkalinity could be self-regulated under proper initial loading. Thankfully, both manure and enriched inoculum used contain low VFA concentration and sufficient alkalinity, therefore, operating the TSHS-AD of cow manure is not required additional buffers to regulate pH in its system. It is interesting to note that operating the TSHS-AD of cow manure without controlling at specified mesophilic or thermophilic temperature could be economically advantageous. Generally, expenses around 8-10 % of biogas energy produced is used for reactor insulation to control constant temperature (Kongjan et al., 2009).

Thus, the TSHS-AD of cow manure operated at uncontrolled mesophilic temperature (ambient temperature) can be afforded by the farmers of the small-scale cow farm. Besides using produced biogas as household gaseous bio-fuel, digested residue discharged from the TSHS-AD could be an excellent organic fertilizer for energy crops as it is rich in organic matter (OM) and very less containing heavy metals such as Cd, Pb, and Cr (Zang et al., 2020). Using organic fertilizer derived from the TSHS-AD for soil amendment is indeed a part of the bio-circular-green (BCG) economy model aimed to drive Thailand's sustainable economic growth. Since the TSHS-AD of cow manure in this investigation is a concept-proof level by simulating the three separate reactors. Further investigation should be an integration of these three-stage process into a practically compact reactor, allowing to have simple control, low maintenance cost, and easy maintenance and handling.

CHAPTER 5
CONCLUSION

The methane derived from TSHS-AD was higher than that of HS-AD due to phase separation that could produce complete microbial activity. The TSHS-AD was started-up by using S: I ratio 2:1, TS 15% and 20g- TS/ L- R1 initial manure concentration provides maximum methane yield (309 mL-CH_4.gVS^{-1}). The lag phase of TSHS-AD is slightly lower than that of the single HS-AD. The methane yield was increased by stepwise increasing initial manure concentrations. TVFA rises quickly, resulting in a lower pH range of 6.5-7.0. Anaerobic microorganisms could produce methane in a comparatively narrow pH range, approximately 7.1 to 7.5. Anaerobic microorganisms could produce methane in a comparatively narrow pH range, approximately 7.1 to 7.5. It indicates the optimum activity in the TSHS-AD process. The bacterial and archaeal population demonstrates Archeae, *Methanoregula* sp. , *Methanosaeta* sp. , and *Methanothrix* sp. containing enriched inoculum were detected substantially. *Cellulosilyticum sp.* , *Hydrogenophaga sp.* , *Lactobacillus sp.* , *Clostridium sp.* , *Methanothermobacter sp.* , *and Methanoplanus sp*, which are essentially bacteria involved in the anaerobic digestion system were also dominantly found Bacteria community in R1. *Lactobacillus* sp. , *and Clostridium* sp. found mainly in R2 are bacteria responsible for the degradation of polysaccharides and monosaccharides. *Dehalogenimonas* sp. found in R2 is an alkane- degrading microorganism and is classified as one of the acetogenic bacteria. Meanwhile, *Hydrogenophaga* sp. , *Alkalitalea* sp. , *and Dehalogenimonas* sp. , as dominant bacteria in R3 can produce propionate and acetate, and sugars. Selenomonas sp. was the one that could convert lactose to major metabolites of ethanol, methanol, acetic acid, and butyric acid. Methanoregula sp., Methanothermobacter sp. and Methanomicrobium sp. are hydrogenotrophic methanogen. Methanoregula sp., Methanothermobacter sp. and Methanomicrobium sp. are hydrogenotrophic methanogens that produce H_2 and CO_2 or formate. Syntrophic acetate oxidation (SAO) is an anaerobic process where two microorganisms are responsible for the degradation of acetate. Hydrogen and formate can serve as interspecies electron carriers (IECs) that are utilized by syntrophic partners.

The contributions of the financial investigation were comprised of those charges related to raw materials, conveyance, digester, equipment correlated parts, employment, operational, preservation, and utility. The amount produced from the economic analysis was positive in terms of sale prices of biogas and solid digestate. The IRR of the TSHS-AD process is more favorable for investments than that of the single-stage HS-AD. The key reason for the better viability comes from different revenues of gas from both processes (Table 15). The project sets a target payback period equal to 10 years. According to IRR NPV and PB, it could be concluded that annual

income from biogas has the most impact on the investment attractiveness of both HS - AD processes. TSHS-AD is economically attractive, according to the technical methane yield obtained from this research investigation and its output. Therefore, the information generated from this research is considerably helpful and beneficial for the manufacturer to further scale up the TSHS-AD process in their production.

REFERENCES

Achak, M., Hafidi, A., Ouazzani, N., Sayadi, S., Mandi, L., 2009. Low cost biosorbent ‘‘banana peel’’ for the removal of phenolic compounds from olive mill wastewater: Kinetic and equilibrium studies. J Hazard Mater 166, 117–125.

Akinshina, N. and Azizov, A., 2019. Co-digestion of Plant Residuals and Chicken Dung in Two-Stage Solid State Anaerobic System with Single and Multiple Hydrolysers. Int. Proc. Chem. Biol. Environ. Eng. 2(1), 1-8.

Alburquerque, J.A., De la Fuente, C., Campoy, M., Carrasco, L., Njera, I., Baixauli, C., et al., 2012. Agricultural use of digestate for horticultural crop production and improvement of soil properties. Eur J Agron. 43, 119-128.

Amlinger, F., Peyr, S., Cuhls, C., 2008. Greenhouse gas emissions from composting and mechanical biological treatment. Waste Manag Res. 26, 47–60.

Amon, B., Kryvoruchko, V., Amon, T., Zechmeister-Boltenstern, S., 2006. Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. Agricult Ecosys Environ. 112, 153–162.

Andreas OttoWagner, Christoph Reitschuler and Paul Illmer., 2014. Effect of different acetate: propionate ratios on the methanogenic community during thermophilic anaerobic digestion in batch experiments. Biochem. Eng. J. 90, 154–161.

Angelidaki, I., Ellegaard, L., 2003. Codigestion of manure and organic wastes in centralized biogas plants-Status and future trends. Appl Biochem Biotechnol. 109, 95–105.

Angelidaki, I. & Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants.Rev. Environ. Sci. Biotechnol. 3(2), 117.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Science & Technology.; 927-934.

Angenent, L.T., Karim, K., Al-Dahhan, M.H., Wrenn, B.A., Domiguez-Espinosa, R., 2004. Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends Biotechnol. 22, 477-485. DOI:10.1016/j.tibtech.2004.07.001.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G., 1976. Inhibition of nitrification by ammonia and nitrous-acid. J Water Pollut Control Fed. 48, 835-852.

APHA, AWWA, WEF., 2012. Standard Methods for the Examination of Water and Wastewater, twenty-second ed. American Public Health Association; American Water Works Association; Water Environment Federation.

Arelli, V., Mamindlapelli, N.K., Juntupally, S., Begum, S., Anupoju, G.R., 2021. Solid-state anaerobic digestion of sugarcane bagasse at different solid concentrations: Impact of bio augmented cellulolytic bacteria on methane yield and insights on microbial diversity. Bioresour. Technol., 340, 125675. DOI 10.1016/j.biortech.2021.125675.

Astals, S., Venegas, C., Peces, M., Jofre, J., Lucena, F., Mata-Alvarez, J., 2012. Balancing hygienization and anaerobic digestion of raw sewage sludge. Water Res 46, 6218-6227.

Azadeh, B., Jalal, S., 2011. Effect of Organic Loading Rates (OLR) on production of methane from anaerobic digestion of vegetables waste. Proc., World Renewable Energy Congress, Linkoping, Sweden.

Azbar, N., Ursillo, P., Speece, R.E., 2001. Effect of process configuration and substrate complexity on the performance of anaerobic processes. Water Res. 35, 817-829.

Badshah, M., 2012. Evaluation of process parameters and treatments of different raw materials for biogas production [PhD thesis], University of Lunds, Sweden.

Baghchehsaraee, B., Nakhla, G., Karamanev, D., Margaritis, A., 2009. Effect of extrinsic lactic acid on fermentative hydrogen production. Int J Hydrogen Energy. 34, 2573-2579. DOI: 10.1016/j.ijhydene.2009.01.01.

Bapteste, E., Susko, E., Leigh, J., MacLeod, D., Charlebois, R.L., Doolittle, W.F., 2005. Do orthologous gene phylogenies really support tree-thinking? BMC Evol. Biol. 5, 33. DOI: 10.1186/1471-2148-5-33.

Banks, C., 2004. “Final Acitivity Report on Renewable energy from crops and agowastes (Project No. SES6-CT-2004-502824),” Tech. Rep.

Barakat, A., Monlau, F., Steyer, J.P., Carrere, H., 2012. Effect of lignin-derived and furan compounds found in lignocellulosic hydrolysates on biomethane production. Bioresour Technol. 104, 90-99.

Battistoni, P., 1997. Pre-treatment, measurement execution procedure and waste characteristics in the rheology of sewage sludges and the digested organic fraction of municipal solid wastes. Water Sci Technol. 36, 33-41.

Belkacemi, S., Cassir, N., Delerce, J., Cadoret, F., Scola, B.L., 2018. Selenomonas massiliensis a new anaerobic bacterial species isolated from human oral microbiota. New Microbes and New Infections. 24, 1-3. https://doi.org/10.1016/j.nmni.2018.03.006

Benbelkacem, H., Garcia-Bernet, D., Bollon, J., Loisel, D., Bayard, R., Steyer, J.P., 2013. Liquid mixing and solid segregation in high-solid anaerobic digesters. Bioresour Technol. 147, 387-394.

Berge, N.D., Reinhart, D.R., Batarseh, E.S., 2009. An assessment of bioreactor landfill costs and benefits. Waste Manage. 29, 1558-1567.

Biey, E.M., Musibono, E.D., Verstraete, W., 2003. Start-up of a multi-stage system for biogas production and solid waste treatment in low-tech countries. Water Sci Technol. 48, 239-243.

Blackall, L.L., Burrell, P.C., Gwilliam, H., Bradford, D., Bond, P.L., Hugenholtz, P., 1998. The use of 16S rDNA clone libraries to describe the microbial diversity of activated sludge communities. Water Science and Technology, 37, Issues 4-5, 451-454. https://doi.org/10.1016/S0273-1223(98)00144-9.

Boe K., Batstone D.J., Steyer J.P. and Angelidaki I., 2010. State indicators for monitoring the anaerobic digestion process. Water Res., 44(20): 5973-5980. DOI 10.1016/j.watres.2010.07.043.

Bollon, J., Benbelkacem, H., Gourdon, R., Buffière, P., 2013. Measurement of diffusion coefficients in dry anaerobic digestion media. Chem Eng Sci 89, 115-119.

Bolzonella, D., Innocenti, L., Pavan, P., Traverso, P., Cecchi, F., 2003. Semi-dry thermophilic anaerobic digestion of the organic fraction of municipal solid waste: focusing on the start-up phase. Bioresour Technol. 86, 123-129. https://doi.org/10.1016/S0960-8524(02)00161-X

Borrel. G., Parisot, N., Harris, H.M.B., Peyretaillade, E., Gaci, N., 2014. Comparative genomics highlights the unique biology of Methanomassiliicoccales, a Thermoplasmatales related seventh order of methanogenic Archaea that encodes pyrrolysine. BMC Genomics. 15:679. DOI:10.1186/1471-2164-15-679.

Borrel G, Toole PW, Harris H, Peyeret P, Brugere JF, Gribaldo S. Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis. Genome Biol E. 5, 1769-1780. DOI:10.1093/gbe/evt128.

Bourriaud, C., Robins, R.J., Martin, L., Kozlowski, F., Tenailleau, E., 2005. Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. J Appl Microbiol. 99. 201-212. DOI:10.1111/j.1365-2672.2005.02605.x

Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K.B., Ramakrishnan, S., 2011. Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. Enzym Res 2011, 787532.

Brown, D., Li, Y., 2013. Solid state anaerobic co-digestion of yard waste and food waste for biogas production. Bioresource Technology. Volume 127, 275-280. https:// doi.org/10.1016/j.biortech.2012.09.081.

Browne, P. and Cadillo Quiroz, H., 2013. Transcription and mRNA regulation patterns of methanogenic Archaea. Archaea, DOI:10.1155/2013/58636.

Burke, D. A., (2000). Anaerobic Treatment Process with Removal of Inorganic Material. US006113786A. United States, Western Environmental Engineering. 11.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol. 94, 223-253.

Bustamante, M.A., Alburquerque, J.A., Restrepo, A.P., De la Fuente, C., Paredes, C., Moral, R., et al., 2012. Co-composting of the solid fraction of anaerobic digestates, to obtain added-value materials for use in agriculture. Biomass Bioenergy 43, 26-35.

Bux, M., Baumann, R., Quadt, S., Pinnekamp, J., Muhlbauer, W., 2002. Volume reduction and biological stabilization of sludge in small sewage plants by solar drying. Drying Technol. 20, 829-837.

Buyukkamaci, N., Filibeli, A., 2004. Volatile fatty acid formation in an anaerobic hybrid reactor. Process Biochem. 39, 1491-1494.

Chandra, R., Takeuchi, H., Hasegawa, T., 2012. Methane production from lignocellulosic agricultural crop wastes: a review in context to second generation of biofuel production. Renewable and Sustainable Energy Reviews. 16(3), 1462–1476.

Chatterjee, B., Debabrata Mazumder, D., 2020. New approach of characterizing fruit and vegetable waste (FVW) to ascertain its biological stabilization via two-stage anaerobic digestion (AD). Biomass and Bioenergy. 139, 105594. https://doi.org/10.1016/j.biombioe.2020.105594.

Chavez-Vazquez, M., Bagley, D.M., 2002. Evaluation of the performance of different anaerobic digestion technologies for solid waste treatment. In: CSCE/EWRI of ASCE Environmental Engineering Conf.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. Bioresour Technol 99, 4044-4064.

Chojnacka, A., Błaszczyk, M.K., Szczęsny, P., Nowak, K., Sumińska, M., Tomczyk-Żak, K., Zielenkiewicz, U., Sikora, A., 2011. Comparative analysis of hydrogen-producing bacterial Biofilms and granular sludge formed in continuous cultures of fermentative bacteria. Bioresour Technol. 102, 10057-10064. DOI:10.1016/j.biortech.2011.08.063.

Chojnacka, A., Szczesny, P., Błaszczyk, M.K., Zielenkiewicz, U., Detman, A., Salamon, A., Sikora, A., 2015. Noteworthy facts about a methane-producing microbial community processing acidic effluent from sugar beet molasses fermentation. PLoS One. 10(5), 0128008. DOI:10.1371/journal.pone.0128008

Cho, S.K., Kim, D.H., Jeong, I.S., Shin, H.S., Oh, S.E., 2013a. Application of low-strength ultrasonication to the continuous anaerobic digestion processes: UASBr and dry digester. Bioresour Technol. 141, 167-173.

Cho, S.K., Im, W.T., Kim, D.H., Kim, M.H., Shin, H.S., Oh, S.E., 2013b. Dry anaerobic digestion of food waste under mesophilic conditions: Performance and methanogenic community analysis. Bioresour Technol. 131, 210-217.

Chong, S., Sen, T.K., Kayaalp, A., Ang, H.M., 2012. The performance enhancements of up flow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment. A state-of-the-art review. Water Res. 46, 3434-3470.

Chynoweth, D., Bosch, G., Earle, J., Legrand, R., Liu, K., 1991. A novel process for anaerobic composting of municipal solid waste. Appl Biochem Biotechnol. 28 (1), 421-432.

Contzen, M., Moore, E.R.B., Blümel, S., Stolz, A., Kämpfer, P., 2000. Hydrogenophaga intermedia sp. a 4-aminobenzene-sulfonate Degrading Organism. Systematic and Applied Microbiology. 23, 4, 487-493. https://doi.org/10.1016/S0723-2020(00)80022-3.

Curtis, T.P., 2010. Low-energy wastewater treatment: strategies and technologies. Environ Microbiol 2.

Dareioti, M.A., Kornaros, M., "Effect of hydraulic retention time (HRT) on the anaerobic co-digestion of agro-industrial wastes in a two-stage CSTR system," Bioresource Technology, vol. 167, pp. 407–415, 2014.

De Baere, L., 2000. Anaerobic digestion of solid waste: state-of-the-art. Water Sci Technol. 41, 283-290.

De Baere, L., 2008. Partial stream digestion of residual municipal solid waste. Water Sci Technol 57, 1073-1077.

De Baere, L.A., Mattheeuws, B., Velghe, F., 2010. State of the art of anaerobic digestion in Europe. In: 12th World Congress on Anaerobic Digestion, AD12. Guadalajara, Mexico.

Demirel, B., Yenign, O., 2002. Two-phase anaerobic digestion processes: a review. J Chem Technol Biotechnol. 77, 743-755.

Dennis, A., Burke, P.E., 2001. Dairy Waste Anaerobic Digestion Handbook. Environmental Energy Company.

Dong, L., Zhenhong, Y., Yongming, S., 2010. Semi-dry mesophilic anaerobic digestion of water sorted organic fraction of municipal solid waste (WS-OFMSW). Bioresour Technol. 101, 2722-2728.

Dridi, B., Fardeau, M.L., Ollivier, B., Raoult, D., Drancourt, M., 2012. Methanomassiliicoccus luminyensis gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. J Syst Evol Microbiol. 1902-7. doi: 10.1099/ijs.0.033712-0.

Duncan, S.H., Louis, P., Flint, H.J., 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol. 70, 5185-5190. DOI:10.1128/AEM.70.10.5810-5817.2004

Eckford, R. E., Newman, J.C., Li, X., Watson, P.R., 2012. Thermophilic anaerobic digestion of cattle manure reduces seed viability for four weed species. Int J Agric & Biol Eng. 5(1), 71-75. https://doi.org/10.3965/j.ijabe.20120501.009

Estevez, M.M., Linjordet, R., Horn, S.J., Morken, J., 2014. Improving nutrient fixation and dry matter content of an ammonium-rich anaerobic digestion effluent by struvite formation and clay adsorption. Water Sci Technol. 70, 337-344.

Evans, P. N., 2015. Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. Science. 350, 434-438, doi:10.1126/science.aac7745.

Fagbohungbe, M.O., Dodd, I.C., Herbert, B.M.J., Li, H., Ricketts, L., Semple, K.T., 2015. High solid anaerobic digestion: Operational challenges and possibilities. Environ. Technol. Innov. 4, 268-284. https://doi.org/10.1016/j.eti.2015.09.003

Fdz-Gulfo, L.A., lvarez-Gallego, C., Sales Marquez, D., Romero Garcia, L.I., 2010. Start-up of thermophilic-dry anaerobic digestion of OFMSW using adapted modified SEBAC inoculum. Bioresour Technol. 101 (23), 9031-9039.

Fernandez, B., Porrier, P. & Chamy, R., 2001. Effect of inoculum-substrate ratio on the start-up of solid waste anaerobicdigesters.Water Sci. Technol.44(4), 103-108.

Fernandez-Rodiquez, J., Prez, M., Romero, L.I., 2013. Comparison of mesophilic and thermophilic dry anaerobic digestion of OFMSW: Kinetic analysis. Chem Eng J 232, 59-64.

Forster-Carneiro, T., Perez, M., Romero, L.I., 2008. Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste. Bioresour Technol 99, 6994–7002.

Fotidis, I. A., Karakashev, D., Kotsopoulos, T. A., Martzopoulos, G. G. & Angelidaki, I., 2013. Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. FEMS Microbiol. Ecol.83, 38-48, doi:10.1111/j.1574-6941.2012.01456.x.

Francisci, D.D., Kougias, P.G., Treu, L., Campanaro, S., Angelidaki, I., 2015. Microbial diversity and dynamicity of biogas reactors due to radical changes of feedstock composition. Bioresour. Technol. Volume 176, 56-64. https://doi.org/10.1016/j.biortech.2014.10.126

Francois, V., Feuillade, G., Matejka, G., Lagier, T., Skhiri, N., 2007. Leachate recirculation effects on waste degradation: Study on columns. Waste Manage 27, 1259–1272.

Fruteau, D.L., H., Desbois, S., Saint-Joly, C., 1997. Anaerobic digestion of municipal solid organic waste: Valorga full-scale plant in Tilburg, The Netherlands. Water Sci Technol 36, 457–462.

Fu, B., Jiang, Q., Liu, H., Liu, H., 2014. Occurrence and reactivation of viable but non-culturable E. coli in sewage sludge after mesophilic and thermophilic anaerobic digestion. Biotechnol Lett 36, 273–279.

Gallert, C., Winter, J., 1997. Mesophilic and thermophilic anaerobic digestion of source-sorted organic wastes: effect of ammonia on glucose degradation and methane production. Appl Microbiol Biotechnol 48, 405–410.

Garcia, B.D., Buffiere, P., Latrille, E., Steyer, J.P., Escudie, R., 2011. Water distribution in biowastes and digestates of dry anaerobic digestion technology. Chem Eng J. 172, 924–928.

Ge, H., Jensen, P.D., Batstone, D.J., 2011. Temperature phased anaerobic digestion increases apparent hydrolysis rate for waste activated sludge. Water Res 45, 1597–1606.

Ge, X., Xu, F., Li, Y., 2016. Solid-state anaerobic digestion of lignocellulosic biomass: Recent progress and perspectives. Bioresour. Technol. 205, 239-249 (). https://doi.org/10.1016/j.biortech.2016.01.050.

Girija, D., Deepa K, Xavier F., Antony, I., Shidhi, P.R., 2013. Analysis of cow dung microbiota—A metagenomic approach. Indian Journal of Biotechnology. 12, 372-378.

Guendouz, A., Brockmann, D., Trably, E., Dumas, C., Delgene`s, J.P., 2012. Total solids content drives high solid anaerobic digestion via mass transfer limitation. Bioresour. Technol. 111, 55-61. https://doi.org/ 10.1016/j.biortech.2012.01.174.

Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van, J. B., 2009. Defining the biomethane potential: proposed protocol for batch assays. Water Sci. Technol. 927-934.

Hadiyarto, A., Johari, S., Hutama, I., Hasyim, W., 2015. The effect of f/m ratio to the anaerobic decomposition of biogas production from fish offal waste. Waste Technol. 3(2), 58-62.

Hanaki, K., Nagase, M., Matsuo, T., 1981. Mechanism of inhibition caused by long-chain fatty-acids in anaerobic-digestion process. Biotechnol Bioeng 23, 1591–1610.

Hansen, T. L., Schmidt, J. E., Angelidaki, I., Marca, E., Jansen, J. C.,Mosbæk, H. & Christensen, T. H., 2004. Measurement ofmethane potentials of solid organic waste.Waste Manage. 24(4), 393 – 400.

Hatamoto, M., Imachi, H., Yashiro, Y., Ohashi, A., Harada, H., 2008. Detection of active butyrate degrading microorganisms in methanogenic sludges by RNA-based stable isotope probing. Appl Environ Microbiol. 74, 3610–3614. DOI:10.1128/AEM.00045-08

Hawkes, D.L., 1980. Factors affecting net energy production from mesophilic anaerobic digestion. In: Stafford, D.A., Wheatley, B.I., Hughes, D.E. (Eds.), Anaerobic Digestion. Applied Science Publishers Ltd., London, UK.

He, P.J., 2010. Anaerobic digestion: An intriguing long history in China. Waste Manage 30, 549–550.

Hendriks, A.T.W.M., Zeeman, G., 2009. Pre-treatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100, 10–18.

Hidaka, T., Wang, F., Togari, T., Uchida, T., Suzuki, Y., 2013. Comparative performance of mesophilic and thermophilic anaerobic digestion for high-solid sewage sludge. Bioresour Technol 149, 177–183.

Ho, D., Jensen, P., Batstone, D., 2014. "Effects of temperature and hydraulic retention time on acetotrophic pathways and performance in high-rate sludge digestion," Environmental Science and Technology. 48, 11, 6468–6476.

Hoibye, L., Clauson-Kaas, J., Wenzel, H., Larsen, H.F., Jacobsen, B.N., Dalgaard, O., 2008. Sustainability assessment of advanced wastewater treatment technologies. Water Sci Technol 58, 963–968.

Hung, C.H., Lee, K.S., Cheng, L.H., Huang, Y.H., Lin, P.J., Chang, J.S., 2007. Quantitative analysis of a high-rate hydrogen-producing microbial community in anaerobic agitated granular sludge bed bioreactors using glucose as substrate. Appl Microbiol Biotechnol. 75, 693–701. DOI:10.1007/s00253-007-0854-7

Iino, T., Tamaki, H., Tamazawa, S., 2013. Candidatus Methanogranum caenicola: a novel methanogen from the anaerobic digested sludge, and proposal of Methanomassiliicoccaceae fam. nov. and Methanomassiliicoccales ord. nov., for a methanogenic lineage of the class Thermoplasmata. Microbes Environ. 28:244-250.

Ilarri, J.R., Elena, M., Clavero, R., Cassiraga, E., 2020. BIOLEACH: A New Decision Support Model for the Real-Time Management of Municipal Solid Waste Bioreactor Landfills. Int. J. Environ. Res. Public Health, 17, 1675. https://doi.org/10.3390/ijerph17051675.

Inka, V., Paul, D.J., Korneel R. Gene, W.T., 2015. Temperature and solids retention time control microbial population dynamics and volatile fatty acid production in replicated anaerobic digesters. Scientific Reports. 5, 8496. DOI: 10.1038/srep08496

Ito, T., Nakashimada, Y., Senba, K., Matusi, T., Nishio, N., 2005. Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process. J Biosci Bioeng. 100, 260-265. DOI:10.1263/jbb.100.260

Jewell, W. J., R. M. Kabrick, et al. (1981). Earthen-Supported Plug Flow Reactor for Dairy Applications. Methane Technology for Agriculture Conference, Ithaca, New York, Northeast Regional Agricultural Engineering Service.

Johnson, J.M., Wawrik, B., Isom, C., Bolingand, W.B., Callaghan, A.V.: Interrogation of Chesapeake Bay sediment microbialcommunities for intrinsic alkane-utilizing potentialunder anaerobic conditionsJamie. FEMS Microbiol Ecol. 91(2), 1-14 (2015). https://doi.org/10.1093/femsec/fiu035

Kafle, G.K., Kim, S.H., 2013. Effects of chemical compositions and ensiling on the biogas productivity and degradation rates of agricultural and food processing by-products. Bioresource Technology. 142, 553–561.

Karim, K., Hoffmann, R., Klasson, T., Al-Dahhan, M.H., 2005. Anaerobic digestion of animal waste: Waste strength versus impact of mixing. Bioresour Technol 96, 1771–1781.

Karim, K., Hoffmann, R., Thomas Klasson, K., Al-Dahhan, M.H., 2005. Anaerobic digestion of animal waste: Effect of mode of mixing. Water Res 39, 3597–3606.

Karthikeyan, O.P., Visvanathan, C., 2013. Bio-energy recovery from high-solid organic substrates by dry anaerobic bioconversion processes: a review. Rev Environ Sci Bio/Technol 12, 257–284.

Kim, D.H., Oh, S.E., 2011. Continuous high-solids anaerobic co-digestion of organic solid wastes under mesophilic conditions. Waste Manage 31, 1943–1948.

Kim, T.H., Lee, Y., Chang, K.H., Hwang, S.J., 2012. Effects of initial lactic acid concentration, HRTs, and OLRs on bio-hydrogen production from lactate-type fermentation. Bioresour Technol. 103, 136–141. DOI:10.1016/j.biortech.2011.09.093

Kongjan, P., Jariyaboon, R., O-thong, S., 2014. Anaerobic digestion of skim latex serum (SLS) for hydrogen and methane production using a two-stage process in a series of up-flow anaerobic sludge blanket (UASB) reactor. Int. J. Hydrog. *Energy*. 39(33), 19343-19348. DOI 10.1016/j.ijhydene.2014.06.057.

Kongjan, P., Min, B., Angelidaki, I., 2009. Biohydrogen production from xylose at extreme thermophilic temperatures (70 °C) by mixed culture fermentation. Water Res., 43(5): 1414-1424. DOI 10.1016/j.watres.2008.12.016.

Koster, I.W., Lettinga, G., 1984. The influence of ammonium–nitrogen on the specific activity of pelletized methanogenic sludge. Agricult Wastes 9 (3), 205–216.

Koster, I.W., Lettinga, G., 1 9 8 8 . Anaerobic-digestion at extreme ammonia concentrations. Biol Wastes 25, 51–59.

Kothari, R., Pandey, A., Kumar, S., Tyagi, V., Tyagi, S., 2014. Different aspects of dry anaerobic digestion for bio-energy: an overview. Renew. Sust. Energ. Rev. 39, 174–195.

Kristina, L., Jörg, S., Andreas, K., Anja, P., Rolf, D., b Andreas B.runecorresponding authora. C., New Mode of Energy Metabolism in the Seventh Order of Methanogens as Revealed by Comparative Genome Analysis of "Candidatus Methanoplasma termitum" Appl Environ Microbiol. 2015 Feb; 81(4): 1338–1352.

Kurade, M.B., Saha, S., Salama, E.S., Patil, S.M., Govindwar, S.P., Jeon, B.H., 2019. Acetoclastic methanogenesis led by Methanosarcina in anaerobic co-digestion of fats, oil and grease for enhanced production of methane. Bioresource Technology. 272, 351-359. https://doi.org/10.1016/j.biortech.2018.10.047

Lane, A.G., 1983. Removal of peel oil from citrus peel press liquors before anaerobic-digestion. Environ Technol Lett 4, 65–72.

Lavagnolo, M.C., Girotto, F., Rafieenia, R., Danieli, L., Alibardi, L., 2018. Two-stage anaerobic digestion of the organic fraction of municipal solid waste - Effects of process conditions during batch tests. Renewable Energy. 126, 14-20. https://doi.org/10.1016/j.renene.2018.03.039.

Lee, S.H., Park, J.H., Kim, S.H., Yu, B.J., Yoon, J.J., Park, H.D., 2015. Evidence of syntrophic acetate oxidation by Spirochaetes during anaerobic methane production. Bioresour Technol. 190, 543-549 DOI:10.1016/j.biortech.2015.02.066

Li, L., He, Q., Wei, Y., He, Q., Peng, X., 2014. Early warning indicators for monitoring the process failure of anaerobic digestion system of food waste. Bioresour. Technol., 171: 491-494. DOI 10.1016/j.biortech.2014.08.089.

Li, Y., Lu, J., Xu, F., Li, Y., Li, D., Wang, G., Li, S., 2018. Reactor performance and economic evaluation of anaerobic co-digestion of dairy manure with corn stover and tomato residues under liquid, hemi-solid, and solid state conditions. Bioresour. Technol., 270: 103-112. DOI 10.1016/j.biortech.2018.08.061.

Li, J., Jha, A.K., Bajracharya, T.R., 2014. Dry anaerobic co-digestion of cow dung with pig manure for methane production. Appl Biochem Biotechnol 173, 1537–1552.

Li, Y., Park, S.Y., Zhu, J., 2011. Solid-state anaerobic digestion for methane production from organic waste. Renewable Sustainable Energy Rev 15, 821–826.

Liang, C.Z., Sun, S.P., Li, F.Y., Ong, Y.K., Chung, T.S., 2014a. Treatment of highly concentrated wastewater containing multiple synthetic dyes by a combined process of coagulation/flocculation and nanofiltration. J Membr Sci 469, 306–315.

Liang, Y.G., Yin, S.S., Si, Y.B., Zheng, Z., Yuan, S.J., Nie, E., et al., 2014b. Effect of pretreatment and total solid content on thermophilic dry anaerobic digestion of Spartina alterniflora. Chem Eng J 237, 209–216.

Lianhua, L., Dong, L., Yongming, S., Longlong, M., Zhenhong, Y., Xiaoying, K., 2010. Effect of temperature and solid concentration on anaerobic digestion of rice straw in South China. Int J Hydrog Energy 35, 7261–7266.

Limam, R.D., Chouari, R., Mazéas, L., Wu, T.D., Li, T., 2014. Members of the uncultured bacterial candidate division WWE1 are implicated in anaerobic digestion of cellulose. Microbiology Open. 2014;3:157–167. DOI:10.1002/mbo3.144

Lin, J. G., Ma, Y. S., Chao, A. C., Huang, C. L. 1999. BMP test onchemically pretreated sludge. Bioresour. Technol.68(2),187-192.

Lissens, G., Vandevivere, P., De Baere, L., Biey, E.M., Verstraete, W., 2001. Solid waste digestors: process performance and practice for municipal solid waste digestion. Water Sci Technol 44, 91-102.

Liu, C., Yuan, X., Zeng, G., Li, W., Li, J., 2008. “Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste,” Bioresour. Technol. 99, 882-888.

Liu, F., Fang, B., 2007. Optimization of bio-hydrogen production from biodiesel wastes by Klebsiella pneumoniae. Biotechnol J. 2, 374-380. DOI:10.1002/biot.200600102

Liu, P., Qiu, Q., Lu, Y., 2011. Syntrophomonadaceae-affiliated species as active butyrate-utilizing syntrophs in paddy field soil. Appl Environ Microbiol. 2011;77:3884–3887. DOI:10.1128/AEM.00190-11

Li, Y., Park, S.Y., Zhu, J., 2011. Solid-state anaerobic digestion for methane production from organic waste. Renewable Sustainable Energy Rev. 15, 821–826. https://doi.org/10.1016/j.rser.2010.07.042.

Llabrés-Luengo, P., Mata-Alvarez, J., 1988. The hydrolytic step in a dry digestion system. Biol Wastes 23, 25–37.

Mace, S., Bolzonella, D., Cecchi, F., Mata, A.J., 2003. Comparison of the biodegradability of the grey fraction of municipal solid waste of Barcelona in mesophilic and thermophilic conditions. Water Sci. Technol. 48(4), 21–28.

Macias, C.M., Samani, Z., Hanson, A., Smith, G., Funk, P., Yu, H., 2008. Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. Bioresour Technol 99, 8288–8293.

Madsen, M., Holm, N.J.B., Esbensen, K.H., 2011. Monitoring of anaerobic digestion processes: A review perspective. Renewable Sustainable Energy Rev 15, 3141–3155.

Mangwandi, C., Jiangtao, L., Albadarin, A.B., Allen, S.J., Walker, G.M., 2013. The variability in nutrient composition of anaerobic digestate granules produced from high shear granulation. Waste Manage 33, 33–42.

Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Renew. Sust. Review on research achievements of biogas from anaerobic digestion. Energ. Rev., 45: 540-555. DOI 10.1016/j.rser.2015.02.032.

Maranon, E., Castrillon, L., Quiroga, G., Fernandez-Nava, Y., Gomez, L., Garcia, M.M., 2012. Co-digestion of cattle manure with food waste and sludge to increase biogas production. Waste Manage 32, 1821–1825.

Marcel, P., Jan, M., Kathrin, H., Edith, N., 2012. Thermo- and mesophilic anaerobic digestion of wheat straw by the upflow anaerobic solid-state (UASS) process. Bioresource Technology. 124 321–327.

Marcel, P., Kathrin, H., Jan, M., 2013. Anaerobic digestion of wheat straw-Performance of continuous solid-state digestion. Bioresource Technology 146, 408-415.

Martin, D.J., Potts, L.G.A., Heslop, V.A., 2003. Reaction mechanisms in solid-state anaerobic digestion: 1. The reaction front hypothesis. Process Saf Environ Prot 81, 171–179.

Martín, M.A., Fernández, R., Serrano, A., Siles, J.A., 2013. Semi-continuous anaerobic co-digestion of orange peel waste and residual glycerol derived from biodiesel manufacturing. Waste Manage 33, 1633–1639.

Martín, M.A., Siles, J.A., Chica, A.F., Martín, A., 2010. Biomethanization of orange peel waste. Bioresour Technol 101 (23), 8993–8999.

Masaru, K., Nobu, T.Narihiro, Kyohei Kuroda, Ran Mei, and Wen-Tso Liu1., 2016. Chasing the elusive Euryarchaeota class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen., ISME J. 10(10), 2478–2487. Published online Mar 4. doi: 10.1038/ismej.2016.33.

Mattocks, R., 1984. Understanding biogas generation, Technical Paper No. 4. Volunteers in Technical Assistance, Virginia, USA, 13.

Mccarty, P.L., 2001. The development of anaerobic treatment and its future. Water Sci Technol 44, 149–156.

McInerney, M.J., Bryant, M.P., 1981. Anaerobic degradation of lactate by syntrophic associations of Methanosarcina barkeri and Desulfovibrio species and effect of H2 on acetate degradation. Appl Environ Microbiol. 41, 346-354. DOI:0099-2240/81/020346-09$02.00/0.

Michael, O.F., 2015. Optimisation of small scale anaerobic digestion technology, 1-342.

Mihoubi, D., 2004. Mechanical and thermal dewatering of residual sludge. Desalination 167, 135–139.

Mihoubi, D., Vaxelaire, J., Zagrouba, F., Bellagi, A., 2003. Mechanical dewatering of suspension. Desalination 158, 259–265.

Mizuki, E., Akao, T., Saruwatari, T., 1990. Inhibitory effect of Citrus Anshu peels on anaerobic digestion. Biol Wastes 33 (3), 161–168.

Morrison, D.J., Mackay, W.G., Edwards, C.A., Preston, T., Dodson, B., Weaver, L.T., 2006. Butyrate production from oligofructose fermentation by the human faecal flora: what is the contribution of extracellular acetate and lactate? British J Nutr. 96, 570–577. DOI: 10.1079/ BJN20061853.

Müller, N., Worm, P., Schink, B., Stams, A.J., Plugge, C.M., 2010. Syntrophic butyrate and propionate oxidation processes: from genomes to reaction mechanisms. Environ Microbiol. 2, 489–99. DOI:10.1111/j.1758-2229.2010.00147.x

Muller, W., Frommert, I. & Jo.rg, R.2004 Standardized methods foranaerobic biodegradability testing.Rev. Environ. Sci.Biotechnol.3(2), 141-158.

Munoz, T.R., Laroche, B., Walter, E., Dore, J., Duncan, S.H., Flint, H.J., Leclerc, M,. 2011. Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. FEMS Microbiol Ecol. 76, 615-624. DOI:10.1111/j.1574-6941.2011.01085.x

Muyzer, G., Waal, E.C.D., Uitierlinden, A.G., 1993. Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16SrRNA. Appl Environ Microbiol. 59, 695-700. DOI 0099-2240/93/030695-06$02.00/.

Nagao, N., Tajima, N., Kawai, M., Niwa, C., Kurosawa, N., Matsuyama, T., 2012. Maximum organic loading rate for the single-stage wet anaerobic digestion of food waste. Bioresour Technol 118, 210–218.

Navaneethan, N., Topczewski, P., Royer, S., Zitomer, D., 2011. Blending anaerobic co-digestates: synergism and economics. Water Sci Technol 63, 2916–2922.

Neves, L., Oliveira, R., Alves, M.M., 2004. Influence of inoculumactivity on the bio-methanization of a kitchen waste underdifferent waste/inoculum ratios.Process Biochem. 39(12), 2019-2024.

Nkemka, V.N., Murto, M., 2013. Two-stage anaerobic dry digestion of blue mussel and reed. Renew Energy 50, 359–364.

Noxolo, T. Sibiya, E.M., Habtom, B.T., 2008. Effect of Temperature and pH on The Anaerobic Digestion of Grass Silage. https://ujcontent.uj.ac.za/vital/access/services/Download/uj:5051/CONTENT1

O-Thong, S., Prasertsan, P., Birkeland, N.K., 2008. Evaluation of methods for preparing hydrogen-producing seed inocula under thermophilic condition by process performance and microbial community analysis. Bioresour. Technol. 100, Issue 2, 909-918. https://doi.org/10.1016/j.biortech.2008.07.036.

Owens, J.M. Chynoweth, D.P., 1993. Biochemical methanepotential of municipal solid-waste (MSW) components.WaterSci. Technol.27(2), 1-14.

Palmqvist, E., Hahn, H., B., 2000. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresour Technol 74, 25–33.

Palmowski, L.M., Müller, J.M., 2000. Influence of the size reduction of organic waste on their anaerobic digestionWater Sci Technol. 41(3): 155-162. DOI 10.2166/wst.2000.0067.

Placha, I., Venglovsky, J., Sasakova, N., Svoboda, I.F., 2001. The effect of summer and winter seasons on the survival of Salmonella typhimurium and indicator micro-organisms during the storage of solid fraction of pig slurry Research Institute of Veterinary Medicine, Kos

Pohl, M., Mumme, J., Heeg, K., Nettmann, E., 2012. Thermo- and mesophilic anaerobic digestion of wheat straw by the upflow anaerobic solid-state (UASS) process. Bioresour Technol 124, 321–327.

Pommier, S., Chenu, D., Quintard, M., Lefebvre, X., 2007. A logistic model for the prediction of the influence of water on the solid waste methanization in landfills. Biotechnol Bioeng 97, 473–482.

Rafieenia, R., Pivato, A., Lavagnolo, M.C., 2018. Effect of inoculum pre-treatment on mesophilic hydrogen and methane production from food waste using two-stage anaerobic digestion. International Journal of Hydrogen Energy. 43, Issue 27, 12013-12022. https://doi.org/10.1016/j.ijhydene.2018.04.170.

Rajagopal, R., Masse, D. I. & Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour Technol 143, 632-641, doi:10.1016/j. biortech.2013.06.030 (2013).

Ramos, L.P., 2003. The chemistry involved in the steam treatment of lignocellulosic materials. Quim Nova 26, 863–871.

Raposo, F., Banks, C. J., Siegert, I., Heaven, S. & Borja, R., 2006. Influence of inoculum to substrate ratio on the biochemicalmethane potential of maize in batch tests.Process Biochem.41(6), 1444-1450.

Raposo, F., de la Rubia, M.A., Borja, R., Alaiz, M., 2008. Assessment of a modified and optimised method fordetermining chemical oxygen demand of solid substrates and solutions with high suspended solid content.Talanta. 76, 448-453.

Raposo, F., Fernández C.V., De la Rubia M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández, P.M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., Wierinck, I., De Wilde, V., 2011. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. J. Chem. Technol. Biotechnol., 86(8): 1088-1098. DOI 10.1002/jctb.2622.

Rehl, T., Müller, J., 2011. Life cycle assessment of biogas digestate processing technologies. Resour Conserv Recy 56, 92–104.

Regueiro, L., Veiga, P., Figueroa, M., Gutierrez, J.A., Stams, A.J.M., Juan M. Lema and Marta Carballa.: Relationship between microbial activity and microbial community structure in six full-scale anaerobic digesters. Microbiological Research 167, 581-589 (2012). https://doi.org/10.1016/j.micres.2012.06.002.

Rhiannon, M., Ben, J., Woodcroft, E.H. Kim, C.K. McCalley, S.B., Hodgkins, P.M., Crill, J.C., Gregory, B.H., Nathan, C.V., Scott, R.S., Philip, H., Virginia, I., Rich, G.W.T., 2014. Discovery of a novel methanogen prevalent in thawing permafrost, Nature Communications ISSN 2041-1723.

Rivard, C.J., Bordeaux, F., Henson, J.M., Smith, P.H., 1998. “Effects of addition of soluble oxidants on the thermophilic anaerobic digestion of biomass to methane,” Appl. Biochem. Biotechnol, 17, 1-3, 245-262.

Riau, V., De la Rubia, M.A., Pérez, M., 2012. Assessment of solid retention time of a temperature phased anaerobic digestion system on performance and final sludge characteristics. J Chem Technol Biotechnol 87, 1074–1082.

Rico, C., Montes, J.A., Munoz, N., Rico, J.L., 2015. Thermophilic anaerobic digestion of the screened solid fraction of dairy manure in a solid-phase percolating reactor system. Clean., Prod., 102: 512-520. DOI 10.1016/j.jclepro.2015.04.101.

Rinzema, A., Boone, M., Vanknippenberg, K., Lettinga, G., 1994. Bactericidal effect of long-chain fatty-acids in anaerobic-digestion. Water Environ Res 66, 40–49.

Roberts, R., Davies, W.J., Forster, C.F., 1999. Two-stage, thermophilic-mesophilic anaerobic digestion of sewage sludge. Process Saf Environ Prot 77, 93–97.

Rotaru, A.E., Shrestha, P.M., Liu, F., Markovaite, B., Chen, S., Nevin, K., 2014. Direct interspecies electron transfer between Geobacter metallireducens and Methanosarcina barkeri. Appl. Environ. Microbiol. 80 4599–4605. 10.1128/aem.00895-14.

Rozzi, A., Remigi, E., 2004. Methods of assessing microbial activityand inhibition under anaerobic conditions: a literature review.Rev. Environ. Sci. Biotechnol.3(2), 93 – 115.

Ruiz, B., Flotats, X., 2014. Citrus essential oils and their influence on the anaerobic digestion process: An overview. Waste Manage http://dx.doi.org/10.1016/j.wasman.2014.06.026.

Sakai, S., Takaki, Y., Shimamura, S., Sekine, M., Tajima, T., 2011. Genome sequence of a mesophilic hydrogenotrophic methanogen Methanocella paludicola, the first cultivated representative of the order Methanocellales. PLoS One. 6(7), e22898. DOI:10.1371/journal.pone.0022898

Schink, B., Stams, A.J.M., 2006. Syntrophism among prokaryotes. In: Dworkin M, editor. The Prokaryotes. 3rd ed. New York: Springer-Verlag. 309–335. DOI:10.1007/978-3-642-30123-0_59

Schlundt, J., 1984. Survival of pathogenic enteric bacteria in anaerobic digestion and on slurry-treated land. Dissertation Abstracts International C45(4), 1025.

Shahriari, H., Warith, M., Hamoda, M., Kennedy, K.J., 2012. Effect of leachate recirculation on mesophilic anaerobic digestion of food waste. Waste Manage 32, 400–403.

Sharma, D., Espinosa-Solares, T., Huber, D.H., 2013. Thermophilic anaerobic co-digestion of poultry litter and thin stillage. Bioresour Technol 136, 251–256.

Shen, L., Zhao, Q., Wu, X., Li, X., Li, Q., Wang, Y., 2016. Interspecies electron transfer in syntrophic methanogenic consortia: from cultures to bioreactors. Renew Sustain Energy. 54, 1358–1367. DOI:10.1016/j.rser.2015.10.102

Sieber, J.R., McInerney, M.J., Gunsalus, R.P., 2012. Genomic insight into syntrophy: the paradigm for anaerobic metabolic cooperation. Annu Rev Microbiol. 66, 429–452. DOI: 10.1146/annurev-micro-090110-102844.

Sikkema, J., Debont, J.A.M., Poolman, B., 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 59, 201–222.

Sikora, A., Błaszczyk, M., Jurkowski, M., Zielenkiewicz, U., 2013. Lactic acid bacteria in hydrogen producing consortia: on purpose or by coincidence. In: Kongo M, editor. Lactic Acid Bacteria-R&D for Food, Health and Livestock Purposes. Rijeka: InTech. 487-514. DOI:5772/50364

Siripatana, C., Sunwanee Jijai, and Prawit Kongjan., 2016. Analysis and extension of Gompertz-type and Monod-type equations for estimation of design parameters from batch anaerobic digestion experiments Citation: AIP Conference Proceedings 1775, 030079 (2016); doi: 10.1063/1.4965199 View online: http://dx.doi.org/10.1063/1.4965199 View Table of Contents: http://scitation.aip.org/content/aip/proceeding/aipcp/1775?ver=pdfcov

Song, Y.C., Kwon, S.J., Woo, J.H., 2004. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilicand thermophilic digestion of sewage sludge. Water Res 38, 1653–1662.

Sosnowski, P., Wieczorek, A., Ledakowicz, S., 2003. Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. Adv EnvironRes 7, 609–616.

Sousa, D.Z., Salvador, A.F., Ramos, J., Guedes, A.P., Barbosa, S., Stams, A.J.M., et al., 2013. Activity and viability of methanogens in anaerobic digestion of unsaturated and saturated long-chain fatty acids. Appl Environ Microbiol 79, 4239–4245.

Sponza, D.T., Ağdağ, O.N., 2004. Impact of leachate recirculation and recirculation volume on stabilization of municipal solid wastes in simulated anaerobic bioreactors. Process Biochem 39, 2157–2165.

Srilatha, H.R., Nand, K., Babu, K.S., Madhukara, K., 1995. Fungal pre-treatment of orange processing waste by solid-state fermentation for improved production of methane. Process Biochem 30, 327–331.

Stams, A.J.M., Plugge, C.M., 2009. Electron transfer in syntrophic communities of anaerobic bacteria and Archaea. Nat Rev Microbiol. 7:568-577. DOI:10.1038/nrmicro2166.

Struchtemeyer, C.G., Ducan, K.E., McInerney, M.J., 2011. Evidence for syntrophic butyrate metabolism under sulfate reducing conditions in a hydrocarbon-contaminate aquifer. FEMS Microbiol Ecol. 76:289–300. DOI:10.1111/j.1574-6941.2011.01046.x

Suksonga, W., Mamiminb, C., Prasertsanb, P., Kongjanc, P., O-Thong, S., 2019. Effect of inoculum types and microbial community on thermophilic and mesophilic solid-state anaerobic digestion of empty fruit bunches for biogas production. Industrial Crops & Products. 133, 193-202. DOI:10.1016/j.indcrop.2019.03.005.

Sung, S.W., Liu, T., 2003. Ammonia inhibition on thermophilic anaerobic digestion. Chemosphere 53, 43–52.

Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81 (5), 577–583.

Tchobanoglous, G., Burton, Franklin, L., and Stensel, H. D., 2010. Wastewater Engineering. Metcalf & Eddy, Inc, ISBN 7-302-05857-1.

Tepsour, M., Usmanbaha, N., Rattanaya, T., Jariyaboon, R., O-Thong, S., Prasertsan, P., Kongjan, P., 2019. Biogas Production from Oil Palm Empty Fruit Bunches and Palm Oil Decanter Cake using Solid-State Anaerobic co-Digestion. Energies., 12(22): 4368. DOI 10.3390/en12224368.

Timmers, P.H.A., Vavourakis, C.D., Kleerebezem, R., Damsté, J.S.S., Muyzer, G., Stams, A.J.M., Sorokin, D.Y., Plugge, C.M., 2018. Metabolism and Occurrence of Methanogenic and Sulfate-Reducing Syntrophic Acetate Oxidizing Communities in Haloalkaline Environments. Front. Microbiol. https://doi.org/10.3389/fmicb.2018.03039

Vaccari, D. A., Strom, P. F. & Alleman, J. E., 2006. Environmental biology for engineers and scientists. Hoboken, NJ: John Wiley & Sons, Inc.

Vandevivere, P., De Baere, L., Verstraete, W., 2003. Types of anaerobic digester for solid wastes. In: Alvarez, J.M. (Ed.), Biomethanization of the Organic Fraction of Municipal Solid Wastes. IWA Publishing, Cornwall, pp. 111–140.

Van, D.P., Fujiwara, T., Tho, B.L., Toan, P.P.S., Minh, G.H., 2020. A review of anaerobic digestion systems for biodegradable waste: Configurations, operating parameters, and current trends. Environmental Engineering Research. 25(1), 1-17. https://doi.org/10.4491/eer.2018.334

Vaneeckhaute, C., Meers, E., Michels, E., Ghekiere, G., Accoe, F., Tack, F.M.G., 2013. Closing the nutrient cycle by using bio-digestion waste derivatives as synthetic fertilizer substitutes: A field experiment. Biomass Bioenergy 55, 175–189.

Vanwonterghem, I., Jensen, P.D., Ho, D.P., Batstone, D.J., Tyson, G.W., 2014. Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. Curr Opin Biotechnol. 27, 55–64. DOI:10.1016/j.copbio. 2013.11.004

Vanwonterghem, I., 2016. Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. Nat. Microbiol 1, 9, doi:10.1038/nmicrobiol.2016.170 (2016).

Vavilin, V.A., Rytov, S.V., Lokshina, L.Y., Pavlostathis, S.G., Barlaz, M.A., 2003. Distributed model of solid waste anaerobic digestion: effects of leachate recirculation and pH adjustment. Biotechnol Bioeng 81, 66–73.

Vaxelaire, J., Bongiovanni, J.M., Mousques, P., Puiggali, J.R., 2000. Thermal drying of residual sludge. Water Res 34, 4318–4323.

Vaxelaire, J., Bongiovanni, J.M., Puiggali, J.R., 1999. Mechanical dewatering and thermal drying of residual sludge. Environ Technol 20, 29–36.

Viana, M.B., Freitas, A.V., Leitão, R.C., Pinto, G.A.S., Santaella, S.T., 2012. Anaerobic digestion of crude glycerol: a review. Environ Technol Rev. 1, 81–92. DOI: 10.1080/09593330.2012.692723.

Vieira da Silva, M.E., Schwarzer, K., Hoffschmidt, B., Rodrigues, F.P., Schwarzer, T., Costa Rocha, P.A., 2013. Mass transfer correlation for evaporation–condensation thermal process in the range of 70–95°C. Renew Energy 53, 174–179.

Vogt, G., Liu, H., Kennedy, K., Vogt, H., Holbein, B., 2002. Super blue box recycling (SUBBOR) enhanced two-stage anaerobic digestion process for recycling municipal solid waste: laboratory pilot studies. Bioresour Technol 85 (3), 291–299.

Watanabe, Y., Tanaka, K., 1999. Innovative sludge handling through pelletization/thickening. Water Res 33, 3245–3252.

Weiss, N.D., Felby, C., Thygesen, L.G., 2019. Enzymatic hydrolysis is limited by biomass-water interactions at high-solids: improved performance through substrate modifications. Biotechnol Biofuels. 12, 3. https://doi.org/10.1186/s13068-018-1339-x

Weng, C., Jeris, J., 1976. Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. Water Res. 10, 9-18. DOI:10.1016/0043-1354(76)90151-2.

Weng, C.N., Jeris, J.S., 1976. Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. Water Res 10, 9–18.

Wijaya, A.S., Jariyaboon, R., Reungsang, A., Kongjan, P., 2020. Biochemical Methane Potential (BMP) of Cattle Manure, Chicken Manure, Rice Straw, and Hornwort in Mesophilic Mono-digestion. International Journal of Integrated Engineering 12 no. 3, 1-8. https://doi.org/10.30880/ijie.2020.12.03.001.

Wilkins, M., Suryawati, L., Maness, N., Chrz, D., 2007. Ethanol production by Saccharomyces cerevisiae and Kluyveromyces marxianus in the presence of orange-peel oil. World J Microbiol Biotechnol 23, 1161–1168.

Williams, R., Davis, U., 2005. Technology assessment for advanced biomass power generation. PIER Consultation Report. California Energy Commission, Sacramento, CA.

Willquist, K., Nkemka, V.N., Svensson, H., Pawar, S., Ljunggren, M., Karlsson, H., Murto, M., Hulteberg, C., Niel, E.W.J., Liden, G., 2012. Design of a novel biohythane process with high H2 and CH4 production rates. International Journal of Hydrogen Energy 37, Issue 23, 17749-17762. https://doi.org/10.1016/j.ijhydene.2012.08.092.

Worm, P., Koehorst, J.J., Visser, M., Sedano-Núñez, V.T., Schaap, P.J., 2014. A genomic view on syntrophic versus non-syntrophic lifestyle in anaerobic fatty acid degrading communities. Biochim Biophys Acta. 1837, 2004–2016. DOI:10.1016/j.bbabio.2014.06.005

Wu, J., Cao, Z., Hu, Y., Wang, X., Wang, G., Zuo, J., Wang, K., and Qian, Y., 2017. Microbial Insight into a Pilot-Scale Enhanced Two-Stage High-Solid Anaerobic Digestion System Treating Waste Activated Sludge Int J Environ Res Public Health. 14(12), 1483. https://doi.org/10.3390/ijerph14121483.

Wu, C., Huang, Q., Yu, M., Ren, Y., Wang, Q., Sakai, K., 2018. Effects of digestate recirculation on a two-stage anaerobic digestion system, particularly focusing on metabolite correlation analysis. Bioresour. Technol. 251, 40-48. https:// doi.org/10.1016/j.biortech.2017.12.020.

Wu, G., Healy, M.G., Zhan, X., 2009. Effect of the solid content on anaerobic digestion of meat and bone meal. Bioresour Technol 100, 4326–4331.

Xu, F., Wang, F., Lin, L., Li, Y., 2016. Comparison of digestate from solid anaerobic digesters and dewatered effluent from liquid anaerobic digesters as inocula for solid state anaerobic digestion of yard trimmings. Bioresour. Technol., 200, 753-760. https://doi.org/10.1016/j.biortech.2015.10.103.

Xu, F., Wang, Z.W., Tang, L., Li, Y., 2014. A mass diffusion-based interpretation of the effect of total solids content on solid-state anaerobic digestion of cellulosic biomass. Bioresour. Technol., 167, 178-185. https://doi.org/10.1016/j.biortech.2014.05.114.

Yadvika, S., Sreekrishnan, T.R., Kohli, S., and V. Rana, “Enhancement of biogas production from solid substrates using different techniques—a review,” Bioresource Technology, vol. 95, no. 1, pp. 1–10, 2004.

Yangin-Gomec, C., Ozturk, I., 2013. Effect of maize silage addition on biomethane recovery from mesophilic co-digestion of chicken and cattle manure to suppress ammonia inhibition. Energy Convers Manage 71, 92–100.

Yang, L.C., Xu, F., Ge, X., Li, Y., 2015. Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. Renewable and Sustainable Energy Reviews, 44, 824-834. https://doi.org/10.1016/j.rser.2015.01.002.

Yao, M., Carsten, J., Jan, M., Kaijun, W., Bernd, L., 2016. Oxygen tolerance capacity of upflow anaerobic solid-state (UASS) with anaerobic filter (AF) system. journal of environme ntal sciences. 45, 200–206.

Yenigun, O., Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: A review. Process Biochem48, 901-911, doi:10.1016/j.procbio.2013.04.012 (2013).

Yue, Z.B., Li, W.W., Yu, H.Q., 2013. Application of rumen microorganisms for anaerobic bioconversion of lignocellulosic biomass. Bioresour. Technol. 128, 738-744. https://doi.org/10.1016/j.biortech.2012.11.073

Zahedi, S., Sales, D., Romero, L.I., Solera, R., 2013a. Optimisation of single-phase dry-thermophilic anaerobic digestion under high organic loading rates of industrial municipal solid waste: Population dynamics. Bioresour Technol 146, 109–117.

Zahedi, S., Sales, D., Romero, L.I., Solera, R., 2013b. Optimisation of the two-phase dry-thermophilic anaerobic digestion process of sulphate-containing municipal solid waste: population dynamics. Bioresour Technol 148, 443–452.

Zang, J., Shih, J.C., Cheng, J.J., Liu, Z., Liu, Y. and Lu, W., 2020. Thermophilic solid state anaerobic digestion of switchgrass for liquid digestate reuse and organic fertilizer production. Renew. Agric. Food Syst., 35(5): 503-512. DOI 10.1017/S1742170519000139.

Zhang, J., Loh, K.C., Li, W., Lim, J.W., Dai, Y., Tong, Y.W., 2017. Three-stage anaerobic digester for food waste. Applied Energy 194 287-295 (). https://doi.org/10.1016/j.apenergy.2016.10.116.

Zhang, Y., Banks, C.J., Heaven, S., 2012. Co-digestion of source segregated domestic food waste to improve process stability. Bioresour Technol 114, 168–178.

Zhang, L., Lee, Y.W., Jahng, D., 2011. Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements. Bioresour Technol 102, 5048–5059.

Zhang, L., Loh, K.C., Zhang, J., Mao, L., Tong, Y.W., Wang, C.H., Dai, Y., 2019. Three-stage anaerobic co-digestion of food waste and waste activated sludge: Identifying bacterial and methanogenic archaeal communities and their correlations with performance parameters. Bioresour. Technol. 285, 121333. https://doi.org/10.1016/j.biortech.2019.121333.

Zhiyang, X., Mingxing, Z., Hengfeng, M., Zhenxing, H., Shumei, G., Wenquan, R., 2014. In situ volatile fatty acids influence biogas generation from kitchen wastes by anaerobic digestion Bioresource Technology 163 (2014) 186–192.

Zhou, H., Wen, Z., 2019. Solid-State Anaerobic Digestion for Waste Management and Biogas Production. Part of the Advances in Biochemical Engineering/Biotechnology book series (ABE, volume 169), Solid State Fermentation, 147-168. https://doi.org/10.1007/10_2019_86.

Zhu, G.F., Jha, A.K., 2013. Psychrophilic dry anaerobic digestion of cow dung for methane production: Effect of inoculum. Scienceasia 39, 500–510.

Zhu, J., Zheng, H., Ai, G., Zhang, G., Liu, D., Liu, X., 2012. The genome characteristics and predicted function of methyl-group oxidation pathway in the obligate aceticlastic methanogens, Methanosaeta spp. PLoS ONE 7:e36756 10.1371/journal.pone.0036756

Zulkifli, Z., Rasit, N., Siddique, M.N.I., Kongjan, P., 2020. Dry mesophilic and thermophilic semi-continuous anaerobic digestion of cow manure: effects of solid loading rate on the process performance. Biointerface Research in Applied Chemistry 10, Issue 4, 5972-5977 (2020). https://doi.org/10.33263/BRIAC104.972977