Examining the methane potential of a fiberbank sediment using two-stage anaerobic digestion system

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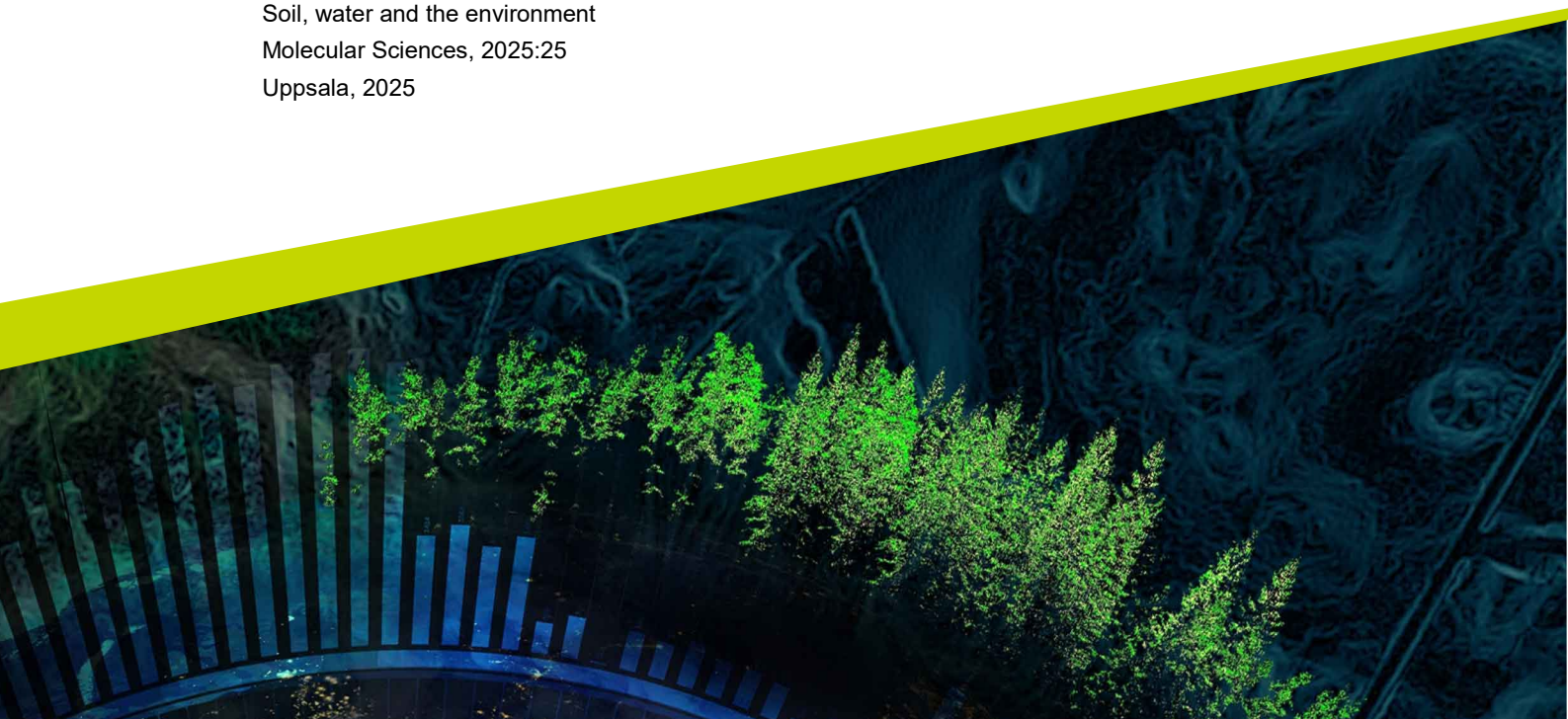

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Abbreviations

NH_4^+	Ammonium
AD	Anaerobic Digestion
CO_2	Carbon Dioxide
CSTR	Continuous Stirred-Tank Reactor
DFB	Digestate Fiberbanks Sediment
FB	Fiberbanks Sediment
FOS/TAC	Concentration of Volatile Fatty Acids to Alkaline Buffer Capacity Ratio
H_2	Hydrogen (gas phase)
H_2S	Hydrogen Sulfide
H	hydrogen ion
HRT	Hydraulic Retention Time
CH_4	Methane
OLR	Organic Loading Rate
PS	Primary Sludge
P&P	Pulp and paper
SMP	Specific Methane Production
TS	Total solid
VFA	Volatile Fatty Acids
VMP	Volumetric Methane Production
VS	Volatile solid

Abstract

Prior to the introduction of the environmental protection act by the Swedish government in 1969, the pulp and paper (P&P) industry discharged its process wastewater directly into the waterbodies, leading to an environmental impact in the form of fiberbank sediment. The fiberbank sediment consists of a high amount of biodegradable material and toxic compounds from industrial processing, posing environmental risks such as greenhouse gas (GHG) emission and the release of toxic substances into the surrounding environment. The traditional remediation methods of contaminated sediment such as dredging and landfilling are not feasible due to the potential of emission of GHG in storage. To address this issue, anaerobic digestion (AD) in two stage configurations is proposed as pre-treatment before storage. The feasibility of the proposed solution was evaluated through laboratory experiments using CSTR reactors and by co-digestion of fiber bank sediment with primary sludge from a wastewater treatment facility. In the acidogenesis stage, an 80:20 ratio of fiberbank sediment (FB) to primary sludge (PS) was found to be optimal for maximizing volatile fatty acid (VFA) production. In the methanogenesis stage, 5% of digestate from the acidogenic reactor was mixed with 95% PS on VS basis. The result showed statistically significantly higher volumetric methane production (VMP) of the test reactor (5.1 ± 0.7 L CH_4 / L) compared to control reactor (4.6 ± 0.7 L CH_4 / L). In contrast, the specific methane production (SMP) showed no significant difference between the test reactor (295 ± 35) compared to control reactor (306 ±37), indicating similar efficiency of biodegradability between the test reactor and control.

Keywords: Fiberbanks sediment, Anaerobic digestion, Remediation, two stage AD

1. Introduction

1.1 Background information

Sweden is one of the leading exporters globally of wood and wood-based materials. The wood industries and its wood derived product such as pulp, and paper (P&P), accounted for approximately 8.5% of Sweden total export (fig1) (business Sweden. 2024). The growth and success of the P&P industry in Sweden are attributed to several factors, including but not limited to unrestrictive access to natural resources such as low cost-energy, abundance of freshwater, and notably access to boreal forests which serve as a key source of raw materials (Bajpai. 2015). According to Swedish Forest Agency it was estimated that about 85 million cubic meters of forest are harvested annually for raw materials (Swedish Forest Agency. 2013). Of this, 35 million m^3 of the harvested forest undergo chemical and mechanical processing by the pulp and paper industry and transform into paper and packages (Sandberg et al. 2014). Furthermore, P&P mill and storage facilities were established alongside northern coastline. This geographic positioning has allowed the P&P industry to take full advantage of domestic water route for efficient transport of raw materials and end-product (Bajpai. 2015).

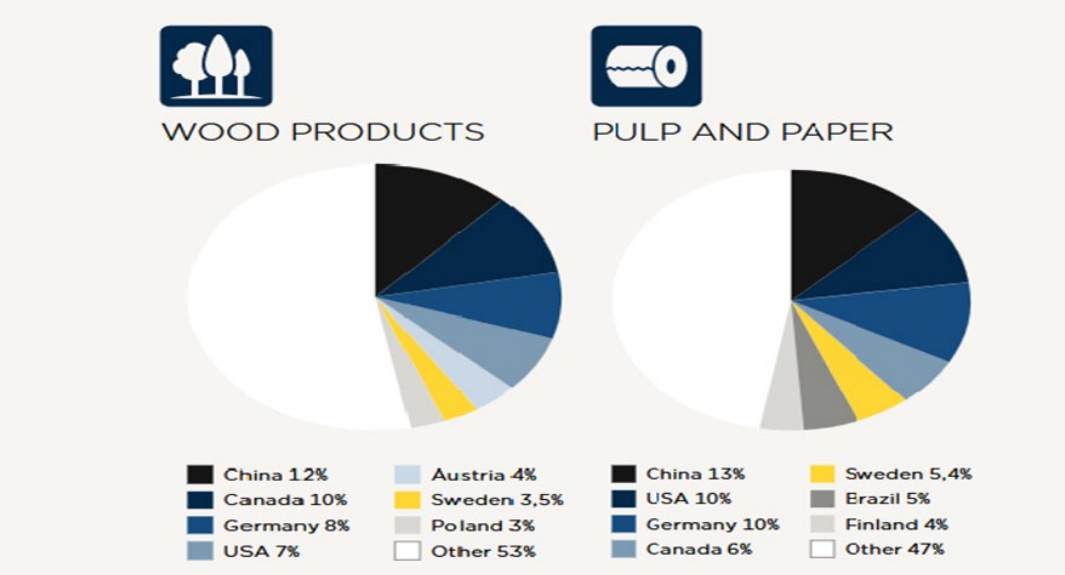

Figure 1: the percentage of wood, pulp and paper product of total export by leading exporter countries of wood and P&P industry around the world (business Sweden. 2024).

The P&P industry utilizes combinations of chemical and mechanical treatments to isolated plant fibers from one another, thus resulting in individual fibers mainly cellulose (Särkkä et al. 2018). During pulping process only about 40%-45% of raw material are utilized, the remaining 55%-65% are discharged as lignocellulosic waste e.g. cellulose, lignin and hemicellulose (Singh and Chandra. 2019). Globally, depending on the process, roughly about 3 billion m^3 of wastewater are discharged annually (Mainardis et al, 2024). Before the introduction of environmental regulations in 1969, effluent discharge from P&P industry was unregulated, leading to devastated environmental impact to aquatic environment such as toxic condition for water-based organism. (Bergquist et al. 2015). The characteristics of P&P wastewater differentiate based on treatment and target quality of the end-product. Several pollutants have been identified in P&P effluents, including heavy metals, inorganic compounds, and a variety of organic substances such as lignin, cellulose, and phenolic compounds (Thompson et al. 2001).

To address the issue of P&P effluents, the Swedish government adopted environmental protection act (EPA) in 1969. The act aimed to regulate and control the quality of effluent from P&P mill into waterbodies, by requiring on-site treatment of the wastewater (Bergquist et al. 2015). Despite the measurements that were taken by P&P industry and government, the environmental Impact of wastewater discharge still exists today, and it manifested by Fiberbanks deposition (Apler et al. 2019).

2. Fiberbanks

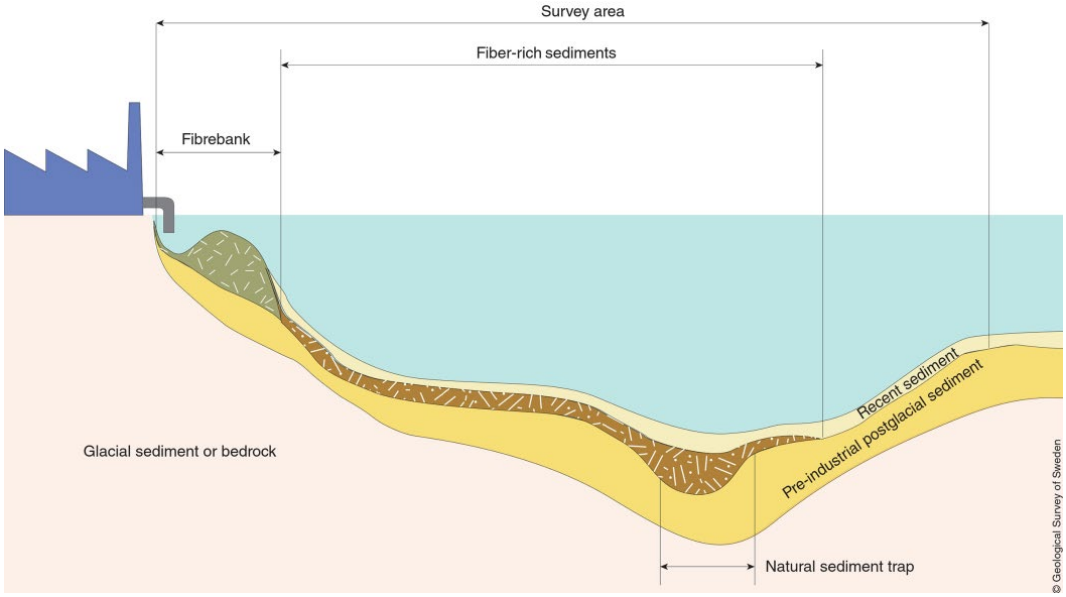

Figure 2: schematic diagram of fiberbanks deposition by P&P and fibred-rich sediment formation in waterbodies. Source Swedish geological survey (SGU).

Fiberbanks form near the point of discharge of P&P mills, resulting in the accumulation of wood and bark chip, and wood fiber such as lignin, cellulose and hemicellulose materials. (Apler et al. 2019). Due to coastal erosion and hydrodynamic processes, fiberbanks disperse into surrounding areas. The dispersed fibred material mixes with natural sediments and settled down in floor of waterbodies forming fiber-rich sediments (fig2) (Göransson et al. 2021). Although there are differences between fiberbanks and fiber-rich sediments in terms of formation process, the literature often refers to both as fiberbanks sediments (Joseph et al. 2016). Furthermore, a survey by Swedish geological survey (SGU) identifies 29 locations of fiberbanks found along the northern coastline of Sweden where previous and active pulp mill operating. These deposits are estimated to cover an area between 2.5 million m^2 and 26.5 million m^2, which corresponds to estimated volume between 7 and 11 million m^3, respectively (Stjärne et al. 2019, Löfroth et al. 2021).

Different chemical substance such as wood-preserver, pesticides and fungicides are used during P&P production processes. Many of these substances eventually accumulate in the fiberbanks sediment after discharge. Fiberbanks sediment had been recognized as potential secondary sources of spreading persistent organic pollutants (POPs) and heavy metal in aquatic environment. Organic compounds such as polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and

dichloro-diphenyl-trichloroethane (DDT) and metals such as zinc (Zn), copper (Cu) and arsenic (As), have been detected in fiberbanks in higher concentration compared to the surrounding environment (Dahlberg et al, 2020, Stjärne et al. 2019). POPs substances and metal elements are known to have toxic and poisonous effect on aquatic organisms and environment including disturbing the natural cycle and reproductive system of fish, reducing the biodiversity of benthic invertebrates and zoobenthos (Singh et al. 2019). Moreover, the toxicity of the POPs and metals substances is not restricted to aquatic organisms, rather it can be transported through the food-chain and posing risk to another organism, due to their ability to bioaccumulates in the tissue of organisms (Ali and Sreekrishnan. 2001).

The deposition of fiber-rich sediments on the sea floor may significantly increase the chemical oxygen demand (COD) due to the high amounts of biodegradable materials within the sediment. This can result in oxygen depletion and the development of an anoxic environment within sediment formation (Burton. 2003). Furthermore, the anoxic environment provides an ideal condition for anaerobic bacteria, which decompose the organic materials of the fiber sediment leading to production of methane (CH_4) and carbon dioxide (CO_2). Both CO_2 and CH_4 are potent greenhouse gases that have great warming influence on climate (Lehoux et al. 2021, Mar et al. 2022). A study by Norlin and Josefsson (2017) estimated that 76% of the 29 fiberbanks show a sign of gas release while 24% show no mark of gas emission. CH_4 emissions from fiberbanks sediment are facilitated by ebullition flux, in which the built-up pressure of gas in the anoxic layer exceeds the hydrostatic pressure of the overburden sediment layer, thus resulting in gas migrating upward toward the water column and forming rising gas bubbles (Bastviken et al. 2004).

The toxicity and GHG emission associated with fiberbanks sediment create environmental and ecological concerns that require effective and efficient remediation that ensure the contamination to be reduced to minimum level and at the same time offer high benefit-cost value. Dredging activities which consist of excavation, transport, and storage, are the established method in remediating contaminated marine sediment (Cecchi et al. 2021). However, in the case of fiberbanks, dredging alone may be insufficient, due to the substantial amount of sediment needed to be excavated, and the potential emission of GHG in post-dredging storage.

These challenges can be overcome by combining excavation with pre-treatment such as anaerobic digestion (AD) before storage. The AD treatment can potentially reduce the risk of methane emissions during sediment storage. Multiple studies have evaluated the biogas production of fibrous sediment co-substrate with different materials using biochemical methane potential (BMP). The BMP tests are used to demonstrate the maximum methane production under anaerobic conditions of substrate. The results show methane yield between 62 and 390 ml of CH4 per

gram of volatile solid (CH_4 mL/gVS), with the variation in yield attributed to fibrous sediment type and inoculum used in the test (Papachristopoulos. 2024, Lehoux et al. 2024). In addition, anaerobic digestion treatment has shown significant ability in terms of reducing pollutants in substrates, due to microbial degradation, sorption, and chemical transformation of contaminants (Blaszczak et al. 2024, Stasinakis. 2012).

3. Anaerobic Digestion

3.1 Introduction

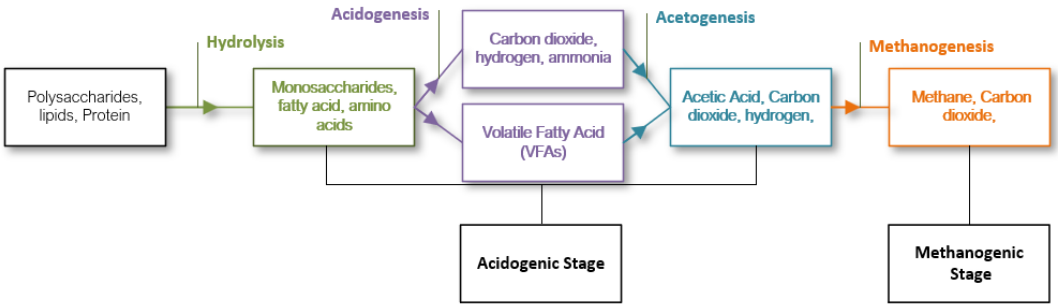

Figure 3: Flowchart showing the steps of anaerobic digestion (AD) in a two-stage configuration (acidogenic and methanogenic stages) and their metabolic products.

Anaerobic digestion (AD) is a biochemical process that utilizes microbial organisms in anoxic conditions to degrade and stabilize organic matter. The final products of AD processes are an organic digestate, and biogas which contain 40%-65 methane (CH_4), 35%-55% carbon dioxide (CO_2), and some trace amount 0.1%-3% of hydrogen sulfide (H_2S) (Ganjifar et al. 2024). The AD process comprises four metabolic stages, hydrolysis, acidogenesis, acetogenesis and methanogenesis (fig 3). Each stage of the process is carried out by distinctive microbial species that utilize different metabolic pathways to generate energy. Additionally, the microorganism species in AD can be classified into two groups, non-methanogenic bacterial species which are active in hydrolysis, acidogenesis, and acetogenesis, and the second type is methanogenic species which are only found in the methanogenesis stage (Chen et al. 2023).

3.2 Overview of acidogenic fermentation and methanogenesis

3.2.1 Hydrolysis

The first step in the AD process is hydrolysis, in which organic polymers such as lipids, polysaccharides and protein are broken into its simplest constitutes e.g., fatty acid, monosaccharides, and amino acids. The hydrolysis processes are carried out by bacteria that secrete extracellular enzymes such as cellulases, lipases, and proteases that catalyze the breakdown of chemical bonds, by bounding to the active site of organic structure (Artha et al. 2019, Song et al. 2005). For example, cellulase facilitates the breaking down of glycosidic bonds of cellulose, thus resulting in release of sugar such as glucose. Similarly, lipase and protease enzymes catalyze the hydrolysis of lipids and proteins, respectively, leading to release of glycerol and amino acid (Bonilla et al. 2018). Furthermore, the rate of hydrolysis reaction depends largely on the composition and complexity of the substrate. For example, lignocellulose is more resistant to degradation anaerobically due to the presence of lignin compound compared to glucose molecules, resulting in slower rate of hydrolysis process (Weinrich& Nelles. 2021).

3.2.2 Acidogenesis

The second step is acidogenesis, in which the derived products of hydrolysis are transformed by fermentative bacteria into VFA, organic acid, alcohol, CO_2, hydrogen (H_2) and some trace amounts of hydrogen sulfide (H_2S) (Weinrich& Nelles. 2021). Fermentative bacteria generate energy through anaerobic respiration utilizing three metabolic pathways: Entner–Doudoroff pathway for glucose substrate, Stickland and Deamination reaction for amino acids, and β-oxidation pathway for long-chain fatty acid (Harirchi et al. 2022).

First, the Enter-Doudoroff pathway oxidizes glucose into pyruvate as an intermediate product, depending on the pathway pyruvate can be further oxidized into VFA with six or less carbon atom (Weinrich& Nelles. 2021). These VFA compounds may have liner configuration such as acetate, propionate, butyrate, and valerate, or a branched-chain configuration namely iso-butyrate, and iso- valerate (Ramos Meyers et al. 2022). The concentration of VFA is influenced by pH, temperature and, importantly, H_2 partial pressure. For example, under high H_2 partial pressure, propionic and butyric acid formation are favorable, as they serve as electron acceptors (Chernicharo. 2007).

Second, the amino acid is metabolized either in pairs via Stickland pathway or individually through deamination reaction. The stickland pathway involves coupled oxidation–reduction reactions in which one amino acid act as an electron donor while the other act as an electron acceptor, producing pyruvate as intermediate product. Pyruvate can undergo further oxidation to yield acetate. In contrast to

stickland, deamination reaction which involves the removal of an amino group from a single amino acid, leading to the release of ammonia (NH_3), and occasionally H_2S. The process continues with the formation of acetyl which undergoes further oxidization generating acetate (Weinrich& Nelles. 2021, Doelle. 1969).

Finally, the acidogenesis of fatty acid is initiated by acyl-CoA enzyme synthetase in the cellular membrane altering the structure of fatty acid to form fatty acid-acyl compounds which then enter the β-oxidation cycle. The β-oxidation cycle comprises four metabolic steps: primary and secondary oxidation, hydration, and thiolysis, generating acetate and propionate acid (Houten and Wanders. 2012). Additionally, the molecular structure of fatty acid determines the organic acid outcome of β-oxidation cycle. For instance, even chain-fatty acid generates acetate while odd-chain fatty acid form propionate (Weinrich& Nelles. 2021).

3.2.3 Acetogenesis

The third step of AD process is acetogenesis, in which the product of acidogenesis e.g. VFA, alcohol, is transformed into acetate, CO_2, and H_2 by acetogenic bacteria (Weinrich& Nelles. 2021). In case of CO_2 and H_2, in strictly anaerobic environment the acetogenic bacteria utilize Wood-Ljungdahl pathway, to generate acetate. This pathway consists of two branches: methyl-branch and carbonyl-branch (Ragsdale & Pierce. 2008), both pathways use carbon-dioxide as a terminal electron acceptor.

In the methyl-branch, CO_2 reduces to formate, which undergoes series of enzymatic reaction forming methyl group bound to co-factor enzyme, iron-Sulphur compound (Co-FeSP). In the carbonyl-branch, CO_2 reduces to carbon monoxide (CO) which is combined with methyl-Co-FeSP by acetyl-CoA synthase, generating acetyl-CoA (Feng et al. 2022, Chen et al. 2017). Finally, acetyl-CoA is transformed into acetate.

The formations of acetate is an exergonic reaction. In contrast the oxidation of intermediate organic acid such as propionate to H_2 and CO_2 are endergonic reaction. Thus, the equilibrium must be shifted toward exergonic to make the reaction thermodynamically favorable. Acetogenic bacteria forms syntrophic relationship with hydrogen utilizing bacteria through interspecies hydrogen transfer (IHT) (Westerholm et al. 2021). As result of this syntrophic relationship, H_2 partial pressure kept at lower rate by hydrogen utilizing bacteria and reaction shifted forward.

3.2.4 Methanogenesis

The final stage of the AD process is methanogenesis, in which the products of acid fermentation e.g. acetate, CO_2 and H_2 are consumed by obligate anaerobic archaea, producing CH_4 and CO_2 (Weinrich& Nelles. 2021). Methane formation is

carried out through two main mechanisms, acetoclastic and hydrogenotrophic methanogenesis. In the acetoclastic reaction, the methyl-carbon of acetate is reduced forming CH_4, while the carboxyl group oxidized to CO_2. In the hydrogenotrophic mechanism, H_2 act as electron donors and CO_2 as electron acceptor, generating methane (Dworkin and Falkow, 2006).

3.3 Configuration and Parameters of AD process

The stability and efficiency of AD process is influenced by multiple factors, notably the substrate composition, environment conditions e.g. temperature, pH, and operating parameter e.g. hydraulic retention time (HRT) and organic loading rate (OLR) (Schnürer et al. 2016, Mao et al. 2015).

3.3.1 Configuration of AD reactor

The AD process in a constructed rector can either be performed in one-stage, or alternatively physically separated into two stage process based on the environmental and operational parameters (E. speece et al. 1997). The two-stage AD system allows the acidogenic fermentation stage to occur independently from the methanogenic stage, in contrast, single-stage AD where all the digestion stages are exposed to the same environment and operating condition. Furthermore, the two stages demonstrate various advantages in comparison to the one-stage system, for instance, the ability to decompose substrate, especially lignocellulose, effectively without limiting the rate of hydrolysis (Jin et al. 2022). Second, the enrichment of various types of bacteria in each stage without disturbing the stability of the process, i.e. the inhibition of methanogens in acidogenesis stage due to acidification or accumulation of VFA (Chakraborty et al. 2022). Furthermore, acidogenic stage are achieved by shortening the HRT and increasing OLR, thus increasing the accumulation of VFA and suppresses methanogenic activity. These conditions are favorable for hydrolytic and fermentative bacteria. In contrast, the methanogenic stage requires optimized conditions that promote the growth of archaea such as longer HRT, lower OLR and alkaline pH (Van et al, 2020)

3.3.2 Substrate characteristics

The composition of the substrates plays a crucial role in supporting efficient microbial growth and development, as they provide essential nutrients. For example, the two most important building blocks for cells are carbon and nitrogen, which are represented by the carbon-to-nitrogen (C/N) ratio (Anderson et al.2003). The typical ratio of C/N that ensure an optimal condition for bacteria, is between 15 and 30 (Schnürer et al. 2016). An Imbalances in the C/N ratio can inhibit bacterial growth. For instance, low C/N ratio indicates excess of nitrogen content in the substrate, promoting ammonia formation, which are toxic to microorganisms,

and specifically to methanogens. While high C/N ratio indicates nitrogen deficiency which can limit the growth of cells. Furthermore, the C/N ratio can be adjusted using co-digestion in which two or more substrates can be combined to improve the nutrient situation (Schnürer et al. 2016).

3.3.3 Environmental parameters

Temperature has substantial impact on the growth of the bacteria population and the rate of degrading organic material and methane production. The temperature in the AD process can be classified into three ranges, psychrophilic (>20ºC), mesophilic (20-45ºC), and thermophilic (55-70ºC) (Tg et al. 2022, Appels et al. 2008). The ideal temperature varies by the process stage. For example, thermophilic temperature was reported to enhance the hydrolytic enzyme activity, and acidogenesis e.g. VFA production, while mesophilic conditions were reported to enrich methanogenic archaea and improve the CH_4 yield (Tg et al. 2022).

Another critical parameter is pH, which can have a significant impact on the microbial population and their metabolic products. pH is regulated by several factors, including substrate composition, VFA accumulation, ammonia levels, and alkalinity (Schnürer et al., 2016). The optimal pH varies depending on the stage of the AD process. for example, hydrolytic and acidogenic bacteria are efficient at a pH between 5.5 and 6.5 (Mao et al., 2015; Kim et al., 2003). In contrast, methanogenesis which are sensitive to pH fluctuations, operates efficiently at pH between 6.5 and 7.8 (Sarker et al., 2019). Moreover, the process stability can be jeopardized by the accumulation of VFA and the release of total ammonia nitrogen (TAN). For instance, during acidogenesis, the accumulation of VFA caused by over-feeding or slower metabolization of intermediate products, can reduce the pH blow 5, leading to process failure. Additionally, protein degradation release ammonia which can increase pH above 7.8, thus inhibiting the methanogenic activity (Sarker et al., 2019).

3.3.4 Operating parameters

The hydraulic retention time (HRT) refers to the average time that organic substrate spends in the reactor. Typically, the HRT in the reactor ranges between 15 and 20 days depending on several factors such as, type of substrate, temperature, and configuration of the reactor (Sarker et al., 2019). Sugar and starch rich substrate are easy to digest requiring less HRT, while protein and lignocellulose rich substrate require comparably longer HRT due to complicated molecular structure (Sarker et al., 2019, Schnürer et al., 2016).

Temperatures, especially under thermophilic conditions, can enhance the hydrolysis rate compared to mesophilic condition thus reduce HRT required. Increasing volume of reactors can extend the HRT, thereby increasing the degradation of organic substances. Additionally, different stages of the AD process

require specific HRT to maintain optimal condition. For instance, methanogens have a slow growth rate and thus require longer HRT (8-10) days are preferred. As compared to what is needed for hydrolytic bacteria which grow more rapidly comparably and therefore require shorter HRT (2-4) day (Cremonez et al. 2021, Van et al, 2020).

Organic loading rate (OLR) is the daily added amount of organic matter per volume of rector. The amount of organic matter is usually expressed as volatile solid (VS) which indicate the total amount of organic matter in the wet substance (Schnürer et al. 2016). Typical OLR lies between 2 – 6 (gVS/l/d) depending on characteristics of the substrate and HRT (Sarker et al., 2019, Li et al. 2018). Substrate with a high-water content and low concentration of organic content require higher input to achieve the target OLR as compared to substrate with high organic content. High OLR is usually associated with high biogas yield. However, excessive feeding reduces the HRT which could potentially result in microbial washout and the accumulation of VFA (Tg et al., 2022).

3.3.5 AD Process Performance

The performance and stability of AD process are evaluated based on a set of indicators that provide a comprehensive assessment of system efficiency. These set of indicators include volumetric methane production (VMP), specific methane production (SMP), degree of digestion and FOS/TAC ratio. First methane production which can be assessed using two measurements, VMP which show the amount of methane produced per volume of reactor per day, demonstrating how efficient the volume digester is utilized (Schnürer et al., 2016). In contrast, the SMP reflect the amount of methane production per gram volatile solid (gVS) added, indicating how efficiently the microbial community are utilizing the feedstock into methane (Schnürer et al., 2016).In the context of two-stage AD system , typical SMP value range between 290 – 500 mL CH_4/gVS, depending on the substrate composition and operational condition (Van et al, 2020). Second, the degree of digestion (expressed as percentage) measure how efficient the system converting organic substrate into biogas assuming the input into the system equal the output (Schnürer et al., 2016). In two-stage system, the degree of digestion typically lies between 53.2 – 80.1% (Van et al, 2020). Finally, the FOS/TAC ratio represents the concentration of VFA (FOS) to alkaline buffer capacity ratio (TAC). The ratio considers to be an essential metric in monitoring the accumulation of organic acid and pH buffer capacity of reactor. A typical range of FOS/TAC ratio is between 0.2 – 0.6 (Nkuna et al. 2021).

4. Aim of The Project

Previous studies have shown promising results when fiberbanks sediment was used as substrate with primary sludge from wastewater treatment plant (WWTP) as co-substrate in one stage anerobic digestion system (Papachristopoulos. 2024). However, there was one primary challenge that affected the efficiency of the process, i.e. protein degradation. The protein degradation decreased when the sludge was combined with the carbohydrate fiberbanks material. Based on these findings, a two-stage anaerobic digestion system was proposed as a solution to overcome this limitation, as this potentially could separate the conversion of carbohydrates and proteins in two different reactors. This study will focus on the following objectives:

- Examine the VFA production of fiberbanks sediment (FB) during co-digestion with primary sludge (PS) in first stage.
- Examine methane potential of the VFA enriched digestate from co-digestion fiberbank sediment with primary sludge (PS) in a second stage reactor.
- Evaluate degree of degradation and protein mineralization in both stages (acidogenic stage and methanogenic stage).

5. Materials And Method

5.1 Substrates and Inoculum

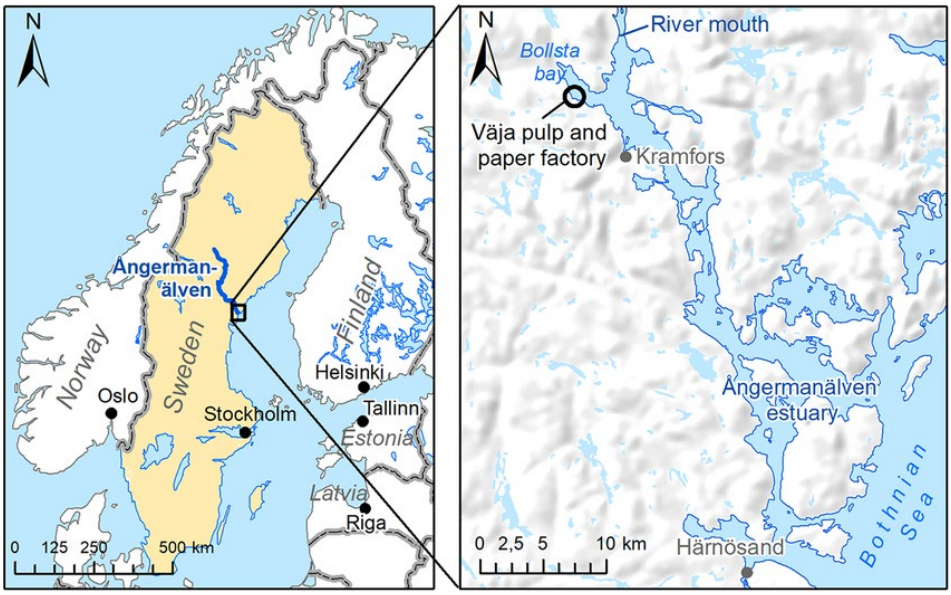

Figure 4: Map showing the location Kramfors municipality in Sweden and the site of the Väja pulp mill along the Ångermanälven River (source: Göransson et al., 2021).

The fiberbanks sediment was obtained by collecting the dredged sediment that originated from sawmill and sulphate pulp mill. The pulp mill was located along Ångermanälven river in Väja, within Kramfors municipality (Fig 5). The collected material was stored in cold storge room +3°C until the start of the project. Additionally, the inoculum and the primary sludge which were used for start-up of the reactors and as co-substrate, were collected from Uppsala wastewater treatment plant. The characteristics of fiberbanks sediment inoculum, and primary sludge including total solid (TS) and volatile solid (VS) are reported in Table 1.

Table 1 the mean and standard deviation (SD) values of total solids (TS) and volatile solids (VS) of the substrate and inoculum.

Substrate	Total Solid (TS%) - (std)	Volatile Solid (VS%)-(std)
Inoculum	3.81 ± 0.05	2.52 ± 0.1
Primary sludge (PS)	4.80 ± 0.01	3.90 ± 0.01
Fiberbanks sediment (FB)	6.66 ± 0.83	3.11 ± 0.27

5.2 Experiment Setup

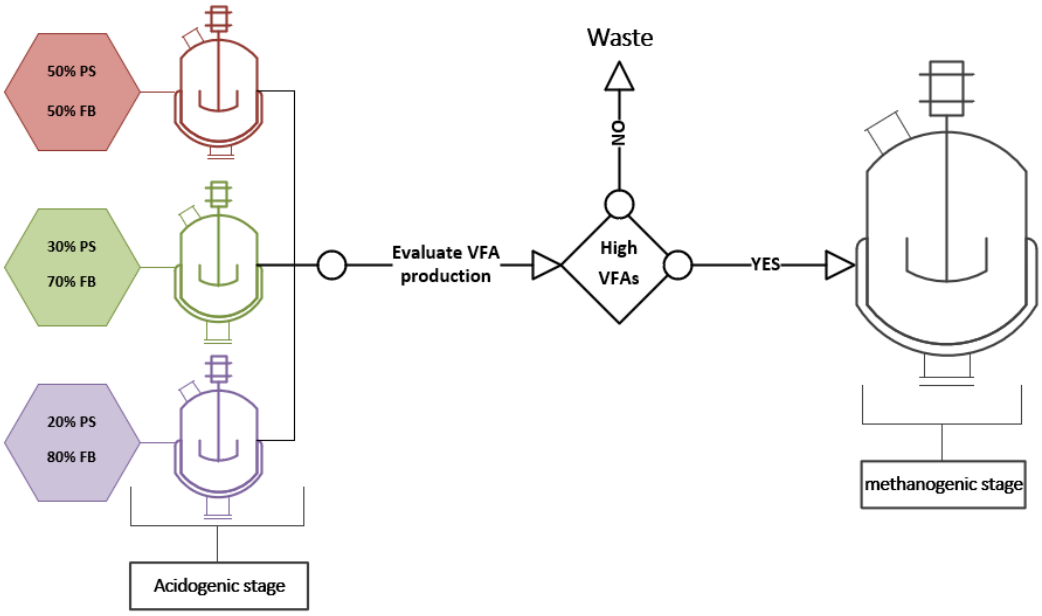

Figure 5: Schematic diagram of the experimental setup. The acidogenic stage consists of three reactors with varying mixtures of primary sludge (PS) and fiberbanks sediment (FB). Volatile fatty acid (VFA) production was evaluated after the acidogenic stage. The output from the substrate mixture giving the highest VFA levels, (20/80%) was collected and fed into the methanogenic stage. (created using Microsoft Visio)

The experiment was caried out in a two-stage configuration using Continuous Stirred-Tank Reactor (CSTR). The first stage was set up as pre-treatment, in which a mixture of different ratio of fiberbanks sediment and primary sludge as shown in (fig 5) were fed into three separate reactors to evaluate the VFA production. The outgoing material of the reactor with the highest VFAs were collected and stored in cold storage rooms to inactivate any further microbial degradation. Later the collected material was combined with primary sludge and fed into the test reactor (methanogenic stage), to evaluate the methane potential. Additionally, a control reactor with primary sludge only was set up as a reference to evaluate the performance of the test reactor (Table 2).

Table 2 AD Process Stages and their Corresponding reactors in this project.

Process Stage	Acidogenesis Stage	Methanogenesis stage	Methanogenesis stage (control)
Representation	Acidogenic reactor	Test reactor	Control reactor

5.2.1 First Stage Setup – (Acidogenic reactor)

Table 3 daily input and substrates composition that was used for each reactor, showing the ratio of fiberbanks sediment (FB) and primary sludge (PS), along with their corresponding volatile solids (VSg) and wet weight (g).

Substrate/Reactor	Q1 – (50% FB,50% PS)	Q2 – (70% FB,30% PS)	P1 – (80% FB,20% PS)
Fiberbanks sediment (FB)	29 VSg	39 VSg	44 VSg
	(938 g)	(1256 g)	(1402 g)
Primary sludge (PS)	29 VSg	15 VSg	10 VSg
	(737 g)	(418 g)	(276 g)
Total	56 VSg	55 VSg	54 VSg
	(1675 g)	(1672 g)	(1678 g)

Three CSTR reactors (Q1, Q2, P1) were used to carry out the acidogenesis stage of the experiment. The acidogenic reactors were filled with four liters of inoculums from a WWTP biogas facility. All acidogenic reactors had HRT of 3-day and were operated at a mesophilic temperature of 37.0 C and stirring speed of 100 rpm. Table 3 summarizes the feed amounts and ratios of fiberbanks sediment and primary sludge for each reactor during the acidogenesis stage. The active volume of the reactors was maintained by discharging a volume equal to the daily input.

In the third week, the active volume and HRT of reactor P1 were adjusted to 3 L and 4 days, respectively. Additionally, the feed to reactor P1 was modified to 619 g of FB and 130 g of PS to accommodate the new volume and HRT. The output from the reactor "P1" was collected and stored in cold storge room for the subsequent test stage.

5.2.2 Second Stage Setup – (Test and Control reactor)

Table 4 daily input of substrates used in P1 (test) and P2 (control) reactors, showing ratio of digestate fiberbanks sediment (DFB) and primary sludge (PS), along with their volatile solids (VSg) and wet weights (g).

Reactors ID/Substrate	P1- Test (5% DFB,95% PS)	P2 – Control (5% Water, 95% PS)
Digested-Fiberbanks sediment (DFB)	3 VSg (118 g)	-
Primary sludge (PS)	14 VSg (386 g)	15 VSg (407 g) + (97 g)
Total	17 VSg (504 g)	15 VSg (504 g)

After the end of the acidogenesis stage, another two reactors (P1, P2) were prepared by transferring 2.5 L the content of the P2 reactor to P1 and adding 2.5 L fresh digestate from the full scale WWTP. Both reactors were operated at an HRT of 10 days. The reactors were operated for a total period of six weeks (3 HRT) under a mesophilic temperature of 37^{o} C and stirring speed of 100 rpm. Like the acidogenic reactor, the active volume was maintained by discharging a volume equal to the daily input before the next feeding. In addition, both reactors were fed 406 g of primary sludge (PS) for 3 days to stabilize process parameters. Table 4 summarized the daily input on VS basis of primary sludge topped with water and stored digestate fiberbanks sediment (DFB) from acidogenic reactor.

5.3 Monitoring

The CSTR reactors in both stages were monitored on a daily and weekly basis to evaluate process performance. The parameters monitored daily included gas production, CO_2 level, and pH, whereas gas composition, FOS/TAC, and VFAs were measured on a weekly basis. First, the CSTR reactors (BELACH BIOTEKNIK, Sweden) came with built-in gas counters. At the beginning of each stage of the experiment, the gas counter was calibrated by attaching a gas bag to the reactor to collect the produced gas. The volume of collected gas was measured using Drum-type gas meters (RITTER, Germany). This process was repeated for five days, and the average value was used to calibrate the gas counter. Second, the CO_2 concentrations were measured using an Einhorn saccharometer. For each measurement, 5 mL of gas was taken out directly from the reactor using a syringe and injected into (7M NaOH) solution. Third, the pH and FOS/TAC were analyzed using TitraLab® AT1000 Series Potentiometric Titrator (Hach, USA). For FOS/TAC sample preparation, 5 mL of reactor output was collected and sieved, followed by adding 50 mL of deionized water.

VFA concentrations were determined using a SHIMADZU I-Series instrument. To prepare the sample for the instrument. 750 (µL) were taken from a 5mL sample of reactor output, using pipette and added into 2 mL tube. The 2 mL tube was

centrifuge for 15 minutes to separate solid from liquid. Then, 450 µL of separated liquid was drawn using a pipette and added to a new 2 mL tube followed by adding 45 µL of 5M sulfuric acid to the same tube. The 2 mL tube was then stored in freezer for at least 4 hours, then centrifuged again for 11 minutes. The sample was filtered through a 0.45 µm syringe filter and stored in a 4 mL glass vial for analysis. Fourth, gas composition including CH_4, CO_2, O_2, and H_2S was monitored using BIOGAS 5000 device (GEOTECH™, USA).

Fifth, The TS and VS was determined at the beginning and end of each stage, by taking measured volume of the substrate and place it in three aluminum trays. After that the trays were dried at 105 °C for 24 hours to obtain the total solids content. After drying, the trays were weighed and moved to 550 °C oven for an additional 24 hours to obtain the volatile solids. The TS and VS value for the first stage of the experiment were corrected using detailed estimation method described by Vahlberg et al (2013), to account for VFA loss during drying process. Next, to assess the performance of each stage, the degree of digestion was calculated using equation that was described by (Schnürer et al, 2016):

$$\text{degree of digestion (\%)} = 1 - \left(\frac{(DS_{in} * VS_{out})}{DS_{substrate} * VS_{substrate}}\right) * 100 = (\text{TS\% of VS})$$

Finally, nutrient concentrations including total carbon, total nitrogen (organic nitrogen and ammonium, NH_4^+)—were analyzed by an external laboratory Agrilab AB (Uppsala, Sweden) at the end of the experiment using standard method described by Perman et al. (2024)

5.4 Statistical analysis and visualization

All the figure presented in the results section and calculation such as the average, standard deviation, and p.value are preformed using JupyterLab (python 3).

6. Result

The VFA production as result of hydrolysis and acidification of different ratio of fiberbanks sediment (FB) and primary sludge (PS), is presented in figure 6. In reactor Q1, the concentration of VFA fluctuated between 0.95 and 1.30 g\L and pH remained at range of 6.7 - 6.9, throughout the entire period without significant change. In the case of reactor Q2, the VFA concentration demonstrated a sharp increase from 1.5 to 3.5 g/L by the end of week 3, while pH gradually decreased from 6.74 to 6.4 over the same period. Meanwhile the P1 reactor showed a gradual increase in VFA concentration to 3.79 g/L and a continuous drop in pH (5.27) until the end of experiment in week four. Reactors Q1 and Q2 operated for three weeks, while reactor P1 operated for an additional week due to significant performance as indicated by the lower pH and high VFA measurements compared to the other reactors.

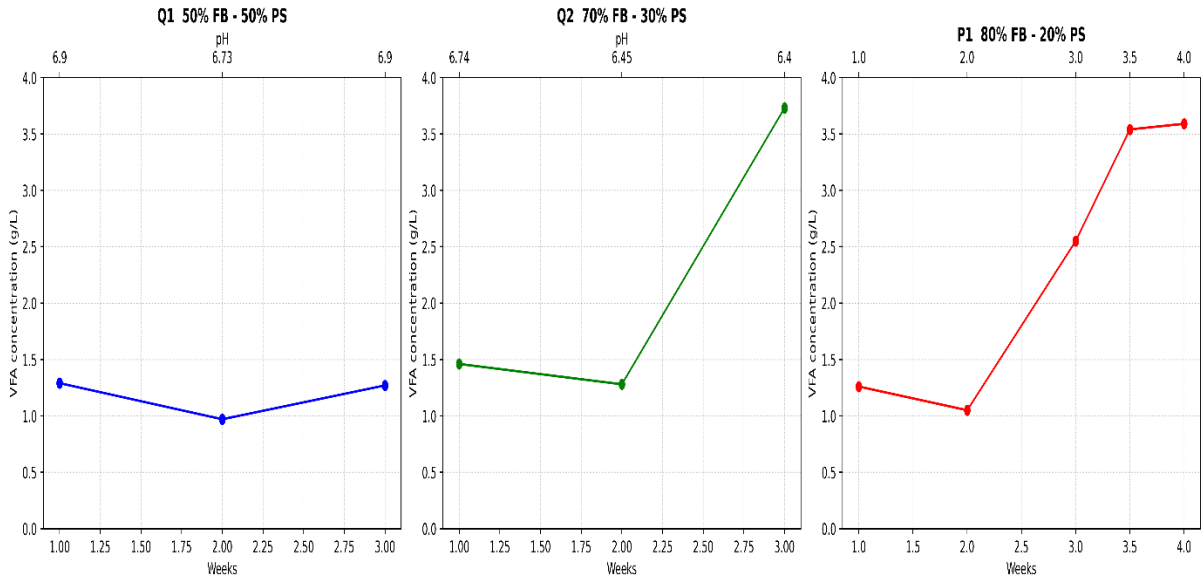

Figure 6: Volatile fatty acid (VFA) concentrations over time. Each graph shows the VFA concentration (g/L) and pH values during the acidogenic stage for different ratio of primary sludge (PS) and fiberbanks sediment (FB)

The result of analyzed acid composition for reactor “P1” are presented in figure 7. The result showed that propionic acid was predominant during the initial weeks (1 -3) with concentration of about 1.3 g/L, while acetic acid concentration was lower. However, from week 3 to the end of the experiment, the acid composition shifted to acetic acid reaching highest level around 1.9 g/L, while propionic acid was constant around 1.5 g/L. The other types of VFA such as butyrate, iso-butyrate, valerate, and iso-valerate stayed below 0.3 g/L for the entire duration. A total of 3.79 g/L of acid was produced during the 4-week period.

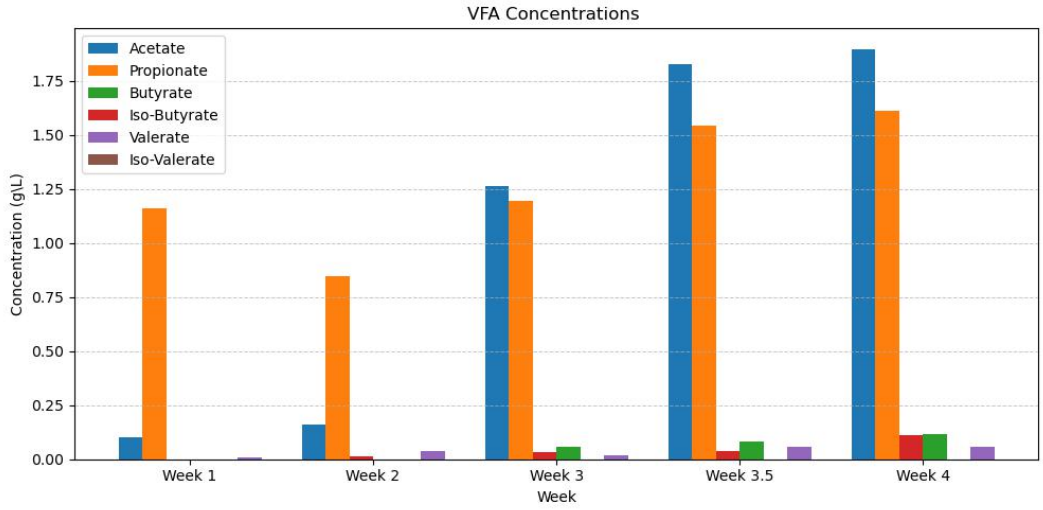

Figure 7: Weekly concentrations of volatile fatty acids (VFAs) in the acidogenic reactor (P1-80% FB – 20% PS), showing changes in the amount of acetate, propionate, butyrate, iso-butyrate, valerate, and iso-valerate over a four-week period.

The weekly volumetric methane production (VMP) of the acidogenic reactor and test reactor, receiving a combination of primary sludge and material from the acidogenic reactor 'P1', are presented in fig 8. The acidogenic reactor was operated for four weeks while the test and control reactor for six weeks. The acidogenic (P1-80% FB - 20% PS) produced an average of 2.4 ± 1.5 L CH_4 / L reactor and showed a decrease trend until end of experiment. In contrast, the test reactor which fed 5% digestate fiberbank (DFB) from the acidogenic reactor and 95% primary sludge on VS basis, produced an average of 5.1 ± 0.7 L CH_4 / L reactor, with an overall increasing trend, with a noticeable decline in week 4 attributed to a technical issue. After that the trend recovered and stabilized towards the end of the experiment. Combined, both the acidogenic and test reactor produced in total 7.5 L CH_4 / L reactor. For comparison, the control reactor was included in fig 8, next to the test reactor. The control produced on average 4.6 ± 0.7 L CH_4 / L reactor. A T.test showed a statistically significant higher difference between the test and control reactor (P.value =0.0119).

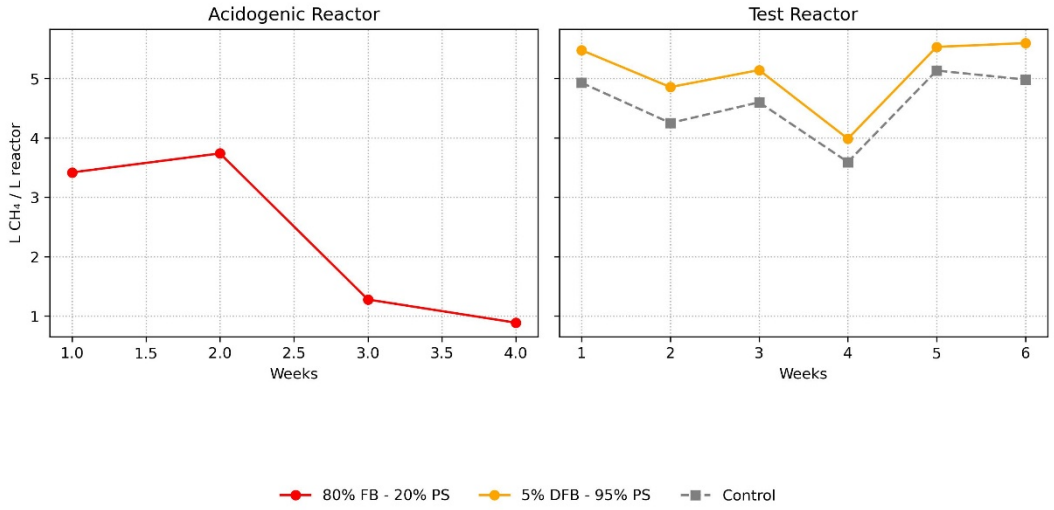

Figure 8: The volumetric methane production (VMP) of the acidogenic reactor (left) and test reactor (right), which also include the control VMP . The acidogenic reactor was fed 80% FB – 20% PS while the test reactor received 95% primary sludge and 5% of digestate from the acidogenic reactor (DFB), on VS basis. The control was fed 100% PS.

The specific methane production (SMP) from the acidogenic and test reactors are presented in (fig 9). The acidogenic reactor (P1-80% FB - 20% PS) had SMP value within the range of 5 – 56 ml CH_4/gVS and an average 36 ± 23. In contrast, the test reactor produced between 282 to 324 ml CH_4/gVS with an average of 295 ± 35 ml CH_4/gVS. Overall, both the acidogenic and test reactor produced in total 351.4 ml CH_4/gVS. The SMP of the control reactor was included alongside the test reactor for comparison. The control reactor yielded SMP value between 283- and 342-ml CH_4/gVS and an average of 306 ± 37.1. The t-test showed no significant difference between the control and test reactors (P.value =0.6)

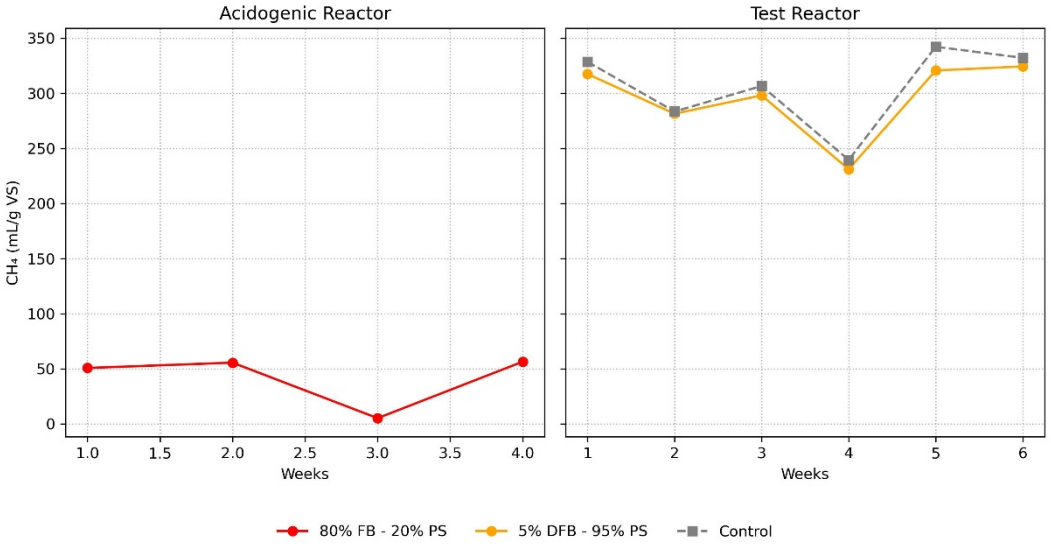

Figure 9: Specific methane production (SMP) for the acidogenic reactor (left) and the test reactor (right), including the control reactor. The acidogenic reactor was fed a mixture of 80% food waste (FB) and 20% primary sludge (PS). The test reactor received 95% PS and 5% digestate from the acidogenic reactor, based on VS basis. The control reactor was fed 100% PS.

Table 5 Monitored parameters in the acidogenic reactor, test reactor, and control reactor. Values are reported as mean ± standard deviation (SD).

Parameter	Acidogenic reactor	Test reactor	Control reactor
pH	6.1 ± 0.3	7.14 ± 0.04	7.18 ± 0.1
TAC (mg/kg)	3015 ± 789	3327 ± 505	3452 ± 370
FOS (mg/kg)	4976 ± 1533	1980 ± 336	1606 ± 356
FOS/TAC	1.7	0.6	0.5
Degree of digestion (%)	24	54	56
NH^{+}_{4} (g/kg wet weight)	0	0.5	0.6

The statistical analysis of different parameters (Table 4) was based on collecting four values of weekly measurements and calculating the mean and standard deviation (SD). The acidogenic reactor showed the lowest pH 6.1 ± 0.3 and TAC (3015 ± 789 mg/kg) followed by test reactor 3327 ± 505 while the control reactor maintained the highest pH and TAC. While the control reactor had the lowest FOS, the acidogenic reactor showed the highest FOS 4976 ± 1533 mg/kg of CH3COOH equivalents, followed by test reactor. The variation between FOS and TAC parameters across reactors is reflected in FOS/TAC ratio, in which acidogenic had the highest ratio 1.7 followed by test and control reactors. The degree of digestion was calculated for each stage of the process, and the acidogenic reactor had the lowest degree 24% of degradation, followed by test 54% and the control reactor 56%. Protein degradation represented by (NH^{4+}) was analyzed, the result showed no detectable amount in acidogenic reactor, while test reactor showed 0.5 g/kg and the highest was found in control reactor 0.6 g/kg.

The results of weekly analyzed gas composition (CO_2, CH_4, and H_2S) for acidogenic, test and control reactors are presented in fig 8. In the acidogenic reactor, the concentration of CO_2 was consistent (between 55 -50%) throughout the whole period, with an average of 53.4% ± 2.3. Meanwhile, CH_4 showed a decreasing trend with a significant drop overtime, with an average of 29% ± 16, while H_2S concentration gradually increased until it reached the highest level 398 ± 163.5 ppm with average 167.3. For test reactor, both the CO_2 and CH_4 measurements showed a relatively stable pattern with a minor decline of around 5% in the second and third week. Additionally, the mean for CO_2 was 37.1% ± 2 and CH_4 was 58.7% ± 3.1. Overall, there were no significant differences ($p = 0.60$) between the test and control reactor in terms of CH_4 or CO_2 production.

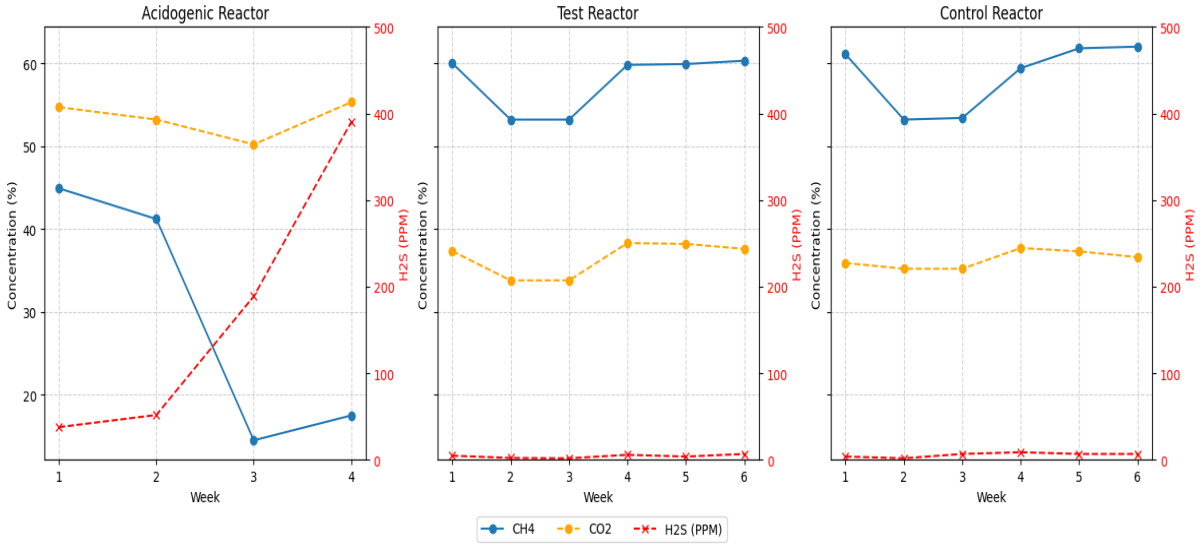

Figure 10: Weekly gas composition—CH_4 and CO_2 expressed in percentages, and H_2S in parts per million measured across Acidogenic, Methanogenic, and Control reactor.

7. Discussion

The result of the acidogenic reactor "P1" indicates that the operational and condition parameters specifically high OLR, and low pH, are the main factors influencing VFA production. The substantial amount of fiberbanks sediment (approximately 80% on VS basis) in the OLR, caused acidification and VFA production. The fiberbank sludge is rich in easily degradable cellulose which undergoes rapid hydrolysis and fermentation into organic acids, leading to acid accumulation, as indicated also by the FOS/TAC ratio (1.6). In contrast, the acidogenic reactor "Q2", which received less amount of fiberbank sludge (70% on VS basis), had a comparably higher pH (6.3) thus allowing methanogens to consume the organic acid, leading to slower acid accumulation and higher CH_4 production.

In regard of acid composition in the acidogenic reactor "P1", the increase in propionic acid concentration in initial week are likely due to ongoing cellulose fermentation and low pH. This interpretation is consistent with finding reported by Wang et al. (2006) who investigated factor that influence propionic acid accumulation in acidogenic reactor. In their study, the acidogenic reactor was fed with organic wastes from beet refinery, OLR (8 kg COD/m3. D) and HRT of (12 hours). The result showed that propionic acid was the predominant at pH (5.5). The similarity between the two studies in terms of operational parameters and substrate type (rich in cellulose and glucose) indicate that low pH and operational conditions play a crucial role in the accumulation of propionic acid. However, the observed shift from propionic to acetate acid in later weeks is likely due to change in the HRT from (3 to 4 days), indicating the longer HRT time enhanced the conversion of intermediate organic acid to acetate. The effect of the prolonged HRT is also reflected in the change in gas composition (Fig 8), where a 3% increase was observed.

Even though the primary goal of the acidogenic reactor was to have hydrolysis and fermentation of cellulose in the fiberbanks sludge, CH_4 was produced at minor quantity (0.46 L CH_4 / L reactor), attributed to methanogenic activity before the drop of pH. Ones the condition became acidic (pH< 6), methanogens become less efficient, as shown by the declining CH_4 concentration (less than 20%). Simultaneously, the increase and accumulation of CO_2 was observed, likely linked to the breaking down of cellulose. In addition, the inhibition of hydrogenotrophic methanogens, which consume H_2 and CO_2 to produce CH_4, can further contribute to the build-up of CO_2 in the acidogenic reactor. Similar conclusions have been reported by Huang et al. (2015), where a synthetic substrate, consisting mainly of sucrose with small concentration of VFA and cellulose, was fed into an acidogenic reactor (pH= 5.5, HRT=1.5 day, and OLR = 5 g/L. d), to investigate the role of hydrogenotrophic methanogens in the acidogenic reactor. In their studies, it was

observed at low pH (5.5), the concentration of CH_4 decreased to almost 0% due to inhibition of hydrogenotrophic methanogens, and simultaneously CO_2 and H_2 increased by approximately 22%.

The gradual increase in H_2S in the acidogenic reactor is likely associated with the solubility of H_2S in low pH condition (Basafa et al. 2020) as shown in the following chemical reaction:

$$H_2S\,(g) \rightleftharpoons H_2S\,(aq) \rightleftharpoons HS^- + H^+ \rightleftharpoons S^{2-} + 2H^+$$

In neutral pH (>7), the equilibrium is shifted toward the right side of reaction, in which H_2S is dissociated into it constitutes ions, sulfide ions (S^{2-}) and Hydrogen ion (H^+). In contrast, as pH decreases (pH< 6), the equilibrium shifts toward H_2S in gas phase and remains undissociated. As illustrated in gas composition (fig 10), the gradual increase in H_2S concentration occurred as pH decreased, indicating the shift in the equilibrium toward gas phase.

It could also have been caused by the increased activity of sulfate-reducing bacteria (SRB), which reduce sulfate (SO^{-2}_4) to H_2S based on the following reaction:

$$4\,H_2 + {SO_4}^{2-} + H^+ \rightarrow H_2S + 4\,H_2O$$

The increase of the activity of SRB is likely linked to the absence of hydrogen consumers such as hydrogenotrophic methanogens, and acetogens under acidic condition (pH< 6) (Mizuno et al. 1998). As shown in (Fig 10), the increase in H_2S concentration occurred simultaneously with a decrease in CH_4 production, indicating shift of the metabolic pathway, where SRB become the consumer of H_2, contributing to H_2S accumulation in the gas phase.

The low concentration of NH_4^+ and the degree of digestion in the acidogenic reactor, compared to the test and control reactors, indicate that low pH has a major impact on these two parameters. First, protein degradation is limited, likely due to the low pH, as suggested by Duong et al. (2019). In their study, an anaerobic batch experiment was conducted in a bottle (2.3 L) for 300 hours using gelatin as feed. The results showed that protein degradation was inhibited due to slow hydrolysis rate of protein to amino acid at low pH. Furthermore, regarding the degree of digestion, the low pH inhibited methanogenic activity, leading to decreased conversion efficiency of VFA to CH_4, thereby impacting the overall degree of digestion. This observation is consistent with findings reported by Paudel et al (2017), in a study of an acidogenic reactor (pH 5 – 5.5) fed with mixture of sorted food waste and brown water. The result showed VS% reduction equal to 26.7 ± 3.31 and hydrogen production equal to 28.38 ± 1.43 mL/gVS, suggesting a correlation between VS% reduction and gas production.

The volumetric methane production (VMP) showed a statistically significant difference between test and control reactor. The test reactor yielded 11.3% more CH_4 compared to the control which can be attributed to the added volume of digestate FB, which contained VFA that are readily converted to CH_4. On the other

hand, the specific methane production (SMP) showed no significant difference, indicating that the biodegradability in both systems was similar in terms of efficiency. Furthermore, this is also supported by the degree of digestion result, where the difference between the control and test reactor was only 2.7%. The small difference (2.7%) is likely linked to two parameters, the protein degradation (NH_4^+) and (FOS/TAC) ratio. The control reactor had higher protein degradation, as illustrated by a comparably higher ammonium level, thus likely giving an overall higher degree digestion. The FOS/TAC ratio (0.6) in the test reactor was also slightly higher than the control reactor and approached the instability range (> 0.6) (Nkuna et al. 2021). This likely indicates overfeeding causing slight VFA accumulation, which in turn led to less efficient conversion of VFA to CH_4, impacting the overall degree of digestion in the test reactor.

Given that no published studies have used a two-stage system configuration (R2) for fiberbank sediment treatment, the results of this study are compared to the one-stage system reported by Papachristopoulos (2024), which used similar substrates (Väja fiberbank sludge). The operating conditions and parameters of the one-stage system (R1) and its control (C1) are summarized in Table 6. The one-stage reactor (R1) received, on a wet basis, 372.9 g of primary sludge, topped up with 22% (99.3 g) fiberbank sludge. The control reactor (C1) operated under similar conditions, except that 99.3 g of water was used as the top-up instead of fiberbank sludge.

Table 6 Operating and condition parameters for the previous one-stage study, One – stage reactor (R1) and its control reactor (C1)

Parameter	One – stage reactor (R1)	Control (C1)
Active volume (L)	5.5	5.5
OLR (gVS/L. d)	2.5	2.5
HRT (d)	11.5	11.5
pH	7.1	7.3

The average weekly volumetric CH_4 production for R1 was reported to be approximately (≈ 5 CH_4 L / L reactor), while for R2 system (acidogenic + test reactor) produced 7.5 CH_4 L / L reactor. As mentioned before, the high volume of CH_4 is likely linked to the composition of the feed (digestate Fiberbank sediment) that R2 system received, compared to the R1 system. The efficiency of the system is highlighted in the specific methane production (SMP) values, in which the collective SMP of (acidogenic reactor + test reactor) for R2 system yielded 351.17 mL CH_4/gVS compared to the R1 system which was reported to have SMP value of 250.32 mL CH_4/gVS while C1 reactor reported higher value (≈ 293 mL CH_4/gVS). It should be noted here that there were differences in the characteristics of sludge. The VS% for the R1 configuration was reported as 1.3%. while for the R2 system it was 2.5%.

Still the result indicates that R2 configuration had more efficient utilization of feeding compared to the R1 and C1 reactors combined. Furthermore, the efficiency of R2 is also reflected in the degree of digestion which had a total of 77.9%. In contrast, The R1 reactor showed a decreasing trend, starting at 60% and by the end of the experiment reached around 50%, while the C1 reactor showed stable digestion at 65%. The enhanced digestion efficiency in R2 is likely attributed to stage separation, where most of cellulose material is hydrolyzed in the acidogenic reactor, allowing the second stage (test reactor) to focus on protein degradation. This is shown in NH_4^+ (0.5 g/kg) concentration compared to (0.28 g/Kg) in R1 and (0.35 g/Kg) in C1 reactor, indicating that microbial community in R1 had preference for cellulose over protein compared to test and C1 reactors. Based on these observations, the R2 system enhanced both the methane yield and digestion efficiency by providing ideal condition for microbial organisms.

Table 7 Summary of operational parameters for large scale acidogenic, methanogenic, one-stage, and control reactors.

Reactor	Volume (m^3)	HRT	OLR (kg VS/m^3·day)	VMP (m^3 CH_4/m^3·day)
Acidogenic	783	3	10.7	0.6
Methanogenic	2800	11	3.4	0.9
One stage	2800	11	2.9	0.7
Control	2800	11	3.1	1.1

The feasibility of this study was evaluated by scaling up the laboratory reactor setup to full-scale. First, the volume of acidogenic reactor was estimated based on the assumption that full-scale reactors would receive a total input of primary sludge (PS) equal to 261 m^3 / day with a reactor volume 2800 m^3 (HRT~ 11 days). If 22% on wet weight basis of PS (57.4 m^3 / day) was diverted and mixed with 78% of fiberbanks sediment (FB) (208.8 m^3 / day), the total input of the acidogenic reactor will be (261 m^3 / day). The HRT of the acidogenic reactor was set to 3 days, thus resulting in volume of the acidogenic reactor to be (783 m^3). Applying a similar top-up approach, only 22% (57.2m^3 out of 261 m^3/day) of the acidogenic reactor output will be utilized in the second methanogenic reactor. Therefore, it is recommended to operate the acidogenic reactor as batch reactor rather than continuously. In the second stage, 22% (57.2m^3) of output of the acidogenic reactor is mixed again with 78% PS (208.8 m^3), resulting in total input of (261 m^3). Assuming a full-scale reactor have HRT ≈ 11 days, then the required volume of second stage reactor was estimated to be ≈ 2800 m^3. Finally, the results of simulation of two-stages are compared with the one-stage study and control reactor (100% PS). The comparison between reactors was made under the assumption that the operational and environmental parameters in the laboratory setting were similar

for both studies (table 7). The two-stage configuration (acidogenic and methanogenic) combined achieved CH_4 yield of approximately 1.5 m^3 $CH_4/m^3 \cdot day$, outperforming the control and the one-stage reactor. This result agrees with laboratory scale studies, which indicate that the two-stage system enhances methane production compared to one-stage configurations.

7.1 Limitations and future research

One of the limitations of this study was the setup of the experiment. The study was originally designed to be conducted as a continuous two-stage system, however, due to shortage of the fiberbanks substrate, the experiment was modified to a pre-treatment using an acidogenic reactor in which the material was collected and stored, rather than feed directly into the second stage. This modification may have affected the microbial adaptation in the test reactor, potentially impacting the overall process performance. Additionally, the shortage of substrates prevented the possibility of setting up one-stage control reactors to use for direct comparison and comparison had to be done with a previous experiment using a different batch of primary sludge.

In term of VFA production in the acidogenic reactor, the shortage of substrates limited the ability to run the acidogenic reactor for longer duration, making it hard to identify the optimal mixing ratio of FB and PS.

The short duration of the experiment specifically in the test reactor, which was run for approximately three HRT (29 day), might be insufficient to draw conclusion about the overall efficiency of the system, since the gas production did not reach stable levels.

The study could be further improved by conducting a continuous two-stage experiment and incorporating additives to prevent methanogens from consuming VFA, or by adding acid to the acidogenic reactor to create acidic conditions at the start of the experiment. Secondly, a different approach could be tested by using less than 5% digested fiberbank sediment, as the 5% on VS basis level affected the FOS/TAC ratio. Finally, extending the HRT of the methanogenic reactor to 25 days may improve system performance specifically CH_4 production, since 10 days may be short when compared to the growth rate of methanogenic archaea.

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8. Conclusion

In this study, the methane potential of fiberbanks sediment co-digested with primary sludge in two-stage anaerobic digestion system were explored, with the goal to address the limitation that were observed in one stage system such as lower protein degradation. The result of the acidogenic reactor demonstrated that 80% FB – 20% PS was the optimal ratio for VFA production, achieving concentration of 3.79 g/L. In the test reactor (methanogenic stage), a top-up approach (5% on VS basis) with digestate fiberbanks sediment mixed with 95% primary sludge, was used to evaluate the methane potential. The result of the second stage configuration indicated on average higher gas production 5.22 L of CH_4 compared to control at 4.69 L of CH_4, while the SMP showed no significant difference between test (295 ± 35 CH_4/gVS) and control (306 ±37 CH_4/gVS). Under two-stage configuration, the overall digestion efficiency (77.9%) and protein degradation (0.5 g/kg) outperformed the results reported for the one stage system. Furthermore, to gain more insight into the practical implication of this study, the laboratory reactor was scaled up to full-size reactor. The scaled-up result showed more gas production in two stages compared to one stage, confirming the result of the laboratory study and the feasibility of this approach.

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Popular science summary

Sweden is a leading producer and exporter of wood and wood-derived products. This position was supported by the country vast boreal forests and network of waterways, especially along the northern coastline. Processing facilities such as pulp mills were established along these coastlines, benefiting from both access to water for industrial use and transport routes for export. However, before the introduction of the Environmental Protection Act in 1969, regulations regarding industrial waste discharge were not strict. As a result, pulp mills released their wastewater directly into nearby water bodies, creating deposits of fiberbank sediment.

Fiberbank sediment consists of plant fibers like cellulose and lignin, as well as toxic chemical compounds used in processing. Over the years, these deposits accumulated and spread with ocean currents and erosion. Due to its high density, the sediment settled on the seabed, forming thick layers of fiber-rich sediments. These layers contain organic compounds that serve as food for microorganisms. As microbes consume organic material, they create oxygen-free conditions, which in turn provide ideal conditions for anaerobic microbes that produce greenhouse gases like methane affecting the surrounding environment. Traditionally, contaminated sediment is excavated and stored in designated locations. However, in this case, simple storage is not enough, as it can still lead to greenhouse gas emissions. To address this issue, this project investigated a two-stage anaerobic digestion (AD) as a pre-treatment method before storage to produce energy in the form of biogas.

The two-stage AD process involved breaking down and fermenting the organic material in an acidogenic reactor, followed by biogas production in a methanogenic reactor (in this project we called Test reactor). The laboratory experiments showed promising results. Mixing 80% fiberbank sediment with 20% primary sludge produced a high amount of organic acids in the first stage, which when fed to the test reactor, it increased the methane production. Finally, the methane production was higher than the control reactor, telling that this two-stage process could be effective and environmentally friendly as a pre-treatment method for managing fiberbank sediment.

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