Evaluating toxicity in the anaerobic treatment of paper pulping effluents and the biological performance of EGSB reactor

By

Sovannmonica Ek

in partial fulfilment of the requirements for the degree of

Master of Science

in Civil engineering

at the Delft University of Technology

Thesis committee:	Assoc. Prof. Dr. Ir. Henri Spanjers (Chairman)
	Prof. Dr. Ir. Jules van Lier
	Asst . Prof . Dr . David Gregory Weissbrodt
Company Supervisor:	Dr. Ana Lucia Morgado Ferreira

TUDelft

Acknowledgement

This thesis research project has greatly expanded my knowledge of the anaerobic treatment technology on industrial wastewater. I have learned a lot from writing this thesis for developing my academic research skill, expressing my ideas, and discussing it scientifically. I will treasure and cherish this not only in my future career but in my whole life. It has become a reality with the kind support and help of many individuals and I would like to take this opportunity to express my immense gratitude to all those people who were a part of this journey.

First and foremost, I would like to express my deep gratitude to the chairman of my committee, Assoc. Prof. Dr. Ir. Henri Spanjers. As my teacher and mentor, he has shown me, by his example, what a good supervisor should be. He has helped me from the beginning of my thesis in searching for companies and topics as well as to develop a broader perspective on this complex thesis.

My sincere thanks go to my thesis committee advisory: Prof. Dr. Ir. Jules van Lier and Asst. Prof. Dr. David Gregory Weissbrodt. They generously devoted their time to give valuable comments, questions, and critical feedbacks to improve my work. In particular, Prof. Dr. Ir. Jules van Lier has provided detailed answers to all my doubts related to anaerobic technology and guided my work in the right direction.

I would like to extend a special thank you to my daily supervisor at Biothane, Dr. Ana Lucia Morgado Ferreira, for continuous support, cooperation, guidance, and for allowing me to execute my research at the company. Thank the Biothane team: Dr. Ran Shang, Anna Katrina Corpus, Rewin Pale, and Patrick van der Linde for being willing to help me out with their abilities in various phases of the completion of this project.

I am most grateful to the collaborators for lending me their expertise and intuition to my scientific and technical problems: Ir. Saqr Abdulrakeeb Al-Muraisy, Ir. Guilherme Lelis Giglio, and Ir. Victor Servando Garcia Rea at TU Delft for motivating and encouraging me to pursue my interests in anaerobic treatment technologies. They never stopped challenging me, helping me to develop new ideas, and always giving me words of encouragement pushing me further than I thought I could go.

Besides these academic supports, I am grateful for a number of friends who went through hard times together, cheers me on, and celebrated each accomplishment: Amirtha Varshiny Arumugam, Sadhna Jagannathan, Eman Rageh, Maitry Phukan, Ranjeev Bhatia, Isabella Schneider, and Mahzad Ansariramandi. They always believe in me and support me in this research journey. Thank you for being there for me both in the moments of despair and excitement, helping me to survive all of the stress from this year, and not letting me give up.

Last but, definitely not least, I am greatly in debt to thank my family for supporting me spiritually throughout my life. It was their unconditional, love, moral support, care, and tolerance that could make me overcome the difficulties during these years.

Abstract

Pulp and paper industries are water-intensive industries and composed of complex production processes. Untreated pulping wastewater is very toxic and lethal to aquatic life if discharged untreated. Anaerobic treatment technology has gained interest in treating these types of wastewater by reducing organic compounds. This thesis research aimed to evaluate the potential toxicity which might present in chemithermomechanical pulp (CTMP) wastewater, through the biological performance of a lab-scale expanded granular sludge bed (EGSB) reactor.

In the theoretical part CTMP process, characteristics of wastewater, toxicants, the functionality of an EGSB reactor, and any limiting factor that influences the treatment were investigated. In the experimental part, CTMP wastewaters from a mill in Sweden were analyzed using a lab-scale EGSB reactor for 182 days. Anaerobic biodegradability and toxicity test were done to measure the extent of anaerobic digestion in this wastewater. Different parameters such as chemical oxygen demand (COD) removal efficiency, volatile fatty acids (VFA), alkalinity, pH, and nutrient uptake were measured to access the biological performance of the EGSB reactor starting from unacclimated anaerobic granular biomass. Data analyzing tools including Excel and PHREEQC modeling were used in this research study. Excel tool was used to plot the graph and curve fitting whereas the PHREEQC model was done to understand the corrosivity of the biogas and calcite precipitation in the effluent discharge pipe.

The results showed that about 60% of the organic compounds in the wastewater were biodegradable, and no significant toxicity was found. Also, the performance of the EGSB was good with COD removal of roughly 50% at the stable phase. The presence of wood cellulose fiber in the wastewater had a negative impact on the performance of the reactor more specifically blockage in the recirculation and reduce methane production. However, based on the experimental results, EGSB alone would not be enough to remove most of the organic pollutants, which require additional post treatment such as aerobic system and membrane filtration to meet the discharge limit.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AGS	Anaerobic Granular Sludge
AMB	Acetotrophic Methanogenic Bacteria
AMPTS	Automatic Methane Potential Testing System
COD	Chemical Oxygen Demand
CTMP	Chemi Thermo Mechanical Pulping
DTPHA	Diethylene Triamine Penta Acetic
EDTA	Ethylene Diamine Tetra Acetic
EGSB	Expanded Granular Sludge Bed
EPS	Extracellular Polymeric Substances
GC	Gas Chromatography
HRT	Hydraulic Retention Time
IBC	Intermediate Bulk Container
IC	Ion Chromatography
SCOD	Soluble Chemical Oxygen Demand
SGW	Stone Ground Wood
SMA	Specific Methanogenic Activity
SRB	Sulfate Reducing Bacteria
TCOD	Total Chemical Oxygen Demand
TMP	Thermo Mechanical Pulping
VFA	Volatile Fatty Acids
VLR	Volumetric Loading Rate

1 Introduction

In recent years, the control of water pollution in the municipal and industrial sectors has become more significant (Cirik et al., 2013). Pulp and paper mills are water-intensive industries and are composed of complex production processes such as raw materials preparation, pulping, bleaching, and paper-making. Water is used in different manufacturing activities ranging from papermaking, cleaning the equipment, cooling, pump sealing, and steam production (Bajpai, 2017). The production of paper would be impossible without water. Aguiñaga-M orales et al. (2017) and Oliveira (2014) reported that this industry polluted 85% of the total water consumed and generated relatively 30 to 60 m^3 per ton of pulp. Due to its potential toxic emission, the pulp and paper industry ranked as the world's sixth-largest polluter after the oil and petroleum, cement, leather, textile, and steel industries (Ali & Sreekrishnan, 2001).

If the wastewater from pulp and paper mills is discharged into the environment untreated, it can cause severe damage to the ecosystem, especially to the aquatic life communities because of its high amount of biochemical oxygen demand (BOD), chemical oxygen demand (COD), halogenated organic compounds (AOX), suspended solids, etc (Ali & Sreekrishnan, 2001; Leach & Thakore, 1976; Rogers, 1973). Billings & DeHaas (1971) reported that the effluent differs between pulp and papermaking. Papermaking effluent contains cellulose fines and other additives which make up 50% of the total mass of the wastewater. The effluent from the paper-making mill that used dye to produce colored paper contains color. Pulping effluent contain dissolved wood substances and color due to the dissolved lignin.

In addition, the effluent concentration and volume depend on the raw materials, paper grade, specific freshwater intake, finished product, and extent of water reuse (Ali & Sreekrishnan, 2001). Each of the pulp and paper mill discharge effluents has its characteristics due to the various technologies used in the different processing units (Ali & Sreekrishnan, 2001). Thus, the choice of effluent treatment method depends on the characteristic of solid matter that needs to be removed, and the discharge limit requirement (Jung & Pauly, 2011). Hamm (2006) reported that anaerobic treatment is suitable for high polluted effluent such as from recycled paper mills. However, after anaerobic treatment, the wastewater has to be treated aerobically to fully biodegraded before discharging into the freshwater stream (Möbius, 2002). High-rate anaerobic treatment systems, which are applied mostly to agro-food and beverage, have

extremely low sludge production and produce granular sludge is an interesting market value for the excess sludge (Bajpai, 2017).

According to Pol & Lettinga (1986), anaerobic digestion is a leading edge technology in many industrial wastewater treatments. Nowadays, the Expanded Granular Sludge Bed (EGSB) reactor is a popular technology because of its robustness, cost-effective, and operation with a fluidized bed that has high treatment efficiencies of about 95% by increasing organic load and cell retention generating renewable energy such as biogas, biomethane, and biohydrogen which is collected in the headspace (Cruz-Salomón et al., 2019).

It is essential to develop a possible solution to treat pulping wastewater, for instance, agro-pulping black liquors contain a high amount of non-biodegradable lignin which contributes 50% of the total COD and has been proved to be toxic by causing the death of aquatic life if treated inadequately (Abhishek et al., 2017; Wang et al., 2006).

The research project concerned a study on pulp mill effluent treatment using a lab-scale EGSB reactor. This study aims to assess the performance of the EGSB in treating pulp mill effluent and to evaluate if there is potential toxicity present within the wastewater.

2   Literature Review

2.1   Introduction to pulp manufacturing

Pulping is the process that converts wood or other plant fiber source (bagasse, bamboo, hemp, flax, straw species, etc) by removing lignin, which acted as a glue that binds the cellulose fiber together, through chemical or mechanical, or a combination of these two techniques (Alén, 2019; Windeisen & Wegener, 2012).

Chemical pulping involves cooking and the use of a different aqueous solution such as soda, sulfate (kraft), or sulfite to extract pulp fiber (Windeisen & Wegener, 2012). Mechanical pulping is the process that produces pulp using mechanical energy which requires only water or steam instead of adding the chemical. The mechanical pulp can be produced by refining or grinding logs of wood, known as groundwood pulping (Bajpai, 2017).

Pulp yield refers to the recovery of pulp obtained from the original wood weight and is generally expressed as a percentage. If 45 oven-dry pounds of pulp is produced from a 100 pound of oven-dry wood, therefore the pulp yield is 45% (Briggs, 1994). Mechanical pulping has a pulp yield range from 90-95% which is almost two times higher than chemical pulping (40-55%). However, there is high content of impurities left in pulp and the grinding action makes short fiber in mechanical pulping which make paper weak and less resistant to aging (Bajpai, 2017; Kincaid, 1998).

2.2   Chemithermomechanical Processing

Chemithermomehanical pulping (CTMP) is the combination of the thermomechanical pulping (TMP) process and chemical impregnation such as sulfite or sodium hydroxide to help soften the adhesive between the fiber. According to Kent (2007), sulfite solution is commonly used to produce CTMP pulp and the pulp quality can be improved over stone groundwood (SGW). Figure 1 illustrates the detailed CTMP process and its waterline.
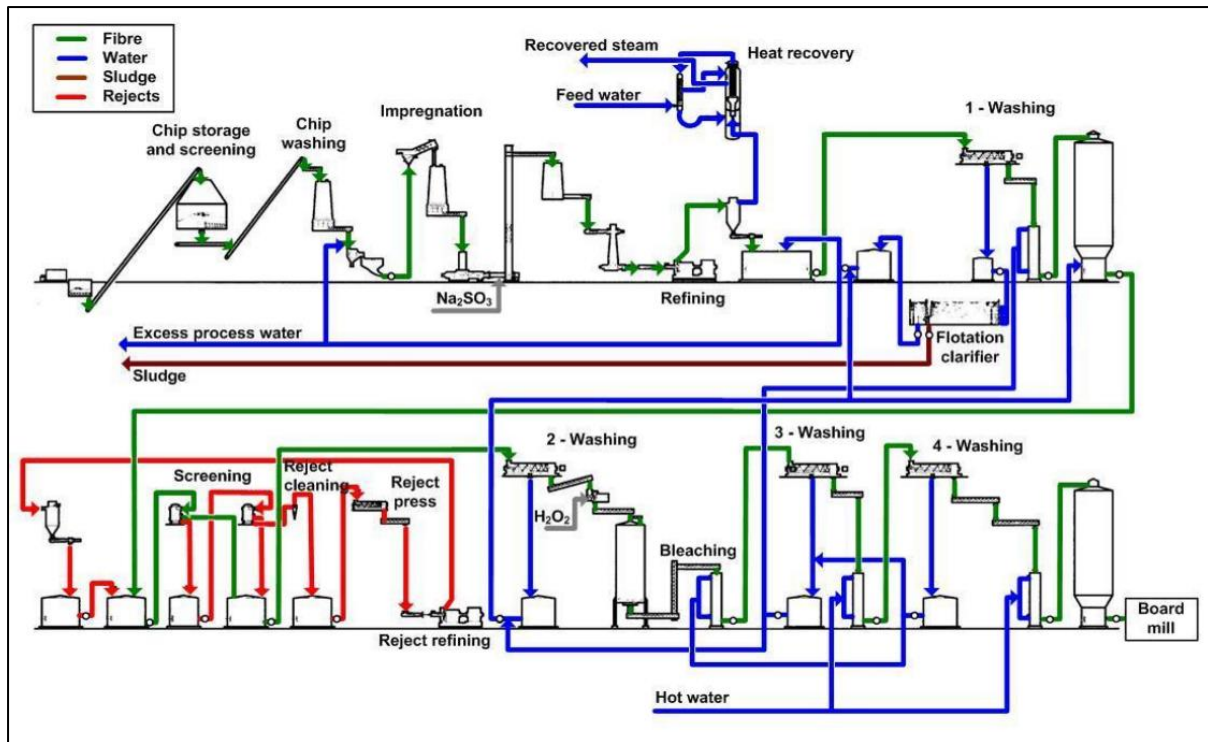
Figure 1. Schematic drawing of CTMP process. The green color indicates fiber line, blue indicates water line, red color indicates rejected stream, and brown color indicates sludge stream (Suhr et al., 2015).

2.3   CTMP Wastewater

In pulping industry, the common unit is in Air Dry tonne (ADt), an arbitrary convention used by the paper maker which is assumed to be 10% water and 90% oven-dry pulp (Briggs, 1994). CTMP process is a water-intensive process ranging from 9.5-30 m^3/ADt of pulp and paper whereas ground wood (GW) and thermomechanical pulping (TMP) use 5-20 m^3/ADt and 4-20 m^3/ADt of pulp and paper respectively (Suhr et al., 2015). Pichon et al. (1986) reported that the CTMP wastewater is composed of organic materials such as carbohydrates (10-15%), organic acids (35-40%), and lignin (30-40%). In the chip washer effluent, there is also the presence of sand and dirt. Figure 2 shows the emissions of wastewater from different process of CTMP mill.
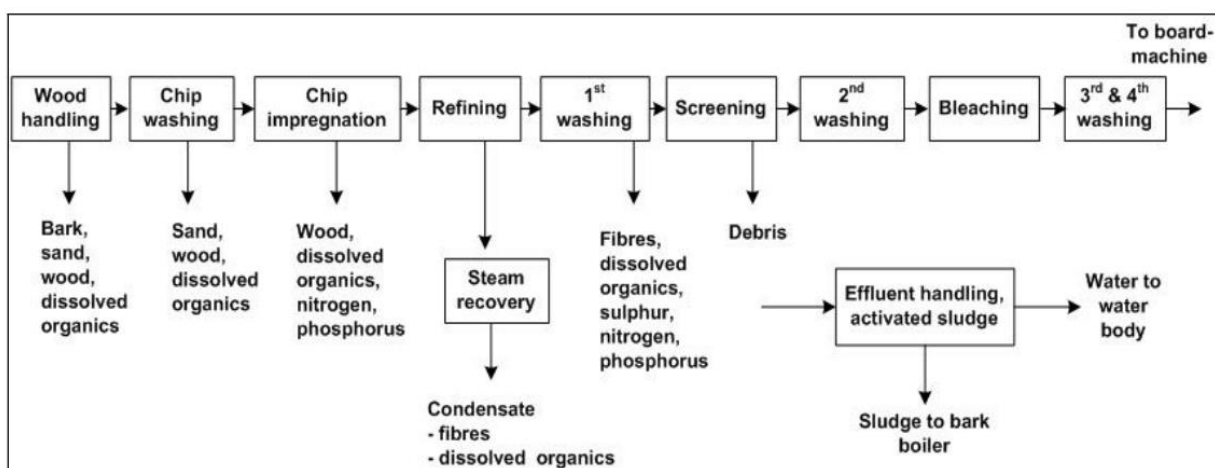
Figure 2. Emission source of wastewater from CTMP mill (Suhr et al., 2015).

Table 1. Summary of typical parameters and pollutants in the CTMP wastewater.

Parameter		Unit	Range	Source
pH		-	5.5- 8	(Stephenson et al., 1994)
Total Organic Carbon		mg/L	650	(Stephenson et al., 1994)
Chemical Oxygen Demand	(COD)	mg/L	2100-13000	(Stephenson et al., 1994)
Resin Acids		mg/L	42-770 [a]	(Stephenson et al., 1994)
Fatty Acid		mg/L	60-420 [a]	(Stephenson et al., 1994)
			60-210 [b]	
Acetate		mg/L	1500	(Stephenson et al., 1994)
Total Suspended Solids	(TSS)	mg/kg	180-5000	(Stephenson et al., 1994)
Total Nitrogen	(TN)	mg/L	14-50	(Ruutiainen, 1987)
Total Phosporous	(TP)	mg/L	0.5-32	(Ruutiainen, 1987)
Sulphate	(SO_4^{2-})	mg/L	181-2700	(Stephenson et al., 1994)
Sulfite	(SO_3^{2-})	mg/L	5-790	(Stephenson et al., 1994)
Sulfide	(S^{2-})	mg/L	0.7-3.3	(Stephenson et al., 1994)
DTPHA [c]	$(C_{14}H_{23}N_3O_{10})$			
EDTA [d]		mg/L	20-500	(Stephenson & Duff, 1996)
	$(C_{10}H_{16}N_2O_8)$			
Hydrogen peroxide	(H_2O_2)	mg/L	50 -1000	(Stephenson & Duff, 1996)

[a] Softwood
[b] Combined hardwood and softwood
[c] Diethylenetriaminepentaacetic acid or Pentetic acid
[d] Ethylenediaminetetraacetic acid

2.4   Anaerobic Digestion

2.4.1 Introduction to Anaerobic Digestion

The purpose of the anaerobic treatment technology of pulping wastewater is to remove the presence of aerobically biodegradable compounds (BOD) and chlorinated organic compounds. However, the presence of the inhibiting compounds or low degradability of the organic matter is the main challenge in the anaerobic treatment of pulping wastewater (Ekstrand, 2019).

The main principles of reaction of anaerobic digestions consist of four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, large organic polymers namely starch, cellulose, and fat are broken down into sugars, amino acids, and long-chain fatty acid (LCFA) by facultative and strict anaerobes fermentative microorganism at an optimum pH of 5-7 and temperature range of 30-50°C (Azman, 2016; Haandel & Lubbe, 2007). Acidogenic microorganism converts the organic into volatile fatty acids (VFA) such as propionic acid, butyric acid, and acetic acids. Acidogenic bacteria have fewer regeneration times of fewer than 36 hours which makes acidogenesis faster than another reaction step in anaerobic digestion (Deublein & Steinhauser, 2008). Within this process, long-fatty acids are also produced by lipids. In acetogenesis, acetoclastic microorganism converts VFAs and other intermediates into acetic acid, hydrogen, and carbon dioxide. In the meantime, lipids produce acetate from glycerol (acidogenesis) and LCFAs (β-oxidation). Methanogenesis is considered as the most critical step in anaerobic digestion because of its slowest biochemical reaction and the sensitivity of methanogenic bacteria to the environment. Acetoclastic methanogens split acetate into methane and carbon dioxide, and hydrogenotropic methanogens produce methane from the reaction of hydrogen and carbon dioxide. These bacteria are strictly anaerobic bacteria and extremely sensitive to the presence of small amount oxygen (Megonigal et al., 2003). In theory, 1 g COD can be converted to 0.35 L of methane (CH_4) at Standard Temperature and Pressure conditions (STP: 0°C and 1 atm).
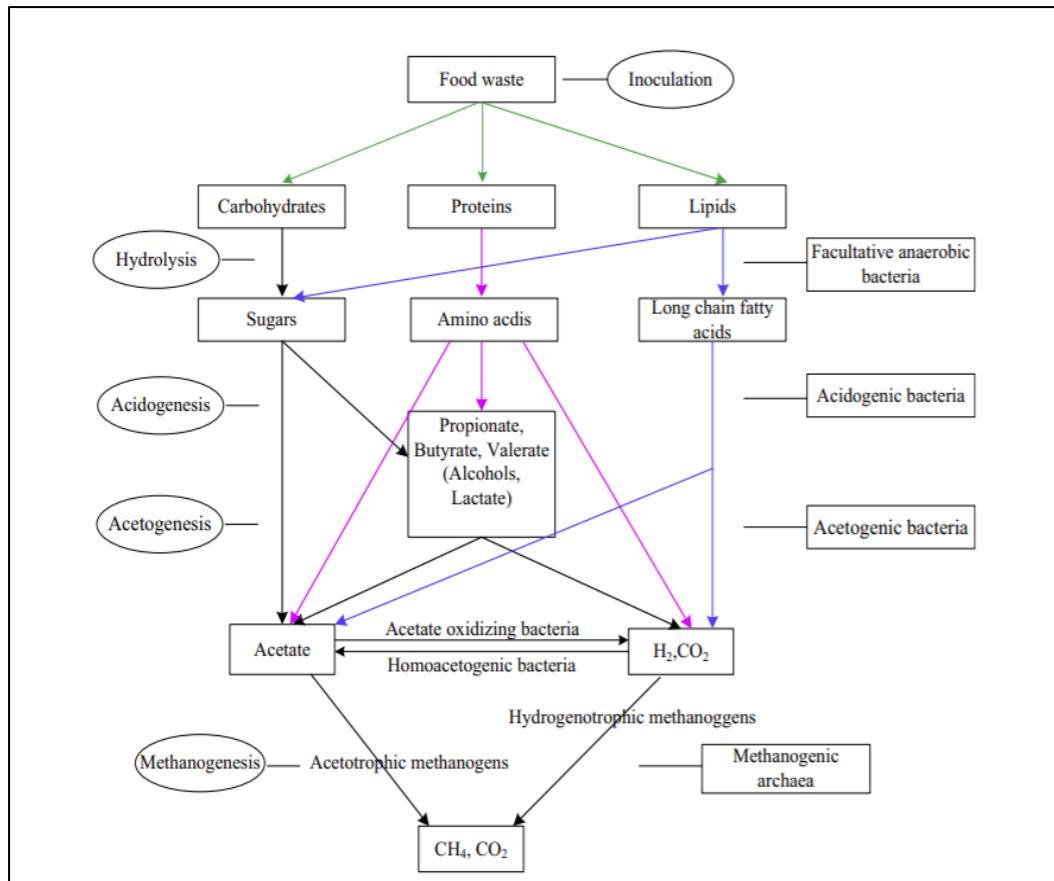
Figure 3. Anaerobic digestion pathways (Kumar Jha et al., 2011).

Astals et al. (2015) and Chen et al. (2008) reported acetoclastic methanogens as the most sensitive to the environmental changes, process conditions, and the presence of inhibitors compared to the other tropic groups in anaerobic digestion. The reduction in the activity of methanogens results in the accumulation of intermediate compounds which later can inhibit the methanogens community in a feedback loop (Astals et al., 2015; Batstone & Jensen, 2011).

Batstone et al. (2002) divide the effect on methanogens into two different categories: biostatic and biocidal. The biostatic effect is known as "inhibition" which is a reversible impairment of biomass function and the anaerobic activity recovers quickly. The biocidal effect is known as "toxicity" which is irreversible impairment of cells leading to decay and biomass taking longer to remedy, to some extent they must be replaced or regrown. Rintala & Puhakka (1994b) reported that the potential inhibitor in CTMP wastewater effluent were resin acids, long chain fatty acids, and sulfur.

2.4.2 Inhibition by sulfur

Khan & Trottier (1978) reported the toxicity of inorganic sulfur compounds varies as follows:

$$SO_4^{2-} < S_2O_3^{2-} < SO_3^{2-} < S^{2-} < H_2S$$
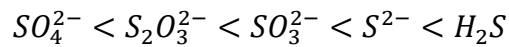
In anaerobic digestion pathways, sulfate-reducing bacteria, namely *Desulfovibreo*, use sulfate and sulfite as electron acceptors to produce carbon dioxide (CO_2) and hydrogen sulfide (H_2S). H_2S is a highly toxic, bad odor, flammable, corrosive, and poisonous gas. Sulfate-reducing bacteria (SRB) cause a reduction in methane production yield per unit and substrate removal because they compete with methanogen for the same organic compounds such as acetate, hydrogen, and methanol (Bajpai, 2017). Visser (1995) assumed that acetate-sulfate reducing bacteria (ASRB) grow faster than acetate methanogenic bacteria at low and high acetate concentrations. Similar to methanogens, SRBs are strictly anaerobic and very sensitive to the presence of oxygen (Cirik et al., 2013). Wang et al. (2009) reported that the growth yield of SRB is 0.2 g VSS/g COD, whereas methanogens have growth yield of 0.03 g VSS/g COD (Schultz, 2005).

According to Eis et al. (1983), Ferguson et al.(1984), Särner (1986), Särner (1989) claimed that an anaerobic reactor can treat sulfite-rich wastewater up to 800 mg SO_3^{2-}/L with a satisfactory result. Rinzima & Lettinga (1988) showed the effect of the COD/SO_4^{2-} ratio on the treatment efficiency of an anaerobic reactor. When COD/SO_4^{2-} was lower than 10, process failure happened in the anaerobic reactor, and at a ratio higher than 10, the treatment process was a success. To prevent sulfide inhibition in the anaerobic reactor, three measurements were suggested by Visser (1995): diluting the wastewater, increasing the reactor's pH, and adding sulfide removal steps before or after anaerobic treatment. In a continuous reactor, sulfite does not cause a serious problem because it is reduced in the less toxic sulfide rapidly.

2.5 Biogas

Biogas is combustible gas produced through anaerobic digestion at a temperature range of 30-65°C. Biogas composes of methane (CH_4) 55-80%, 20-45% carbon dioxide (CO_2), 5-10% hydrogen (H_2), and other impurities gases such as hydrogen sulfide (H_2S), oxygen (O_2), nitrogen (N_2), and ammonia (NH_3) (Truong & Abatzoglou, 2005). Hydrogen sulfide (H_2S) is a colorless, lipophilic, volatile, rotten egg odor, flammable and poisonous gas that may lead to death at an exposure of 500 ppm (Li & Lancaster, 2013). According to International Union of

Pure and Applied Chemistry (IUPAC), hydrogen sulfide also got a new systematic name "sulfane" (Mc Naught & Wilkinson, 2019).

The general industry peak for H_2S running in the internal combustion engines is 50 ppm (Wellinger & Lindeberg, 1999). At a range of 50-1000 ppm, H_2S can cause corrosion to the engine and metal part via emission of sulfur dioxide (SO_2) from combustion and adsorbed or oxidized by autotropic aerobic *Thiobacillus* bacteria which grow on a wet metal surface (Chambers & Potter, 2002).

2.6   Expanded Granular Sludge Bed bioreactor (EGSB)

In the mids-1980, the EGSB reactor was developed in the Netherlands to increase contact between anaerobic granular sludge and wastewater, and reduction of the dead zones. The advantages of the EGSB bioreactor are a simple design, low anaerobic granular sludge production, high treatment efficiency, low operational cost, and especially generation of renewable energy (Cruz-Salomón et al., 2019). The characteristics of EGSB are the combination of Upflow Anaerobic Sludge Blanket (UASB) bioreactor and fluidized bed bioreactor (FBR) technology. It can handle high upflow velocity, low (<5 g COD/L) and high strength loading rate (>30 g COD/L), and wastewater that contains recalcitrant compounds such as aromatic compounds, aldehyde, lipids, fatty acids, etc (Lettinga et al., 1997).

The upflow velocities (V_{up}) of the EGBS can reach 30 m/h for liquid and 7 m/h for gases. The high V_{up}, optimal height to width ratio of 4-5, and the presence of external recirculation expand the contact between anaerobic granular sludge (AGS) and wastewater and increase internal mixing efficiency (Kato et al., 2003; Lim, 2011; López & Borzacconi, 2011). Additionally, the recirculation of the medium help to dilute the wastewater containing biodegradable inhibitor and toxic substance which make EGSB can be used to handle pharmaceutical and chemical wastewater (Teixeira-Correia, G.; Pérez-Pérez et al., 2014).

The EGSB reactor consists of three zones: settling zone, transition zone, and digestion zone. The settling zone is located at the upper part of the reactor which consists of a gas-liquid-solid separator (GLS) to separate the treated effluent from the anaerobic granular sludge (AGS). In the transition zone, there is the preservation of substrate for biodegradation and size distribution. The area where the chemical transformation of the wastewater pollutant by AGS is called the digestion zone. Figure 4 illustrates the schematic drawing of a conventional EGSB bioreactor.
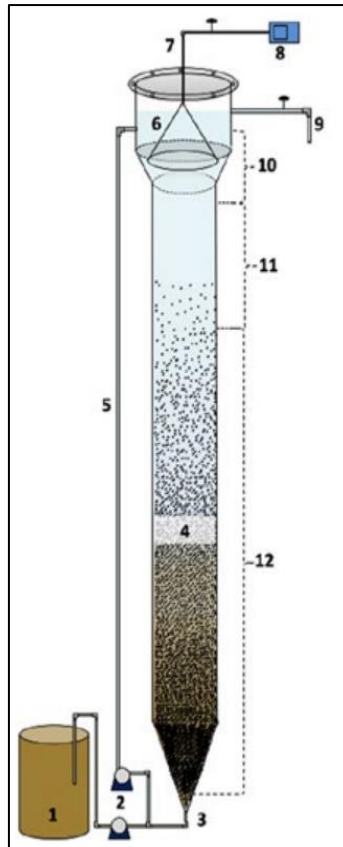
Figure 4. Schematic drawing of conventional EGSB bioreactor. (1) Feed tank, (2) Peristaltic pump, (3) Influent, (4) EGSB bioreactor, (5) Recirculation, (6) Bell separation, (7) Biogas outlet, (8) Gas flow meter, (9) Effluent, (10) Settling zone, (11) Transition zone, (12) Digestion zone (Cruz-Salomón et al., 2019).

2.7   Parameters influencing the performance of EGSB bioreactor

The important parameters that influence the performance of EGSB reactor are pH, bioreactor configuration, temperature, substrate type, volumetric loading rate (VLR), particle size distribution (PSD), hydraulic retention time (HRT), solid retention time (SRT), upflow velocity (V_{up}), wastewater characteristics, and the acclimatization of the anaerobic granular sludge (Cruz-Salomón et al., 2019).

2.7.1 pH

The optimum pH for the operation of an anaerobic EGSB reactor is between 6.7-7.4, where the metabolic activity of methanogenic archaea is high at this pH range (Figure 5). At pH < 6 results in the accumulation of volatile fatty acid and hydrogen. When pH >7.5, the nutrients and trace metals are precipitated which slow down the metabolism rate of methanogen and will further cause methane production inhibition (Leitão et al., 2006). Visser (1998) reported that

acetate-sulfate reducing bacteria (ASRB) have a higher growth rate than acetotrophic methanogenic bacteria (AMB) and will outcompete with AMB and become predominant within the system.
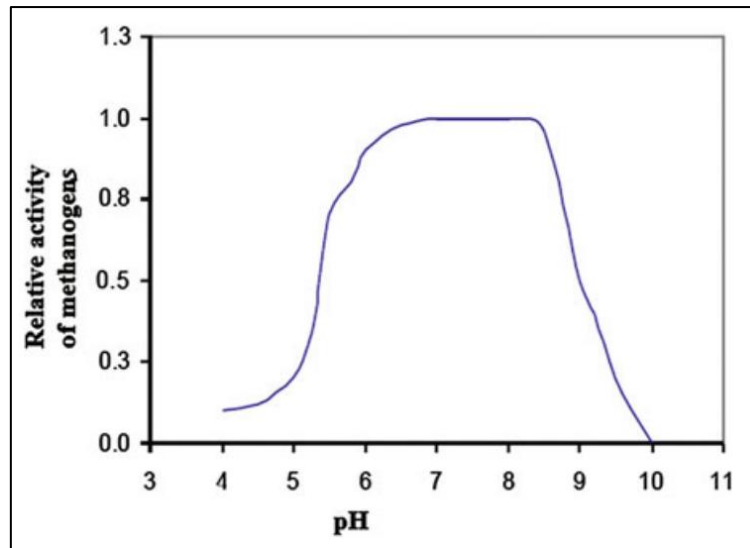
Figure 5. The relative activity of methanogen to different pH levels (Mata-Alvarez, 2003; Seadi et al., 2008).

2.7.2 Temperature

Temperature is an important parameter that affects operation conditions in anaerobic digestion. Yuan et al. (2011) described that temperature effect the biochemical reaction such as reaction rate, reaction pathway, microbial yield, and death. Mesophilic (35-40°C) and thermophilic (50-55°C) are the two optimum temperature ranges for anaerobic biodegradation (Bajpai, 2017; Verma, 2002). Henze & Harremoes (1983) reported that the maximum anaerobic digestion in the mesophilic range happens between 35-40°C. When the temperature is between 40-50°C, inhibition may happen to the methanogens which lower biogas production yield. Jaiswal et al. (2021) reported that mesophilic condition is likely used to treat rich suspended organic materials wastewater discharge from the paper industry. Additionally, the bacteria that grow in mesophilic conditions are diverse, robust, and easily adapt to the changing environment which makes operation more stable than thermophilic (The Wales Centre of Excellence for Anaerobic Digestion, 2008). Cruz-Salomón et al. (2019) reported that most EGSB has been operated under a mesophilic temperature range of 35-37°C.
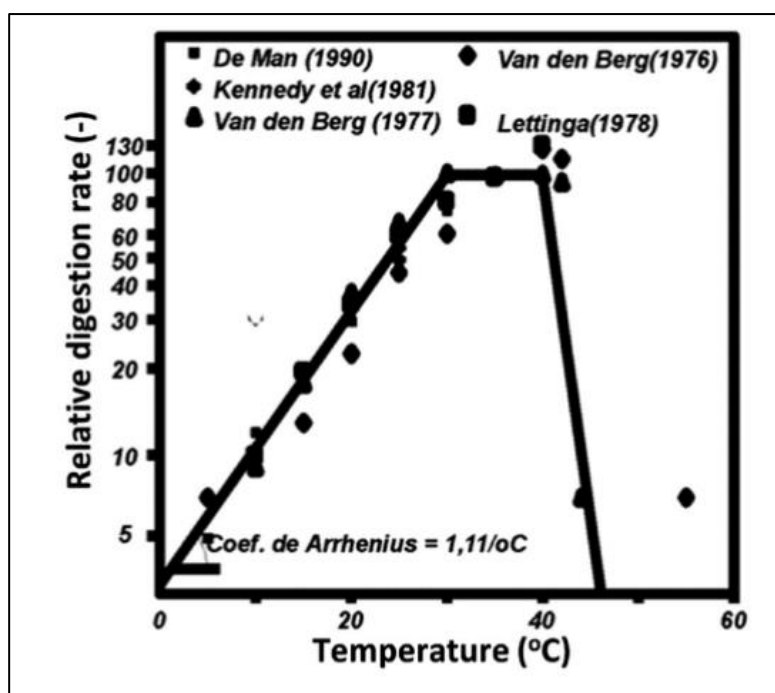
Figure 6. Influence of temperature on digestion efficiency in mesophilic range (Henze & Harremoes, 1983).

2.7.3 Volumetric Loading Rate

The applied volumetric loading rate (VLR) in the EGSB bioreactor is up to 40 kg COD/m^3.d. However, Cruz-Salomón et al. (2019) reported that if VLR exceeds 40 kg COD/m^3.d, it will cause an operational problem and affect the performance of EGSB. Moreover, high VLR will increase the VFA production, and decrease pH leading to acidification which inhibits the metabolism of methanogenic archaea in converting VFA to methane (Gou et al., 2014; Kougias et al., 2013; Mao et al., 2015; Nagao et al., 2012).

2.7.4 Hydraulic Retention Time

Hydraulic retention time (HRT) refers to the average time of the wastewater remaining in the bioreactor. The optimal operational HRT depends on the wastewater composition and VLR. Johansson (2012) and Lin et al. (2012) reported that the typical HRT for anaerobic treatment of paper and pulp wastewater is between 2-10 days and for aerobic treatment is between 0.5-3 days. Too long HRT will affect the sludge granulation process and the biomass might come out with the effluent for too short HRT (Kaviyarasan, 2014).

2.7.5   The Effect of suspended solids on granular biomass

Zinatizadeh et al. (2007) conducted experiments with an Upflow Anaerobic Sludge Fixed-Film Bioreactor (UASFF) treating palm oil mill effluent (POME) and the effect of overload suspended solids on the granular sludge has been observed. Suspended solids (SS) had a slower hydrolysis rate compared to the soluble fraction of other organic matter, and at the loading rate of 5.24 g SS/L.d, it was observed that biogas production got trapped in the sludge blanket. After several operational hours, the biogas buoyed and caused a sludge washout.

Ghangrekar et al. (2005) reported the granules that developed under high VFA contents are fragile and easily wash out from the reactor. An acidic and high temperature at high soluble organic loading rate conditions leads to the partial disintegration of the granule outer surface. The strength of the granule decrease when the VFA in the reactor is more than 200 mg/L, which causes low COD removal efficiency (Ghangrekar et al., 2005).

Once the suspended solids entered the reactor from the bottom, they first settled at the bottom of the reactor and some of them slowly moved upward above the sludge bed meanwhile some remain diluted with the biomass at the bottom of the reactor. These suspended solids have higher specific gravity than water which account for their settleability (Zhang et al., 2008).

2.8   Anaerobic Biodegradability of CTMP wastewater

Pokhrel & Viraraghavan (2004) reported that the anaerobic biodegradability of CTMP pulping which contain COD in the range of 2500-13000 mg/L is between 40-60%. Sierra-Alvarez et al. (1990) found out that wood toxins such as resin, and terpenes remained persistent under methanogenic conditions.

2.9   Anaerobic Granular Sludge

In anaerobic treatment, granular seeding sludge became a valuable raw material because it provides optimal wastewater treatment, requires a short start-up time (Alphenaar, 1995), and can be stored unfed for a long period (Hulshoff Pol, 1989; Wu Wei-min et al., 1985).

Malik et al. (2020) mentioned that the effectiveness of the anaerobic degradation process within an anaerobic reactor depends on granule formation. They support the active biofilm and maximize the contact between anaerobic microorganisms and wastewater. The granulation process depends on several factors such as the morphology of the bacteria, substrate specificity,

surface charge compatibility, bacteria's growth and decay rate, hydrophobicity, and ability to produce specific polymers to form flocs or granules (Lettinga et al., 1997).

The startup period is an adaptation of microbiological agents and the granulation process of bacteria (Malik et al., 2020). Tian et al.(2015) denoted that the start-up period is depend on the characteristics of the seed sludge, feed wastewater characteristics, and sludge retention time in the reactor. Figure 7 illustrates the granular growth model and range of granule size.
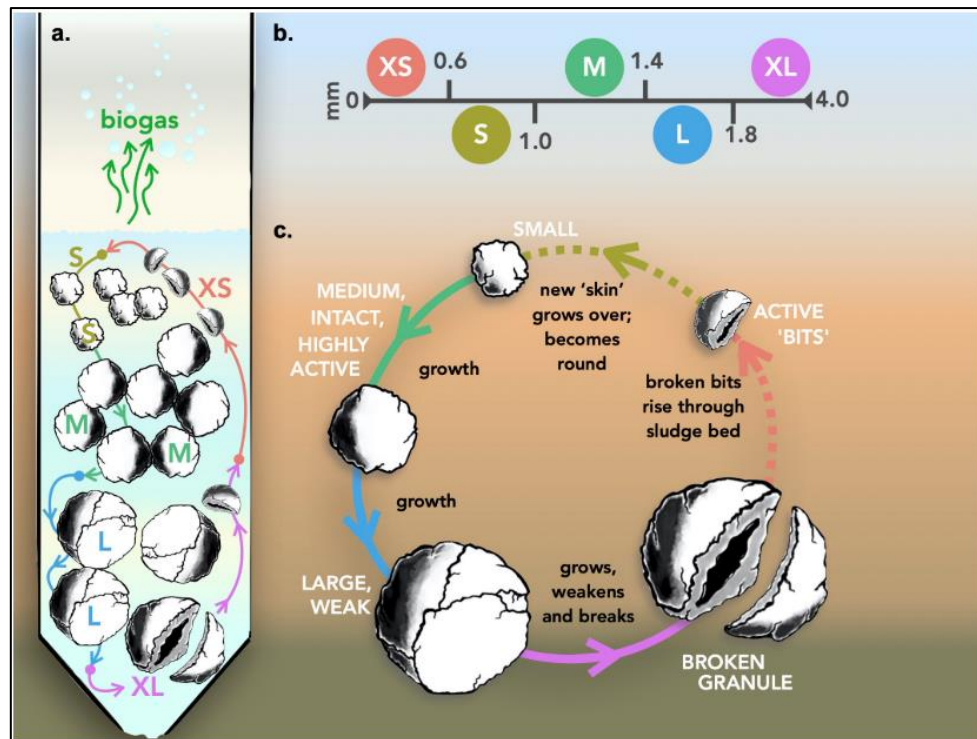
Figure 7. Granular growth model and biofilm development model. (a) Operation model in an anaerobic reactor;(b) size fraction parameters; (c) the growth cycle includes the breakup form new and smaller granules (Trego et al., 2020).

3 Knowledge Gaps, Research Objectives, and Research Questions

3.1 Knowledge Gaps

Earlier research studies reported challenges in implementing anaerobic digestion to treat CTMP pulping wastewater due to the presence of resin (Larsson et al., 2017). Moreover, there is a limited report concerning the effect of the presence of suspended solids on the performance of the reactor. According to Walden et al. (1971), one of the problems with bioassays of pulp mill effluent is the variability of effluents within given plants and among plants. The impact of volumetric loading rate (VLR) and sludge loading rate (SLR) on the COD removal efficiency for this type of wastewater has not yet been reported. Additionally, there are limited studies about the anaerobic sludge treating pulp mill effluents, and the impact of suspended solids, especially wood cellulose fibers, on the microorganisms involved in hydrolysis, fermentation, and methanogenic are reported poorly understood (Lin & Hsiu, 1997).

Therefore, this research will be focusing on the treatment of CTMP wastewater, in terms of COD removal, and digestion efficiency, as well as evaluate the biodegradability and toxicity of this wastewater stream.

3.2 Research Objectives

The overall aim of this research is to study the effect of CTMP pulp mill effluents on the EGSB reactor performance.

Two specific research objectives include:

1. Assess the biodegradability and toxicity of the raw wastewater
2. Evaluate direct anaerobic treatment of paper pulping wastewater in an EGSB system and assess biological performance. The focus will be on the volumetric loading rate of the EGSB reactor.

3.3 Research Questions

Main research question:

How can the advanced understanding of anaerobic digestion of EGSB bioreactor under mesophilic conditions (35-36°C) help in improving the treatment of CTMP wastewater?

Research Question 1: What is the anaerobic biodegradability of CTMP wastewater?

Hypothesis: There is a high amount of poorly biodegradable organic compounds of approximately 50% present as resin and terpenes within the total organic matter of the wastewater, causing low COD removal.

Research Question 2: What is the methanogenic toxicity of CTMP wastewater?

Hypothesis: The presence of wood resins and terpenes in the pulp wastewater may cause high toxicity for the methanogenic bacteria. The 50% inhibition concentrations can range from 2.8-4.8 g COD/L.

Research Question 3: What is the biological performance of the EGSB in terms of digestion efficiency, inhibition, and biogas production while treating raw pulp mill effluent under the mesophilic condition (35-36°C) during the start-up phase with unacclimated biomass?

Hypothesis: The methane (CH_4) percentage within 50-75%, COD removal efficiency of 40-60%, and VFA in the effluent below 5 meq/L. The start-up phase might take up to 3 months for biomass to acclimate to the wastewater.

Research Question 4: What is the biological performance of the EGSB treating raw pulp mill effluent under the mesophilic condition (35-36°C) during the different phases with acclimated biomass?

Hypothesis: As VLR increases, causing slight overloading of the methanogenic capacity of the reactors leading to high VFA in the effluent.

Sub question:

- What is the major accumulated VFA in the effluent?
- What is the COD removal efficiency and daily biogas production in each phase?
- What is the sludge yield at the end of each phase?
- What percentage of sulfate is converted into sulfide?

4 Methodology

4.1 Reactor set-up

The experiments were performed in a lab-scale expanded granular sludge bed reactor (Figure 8). The EGSB reactor is a double-glass wall, cylindrical shaped, tall, and slender with a volume of 4.2 L, height of 1.5 m, diameter of 0.05 m, and cross-sectional surface area of 0.00196 m^2. A black rope string was attached inside the reactor to prevent the flush out of biomass when there is a big bubble of biogas production.

A conditioning tank with a volume of 3 L and equipped with a mechanical stirrer (Type KYC 26A2, Bodine Electric Company, The United States of America) on top to ensure well mixing with a stirring speed of 50 r/min was used as a pretreatment step and temperature of the feed raw wastewater. An influent pump (Shenzhen Peristaltic Pump YZ1515x, China) was used to pump the feed flow into the conditioning tank, and the cross-flow pump (Watson Marlow Qdos 30 Peristaltic Pump, United Kingdom) was be used to recirculate the mixed liquor of the feed and effluent to attain a homogenous body and to enhance the contact between substrate and biomass. A dosing pump (Model 77240-20, Cole Parmer, The United States of America) to dose 0.5 M of caustic soda (NaOH) into the conditioning tank to regulate the pH in the tank to not go below 7. A pH probe connected to a digital pH reader (Hach sc 200 Universal Controller) was plugged into the conditioning tank. A drum-type gas meter was used to measure daily biogas production (Ritter, Germany). A digital temperature sensor (Aqua Medic T-Meter II) was attached to the wall of the EGSB reactor to monitor the real-time temperature. A water bath (Thermo Scientific: Type 003-2859, The United State of America) was used to maintain the reactor at a temperature range of 35-36°C. The effluent recirculation was used to set the up-flow velocity (V_{up}) of 3-3.5 m/h.
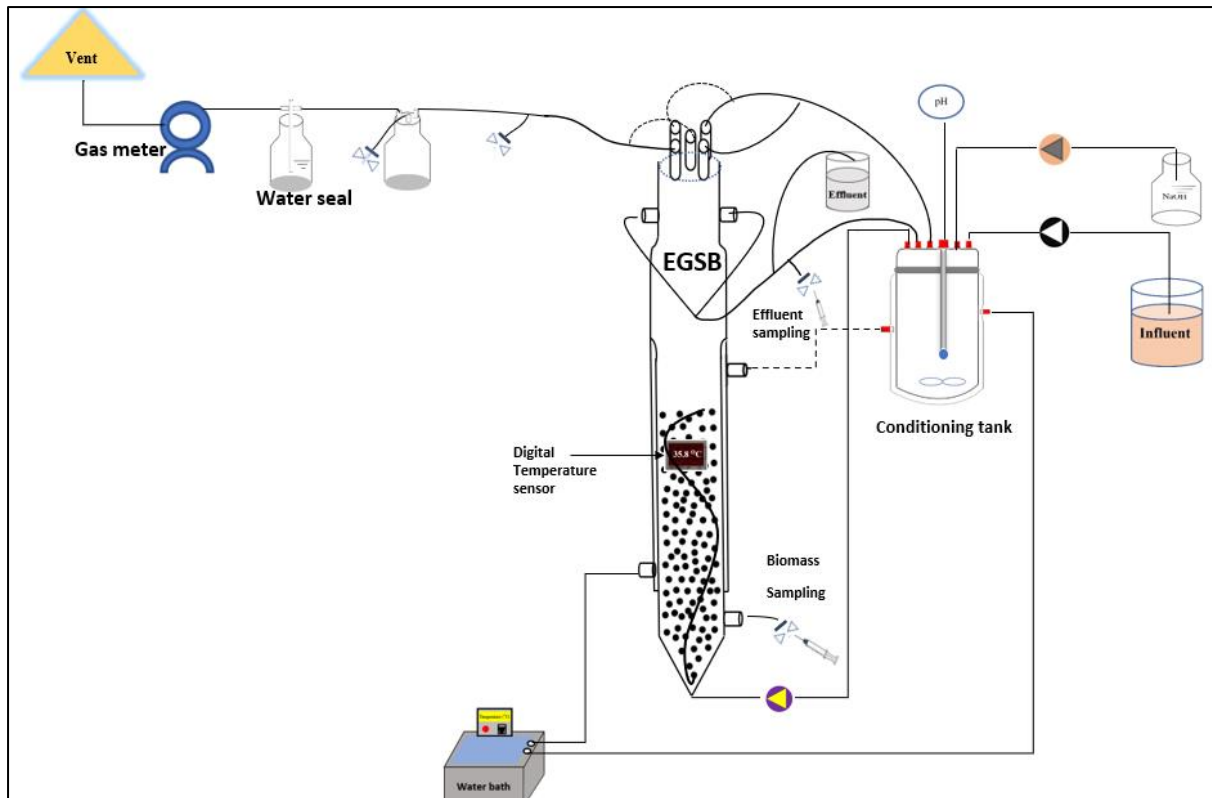
Figure 8. Schematic drawing of a lab-scale EGSB reactor.

4.2   Inoculum and Wastewater characteristics

4.2.1 Morphology of the inoculum

The EGSB reactor was inoculated with mesophilic granular sludge obtained from an industry-producing chemical and biobased chemical in the Netherlands. The seed anaerobic granular biomass was diluted with 20 mmol/L $NaHCO_3$ in the reactor to stabilize the system and prevent any acidification happening during the hydrolysis stage in anaerobic digestion in which fermentative microorganisms consume nutrients and produce organic acid which lower pH (Trego et al., 2020). Figure 9 shows the image of granules that were observed under a digital microscope (Toolcraft Lab5.0 USB Microscope) and the summary of their morphology was described in Table 2. The granule's core was not hollow, but contain different inorganic layers which was the reason for their good settling.

The majority of the granule had black, white, and grey color with a diameter of 0.8-3.5 mm. Kosaric et al. (1990) explained that the elemental composition within granule caused the color difference. The white and grey granules are composed of about 30% sulfur (S), 25% phosphorus (P), 2% silicon (Si), and 2% aluminum (Al). The black granule is composed of about 15% sulfur, 20% phosphorus, 14% silicon, and 15% aluminum. Kosaric et al. (1990) added that iron sulfide and other related compounds may be the reason for the black color granule. The sulfur to iron ratio (S:Fe) of black, grey, and white color granule is about 1.8, 5.5, and 8, respectively. Granular biomass contains mainly bacterial cells and EPS (extracellular polymeric substances) which all have negative charges, which means other cations (mono-, di-, and trivalent) can be bound easily (Tawfik et al., 2008).
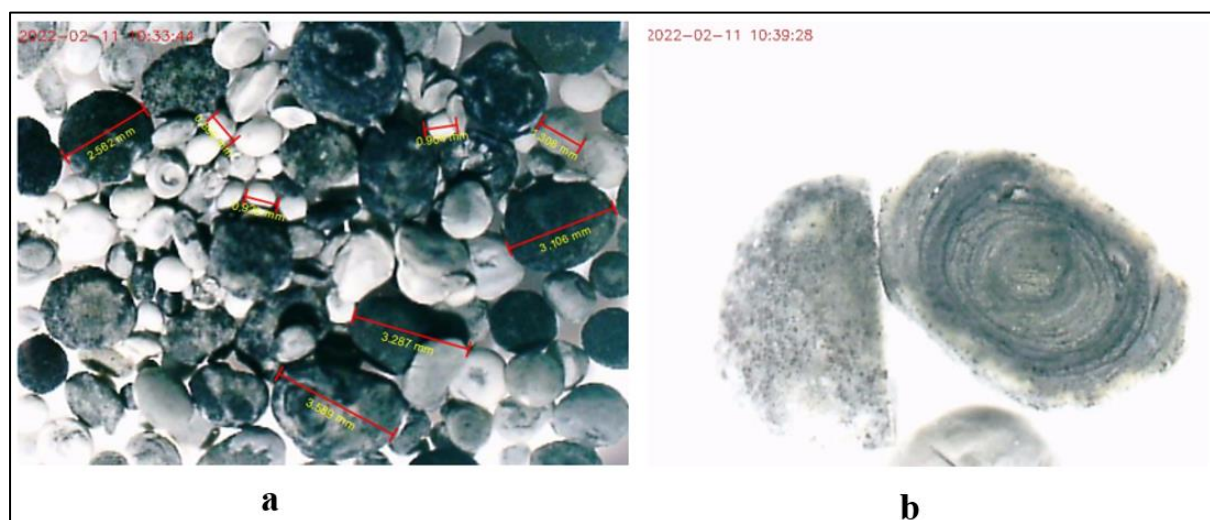
Figure 9. Microscopic view of granule; (a) mixed granules, (b) A cut-through shows different layers inside the granule.

Table 2. Summary of the morphology of inoculum.

Structural strength of the granules[1]	strong
Stickiness of the granules	non-aggregated
Presence of hollow chambers in the granules	none
Presence of inorganic precipitates - granules with gritty cores - granules with inorganic layers/scale	 none a lot
Presence of fines [2]	a lot
Presence of flocculent matter[3]	none

[1] Weak granules fall apart when mildly pressed; strong granules are even hard to squash

[2] Refers to the relative share of non-granular mud/sludge/sediment in the sludge

[3] Refers to the relative share of flocculent matter or slime in the sludge matrix

4.2.2 Wastewater Characteristics

1 m³ sample of CTMP wastewater was collected and stored in an IBC (Intermediate Bulk Container) from a paper pulping industry and transported to the laboratory in one week. The color of the fresh delivered wastewater new sample is yellow-brownish. After 6 months, the color has changed to a more brownish color (Figure 10). According to Slade et al. (2007), this color is due to the presence of lignin derivatives compounds such as humic and fulvic acids compounds. Two submerged pumps were used to stir vigorously the IBC for 3 hours before pouring it into 25 L plastic containers as subsamples and all the containers were stored at 4°C. Throughout the operation, wastewater was used in two different batches, the first batch (Day 1-106) and the second batch (Day 107-182) in which their physicochemical characteristics are shown in Table 3.
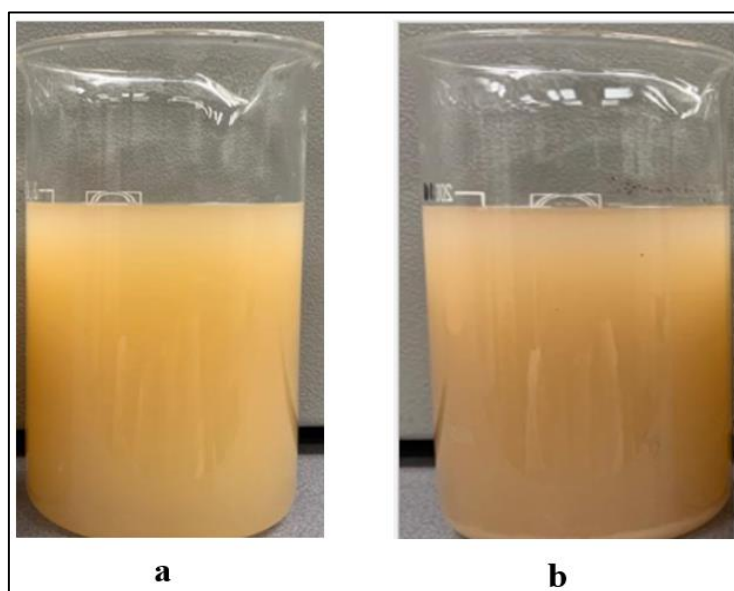
Figure 10. Color of the wastewater from the IBC; (a) fresh sample, (b) sample after 6 months.

Table 3. Physicochemical characteristics for first and second batch wastewater.

Parameter		Unit	1st Batch	2nd Batch
			Value	
pH		-	6.2	5.6
Conductivity		mS/cm	2.7	2.5
Total Organic Carbon		mg/L	1871	1648
Total alkalinity		meq/L	6.94	7.7
Density		g/mL	1	1
Total COD	(TCOD)	mg/L	5156	4844
Soluble COD	(SCOD)	mg/L	3790	3671
Acetic acid	(C2)	mg/L	955	878
Propionic acid	(C3)	mg/L	12	174
iso-Butyric acid	(iC4)	mg/L	0	0
Valeric acid	(C5)	mg/L	0	140
Hexanoic acid	(C6)	mg/L	0	0
Total Solids	(TS)	mg/kg	4330	4030
Volatile Solids	(VS)	mg/kg	2510	2343
Total Suspended Solids	(TSS)	mg/kg	130	127
Volatile Suspended Solids	(VSS)	mg/kg	128	122
Total Kjeldahl Nitrogen	(TKN)	mg N/L	35	19
Soluble Kjeldahl Nitrogen	(SKN)	mg N/L	33	9
Ammoniacal Nitrogen	(NH_4^+)	mg N/L	8	2
Total Phosphorous	(TP)	mg P/L	3.8	4.1
Ortho-Phosphate	(PO_4^{3-})	mg P/L	1.1	4.2
Sulphate	(SO_4^{2-})	mg/L	660	433
Nitrate	(NO_3^-)	mg N/L	6.83	8.22
Nitrite	(NO_2^-)	mg N/L	0	0
Chloride	(Cl^-)	mg/L	18	37
Sodium	(Na^+)	mg/L	850	760
Magnesium	(Mg^{2+})	mg/L	6	5.6
Potassium	(K^+)	mg/L	25	27
Calcium	(Ca^{2+})	mg/L	27	26
COD/TOC		g COD/g TOC	2.76	2.94
PCOD/VSS		g COD/g VSS	4.23	9.61
COD/VS		g COD/g VS	1.85	2.07
COD/SO4		g COD/g SO4	7.81	10.09
VFA-COD/SCOD		gCOD/gCOD	0.27	0.40

4.2.3 Solids in the wastewater

The CTMP wastewater has a high content of suspended solids, mainly fiber or fiber debris, compared to the mechanical pulping wastewater (Mathys, 1991). Figure 11 shows the microscopic view (Olympus BX51, The United Stated of America) of the presence of suspended solids in the wastewater before sieving.
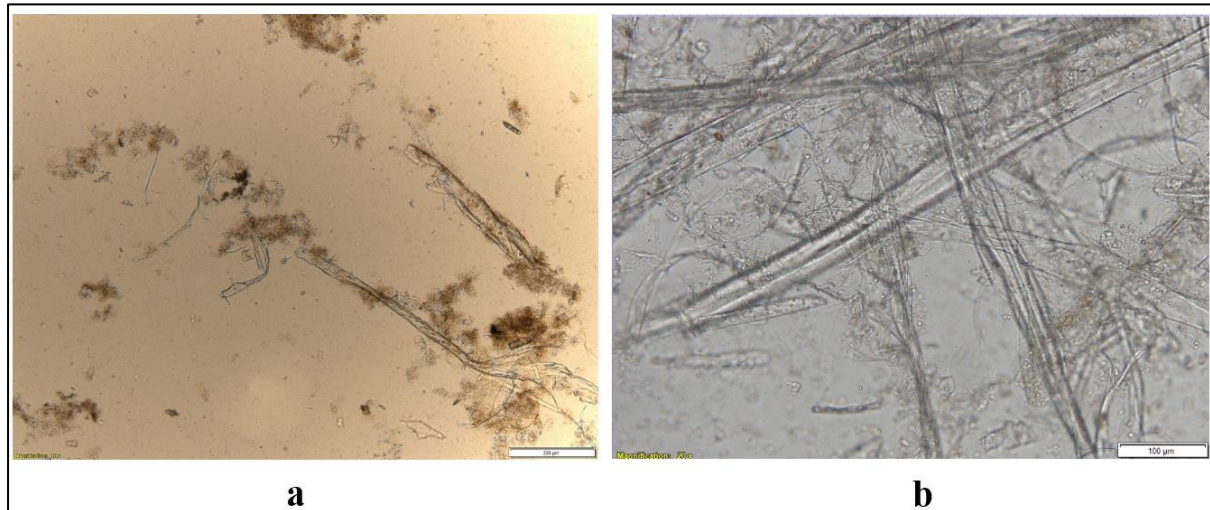
Figure 11. Microscopic observation of the solids in the wastewater before sieving; (a) magnification 100x , (b) magnification 200x.

4.3   Feed preparation

Feed wastewater was prepared by filtering the wastewater with a sieve size of 0.125 mm (Haver&Boecker Analysensieb Prüfsieb DIN 4188, Germany) and 0.090 mm (Analysensieb-retsch-5657 HAAN W, Germany) to remove the solids. The feed was stored with constant mixing in the fridge (10°C) during the weekday and at room temperature (20-25°C) during the weekend with a digital overhead stirrer (Model RW 20 D S000, IKA RW 20 digital, Germany) at a control stirrer speed of 150 rpm. In anaerobic treatment, 10 mg of nitrogen per 100 mg of biomass is typically needed (Rantanen et al., 2018), therefore macronutrients and micronutrients were supplied in accordance with a COD:N:P ratio of 500:5:1, shown in Table 4.

Table 4. Micro and Macronutrients receipts per gCOD of wastewater.

Name	Original concentration (%)	Molar mass (g/mol)	Elemental dosage per gCOD
Urea ($CO(NH_2)_2$)		60	5.90
Phosphoric acid (H_3PO_4) (85%)	85	98	1.2
Potassium sulfate (K_2SO_4)		174	2.10
Sulfuric acid (H_2SO_4) 96%	96		1.50
Magnesium Chloride Hexahydrate ($MgCl_2.6H_2O$)		203	0.07
Calcium Chloride Dihydrate ($CaCl_2.2H_2O$)		147	140
Ferrous Chloride ($FeCl_2$ 41%)	40	162	0.25
Vithane			

4.4 Analytical Methods

During the daily routine, the pH of the feed, and effluent was measured using a digital pH meter (WTW pH 3110, Germany). Effluent pH had to be measured immediately after taking a sample from the reactor to avoid the CO_2 gas escaping from the effluent sample which causes a rise in pH level.

The caustic soda consumption and water bath temperature were recorded daily. Total solids (TS) and suspended solids (SS) were measured based on the standard methods (Federation, 1999). Chemical oxygen demand (COD), total phosphorous (TP), phosphate (PO_4^{3-}), total nitrogen (TN), and ammonium nitrogen (NH_4-N) were measured spectrophotometrically (Hach Lange DR 2800) using Hach-Lange test kits (Germany) according to the manufacturer's instructions. The VFAs were analyzed using the Gas Chromatograph system (Agilent Technologies 7820A). Daily production was monitored by a drum-type gas meter (Ritter type TG 0.5/5, Germany) connected to the headspace of the EGSB reactor. Biogas composition (% CH_4, CO_2, O_2, and other gases) was evaluated daily using a portable Biogas Check Analyzer (Biogas 5000, Geotech, UK) at standard temperature and pressure (STP).

Total Liquid Sulfide (TLS) in the effluent and biogas was measured following the direct methylene-blue method (van Loosdrecht et al., 2016) by adding a known concentration of the sample, 20 mL zinc acetate to ensure sulfide fixation, 10 mL of N-dimethyl-p-phenylenediamine (DMP), 0.5 mL of iron (III) ammonium sulfate ($FeNH_4(SO_4)_2 \cdot 12H_2O$) and

filled up to 100 mL with demineralized water. After 45 minutes, the solution turned into methylene blue due to the reaction of zinc sulfide under acidic conditions. The solution was read colorimetrically by a spectrophotometer (Hach Lange DR 2800, Germany) as methylene blue at 670 nm with an absorption coefficient (α) of 1.0203, after the reaction of zinc sulfide under acidic conditions with N, N-dimethyl-p-phenylenediamine and iron (III) ammonium sulfate ($FeNH_4(SO_4)_2 \cdot 12H_2O$). The analysis was done with two different concentrations of both biogas and effluent.

Sulfate measurements were done by the Ion Chromatography (IC)(Metrohm, Switzerland) with anion column method A Supp 5 150/4.0 (818 anion system) containing 3.2 mM Na_2CO_3, 1 mM $NaHCO_3$ (run at 0.7 ml/min), and 150 mM H_3PO_4. The soluble sample was filtered with a 0.45 µm membrane filter (Agilent Captiva Syringe Filter, The United States of America). The Hach Lang Test kit (LCK 153) was found to be uncertain in measuring feed wastewater and reactor's effluent which may be due to the color, turbidity or any compound presence in the solution cause interference. Additional information about the comparison between Hach Lang Test kit and IC can be found in the appendix C (Table C).

The measurement of effluent COD without sulfide was done by acidified the soluble sample with 1% H_2SO_4 to pH of about 5.5, in which H_2S act as unreactive gas with moderate solubility, and if pH rise above 6, H_2S will dissociate into bisulfide (HS^-) and sulfide (S^{2-}) ion, shown in (Figure 12). The sample was then flushed with nitrogen gas to strip out H_2S for 30 minutes and COD was measured using the Hach Lang test kit and dilution factor was considered. The focus of this study is mainly on the variable related to the characteristics of biomass as shown in Table 5 and their measurement frequency.
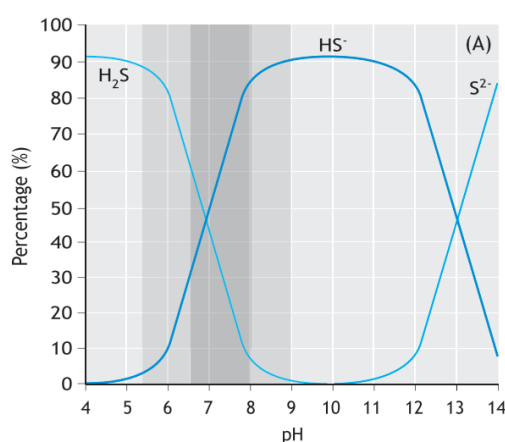
Figure 12. Equilibrium of H_2S and the effect of pH on their speciation at 25°C (Rintala & Puhakka, 1994a).

Table 5. Analytical parameters, measuring frequency (expressed per week), and the objective during start-up and operational phase.

Parameter	Sample	Frequency/week Start-up	Frequency/week Operational Phase	Objective
pH	CT tank, Feed, effluent	Daily		Reflect on the digestion process and accumulation of VFA
TS, VS,TSS,VSS	Biomass, Feed, Effluent	-	1	Concentration of Microorganism
Alkalinity	Feed, Effluent	-	1	To maintain sufficient alkalinity to buffer the effect of acids
TCOD, SCOD	Feed, Effluent	Daily	3	COD removal efficiency
TP, PO_4-P, TN, NH_4-N,NO_3-N,NO_2-N	Feed, Effluent	-	1	Biological performance, and nutrient uptake for biomass growth
Biogas production/composition	Biogas	Daily		Methane yield and quantity
SO_4^{2-}	Feed, Effluent	2		Evaluate sulfate inhibition on anaerobic digestion
TLS	Biogas, Effluent	2		
VFA	Effluent	5	2	Indicators of inhibition and anaerobic biological treatment
SMA	Biomass	Intermediate		To evaluate anaerobic sludge capability and quantity
BDG	Raw WW	During start-up		The anaerobic degradability of wastewater, the percentage of the COD in a sample eventually converted to methane, and possible toxicity and/or biomass adaptation
Toxicity	Raw WW	During start-up		Expressed as IC50%, the concentration that results in 50% inhibition of the activity

4.4.1 Specific Methanogenic Activity

Specific methanogenic activity (SMA) was measured to evaluate the anaerobic sludge capability and quantity. The test bottles were prepared with a known amount of biomass and sodium bicarbonate ($NaHCO_3$) medium. Sodium acetate (CH_3COONa) was used as a sole substrate for the biomass in a sealed serum bottle with black rubber and a digital gas meter (Oxitop) was attached to the top of the bottle. Then, the gas headspace of the bottles was flushed with a 70%/30% N_2/CO_2 mixture providing oxygen-free condition and a neutral HCO_3^-/CO_2 pH buffer. The bottle was incubated and shaken slowly to prevent the breakdown of biomass under mesophilic conditions (37°C). The build-up gas pressure data was generated by the

Oxitop device every 20 minutes. The biomass was fed with sodium acetate twice during the test.

According to Sierra-Alvarez et al. (1991), the second feeding gives more reliable results than the first feeding because of the different rates and levels of acidification. The specific acetoclastic methanogenic activity (SMA) of the biomass was calculated from the maximum slope of the pressure versus time curve and expressed as the amount of CH_4 produced by 1 g of sludge VS per day (g CH_4-COD/g VS day).

4.4.2 Anaerobic Biodegradability Assay

The objective of performing the anaerobic biodegradability test is to measure the extent of anaerobic digestion for a wastewater sample. To achieve optimum performance, it is important to maintain consistent environmental conditions such as temperature-controlled, proper mixing, and sufficient incubation time for the microbiology and biochemistry for anaerobic digestion to maximize the chances of achieving optimum performance (Filer et al., 2019).

The batch of anaerobic biodegradability assays was incubated with anaerobic seed biomass and the waste stream to be tested in six glass bottles: one substrate-free blank, two total solids, and one soluble solid for biogas pressure monitoring, one total solid and one soluble solid for liquid sampling. The raw wastewater pH was neutralized using a 0.5 M caustic soda (NaOH) solution. The flasks were sealed with butyl rubber stoppers and an aluminum crimp seal and flashed with $N_2:CO_2$ (70%/30%) for one minute through a needle to obtain oxygen-free headspace. Subsequently, the bottles were incubated and shaken slowly to assure good contact between wastewater and anaerobic granular biomass and to prevent the breakdown of biomass in a temperature-controlled shaker at the mesophilic temperature of $36 \pm 1^{\circ}C$. The pressure in the headspace of each serum flask was monitored twice a day during the assay using a hand-held precision pressure transducer device (Model PSI-30, Centrepoint electronics). Soluble COD and VFA composition were measured from the sample withdrawn from the test bottles. At the end of the test, the methane composition was measured using the NaOH displacement method with the pH indicator Thymolphthalein.

The result of this test showed the anaerobic degradability of pulp mill wastewater under methanogenic conditions, the percentage of the COD in a sample eventually converted to methane, possible toxicity, and biomass adaptation.

4.4.3 Anaerobic Toxicity Assay

The objective of this test was to determine the impact of wastewater on anaerobic degradation and to understand the relationship between dose and effect. The set up of toxicity test was done with different concentrations of wastewater such as 5 times, 10 times dilution, and non-diluted wastewater. The positive control bottle contain biomass, substrate (sodium acetate), and medium (sodium bicarbonate), and no wastewater was added to test the quality of the inoculum. The negative control bottle contains only biomass and tap water to compensate for the amount of biogas produced by the inoculum itself. This test was conducted using an AMPTS II machine (automatic methane potential testing system) from Bioprocess Control AB (Sweden) version 3.0 (Figure 13), which can measure daily methane volume produced from anaerobic digestion of substrate based on the principle of liquid displacement and buoyancy. Each reactor consists of glass bottles with a working volume of 400 mL and 250 mL of headspace for the accumulation of biogas. The biogas produced in each of the reactors goes through bottles that contain 80 mL of 3 M NaOH and 0.4 % Thymolphthalein pH-indicator solution, which is used to remove acidic gases such as CO_2 and H_2S, and only CH_4 passed through the solution and analyzed using wet gas flow measuring device which works according to liquid displacement methods. After a certain volume of produced biogas accumulated under each mould flow cell, the magnetic metal pieces lifted open and released trapped methane goes through the water and clicks back shut. The standard minimum volume to make one click is 10 mL. The digital pulse was connected to a data acquisition unit and computer to record the results. All the experimental tests were done in duplicates to evaluate analytical or measurement precision. All the values of the activity are expressed in g CH_4-COD/g VS/day.
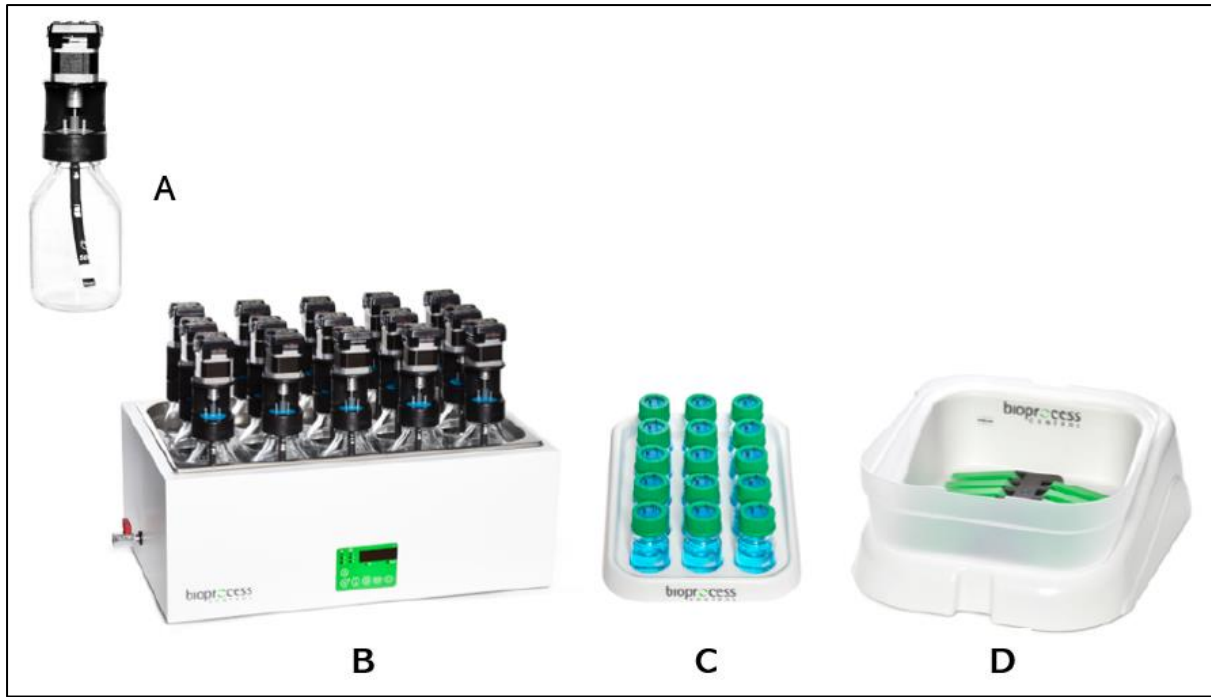
Figure 13. AMPTS II machine (automatic methane potential testing system) (pictures adapted from Bioprocess control's manual), (A) Reactor Bottle with an automatic stirrer, (B) Water Bath, (C) CO$_2$-Absorption Unit, (D) Gas Volume Measuring Device.

4.4.4   Mass Balances and Calculation

4.4.4.1   COD Mass Balance

The total mass balance of the COD was calculated daily to monitor the reliability of the experiment considering the input COD (influent feed wastewater), output COD (effluent, methane, hydrogen sulfide in the biogas), and estimated sludge growth. The conversion rate of COD into methane was measured according to the theoretical oxidation of methane in which 1 kg of COD degraded in the wastewater result in the production of 0.35 m^3 methane (Radian Beluc, 2019). Lens et al. (1998) noted that in theory, 1g of SO$_4$-S consume 2 g of COD.

The COD used for growth was assumed based on the true sludge yield of acetoclastic methanogens of 0.04 gVSS/g biodegradable COD (Rittmann & McCarty, 2001).

$$\sum COD_{input} = \sum COD_{output} \tag{1}$$

$$COD_{total,influent} = COD_{total,effluent} + COD_{CH_4} + COD_{growth} + COD_{H_2S,biogas} \tag{2}$$

$$COD\ mass\ balance\ (\%) = \frac{COD_{total,effluent} + COD_{CH_4} + COD_{growth} + COD_{H_2S,biogas}}{COD_{total,influent}} \quad (3)$$

4.4.4.2 Sulfur Mass Balance

The activity of SRB which reduces sulfate is the main factor that determines the formation of sulfide. Other factors such as available carbon source, initial sulfate concentrations in the wastewater, and the amount of SRB genera will determine the variability of the rate coefficients (van Loosdrecht et al., 2016).

The sulfur balance was calculated based on the equation below:

$$S_{SO_4,influent} = S_{SO_4,effluent} + S_{TLS,effluent} + S_{H_2S,biogas} \quad (4)$$

$$S\ balance\ (\%) = \frac{S_{SO_4,effluent} + S_{TLS,effluent} + S_{H_2S,biogas}}{S_{SO_4,influent}} \quad (5)$$

4.4.4.3. Sludge Yield

A reported by Eddy (2014), sludge yield, or biomass yield, was defined by the amount of biomass produced to the amount of total substrate consumed (g VS/g COD).

The sludge yield was calculated based on the equation below:

$$Sludge\ yield = \frac{VS_{end\ sludge} - VS_{initial\ sludge} + VSS_{effluent} - VSS_{influent}}{TCOD_{feed} - TCOD_{effluent}} \quad (6)$$

4.4.4.4. Digestion efficiency

Digestion efficiency was calculated based on equation (*7*), in which COD used for growth was considered as 0.04 gVSS/g biodegradable COD.

$$Digestion\ efficiency = \frac{TCOD_{in} - TCOD_{out} - COD_{growth} - COD_{H_2S}}{TCOD_{in}} \quad (7)$$

4.4.5 PHREEQC Modelling

PHREEC is a multipurpose geochemical software written in C and C++ programming languages. In the research, Notepad ++ was used as an interface to run PHREEQC version 3 to predict scaling and potential corrosion in the reactor. Different database was used for the stimulation. minteq.v4 dat was used for the corrosion of caused by hydrogen sulfide (H_2S) and phreeqc.dat was used for the calcite precipitation.

5   Results and Discussion

5.1   Observation of effluent color formation

The effluent sample color turned dark brown after a few hours of sampling. It is unclear at this point whether the color change was due to anaerobic metabolism, or any reducing condition that happens during the anaerobic digestion. Wingate (2002) reported that the presence of quinone and quinoid derivation can be the reason for the changes in the effluent chromophore. Kortekaas et al. (1998) explained the changes of color under anaerobic condition is due to the biological transformation of organic precursor and autoxidative polymerization. Acetogenic bacteria groups such as *Clostridium thermoaceticum*, *Acetobacterium woodii*, and *Eubacterium limosum* have the capability to demethylate lignin model compounds causing free hydroxylated aromatic structure, which makes lignin susceptible to autooxidation. In addition to that, with the presence of air and increasing pH from neutral to alkaline conditions, lignin breaks down into fragments and combines with the hydroxy group to form dark brown quinone complexes.

Shan et al.(2016) stated that the high chroma in the biologically treated effluent comes from humic materials which contributed to a great fluorescence portion of about 67.1%. Additionally, according to Gallert & Winter (2005), the formation of humic acid-like residues generally appears after the reaction of biodegradable compounds (cellulose and starch) and non-biodegradable compounds (carbohydrate-derived cellular).
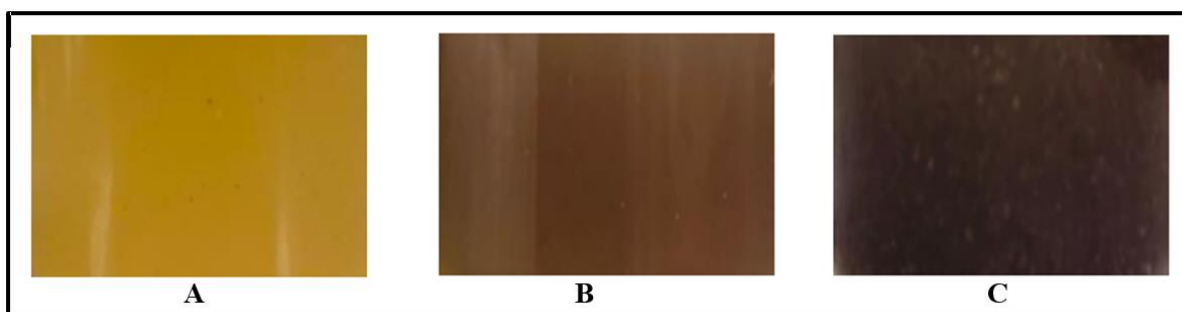
Figure 14. Change in effluent color; (A) fresh sample, (B) after few hour, (C) after 5 days.

5.2   Anaerobic Biodegradability Tests

Figure 15 and   Figure 16 show the results of the anaerobic biodegradability test with the wastewater. Figure 15 illustrates the fast conversion of COD into methane about 1334 mg CH_4-COD/L during the first day of incubation. After the first day, some additional biological degradation took place but only last for two weeks then there is hardly anything happening for the remaining time. This figure display a fast conversion, with more than 90% of its final conversion reached after six to seven days of incubation. The subsequent days of incubation periods show minor further conversion which is reflected by the decrease in SCOD and production of methane. The test stopped on day 24 after no further removal of SCOD and no more methane production. The methane production data were corrected for the methane production in the substrate-free controls. All values are expressed in mg COD per liter liquid phase.

Figure 16 shows that approximately 70% of wastewater TCOD and SCOD was converted to methane and the removal efficiency was 60%. However, compared to the apparent COD removal in the long-run actual EGSB reactor (Figure 19) in which the removal efficiency was invariably in the range of 45% to 55% for total COD and 50%-60% for soluble COD. Ho et al (1984) explained the particulates-plant cell debris and fragments contain high lignin which appears to be not easily hydrolyzable leading to low digestibility and Sìerra-Alvarez (1990) noted that anaerobic bacteria have a very limited capacity to degrade lignin.

Theoretically, methane-COD production should be a few percent lower than COD removal (because a fraction of the removed COD is used for growth). However, this is not the case in this sample, which may suggest an underestimate of the measured COD or there is a presence of any other compounds which were not measured. The calculation of methane production was calculated theoretically based on the assumption that all the COD removed are converted to methane in the biogas and other gases such as carbon dioxide, trace gases have no COD. In the actual practice of reactor operating conditions, the process generates $0.25 - 0.30\ m^3$ of methane per one kg of COD degraded (Radian Beluc, 2019).
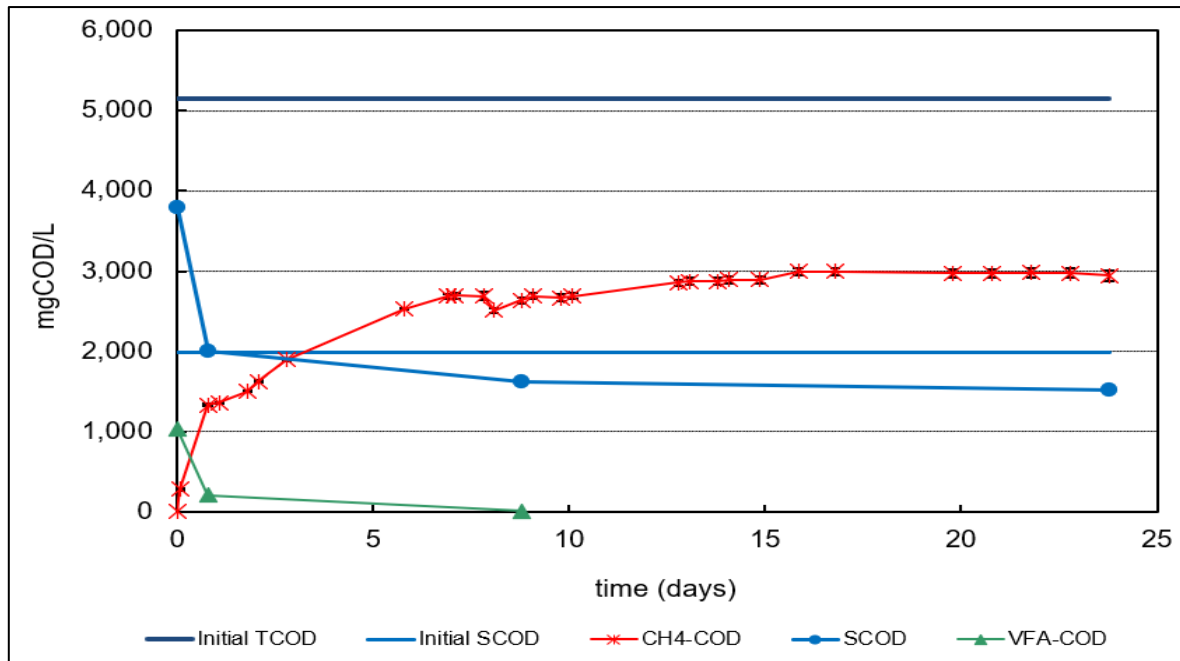
Figure 15. The course of SCOD, VFA, and CH$_4$ during incubation of the wastewater (non-diluted wastewater with initial TCOD of 5156 mg/L) with non-adapted anaerobic granular sludge (6.9 g VSS/L).
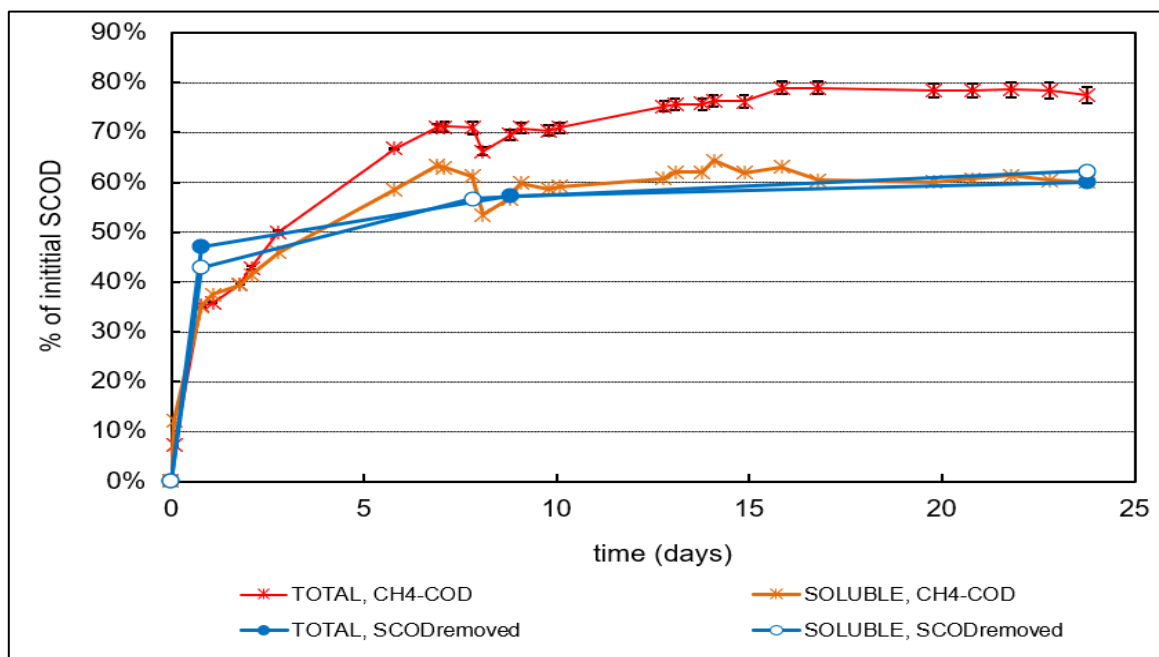
Figure 16. SCOD removal and methane production (expressed as a percentage of the initial SCOD) during incubation of the wastewater with non-adapted anaerobic granular sludge (6.9 g VSS/L).

Various research studies from Jurgensen et al. (1985), Pichon et al. (1987), and Welander (1988) reported the COD reduction of CTMP wastewater from 45-60%. Sierra-Alvarez et al. (1990) indicated that the anaerobic treatment has a limited capacity to mineralize resin, volatile terpenes, and other wood toxins compounds.

5.3   Anaerobic Toxicity Test

Figure 17 and Figure 18 show the results of the toxicity test with the wastewater with the positive control, non-diluted wastewater, and with different dilution gradients. The negative control bottle has no gas production. Figure 17 depicts the cumulative gas production and the bottle with 5 times dilution produces more biogas during the first day than the positive control bottles. Non-diluted wastewater bottle shows significant cumulative biogas production for 3.5 days, whereas positive control bottle biogas production stopped after one day.

Figure 18 displays the specific methanogenic activity (SMA) in which acetate was provided as the sole substrate. The shapes of the 5 times dilution curve show a higher peak with an average SMA of 0.70 gCH$_4$-COD/gVS/day than other bottles. From this graph, all the bottles containing wastewater have higher activities than the positive control bottle. Table 6 shows the ultimate anaerobic toxicity test results.
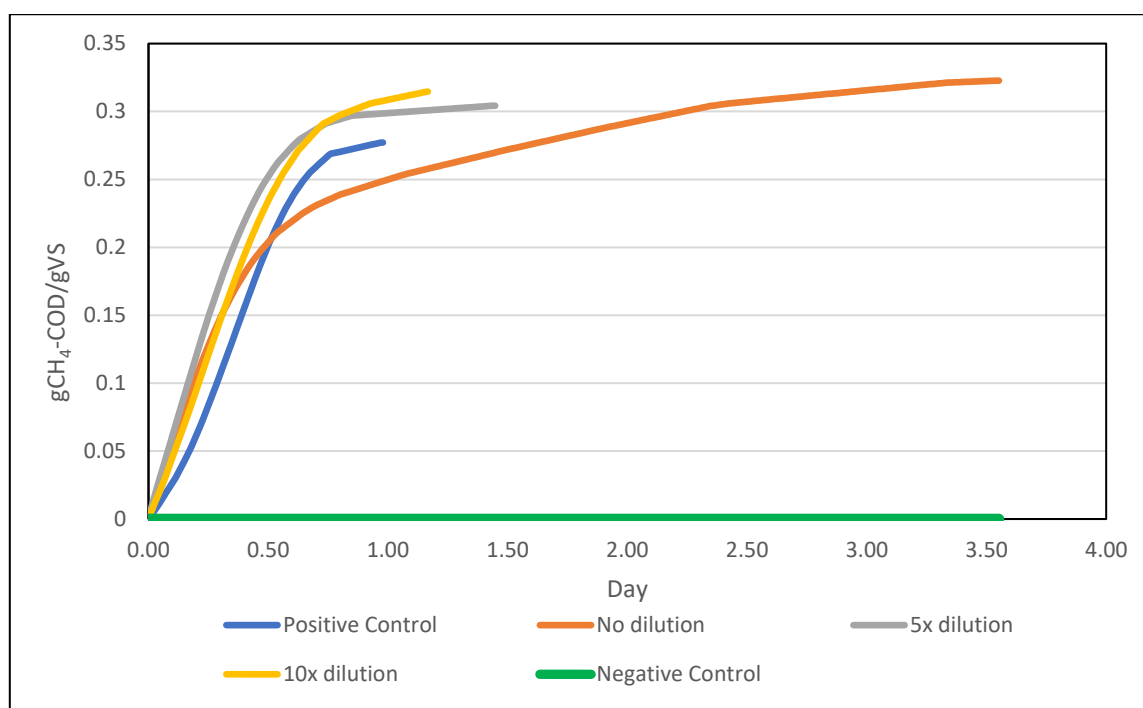
Figure 17. Cumulative biogas production of positive control bottle, non-diluted, 5 times and 10 times diluted wastewater.
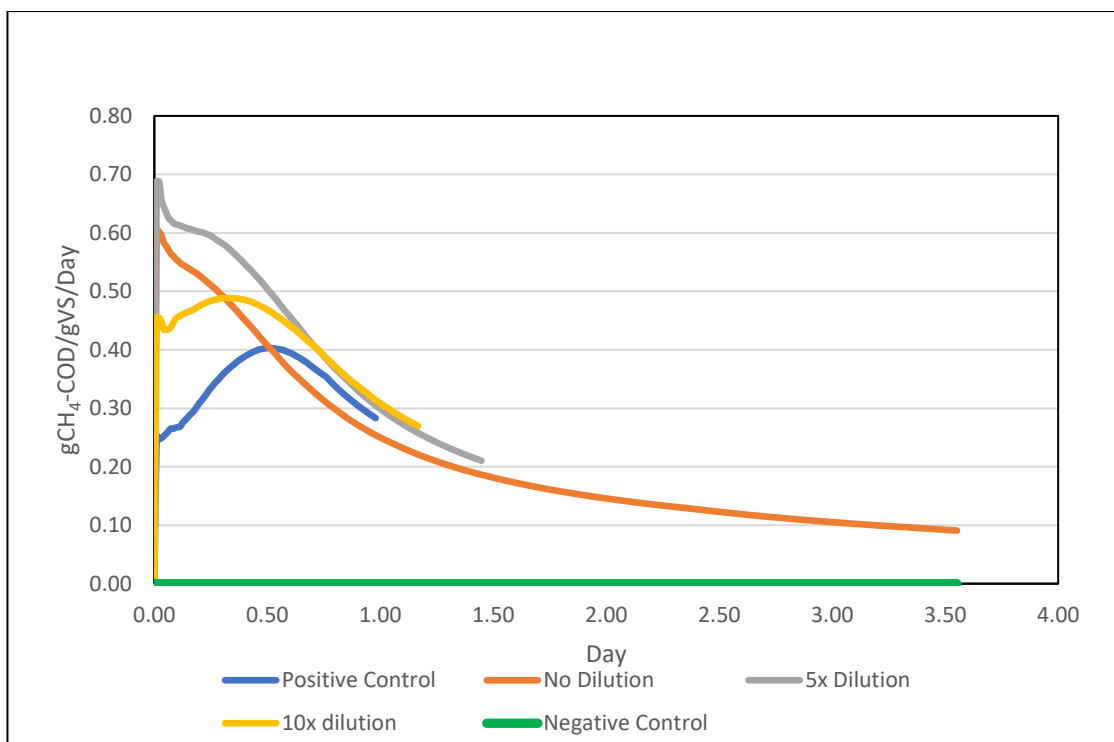
Figure 18. Specific Methanogenic Activity Test results of positive control bottle, non-diluted, 5 times and 10 times diluted wastewater.

Table 6. Result of toxicity based on SMA test, each bottle test was done in duplicate.

Bottle Number	SMA (gCH₄-COD/gVS/day)	Dilution
1-1	0.44	Positive Control
1-2	0.4	
2-1	0.59	Non-diluted
2-2	0.63	
3-1	0.71	5-times
3-2	0.69	
4-1	0.53	10-times
4-2	0.55	
5-1	0	Negative Control
5-2	0	

5.4   Biological Performance

5.4.1 Start-up phase

The start-up phase was operated from day 1 to 29 with a rapid increase of VLR only when there is no accumulation of VFA in the effluent (VFA<5 meq/L). During this period, the HRT ranged from 8 to 30 h with influent wastewater to favor biomass acclimation. Figure 19 shows the COD removal efficiency for both total and soluble COD concentration in the wastewater. The system was paused twice due to the Christmas break (first pause: Day 30-56) and another pause to mimic the situation during Christmas (second pause: Day 128-137). On the first day of operation, there was an incident with the water bath that cause reactor temperatures to go up to about 40 $^{\circ}$C beyond the set point (35±1°C) which affect the activity of the inoculum (0.29 g CH_4-COD/g VS.d). After seeding the reactor, the system was let recirculate at a flow velocity of 3 m/h overnight. On day 2, the reactor was fed with 7.5 g TCOD/L/d, and over the weekend (Day 3 and 4), there was an issue with the inlet pump, which left the reactor unfed. It can be seen that during the start-up period (Day 1-28), the COD removal efficiency was between 50-60%. On day 29, a part of the reactor's recirculation line was broken which cause air intrusion into the reactor. Before this pause, an SMA test was done on day 29 to check the activity of the biomass, and the result showed very low activity of the biomass 0.06 g CH_4-COD/g VS.d.

Over the pausing period from day 30-56, the reactor was left unfed with only recirculation, and day 30-37, the reactor temperature was left at 35±1°C and from day 37-56, the temperature was decreased to 24°C.
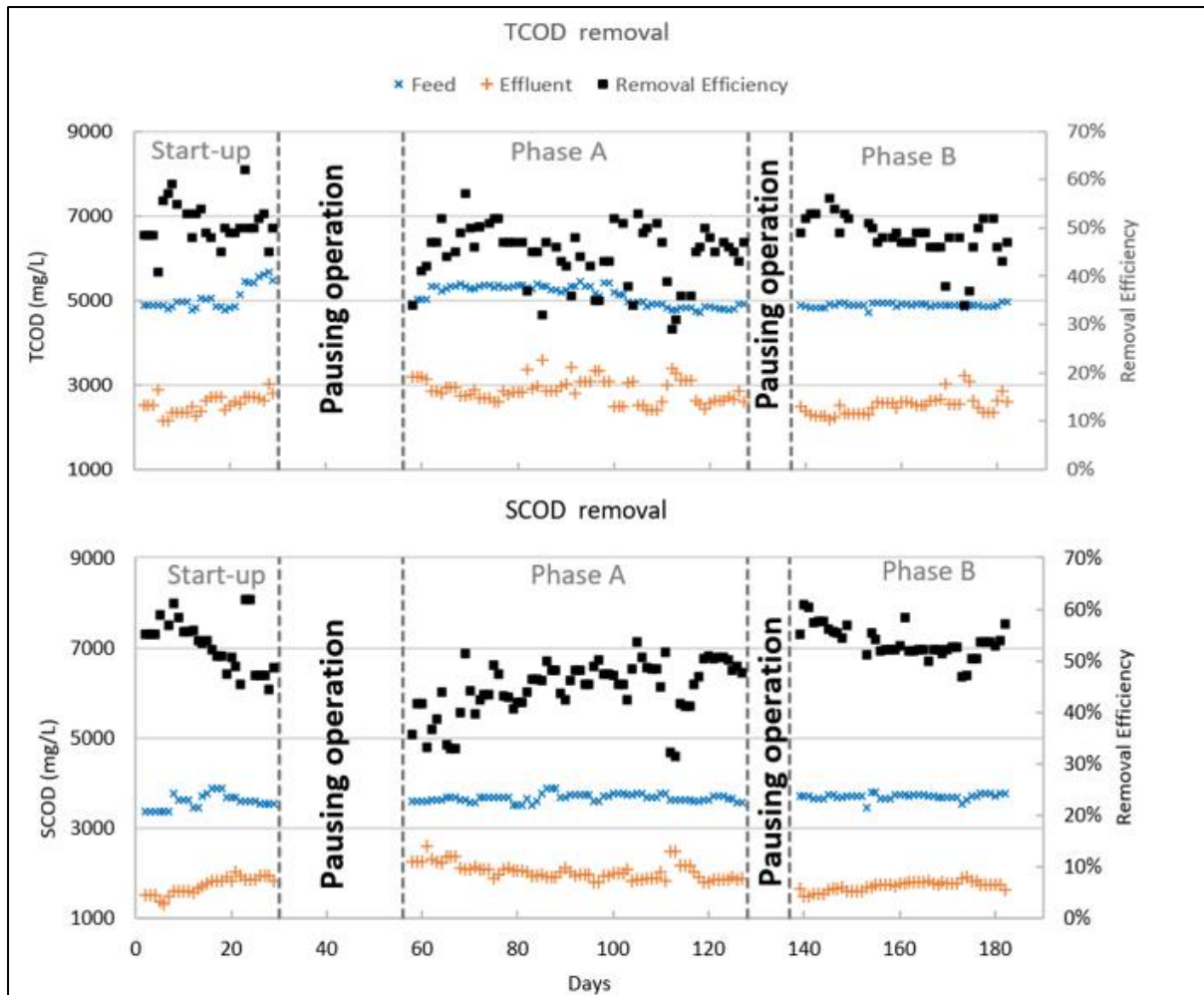
Figure 19. Feed COD (mg/L), Effluent COD (mg/L) and COD removal efficiency (%) of the reactor.

Figure 20 depicts the result of volatile fatty acids (VFA) in the effluent with respect to the volumetric loading rate (VLR). VFA depicted in the figures was the total result ranging from acetic acid (C2), propionic acid (C3), isobutyric acid (iC4), butyric acid (C4), isovaleric acid (iC5), valeric acid (C5) and hexanoic acid (C6) expressed in meq/L. The accumulation of VFA in the effluent could be a sign that the anaerobic bacteria, especially methanogens got inhibited. Throughout the entire evaluation (Day 1-29), acetate was the primary constituent found in the effluent sample.
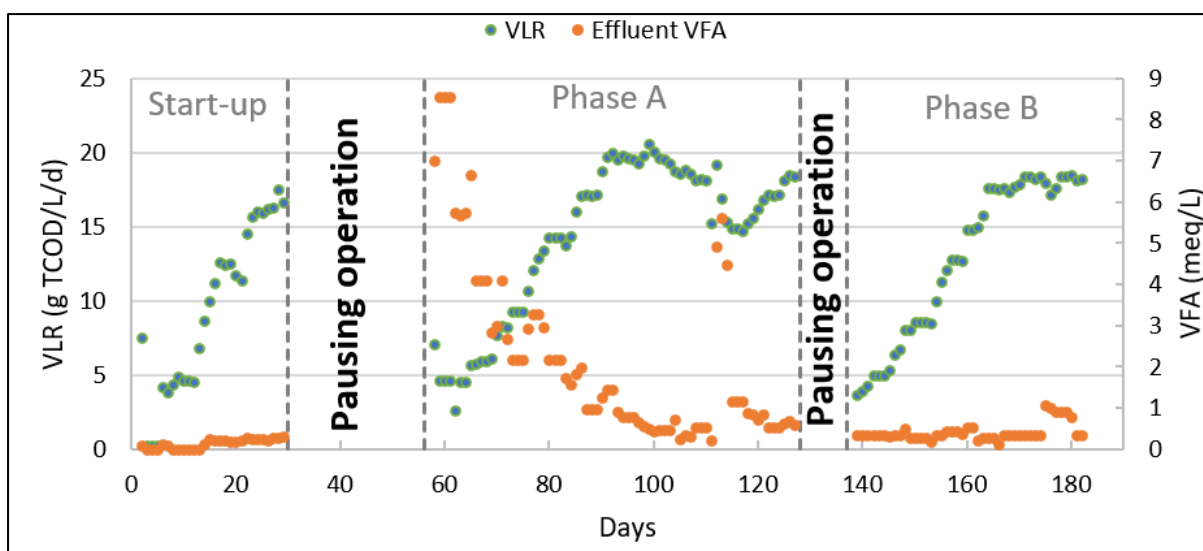
Figure 20. Volatile Fatty Acids concentration in the effluent (meq/L) corresponding to Volumetric Loading Rate (g TCOD/L/d).
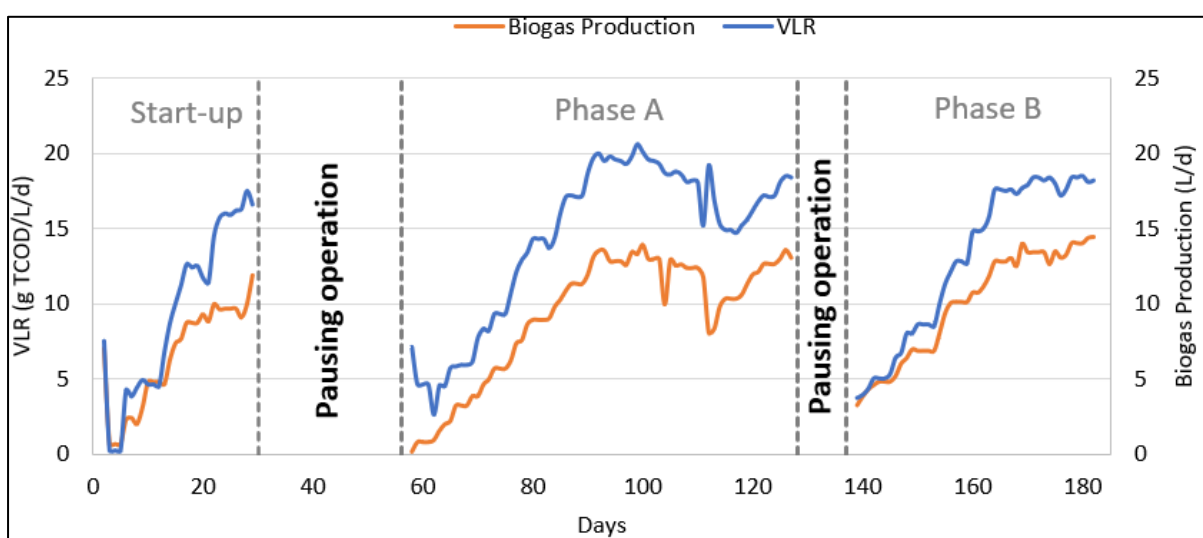
Figure 21. Biogas production rate (L/d) under mesophilic conditions (35±1ºC).

Figure 21 and Figure 22 show the daily biogas production and the composition of methane (CH_4), carbon dioxide (CO_2), oxygen (O_2), and hydrogen sulfide (H_2S). During the first stage of start-up and later part, there was a significant increase in biogas production with the increasing VLR including the rising of methane production. From day 2-20, the production of H_2S in the biogas was 0.2% (2000 ppm) and increased to 1.1% (11000 ppm). This increase in H_2S could be due to the presence of oxygen in the reactor which oxidized sulfate and elemental sulfur and further reduced by chemolithotrophic sulfate reducing bacteria (SRB) (Greer, 2010).

Table 7 summarizes the result of the COD mass balance calculations obtained from the start-up phase during the stable operation (VLR 18 g TCOD/L/d). About 25% of the influent biodegradable COD was recovered as methane gas. The "loss" 10% in the balance can be explained loss by the presence of recalcitrant compounds such as lignin and its derivatives may oxidize with the presence of air intrusion in the reactor. Salehi (2016) discussed that the reduction of sulfate could contribute to the loss of COD in the balance.
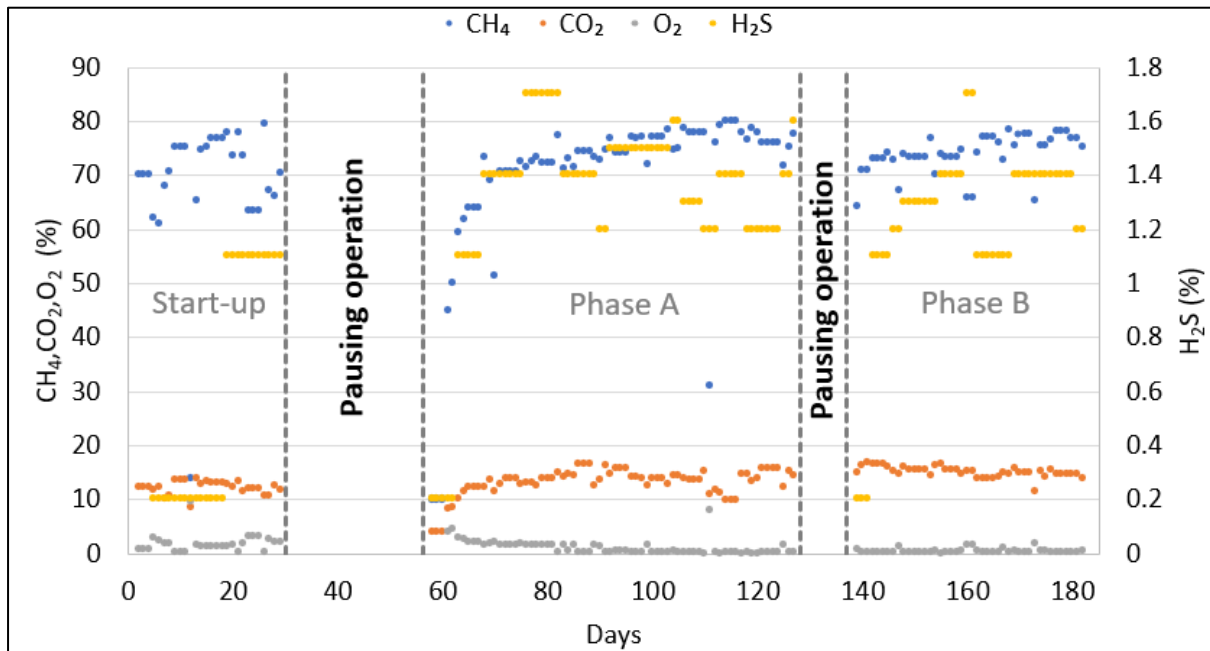
Figure 22. Biogas composition (%), methane (CH_4), carbon dioxide (CO_2), oxygen (O_2), and hydrogen sulfide (H_2S).

Table 7. Summary of the result from the start-up phase (Day 1-29). All values are expressed in average of stable operation period (Day 23-29).

Parameter	Unit	Value
HRT	h	8 ± 0.16
VLR	gTCOD/L/d	18 ± 0.59
Effluent VFA	meq/L	0.25 ± 0.03
TCOD removal efficiency	%	50 ± 2.76
SCOD removal efficiency	%	46.81 ± 1.82
Digestion efficiency	%	45.61 ± 2.94
Methane production[a]	gCOD/d	17.02 ± 2.32
Effluent pH	-	7.54 ± 0.22

[a] At standard temperature and pressure

Table 8. COD mass balance for the start-up phase (Day 1-29). All values are expressed in an average stable operation period (Day 23-29).

Stream	Units	Value
Influent	g COD/d	68.86 ± 2.51
Effluent	g COD/d	34.43 ± 3.18
Growth	g COD/d	2.75 ± 0.10
Methane	g COD/d	17.28 ± 2.42
Hydrogen sulfide	g COD/d	0.31 ± 0.03
Balance	%	80

5.4.2 Operational Phase A

In operational phase A (Day 57-127), after restarting the system, the reactor with HRT ranging from 8 to 48 h at VLR of 2 -18 g TCOD/L/d. Before the beginning of this phase, on day 56, all sludge was removed from the main EGSB reactor and mixed well to get a representative sample for TS, VS, and SMA analysis. The result (Table 12) showed a significant decrease of biomass within the reactor with VS of 80 gVS/kg with the activity of 0.02 g CH_4-COD/gVS.d.

The reactor was restarted again on day 58 with a VLR of 7.1 gTCOD/L/d and VFA began to accumulate (7 meq/L). Hence, VLR was further reduced to 2 gTCOD/L/d and slowly increased to design load (VLR of 18 gTCOD/L/d) only if the VFA was below 5 meq/L. The biomass took approximately 10 days to recover. The accumulation of VFA in the effluent can be attributed to the limited growth of methanogens combined with the inhibitory effect of air intrusion on microbial activity.

At VLR of 18 ± 0.2 gTCOD/L/d, the system was performing well with low VFA in the effluent (Figure 20) and biogas production met with the expected gas production which was calculated based on influent SCOD, methane production, and SCOD removal efficiency. However, the calculated estimated biogas production based on TCOD was higher than the actual biogas production. The sludge yield at the end of this phase was 0.04 g VS/g COD.
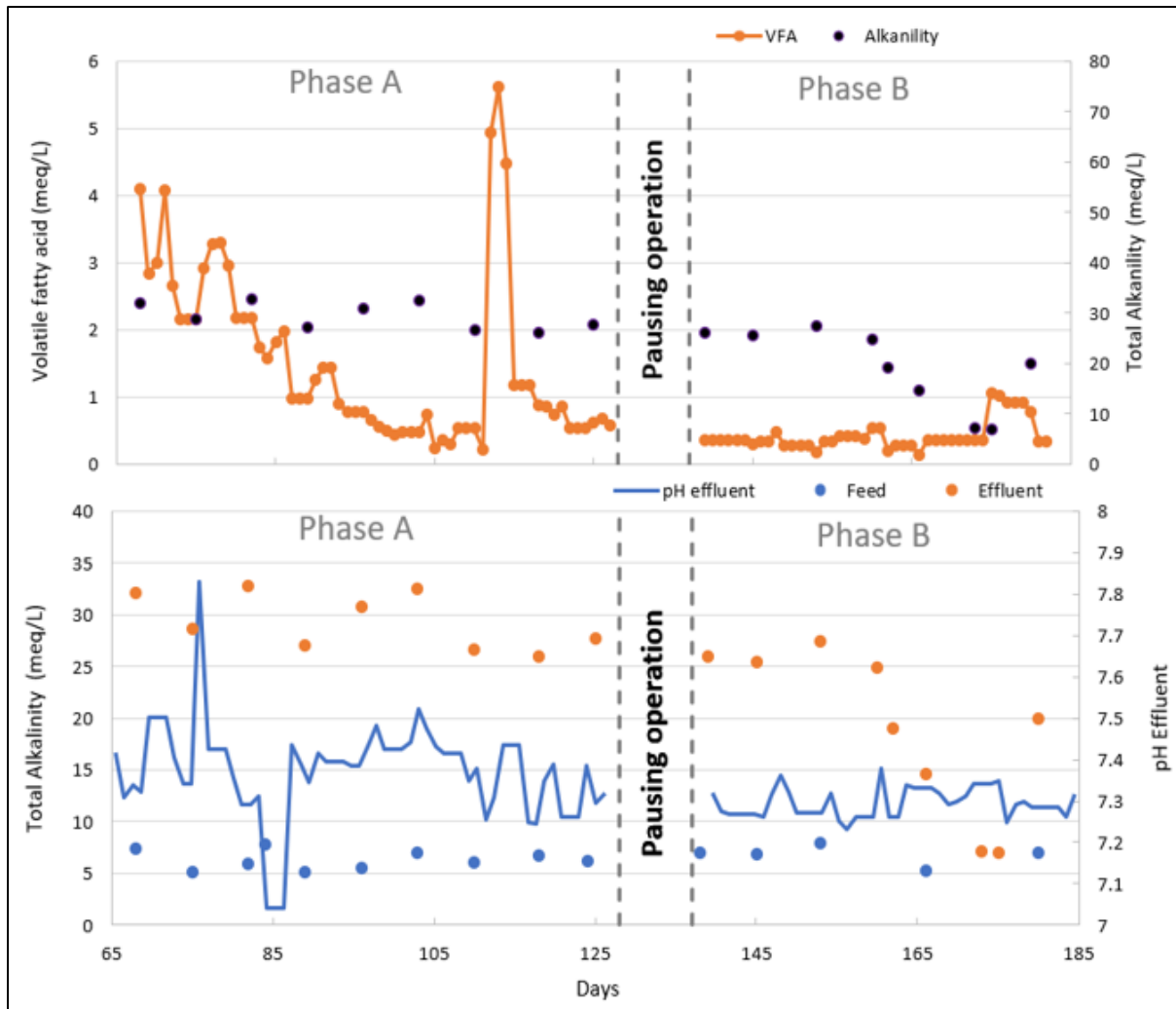
Figure 23. Changes in VFA with respect to alkalinity and changes in alkalinity with respect to pH in the effluent.

Changes in acidity and alkalinity in the effluent from the EGBS reactor from day 68 to 182 are shown in Figure 23. It can be seen from the graph that on day 112-114, there were high VFA in the effluent. This can be related to the event of air intrusion on day 111 leading to a VFA/TA of 5. Then, the system recovered quickly showing a VFA/TA ratio of less than 0.4, which was reported by Hernández et al.(2014) as a safe range for anaerobic digestion with a lower risk of acidification.
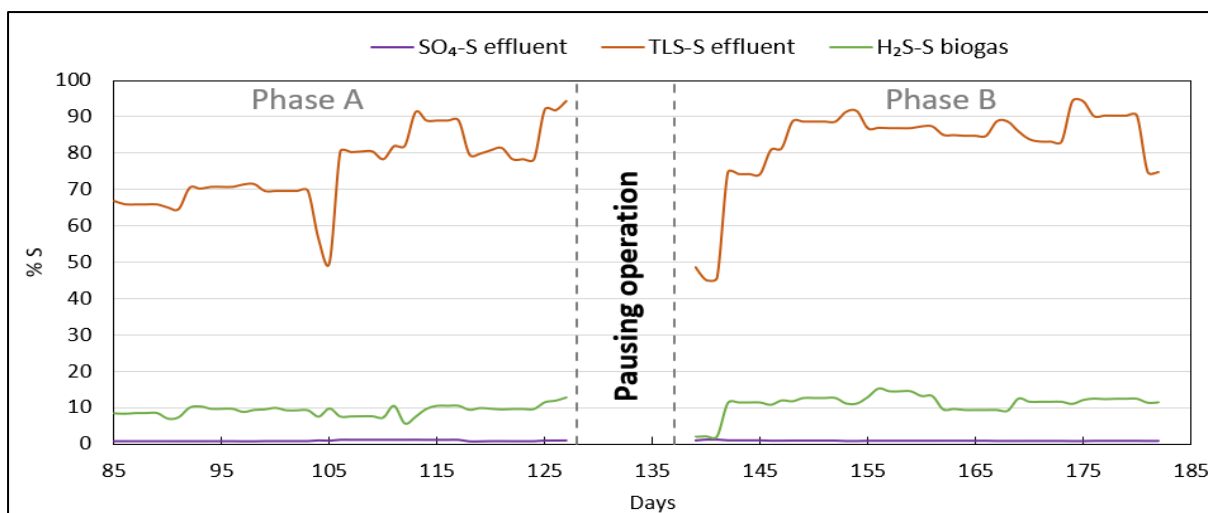
Figure 24. Elemental sulfur (S) presented in the liquid and gaseous phase after sulfate reduction.

Table 9 and Table 13 shows the concentration distribution of sulfur compounds in the system in two different states: liquid (feed and effluent) and gaseous phase (biogas). The outputs of sulfur accounted for 88.85% and 98.2 % of the inputs, for phase A and phase B respectively. The "lost" sulfur was not analyzed in the input and output causing the lack of sulfur balance. Ruan et al. (2017) discussed that the sulfur content in the biogas is not limited to H_2S only, there is more possible gaseous form of sulfur such as dimethyl sulfide, carbon disulfide, mercaptans, etc. In addition to that, the elemental sulfur determination may have been biased in total liquid sulfide content, which could have volatized during the sampling procedure.

Table 11 summarizes the COD mass balance calculation obtained from operational phase A on average of stable operation period VLR 18 g TCOD/L/d (Day 104-110). The COD mass balance had increased to 88.85%, which was 8.85% higher than the start-up phase.

Table 9. Sulfur balance in operational phase A (Day 58-127) at VLR 18 g TCOD/L/d (Day 104-110).

Parameter	g/d	%
SO_4-S feed	2.94 ± 0.04	-
SO_4-S effluent	0.03 ± 0	1.17 ± 0
TLS-S effluent	2.35 ± 0	79.89 ± 0.93
H_2S-S biogas	0.07 ± 0	7.49 ± 0.85
Balance		88.55

Table 10. Summary of the result for the operational phase A (Day 58-127). All values are expressed on average of stable operation period VLR 18 gTCOD/L/d (Day 104-110).

Parameter	Unit	Value
HRT	h	6.40 ± 0.08
VLR	g TCOD/L/d	18.00 ± 0.30
Effluent VFA	meq/L	0.46 ± 0.17
TCOD removal efficiency	%	50.00 ± 2.24
TCOD-TLS removal efficiency	%	55.97 ± 1.51
SCOD removal efficiency	%	49.07 ± 2.91
SCOD-TLS removal efficiency	%	54.37 ± 1.42
Digestion efficiency	%	45.65 ± 1.80
Methane production[a]	g COD/d	23.72 ± 2.20
Effluent pH	-	7.44 ± 0.06

[a] At standard temperature and pressure

Table 11. COD mass balance for the operational phase A (Day 58-127). All values are expressed on average of stable operation period VLR 18 g TCOD/L/d (Day 104-110).

Stream	Units	Value
Influent	g COD/d	77.44 ± 1.12
Effluent	g COD/d	40.37 ± 5.10
Growth	g COD/d	3.10 ± 0.04
Methane	g COD/d	23.72 ± 2.20
Hydrogen sulfide	g COD/d	0.46 ± 0.05
Balance	%	87

5.4.3 Sludge profile at the end of phase A

Figure 25 illustrates the nitrogen compounds within influent and effluent in the form of total, soluble, ammonium, and nitrate. In Phase A, the total nitrogen in the effluent was lower than the feed except for the day 91-97 and 112-118 it became higher than the feed. However, in the soluble part, nitrogen was lower in the effluent compared to the influent. The suspended solids present in the reactor hold organic matter, nutrients, and other substances (Di Costanzo et al., 2021). Goto et al. (2021) pointed out that nitrogen, also known as "natural N-contents" is so common in the natural wood cellulose.

In ammonium and nitrate- nitrogen was consistently higher in the effluent as shown in Figure 25. This can be explained by McCarty (1964) that the ammonium and ammonia form in the anaerobic treatment process results from the degradation of organic wastes containing protein, urea, or amino acids. Ammonium ions exist in equilibrium with free ammonia and hydrogen ion (Equation 8) and ammonia can present in form of ammonium ion or ammonia gas (Equation 9). The ammonium-nitrogen concentration within the anaerobic system was indicated by Lay et al. (1998) as a significant factor in affecting the activity of methanogenic bacteria as well as in an acclimatized system. It was reported by McCarty (1964) that at a concentration range from 1500-3000 mg/L, ammonium nitrogen can inhibit the anaerobic process at a pH range of 7.4-7.6 and were expected to be toxic to anaerobic microorganisms when the concentration exceeds 3000 mg/L at all pH value. Table D in appendix A provides more details on the ammonium concentration affecting the anaerobic process.

$$NH_4^+ \rightarrow NH_3 + H^+ \tag{8}$$

$$NH_{3(aq)} + H_2O_{(l)} \leftrightarrow NH_{4(aq)}^+ + OH^- \tag{9}$$
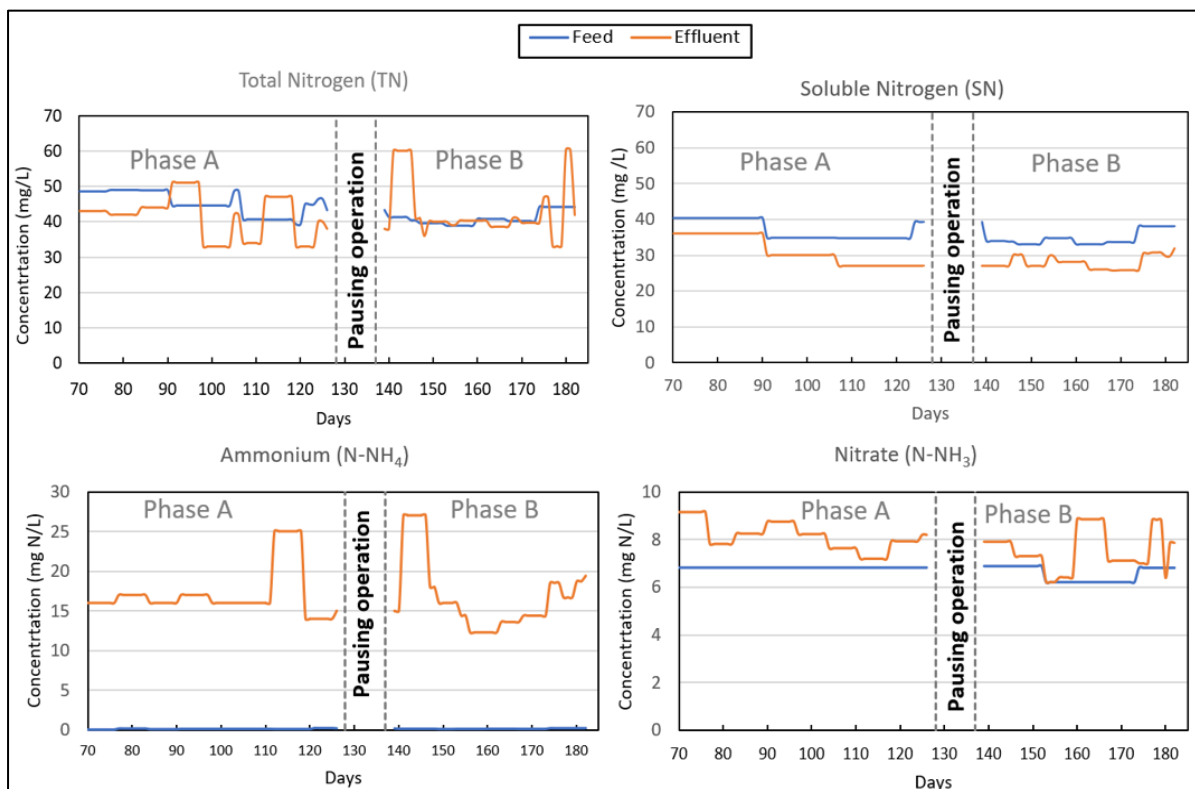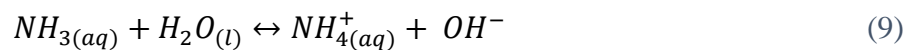
Figure 25. Total Nitrogen (TN), Soluble Nitrogen (SN), Ammonium (NH4-N), and Nitrate (NO3-N).

Figure 26 illustrates the total phosphorus and orthophosphate in the effluent and feed. It can be seen that total phosphorus effluent in phase A was lower than the feed except for the days 91-97 and 112-118 which became higher than the feed.
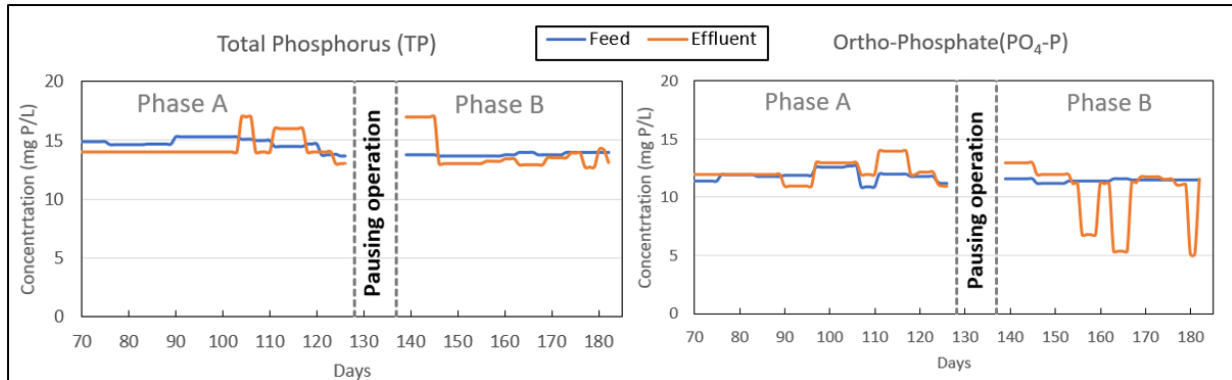
Figure 26. Total Phosphorus (TP), Ortho-Phosphate (PO$_4$-P)

Figure 27 depicts the photo of the reactor taken on day 68. The upper part (A and B) shows accumulated suspended solids and it mixed with the anaerobic granule in the sludge bed (C and D). At the lower part of the reactor, it can be seen that those solids were attached to the wall of the reactor which makes it difficult to wash out.
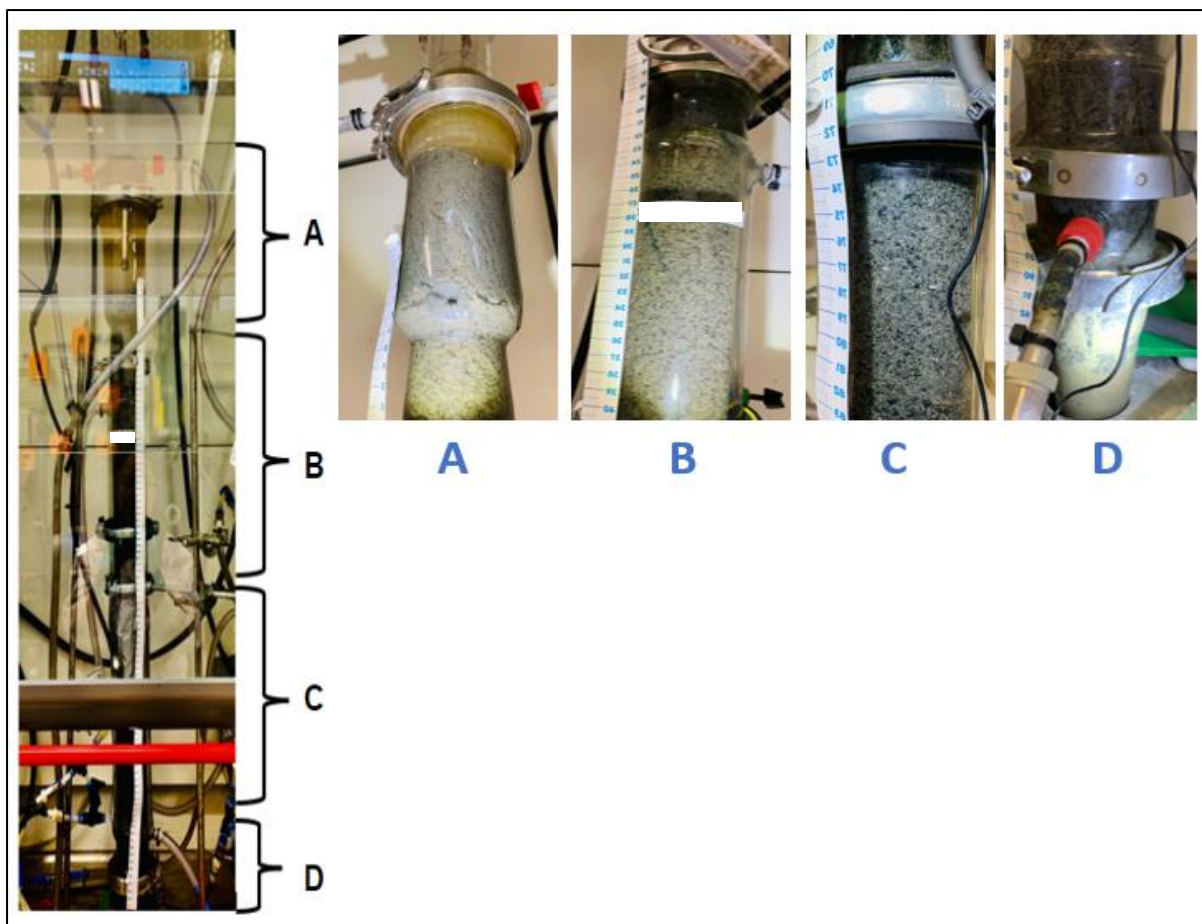
Figure 27. Reactor's photo taken on day 68; (A) Upper part, (B) Upper middle part, (C) Lower middle part, (D) Bottom part.

Figure 28 displays images of the biomass under microscopic view (Keyence VH-Z20UR, Japan). It was observed that sludge contains suspended solids and the biomass had different shape and size. Some of the granules remained intact while others disintegrated into pieces.

Figure 28. Microscopic view of the reactor's sludge on day 127;(a) unwashed sludge (X20), (b) cross-sectional view of granule (X160), (c) & (d) biomass after washing with tap water and sieve size 0.425 mm (X20).

5.4.4 Operational Phase B

The presence of solids in the reactor had a large impact on its performance of the reactor. Most of the solid remained in the upper part of the sludge bed and some stayed in between the granule. On day 104, solids had been washed out from the main EGSB reactor and a part of it ended up in the conditioning tank and effluent. A significant drop was observed in the sludge bed (5 cm) which shows a loss of biomass went along with the solid out of the main reactor. Some of the biomass was also seen to be left on the biogas line which connected to the top of the conditioning tank. The sludge yield at the end of this phase was 0.09 g VS/g COD which

is about 56% higher than phase A. This can be explained by the stability of the reactor during phase B compared to phase A.

Table 12. Solids in the EGSB reactor from the start-up day (Day 1) to the end of the trial (Day 182); Total Solids (TS) and Volatile Solids (VS).

Day	TS (g)	VS (g)	VS/TS (%)
1	232	140	60
58	242	119	49
127	108	80	74
182	176	125	71

Table 12 shows the number of solids in the reactor from the start-up day (Day 1) to the end of the trial (Day 182). On day 58, VS content of the sludge dropped 15% compared to the start-up day. This could be because of the air intrusion in the reactor or the hydrogen sulfide present within the reactor. On day 127, the VS continue to drop 32.77% and increased by 36% on the last day of operation. The ratio of volatile solids to total solids (VS/TS) indicated the organic matter content in the sludge and can be used to access the stability of the effluent. Rajagopal et al.(2019) witnessed the effects of accumulated non-degraded suspended solids on the performance of UASB reactor treating sewage leading to the reduction of active biomass, low biogas production, and high acid in the effluent. Table 15 summarizes the COD mass balance calculation obtained from operational phase B (Day 138-182). All values are expressed on average of the stable operational phase (164-182). The COD mass balance had increased to 91%, which was 11% higher than the start-up phase and 3% higher than operational phase B.

Table 13. Sulfur balance in operational phase B (Day 138-182).

Parameter	g/d	%
SO_4-S feed	2.27 ± 0.07	-
SO_4-S effluent	0.02 ± 0.00	0.77 ± 0.03
TLS-S effluent	1.96 ± 0.15	86.24 ± 5.40
H_2S-S biogas	0.25 ± 0.03	11.19 ± 1.26
Balance		98.20

Table 14. Summary of the result for the operational phase B (Day 138-182). All values are expressed on average in the stable operational phase (Day 164-182).

Parameter	Unit	Value
HRT	h	7.00 ± 0.16
VLR	gTCOD/L/d	18.00 ± 0.41
Effluent VFA	meq/L	0.51 ± 0.16
TCOD removal efficiency	%	47.07 ± 3.49
TCOD-TLS removal efficiency	%	52.54 ± 3.54
SCOD removal efficiency	%	52.54 ± 1.72
SCOD-TLS removal efficiency	%	58.78 ± 1.18
Digestion efficiency	%	42.78 ± 3.44
Methane production rate[a]	gCOD/d	25.69 ± 1.43
Effluent pH	-	7.30 ± 0.03

[a] At standard temperature and pressure

Table 15. COD mass balance for the operational phase B (Day 138-182). All values are expressed in an average of the stable operational phase (Day 164-182).

Stream	Units	Value
Influent	gCOD/d	75.38 ± 1.87
Effluent	gCOD/d	40.64 ± 4.04
Growth	gCOD/d	3.02 ± 0.07
Methane	gCOD/d	25.69 ± 1.43
Hydrogen sulfide	gCOD/d	0.51 ± 0.06
Balance	%	91

Figure 25 shows that in phase B, total nitrogen in the effluent was higher than the feed from day 141 to 145. At the beginning of phase B, the effluent phosphorus was higher than the feed for only a few days and remain lower in the remaining days as depicted in Figure 26.

5.4.5 Sludge Profile at the end of the trial

At the end of the operation (Day 182), the sludge has been removed from the reaction and it was observed from the microscope that anaerobic granules from EGSB were disintegrated into smaller fragmented granules and small debris (Figure 29). In addition to that, sludge contains short and long cellulose fiber resulting from the inlet feed wastewater (Figure 30).
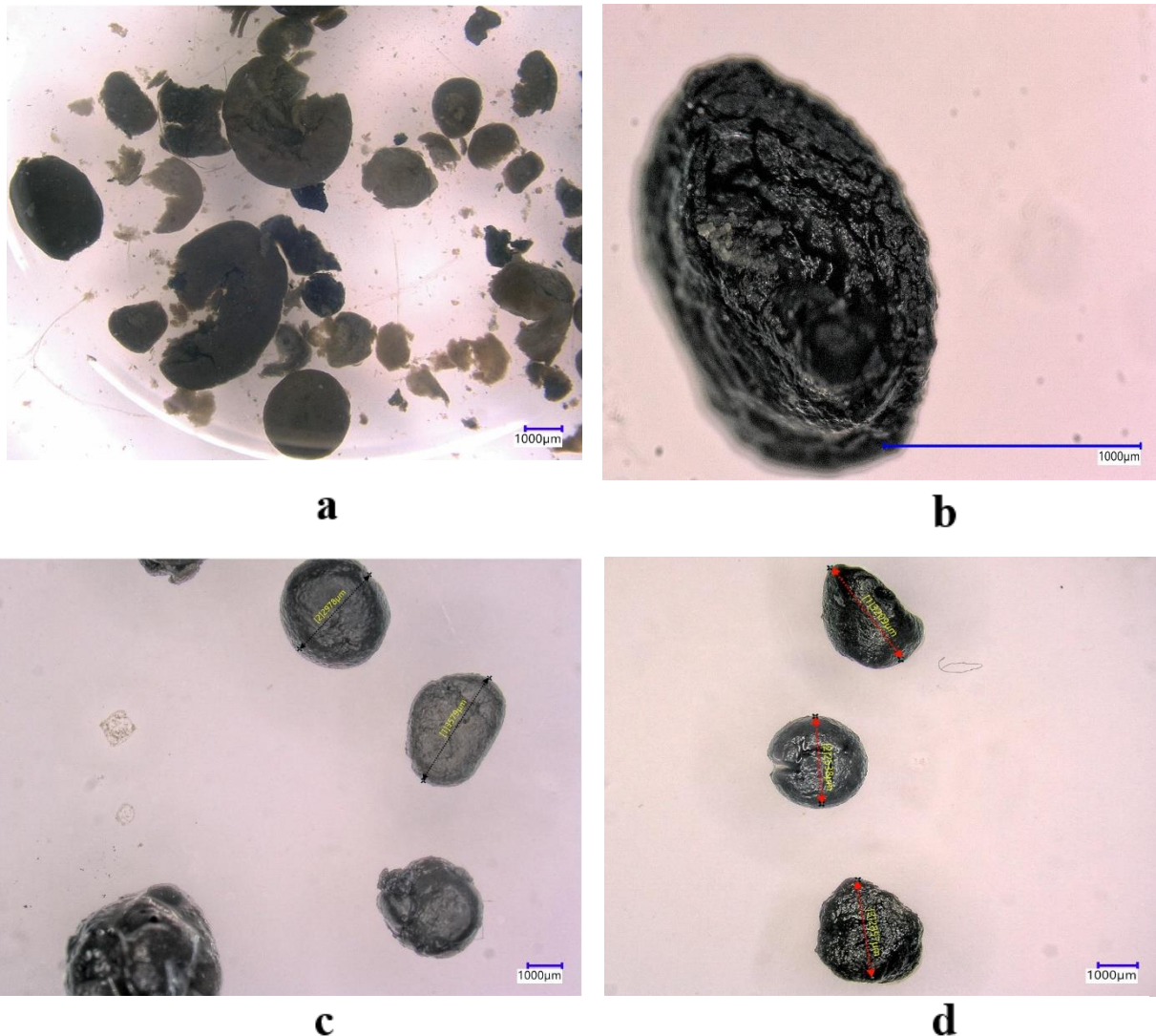
Figure 29. Microscopic view of the reactor's sludge on day 182;(a) unwashed sludge, (b) cross-sectional view of granule, (c) biomass after washing with tap water and sieve size 0.425mm, (d) disintegrated granule.
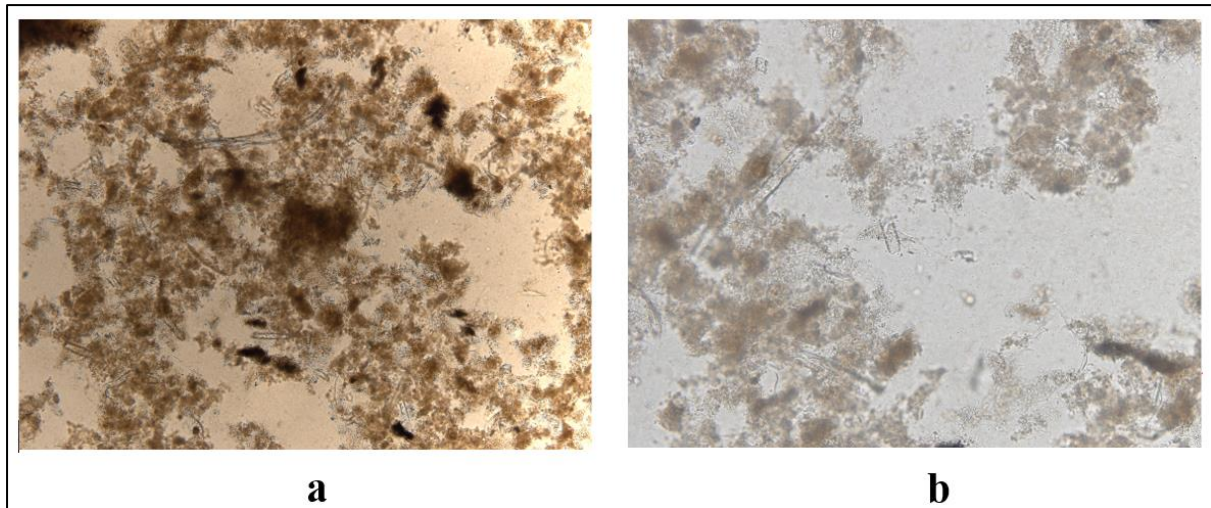
Figure 30. Microscopic view of the reactor's sludge; (a) magnification of 100x, (b) magnification of 200x.

5.5   Operational Problem and Countermeasure

The presence of solids in the wastewater caused the blockage in the influent, effluent, and recirculation lines. Therefore, regular maintenance was done to prevent blockage and overpressure within the reactor. Daily measure the effluent flow rate to check whether up flow velocity met with the set-point or not. If the flow rate was not matched with the set point, this could be an indication that blockage happen within the tube or in the nozzle, which required squeezing the tube to push the solids. The water bath was filled with polypropylene anti-evaporation spheres (diameter of 20 mm) and refilled every day with demineralized water to prevent scaling and evaporation which can cause a decrease in the reactor's temperature leading to less biogas production. The white plastic stopper located along the biogas line was changed monthly to prevent leakage of biogas.

At the end of the operation, on the last day (Day 182), while emptying the conditioning, the solids accumulate, and with the rotor mixing resulting in muddy-like (Figure 31). It was observed that there was a white color deposit on the wall of the upper part of the conditioning tank. Zhou et al. (2022) and Ruan et al. (2017) described that this white deposit color was caused by the oxidation of sulfide to elemental sulfur under microaerobic or anoxic conditions by sulfur oxidizing bacteria. The majority of the research on anaerobic digesters reported that the yellowish-white deposits due to the production of elemental sulfur were commonly found in the reactor's headspace, wall, and ceiling (Kobayashi et al., 2012; Ramos & Peña, 2014; Rodríguez et al., 2012).
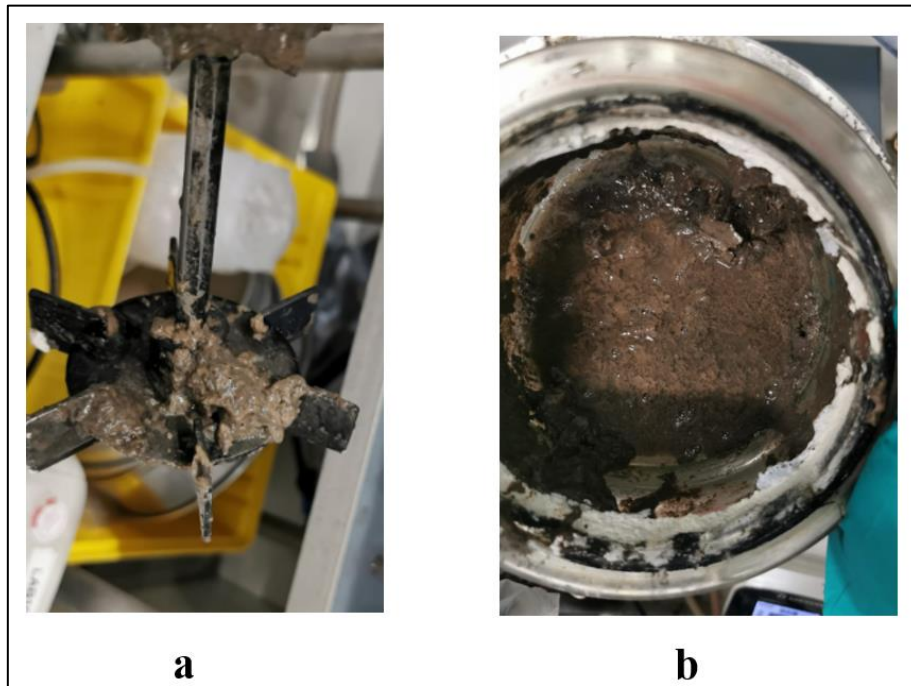
Figure 31. The accumulation of solids in the conditioning tank; (a) solids attached to the mixing rotor, (b) solids in the conditioning tank, and elemental sulfur deposit on the upper part (white color).

5.5.1   Effluent precipitation

Due to the precipitation of calcium carbonate and/or calcium phosphate, hydrogen sulfide precipitation and the formation of elemental sulfur, the effluent discharge tube got clogged (Langerak, 1998). A simulation code developed using the PHREEQC model based on effluent composition using Notepad++ showed the relationship between saturation index with respect to calcite (SI_calcite), equilibrium CO_2 partial pressure, and effluent pH. According to the definition from Langelier (1936), positive saturation index ($SI > 0$), which mean the ability of water to precipitate (supersatured water) which cause scaling, negative value ($SI < 0$), correspond to calcium carbonate ($CaCO_3$) dissolve to form calcium ion (Ca^{2+}) and bicarbonate ions (HCO_3^-), and at value of zero ($SI = 0$), the water and minerals are at chemical equilibrium. Equation (*10*) and (*11*) illustrates the carbon dioxide equilibria.

$$CO_{2\,(g)} \leftrightarrow CO_{2\,(aq)} \tag{10}$$

$$CO_{2\,(aq)} + H_2O_{(l)} \leftrightarrow H_2CO_{3(aq)} \leftrightarrow HCO_{3(aq)}^- + H_{(aq)}^+ \tag{11}$$
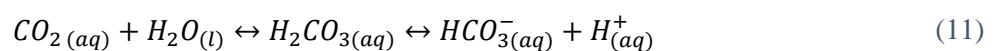
Figure 32 shows that SI has significantly increased above zero and pH also increase as a result of the escape of CO_2 from the solution.
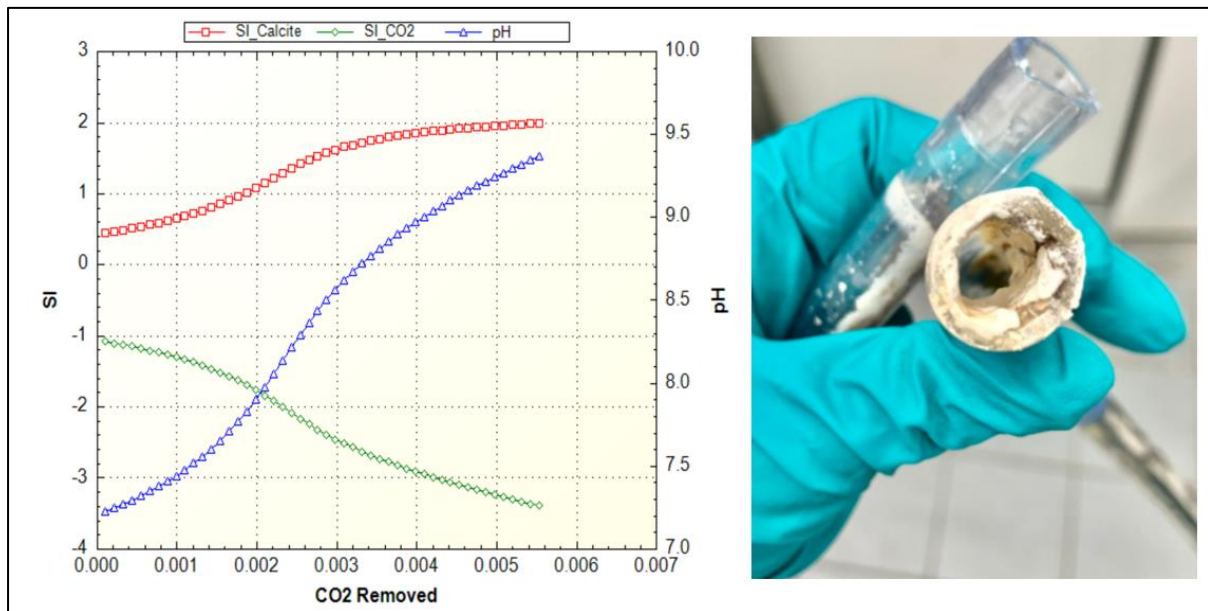
Figure 32. PHREEQC modeling on the relationship between pH and CO_2 level (left), and scaling in the effluent tube (right).

5.5.2   Potential Corrosion in biogas pipeline

Originally, the water seal was filled with demineralized water, and later due to reactor operation the water become acidic due to the dissolution of $CO_{2(g)}$ (Equations 10 and 11) and the oxidation of H_2S (Equation 13) in the biogas pipelines. It is clear from the equation of sulfide biological oxidation carried out by chemolithoautotrophic sulfur oxidizing bacteria (SOB) in which oxygen act as an electron acceptor. This can be confirmed by the air intrusion into the reactor on days 12, 29, 111, and 124.

$$HS^- \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_4^{2-} \tag{12}$$

$$H_2S + 0.5O_2 \rightarrow S^0 + H_2O \qquad \Delta G^0 = -209.4 \, kJ/reaction \tag{13}$$

$$S^0 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \qquad \Delta G^0 = -587.41 \, kJ/reaction \tag{14}$$

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \qquad \Delta G^0 = -798.2 \, kJ/reaction \tag{15}$$

$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+ \qquad \Delta G^0 = -818.3 \, kJ/reaction \tag{16}$$

This is the evidence from the last day of operation (Day 182), the pH in the water seal along the biogas line was checked using the digital pH meter (WTW pH 3110, Germany) with a result of 3.31.

In this research, the biogas line was made with rubber plastic material (Neoprene) which is corrosion-proof. However, in most full-scale plants, the biogas pipe was made with iron and steel which are susceptible to corrosion. A PHREEQC model was done to understand the corrosivity of the biogas. A set of data was taken from day 181 to run this stimulation (Figure 33). The air intrusion within the reactor indicated that there positive potential in the system i.e.; oxidizing condition. From the Pourbaix diagram, it can be observed that at pH range 4-5, iron (Fe) is stable in its +2 oxidation state between potential 0 and 0.8 V, and +3 oxidation state is stable between potential 0.8 to 2 V, which are corrosive conditions. Moreover, Landrum (1989) described that the maximum allowable corrosion rate for the equipment in the chemical industry is 0.50 mm/year, as shown in Table 16.
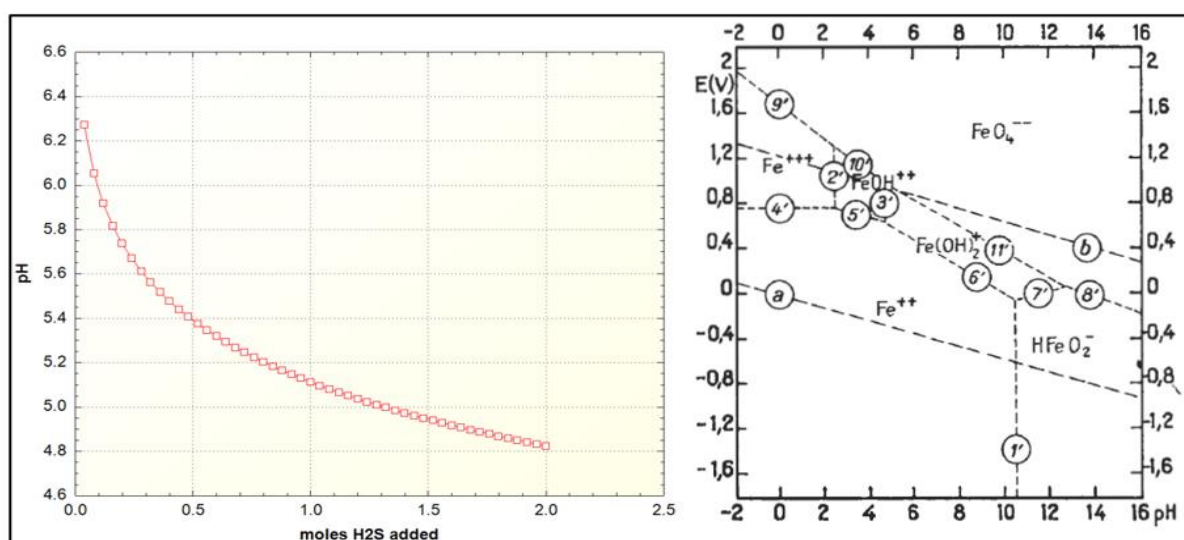
Figure 33. PHREEQC modeling on the relationship between pH and addition of H_2S (left), and Pourbaix diagram of equilibrium potential E(V) of iron as a function of pH (right) adapted from Marcel Pourbaix (1974).

Table 16. Characteristic of corrosion rates for equipment in the chemical industry (Landrum, 1989).

Maximum corrosion rate (mm/year)	Characteristic
0.025	Very low corrosion
0.25	Low corrosion
0.50	Fairly low corrosion and can be considered to be "normal" maximum allowed in chemical equipment
1.25	High corrosion
>1.25	Excessive corrosion

6   Conclusion and Outlook

6.1   Conclusion

1. Overall, the performance of EGSB treating CTMP wastewater showed good stability and robustness while clogging problems caused by the suspended solids represented a major drawback in the system. There was no accumulation of VFA in the effluent. In this research no toxicity was found at 3.4 g COD/L and 75% CH_4 found in biogas. Sierra-Alvarez et al., 1991 found that %IC_{50} happen at concentration of 2.1-5.4 g COD/L of wastewater

2. Anaerobic biodegradability wastewater showed that the maximum COD removal efficiency was 60%.

3. Methanogenic toxicity test indicated that the compounds present in the wastewater did not have any toxic impact on the granule sludge.

4. At the beginning of the start-up after seeding the reactor, the start-up phase took about 30 days and along with the increase of VLR, there was no accumulation of VFAs in the effluent. The methane composition in the biogas was between 60-75% and at the end of the start-up phase, at VLR of 18 gTCOD/L/d, the COD removal efficiency was 50.00 $\pm$ 2.76 %, and 46.81 $\pm$ 1.82 % for total and soluble COD, respectively. The digestion efficiency was 45.61 $\pm$ 2.94 % and daily methane production was 17.02 $\pm$ 2.32 g COD/d.

5. In Phase A, the methane composition in the biogas was between 75-78% and at VLRs of 18 g TCOD/L/d, the COD removal efficiency was 50.00 $\pm$ 2.24% and 49.07 $\pm$ 2.91% for total and soluble COD respectively. The digestion efficiency was 45.65 $\pm$1.80% and daily methane production rate was 23.72 $\pm$ 2.20 g COD/d. The sludge yield at the end of this phase was 0.04 g VS/g COD. The sulfur balance was 89%, in which most of the sulfate has been reduced by SRB and was present in liquid (TLS-S 80%, SO_4-S 1%) and gases (H_2S-S 8%).

    In Phase B, the methane composition in the biogas was between 75-78% and at VLRs of 18 g TCOD/L/d, the COD removal efficiency was 47.07$\pm$3.49% and 52.54 $\pm$ 1.86 % for total and soluble COD respectively. The digestion efficiency was

42.78 ± 3.44 % and daily methane production was 25.69 ± 1.43 g COD/d. The sludge yield at the end of this phase was 0.09 g VS/g COD. The sulfur balance was 98 %, in which most of the sulfate was reduced by SRB and was present in liquid (TLS-S 86%, SO_4-S 1%) and gases (H_2S-S 11%). Acetate (C_2) was found as the major VFA compound in the effluent for the entire reactor operation (Day 1-182).

6.2   Recommendations for Further Study

1. It is recommended to further reduce the organic pollutant in the wastewater, using an aerobic system as a post-treatment after an anaerobic system was found to be efficient in the removal of non-biodegradable compounds. Tertiary treatments such as fungal treatment, coagulation, chemical oxidation, and ozonation can efficiently be used to remove color. Other techniques include adsorption, ozonation, and membrane filtration can be used to remove Chlorinated phenolic compounds and adsorbable organic halides (AOX) (Pokhrel & Viraraghavan, 2004). Jackson-Moss et al.(1992) reported that anaerobic biological granular activated carbon can reduce 50% of COD and color and membrane filtration can reduce more than 90% of color.

2. H_2S can be removed via zero valent iron (Mamun & Torii, 2015), chemical absorption into liquid such as water or alkaline solution (i.e. NaOH), desorption with iron oxide-coated ($Fe(OH)_3$ or Fe_2O_3) material, activated carbon or impregnated activated carbon with catalyzer potassium iodide (KI), potassium carbonate (K_2CO_3) or zinc oxide (ZnO), biological treatments via adding air to oxidize the H_2S into elemental sulfur by sulfur oxidizing bacteria genera such as Thiobacillus and Sulfolobus (Mamun & Torii, 2015; Petersson & Wellinger, 2009).

3. The presence of H_2S in the biogas cause corrosion and the use of corrosion-resistance material is suggested such as an anti-corrosive gas meter.

4. It is recommended to conduct elemental analysis on the cellulose pulps to have a better understanding of the composition of carbon, nitrogen, phosphorus, etc.

5. It is recommended to analyze the biogas composition using gas chromatography (GC), which is more reliable than portable gas analyzer.

6. It is recommended to analyze sulfate using the ion chromatography (IC) because HACH LANGE test tubes were found to be not accurate.

7. It is recommended to use a scanning electron microscope (SEM) to look into the internal structure of the anaerobic granule.

8. It is recommended to have 24/7 automated system control to maintain the stability of the reactor.

9. It is recommended to further reduce the amount of suspended solids present in the wastewater before adding it to the reactor.

References

Abhishek, A., Dwivedi, A., Tandan, N., & Kumar, U. (2017). Comparative bacterial degradation and detoxification of model and kraft lignin from pulp paper wastewater and its metabolites. *Applied Water Science*, *7*(2), 757–767. https://doi.org/10.1007/s13201-015-0288-9

Aguiñaga-Morales, F., Padrón-Páez, J. I., & Román-Martínez, A. (2017). Model identification, calibration and validation of the aerobic stage in water remediation of a paper mill effluent. *Computer Aided Chemical Engineering*, *40*, 547–552. https://doi.org/10.1016/B978-0-444-63965-3.50093-3

Alén, R. (2019). Pulp and paper. In *Encyclopedia of Analytical Science* (3rd ed.). Elsevier Inc. https://doi.org/10.1016/B978-0-12-409547-2.14014-4

Ali, M., & Sreekrishnan, T. R. (2001). Aquatic toxicity from pulp and paper mill effluents: A review. *Advances in Environmental Research*, *5*(2), 175–196. https://doi.org/10.1016/S1093-0191(00)00055-1

Alphenaar, A. (1995). *Anaerobic granular sludge, Ph.D. Thesis*.

Astals, S., Batstone, D. J., Tait, S., & Jensen, P. D. (2015). Development and validation of a rapid test for anaerobic inhibition and toxicity. *Water Research*, *81*, 208–215. https://doi.org/10.1016/j.watres.2015.05.063

Azman, S. (2016). *Anaerobic digestion of cellulose and hemicellulose in the presence of humic acids*.

Bajpai, P. (2017). *Anaerobic Technology in Pulp and Paper Industry*. https://doi.org/10.1007/978-981-10-4130-3

Batstone, D. J., & Jensen, P. D. (2011). Anaerobic Processes. *Treatise on Water Science*, *4*, 615–639. https://doi.org/10.1016/B978-0-444-53199-5.00097-X

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T., Siegrist, H., & Vavilin, V. A. (2002). The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science and Technology : A Journal of the International Association on Water Pollution Research*, *45*(10), 65–73.

https://doi.org/10.2166/wst.2002.0292

Billings RM, & DeHaas GG. (1971). Pollution control in the pulp and paper industry. *Oecd Observer*, *no. 66*, 13–15.

Briggs, D. G. (1994). Chapter 8: Pulp and Paper. *Forest Product Measurements and Conversion Factors: With Special Emphasis on the U.S. Pacific Northwest*, 96–100.

Chambers, A., & Potter, I. (2002). Gas utilization from sewage waste. *Carbon and Energy Management, Alberta Research Council.*, *1*, 1–9. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.476.516&rep=rep1&type=pdf

Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, *99*(10), 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

Cirik, K., Kitis, M., & Cinar, O. (2013). The effect of biological sulfate reduction on anaerobic color removal in anaerobic-aerobic sequencing batch reactors. *Bioprocess and Biosystems Engineering*, *36*(5), 579–589. https://doi.org/10.1007/s00449-012-0813-2

Cruz-Salomón, A., Ríos-Valdovinos, E., Pola-Albores, F., Lagunas-Rivera, S., Meza-Gordillo, R., Ruíz-Valdiviezo, V. M., & Cruz-Salomón, K. C. (2019). Expanded granular sludge bed bioreactor in wastewater treatment. *Global Journal of Environmental Science and Management*, *5*(1), 119–138. https://doi.org/10.22034/gjesm.2019.01.10

Deublein, D., & Steinhauser, A. (2008). Biogas from Waste and Renewable Resources: An Introduction, Second Edition. *Biogas from Waste and Renewable Resources: An Introduction, Second Edition*. https://doi.org/10.1002/9783527632794

Di Costanzo, N., Cesaro, A., Di Capua, F., & Esposito, G. (2021). Exploiting the nutrient potential of anaerobically digested sewage sludge: A review. *Energies*, *14*(23), 1–25. https://doi.org/10.3390/en14238149

Eddy, M. (2014). Wastewater Engineering: Treatment and Reuse (Book). In *Chemical engineering* (Issue 7, p. 421).

Eis, B. J., Ferguson, J. F., & Benjamin, M. M. (1983). The fate and effect of bisulfate in anaerobic treatment. *Journal of the Water Pollution Control Federation*, *55*(11), 1355–1365.

Ekstrand, E.-M. (2019). *Anaerobic digestion in the kraft pulp and paper industry – benefits and strategies for implementation* (Issue 769). https://doi.org/10.3384/diss.diva-156667

Federation, W. E. (1999). Standard Methods for the Examination of Water and Wastewater Standard Methods for the Examination of Water and Wastewater. *Public Health*, *51*(1), 940–940. https://doi.org/10.2105/AJPH.51.6.940-a

Ferguson, J. F., Eis, B. J., & Benjamin, M. M. (1984). Neutralization in anaerobic treatment of an acidic waste. *Water Research*, *18*(5), 573–580. https://doi.org/10.1016/0043-1354(84)90206-9

Filer, J., Ding, H. H., & Chang, S. (2019). Biochemical methane potential (BMP) assay method for anaerobic digestion research. *Water (Switzerland)*, *11*(5). https://doi.org/10.3390/w11050921

Gallert, C., & Winter, J. (2005). Bacterial Metabolism in Wastewater Treatment Systems. In *Environmental Biotechnology Concepts and Applications* (pp. 1–41). https://doi.org/10.1002/9783527620999.ch2l

Ghangrekar, M. M., Asolekar, S. R., & Joshi, S. G. (2005). Characteristics of sludge developed under different loading conditions during UASB reactor start-up and granulation. *Water Research*, *39*(6), 1123–1133. https://doi.org/10.1016/j.watres.2004.12.018

Goto, T., Zaccaron, S., Bacher, M., Hettegger, H., Potthast, A., & Rosenau, T. (2021). On nitrogen fixation and "residual nitrogen content" in cellulosic pulps. *Carbohydrate Polymers*, *253*. https://doi.org/10.1016/j.carbpol.2020.117235

Gou, C., Yang, Z., Huang, J., Wang, H., Xu, H., & Wang, L. (2014). Effects of temperature and organic loading rate on the performance and microbial community of anaerobic co-digestion of waste activated sludge and food waste. *Chemosphere*, *105*, 146–151. https://doi.org/10.1016/j.chemosphere.2014.01.018

Greer, D. (2010). Biogas conditioning and upgrading in action. *BioCycle*, *51*(3), 53–56.

Haandel, A. van, & Lubbe, J. van der. (2007). *Handbook Biological Wastewater Treatment*. http://www.wastewaterhandbook.com/

Hamm, U. (2006). Environmental aspects. In *Handbook of Paper and Board* (pp. 208--218).

Henze, M., & Harremoes, P. (1983). Anaerobic treatment of wastewater in fixed film reactors - A literature review. *Water Science and Technology*, *15*(8–9), 1–101. https://doi.org/10.2166/wst.1983.0161

Hernández, M. A., Rodríguez Susa, M., & Andres, Y. (2014). Use of coffee mucilage as a new substrate for hydrogen production in anaerobic co-digestion with swine manure. *Bioresource Technology*, *168*, 112–118. https://doi.org/10.1016/j.biortech.2014.02.101

Ho, C. C., Tan, Y. K., & Wang, C. W. (1984). The distribution of chemical constituents between the soluble and the particulate fractions of palm oil mill effluent and its significance on its utilisation/treatment. *Agricultural Wastes*, *11*(1), 61–71. https://doi.org/10.1016/0141-4607(84)90055-6

Hulshoff Pol, L. W. (1989). *The phenomenon of anaerobic sludge*. University Wageningen.

Jackson-Moss, C. A., Maree, J. P., & Wotton, S. C. (1992). Treatment of bleach plant effluent with the biological granular activated carbon process. *Water Science and Technology*, *26*(1–2), 427–434. https://doi.org/10.2166/wst.1992.0422

Jaiswal, S., Kumar Gupta, G., Panchal, K., Mandeep, & Shukla, P. (2021). Synthetic Organic Compounds From Paper Industry Wastes: Integrated Biotechnological Interventions. *Frontiers in Bioengineering and Biotechnology*, *8*(January), 1–13. https://doi.org/10.3389/fbioe.2020.592939

Johansson, T. (2012). *Application of membrane bioreactors in the pulp and paper industry*. Uppsala University.

Jung, H., & Pauly, D. (2011). Water in the Pulp and Paper Industry. *Treatise on Water Science*, *4*, 667–683. https://doi.org/10.1016/B978-0-444-53199-5.00100-7

Jurgensen, S. L., Benjamin, M. M., & Ferguson, J. F. (1985). Treatability of Thermomechanical Pulping Process Effluents With Anaerobic Biological Reactors. *Environmental Conference, Proceedings of the Technical Association of the Pulp and Paper Industry*, 83–92.

Kato, M. T., Florencio, L., & Arantes, R. F. M. (2003). Post-treatment of UASB effluent in an expanded granular sludge bed reactor type using flocculent sludge. *Water Science and Technology*, *48*(6), 279–284. https://doi.org/10.2166/wst.2003.0413

Kaviyarasan, K. (2014). Application of UASB Reactor in Industrial Wastewater Treatment–

A Review. *International Journal of Scientific & Engineering Research*, 5(1), 584–589. http://www.ijser.org/researchpaper/Application-of-UASB-Reactor-in-Industrial-Wastewater-Treatment.pdf

Kent, J. A. (2007). Kent and Riegel's Handbook of Industrial Chemistry and Biotechnology. In J. A. Kent (Ed.), *Kent and Riegel's Handbook of Industrial Chemistry and Biotechnology* (Vol. 1). Springer US. https://doi.org/10.1007/978-0-387-27843-8

Khan, A. W., & Trottier, T. M. (1978). Effect of sulfur-containing compounds on anaerobic degradation of cellulose to methane by mixed cultures obtained from sewage sludge. *Applied and Environmental Microbiology*, 35(6), 1027–1034. https://doi.org/10.1128/aem.35.6.1027-1034.1978

Kincaid, J. (1998). *North American Pulp & Paper Fact Book*.

Kobayashi, T., Li, Y. Y., Kubota, K., Harada, H., Maeda, T., & Yu, H. Q. (2012). Characterization of sulfide-oxidizing microbial mats developed inside a full-scale anaerobic digester employing biological desulfurization. *Applied Microbiology and Biotechnology*, 93(2), 847–857. https://doi.org/10.1007/s00253-011-3445-6

Kortekaas, S., Vidal, G., Yan-Ling, H., Lettinga, G., & Field, J. A. (1998). Anaerobic-aerobic treatment of toxic pulping black liquor with upfront effluent recirculation. *Journal of Fermentation and Bioengineering*, 86(1), 97–110. https://doi.org/10.1016/S0922-338X(98)80041-X

Kosaric, N., Blaszczyk, R., Orphan, L., & Valladarfs, J. (1990). The characteristics of granules from upflow anaerobic sludge blanket reactors. *Water Research*, 24(12), 1473–1477. https://doi.org/10.1016/0043-1354(90)90081-G

Kougias, P. G., Boe, K., & Angelidaki, I. (2013). Effect of organic loading rate and feedstock composition on foaming in manure-based biogas reactors. *Bioresource Technology*, 144, 1–7. https://doi.org/10.1016/j.biortech.2013.06.028

Kumar Jha, A., Li, J., Nies, L., & Zhang, L. (2011). Research advances in dry anaerobic digestion process of solid organic wastes. *African Journal of Biotechnology*, 10(65), 14242–14253. https://doi.org/10.5897/AJB11.1277

Landrum, R. J. (1989). Fundamentals of Designing for Corrosion Control: A Corrosion Aid for the Designer. In *R. J. Landrum, published 1989 by NACE, 350*.

Langelier, W. F. (1936). The Analytical Control of Anti-Corrosion Water Treatment. *American Water Works Association*, *28*(10), 1500–1521.

Langerak, E. (1998). *Control of calcium carbonate precipitation in anaerobic reactors* (Issue november). http://library.wur.nl/WebQuery/wda/959640

Larsson, M., Truong, X. Bin, Björn, A., Ejlertsson, J., Svensson, B. H., Bastviken, D., & Karlsson, A. (2017). Anaerobic digestion of wastewater from the production of bleached chemical thermo-mechanical pulp – higher methane production for hardwood than softwood. *Journal of Chemical Technology and Biotechnology*, *92*(1), 140–151. https://doi.org/10.1002/jctb.4980

Lay, J.-J., Li, Y.-Y., & Noike, T. (1998). The influence of pH and ammonia concentration on the methane production in high-solids digestion processes. *Water Environment Research*, *70*(5), 1075–1082. https://doi.org/10.2175/106143098x123426

Leach, J. M., & Thakore, A. N. (1976). Toxic constituents in mechanical pulping effluents. *Tappi*, *59*(2), 129–132.

Leitão, R. C., Van Haandel, A. C., Zeeman, G., & Lettinga, G. (2006). The effects of operational and environmental variations on anaerobic wastewater treatment systems: A review. *Bioresource Technology*, *97*(9), 1105–1118. https://doi.org/10.1016/j.biortech.2004.12.007

Lens, P. N. L., Visser, A., Janssen, A. J. H., Hulshoff Pol, L. W., & Lettinga, G. (1998). Biotechnological treatment of sulfate-rich wastewaters. *Critical Reviews in Environmental Science and Technology*, *28*(1), 41–88. https://doi.org/10.1080/10643389891254160

Lens, P. N. L., Visser, A., Janssen, A. J. H., Pol, L. W. H., & Lettinga, G. (1998). Biotechnological Treatment of Sulfate-Rich Wastewaters. *Critical Reviews in Environmental Science and Technology*, *28*(1), 41–88. http://www.tandfonline.com/doi/abs/10.1080/10643389891254160

Lettinga, G., Field, J., Van Lier, J., Zeeman, G., & Hulshoff Pol, L. W. (1997). Advanced anaerobic wastewater treatment in the near future. *Water Science and Technology*, *35*(10), 5–12. https://doi.org/10.1016/S0273-1223(97)00222-9

Li, Q., & Lancaster, J. R. (2013). Chemical foundations of hydrogen sulfide biology. *Nitric*

Oxide - Biology and Chemistry, 35, 21–34. https://doi.org/10.1016/j.niox.2013.07.001

Lim, S. J. (2011). Comparisons between the UASB and the EGSB reactor. *Iowa State University Publication*, *17*(1969), 1–17.

Lin, C., & Hsiu, M. (1997). Effects of sulfide, sulfite and sulfate on acidogenesis in upflow anaerobic sludge blanket process. *Journal of Environmental Science and Health . Part A: Environmental Science and Engineering and Toxicology*, *32*(4), 1171–1184. https://doi.org/10.1080/10934529709376603

Lin, H., Gao, W., Meng, F., Liao, B. Q., Leung, K. T., Zhao, L., Chen, J., & Hong, H. (2012). Membrane bioreactors for industrial wastewater treatment: A critical review. *Critical Reviews in Environmental Science and Technology*, *42*(7), 677–740. https://doi.org/10.1080/10643389.2010.526494

López, I., & Borzacconi, L. (2011). Modelling of an EGSB treating sugarcane vinasse using first-order variable kinetics. *Water Science and Technology*, *64*(10), 2080–2088. https://doi.org/10.2166/wst.2011.697

Malik, R. A., Vistanty, H., Mukimin, A., & Zen, N. (2020). Rapid start-up of expanded granular sludge bed (EGSB) reactor using granulated anaerobic bacteria in pharmaceutical wastewater treatment: pilot scale. *IOP Conference Series: Materials Science and Engineering*, *980*(1). https://doi.org/10.1088/1757-899X/980/1/012078

Mamun, M. R. Al, & Torii, S. (2015). Removal of Hydrogen Sulfide (H2S) from Biogas Using Zero-Valent Iron. *Journal of Clean Energy Technologies*, *3*(6), 428–432. https://doi.org/10.7763/jocet.2015.v3.236

Mao, C., Feng, Y., Wang, X., & Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *45*, 540–555. https://doi.org/10.1016/j.rser.2015.02.032

Marcel Pourbaix. (1974). *Atlas of electrochemical equilibria in aqueous solutions - Marcel Pourbaix - Google Books* (p. 644).

Mata-Alvarez, J. (2003). Fundamentals of the Anaerobic Digestion Process. *IWA Publishing Company, Biomethani*(Biomethanization of the Organic Fraction of Municipal Solid Wastes), 202–209.

Mathys, R. G. (1991). *Aerobic Treatment of a CTMP Wastewater Using a Rotating*

Biological Contactor.

Mc Naught, a. D., & Wilkinson, a. (2019). The IUPAC Compendium of Chemical Terminology. *The IUPAC Compendium of Chemical Terminology*, 1670. https://doi.org/10.1351/goldbook

McCarty, P. L. (1964). Anaerobic Waste Treatment Fundamentals. *Public Works*, *95*, 107–112.

Megonigal, J. P., Hines, M. E., & Visscher, P. T. (2003). Anaerobic Metabolism: Linkages to Trace Gases and Aerobic Processes. *Treatise on Geochemistry*, *8–9*, 317–424. https://doi.org/10.1016/B0-08-043751-6/08132-9

Möbius CH. (2002). *Waste Water of the Pulp and Paper Industry*.

Nagao, N., Tajima, N., Kawai, M., Niwa, C., Kurosawa, N., Matsuyama, T., Yusoff, F. M., & Toda, T. (2012). Maximum organic loading rate for the single-stage wet anaerobic digestion of food waste. *Bioresource Technology*, *118*, 210–218. https://doi.org/10.1016/j.biortech.2012.05.045

Oliveira, D. V. M. de. (2014). Evaluation of a MBBR (Moving Bed Biofilm Reactor) Pilot Plant for Treatment of Pulp and Paper Mill Wastewater. *International Journal of Environmental Monitoring and Analysis*, *2*(4), 220. https://doi.org/10.11648/j.ijema.20140204.15

Petersson, A., & Wellinger, A. (2009). Biogas upgrading technologies–developments and innovations. *IEA Bioenergy*, 20. http://typo3.dena.de/fileadmin/biogas/Downloads/Studien/IEA-BiogasUpgradingTechnologies2009.pdf

Pichon, M., Rouger, J., & Junet, E. (1986). Anaerobic Treatment of CTMP wastewater. *EUCEPA Symposium*.

Pichon, M., Rouger, J., & Junet, E. (1987). Anaerobic treatment of sulfur containing effluents. *Second IAWPRC Symposium on Forest Industry Wast*.

Pokhrel, D., & Viraraghavan, T. (2004). Treatment of pulp and paper mill wastewater - A review. *Science of the Total Environment*, *333*(1–3), 37–58. https://doi.org/10.1016/j.scitotenv.2004.05.017

Pol, L. H., & Lettinga, G. (1986). New Technologies for Anaerobic Wastewater Treatment. *Water Science and Technology*, *18*(12), 41–53. https://doi.org/10.2166/wst.1986.0162

Radian Beluc. (2019). Biogas Production. In *Energy Storage, Grid Integration, Energy Economics, and the Environment* (p. 131). CRC Press.

Rajagopal, R., Choudhury, M. R., Anwar, N., Goyette, B., & Rahaman, M. S. (2019). Influence of pre-hydrolysis on sewage treatment in an Up-Flow Anaerobic Sludge BLANKET (UASB) reactor: A review. *Water (Switzerland)*, *11*(2), 3–7. https://doi.org/10.3390/w11020372

Ramos, I., & Peña, M. (2014). Bioresource Technology Where does the removal of H 2 S from biogas occur in microaerobic reactors ? *BIORESOURCE TECHNOLOGY*, *166*, 151–157. https://doi.org/10.1016/j.biortech.2014.05.058

Rantanen, S., Sc Mika Mänttäri Sc Oskari Frösén Advisor, D. M., & Sc Oskari Frösén, M. (2018). *Evaluation of CTMP mill wastewaters and handling methods*. 95.

Rintala, J. A., & Puhakka, J. A. (1994a). Anaerobic treatment in pulp- and paper-mill waste management: A review. *Bioresource Technology*, *47*(1), 1–18. https://doi.org/10.1016/0960-8524(94)90022-1

Rintala, J. A., & Puhakka, J. A. (1994b). Anaerobic treatment in pulp- and paper-mill waste management: A review. *Bioresource Technology*, *47*(1), 1–18. https://doi.org/10.1016/0960-8524(94)90022-1

Rinzima A, & Lettinga G. (1988). Anaerobic treatment of sulfate containng wastewater. *Biotreatment Systems*, *3*, 65–109.

Rittmann, B. E., & McCarty, P. L. (2001). *Environmental Biotechnology: Principles and Applications*.

Rodríguez, E., Lopes, A., Fdz.-Polanco, M., Stams, A. J. M., & García-Encina, P. A. (2012). Molecular analysis of the biomass of a fluidized bed reactor treating synthetic vinasse at anaerobic and micro-aerobic conditions. *Applied Microbiology and Biotechnology*, *93*(5), 2181–2191. https://doi.org/10.1007/s00253-011-3529-3

Rogers, I. H. (1973). Isolation and Chemical Identification of Toxic Components of Kraft Mill Wastes. *Can Pulp and Pap Assoc, Tech Sect, Proc*, 111–116.

Ruan, R., Cao, J., Li, C., Zheng, D., & Luo, J. (2017). The influence of micro-oxygen addition on desulfurization performance and microbial communities during waste-activated sludge digestion in a rusty scrap iron-loaded anaerobic digester. *Energies*, *10*(2). https://doi.org/10.3390/en10020258

Ruutiainen, R. (1987). *CTMP-jäteveden anarobipuhdistuksen edellytykset*. Lappeenrannan teknillinen korkeakoulu.

Salehi, R. (2016). *COD "loss" in enhanced biological phosphorus removal systems*.

Särner E. (1986). Influence and control of H2S on full Scale plants and piloplant experiments. *Anaerobic Wastewater Treatment, a Grown-up Technology*, 189–204.

Särner E. (1989). Removal of sulphate and sulphite in an anaerobic trickling (antric) filter. *Recent Developments : Technological Advances in Biofilm Reactors*, 405–414.

Schultz, T. E. (2005). Biological wastewater treatment. In *Chemical Engineering* (Vol. 112, Issue 10).

Seadi, T. Al, Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S., & Janssen, R. (2008). *Biogas handbook*. http://www.lemvigbiogas.com/BiogasHandbook.pdf

Shan, L., Liu, J., Yu, Y., Ambuchi, J. J., & Feng, Y. (2016). Characterization of persistent colors and decolorization of effluent from biologically treated cellulosic ethanol production wastewater. *Environmental Science and Pollution Research*, *23*(10), 10215–10222. https://doi.org/10.1007/s11356-016-6220-5

Sìerra-Alvarez, R. (1990). *The Role of Natural Wood Constituents on the Anaerobic Treatability of Forest Industry Wastewaters*.

Sierra-Alvarez, R., Kortekaas, S., Van Eekert, M., & Lettinga, G. (1991). The anaerobic biodegradability and methanogenic toxicity of pulping wastewaters. *Water Science and Technology*, *24*(3–4), 113–125. https://doi.org/10.2166/wst.1991.0468

Sierra-Alvarez, R., Kato, M., & Lettinga, G. (1990). The anaerobic biodegradability of paper mill wastewater constituents. *Environmental Technology (United Kingdom)*, *11*(10), 891–898. https://doi.org/10.1080/09593339009384941

Slade, A. H., Thorn, G. J. S., & Dennis, M. A. (2007). *Colour Formation in Pulp and Paper Wastewater Treatment Systems*. *3046*.

https://www.waternz.org.nz/Attachment?Action=Download&Attachment_id=1124#:~:te
xt=Pulping wastewaters contain lignin derivatives,its characteristic yellow-brown
colour.&text=The most highly coloured waste,are from the bleach plant

Stephenson, R. J., Branion, R. M. R., & Pinder, K. L. (1994). Anaerobic 35°C and 55°C
Treatment of a BCTMP/TMP Effluent: Sulphur Management Strategies. *Water Science
and Technology*, *29*(5–6), 433–445. https://doi.org/10.2166/wst.1994.0736

Stephenson, Robert J., & Duff, S. J. B. (1996). Coagulation and precipitation of a mechanical
pulping effluent - I. Removal of carbon, colour and turbidity. *Water Research*, *30*(4),
781–792. https://doi.org/10.1016/0043-1354(95)00213-8

Suhr, M., Klein, G., Kourti, I., Rodrigo Gonzalo, M., Giner Santonja, G., Roudier, S., &
Delgado Sancho, L. (2015). *Best Available Techniques (BAT) Reference Document for
the Production of Pulp, Paper and Board*. https://doi.org/10.2791/370629

Tawfik, A., El-Gohary, F., Ohashi, A., & Harada, H. (2008). Optimization of the
performance of an integrated anaerobic-aerobic system for domestic wastewater
treatment. *Water Science and Technology*, *58*(1), 185–194.
https://doi.org/10.2166/wst.2008.320

Teixeira-Correia, G.; Pérez-Pérez, T. ., Pereda-Reyes, I. ., Oliva-, Merencio, D. ., Zaiat, M. .,
& Hong-Kwong, W. (2014). Mathematical Modeling of the Hydrodynamics of an EGSB
Reactor. *Journal of Chemistry and Chemical Engineering*.

The Wales Centre of Excellence for Anaerobic Digestion. (2008). *Mesophilic and
Thermophilic Systems*. Http://Www.Walesadcentre.Org.Uk/.
http://www.walesadcentre.org.uk/technologies/mesophilicandthermophilicsystems.aspx

Tian, Z., Zhang, Y., Li, Y., Chi, Y., & Yang, M. (2015). Rapid establishment of thermophilic
anaerobic microbial community during the one-step startup of thermophilic anaerobic
digestion from a mesophilic digester. *Water Research*, *69*, 9–19.
https://doi.org/10.1016/j.watres.2014.11.001

Trego, A. C., Galvin, E., Sweeney, C., Dunning, S., Murphy, C., Mills, S., Nzeteu, C.,
Quince, C., Connelly, S., Ijaz, U. Z., & Collins, G. (2020). Growth and Break-Up of
Methanogenic Granules Suggests Mechanisms for Biofilm and Community
Development. *Frontiers in Microbiology*, *11*(June), 1–12.

https://doi.org/10.3389/fmicb.2020.01126

Truong, L. V. A., & Abatzoglou, N. (2005). An adsorption/reaction process for the purification of biogas prior to its use as energy vector. *Biomass Bioenergy*, *29*(2), 142–51.

van Loosdrecht, M. C. M., Halkjær Nielsen, P., Lopez-Vazquez, C. M., & Brdjanovic, D. (2016). *Experimental Methods in Wastewater Treatment*. IWA Publishing.

Verma, S. (2002). *Anaerobic Digestion of Biodegradable Organics in Municipal Solid Wastes*. Columbia University.

Visser, A. (1995). *The anaerobic treatmento of sulfate containing wastewater*.

Walden, C. C., Howard, T. E., & Sheriff, W. J. (1971). The relation of kraft pulp mill operating and process parameters to pollution characteristics of the mill effluents. *Pulp Paper Mag. Can.*, *72*(2), T81–T87.

Wang, J., Lu, H., Chen, G.-H., Lau, G. N., Tsang, W. L., & van Loosdrecht, M. C. M. (2009). A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. *Water Research*, *43*(9), 2363–2372. https://doi.org/10.1016/j.watres.2009.02.037

Wang, L. K., Hung, Y.-T., Lo, H. H., & Yapijakis, C. (2006). *Waste Treatement in the process industries* (illustrate). CRC Press, 2005.

Welander, T. (1988). Anaerobic process for treatment of CTMP effluent. *Water Science and Technology*, *20*(1), 143–147. https://doi.org/10.2166/wst.1988.0017

Wellinger, A., & Lindeberg, A. (1999). Biogas upgrading and utilization. *Task 24: Energy from Biological Conversion of Organic Wastes*, 1–19.

Windeisen, E., & Wegener, G. (2012). Lignin as Building Unit for Polymers. *Polymer Science: A Comprehensive Reference, 10 Volume Set*, *10*, 255–265. https://doi.org/10.1016/B978-0-444-53349-4.00263-6

Wingate, K. G. (2002). *Novel Methods for the Removal of Colour from Pulp and Paper Mill \ Mastewater* (Vol. 1994). The University of Auckland.

World Bank. (2007). *Environmental, Health, and Safety Guidelines Pulp and Paper Mills*.

Wu Wei-min, Hu Ji-cui an, & Xia-sheng, G. (1985). Properties of granular sludge in upflow

anaerobic sludge blanket (UASB) reactor and its formation. *Proc. 4th Int. Symp. on Anaerobic Digestion.*

Yuan, Q., Sparling, R., & Oleszkiewicz, J. A. (2011). VFA generation from waste activated sludge: Effect of temperature and mixing. *Chemosphere*, *82*(4), 603–607. https://doi.org/10.1016/j.chemosphere.2010.10.084

Zhang, Y., Yan, L., Chi, L., Long, X., Mei, Z., & Zhang, Z. (2008). Startup and operation of anaerobic EGSB reactor treating palm oil mill effluent. *Journal of Environmental Sciences*, *20*(6), 658–663. https://doi.org/10.1016/S1001-0742(08)62109-9

Zhou, X., Fernández-Palacios, E., Dorado, A. D., Gamisans, X., & Gabriel, D. (2022). Assessing main process mechanism and rates of sulfate reduction by granular biomass fed with glycerol under sulfidogenic conditions. *Chemosphere*, *286*, 131649. https://doi.org/10.1016/J.CHEMOSPHERE.2021.131649

Zinatizadeh, A. A. L., Mohamed, A. R., Mashitah, M. D., Abdullah, A. Z., & Isa, M. H. (2007). Characteristics of Granular Sludge Developed in an Upflow Anaerobic Sludge Fixed-Film Bioreactor Treating Palm Oil Mill Effluent. *Water Environment Research*, *79*(8), 833–844. https://doi.org/10.2175/106143007x156646

Appendix

A   Sludge elemental analysis

The elemental composition of the granule sludge taken from the EGSB reactor on day 182 was sent for analysis at an external lab. The sludge sample was first dried at $105 \pm 3°C$ in the stove with a porcelain cup overnight to get dry weight. The result of the composition of the metal within the sludge is presented in Table A.

Table A. Sludge elemental analysis expressed in mg/kg of dry matter.

Metals	Symbol	Unit	Value
Dry matter	(DM)	%	100
Aluminium	(Al)	mg/kg DM	1200
Antimony	(Sb)	mg/kg DM	1
Arsenic	(As)	mg/kg DM	1.2
Barium	(Ba)	mg/kg DM	61
Beryllium	(Be)	mg/kg DM	< 0.2
Boron	(B)	mg/kg DM	<10
Cadmium	(Cd)	mg/kg DM	1.5
Calcium	(Ca)	mg/kg DM	74000
Chromium	(Cr)	mg/kg DM	17
Cobalt	(Co)	mg/kg DM	3.5
Copper	(Cu)	mg/kg DM	130
Gold	(Au)	mg/kg DM	< 5
Iron	(Fe)	mg/kg DM	7200
Lanthanium	(La)	mg/kg DM	3.2
Lead	(Pb)	mg/kg DM	26
Lithium	(Li)	mg/kg DM	0.6
Magnesium	(Mg)	mg/kg DM	1700
Manganese	(Mn)	mg/kg DM	390
Molybdenum	(Mo)	mg/kg DM	56
Nickel	(Ni)	mg/kg DM	23
Palladium	(Pd)	mg/kg DM	<5
Phosphorus	(P)	mg/kg DM	3600
Platinum	(Pt)	mg/kg DM	< 5
Potassium	(K)	mg/kg DM	1300

Scandium	(Sc)	mg/kg DM	< 0.2
Selenium	(Se)	mg/kg DM	< 1
Silicium	(Si)	mg/kg DM	< 20
Silver	(Ag)	mg/kg DM	1.3
Sodium	(Na)	mg/kg DM	7300
Strontium	(Sr)	mg/kg DM	120
Sulfur	(S)	mg/kg DM	10000
Tellurium	(Te)	mg/kg DM	< 0.1
Tin	(Sn)	mg/kg DM	0.5
Titanium	(Ti)	mg/kg DM	13
Tungsten	(W)	mg/kg DM	< 50
Vanadium	(V)	mg/kg DM	3.9
Zinc	(Zn)	mg/kg DM	1300
Zirconium	(Zr)	mg/kg DM	< 2

B   Discharge Limit

Table B. Guidelines for CTMP effluent (World Bank, 2007)

Parameter	Units	Guideline
Flow[a]	m^3/ADt	20
pH	-	6-9
TSS	kg/ADt	1
COD	kg/ADt	5
BOD$_5$	kg/ADt	1
Total N	kg/ADt	0.2
Total P	kg/ADt	0.01

[a] Cooling water and other clean water are discharged separately and are not included.
kg/ADt : kilograms of pollutant per 1,000 kg of air dry pulp

C   Comparison of the sulfate analysis

Table C. The analytical comparison between Ion Chromatography and Hach Lang Test kit (LCK153) of the feed wastewater and EGSB effluent.

Analytical Method	Ion Chromatography		Hach Lang Test kit	
Filter size	0.45 µm	0.2 µm	0.2 µm	0.45 µm
Feed	433.07	438.88	324.5	414
	433.15	439.35	322.5	414
Effluent	5.16	5.292	62.3	112
	5.188	5.209	62	109
Clean sample (without wastewater)	98.595		99.5	
			100	

D   The effect of Ammonium-nitrogen in Anaerobic Digestion

Table D. The effect of ammonium-nitrogen in anaerobic digestion (McCarty, 1964).

Ammonia N concentration	Units	Effect on anaerobic Process
50-200	mg/L	Beneficial
200-1000	mg/L	No adverse effect
1500-3000	mg/L	Inhibitory at higher pH value (7.4-7.6)
Above 3000	mg/L	Toxic

E   PHREEQC code for scaling

```
1   Database c:\phreeqc\database\phreeqc.dat
2
3   Solution 1                              # effluentComposition #data date: Day 181
4   -units mg/l
5   -temp 35
6   pH 7.2
7   Alkalinity 1220 as HCO3                 #999.5 mgCaCO3/l
8   Ca 34
9   Mg 14
10  S(6)  3
11  END
12
13  use solution 1
14  Equilibrium_Phases 1
15  CO2(g) -3.4                             #400ppm#log((400/1000000)*1atm)
16  end
17
18  use solution 1
19  reaction 1
20  CO2(g) 1
21  -0.005534 moles in 50 steps
22
23  User_graph 1                            #Plot Graph
24  -headings  CO2_RE SI_Calcite SI_CO2 pH
25  -axis_titles "CO2 Removed" "SI" "pH"
26  -start
27  10 graph_x -RXN
28  20 graph_y SI("Calcite") SI("CO2(g)")
29  30 graph_sy -la("H+")
30  -end
31  end
32
33  user_graph 1
34  -detach
35  end
```

F   PHREEQC code for corrosion

```
Database c:\phreeqc\database\minteq.v4.dat

Solution 1                                # Effluent Composition #data date: 17/5/22
-units mg/l
-temp 35
pH 7.2
Alkalinity 1220 as HCO3                   #261mgCaCO3/l   #261*1.22=318.42
Ca 34
Mg 14
S(6) 3
END

Use solution 1
Gas_Phase 1
-fixed pressure
-pressure 1.0
H2S(g) 0.014 #partial pressure#14000ppm
end

use solution 1
Equilibrium_Phases 1
CO2(g) -3.4   #400ppm#log((400/1000000)*1atm)
end

Use solution 1
reaction 1
H2S(g) 1
2 mol in 50 steps #2/50
User_Graph 1
-headings pH
-axis_titles "moles H2S added" "pH"
-start
10 graph_x RXN
20 graph_y -la("H+")
-end
```