Anaerobic selective fermentation of swine lagoon wastewater to valorize carbon into organic acids

by

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Abstract

Volatile fatty acids (VFAs) are short-chain organic acids naturally generated from complex organic compounds through intermediate fermentation reactions during anaerobic digestion. VFAs serve as building blocks for many valuable commercial chemical products. This study aims to develop a modified fermentation biotechnology platform from swine wastewater coupled with membrane filtration aided by microbial electrochemistry. Controlled swine wastewater fermentation experiments were conducted with two different inocula (wastewater sludge and cattle rumen fluid), with or without a microbial bioanode. The key variables were temperature, pH, solid retention time (SRT)/hydraulic retention time (HRT), and the anode potential. A series of experiments were performed at different SRT conditions. Performance was characterized by electric current production, VFA quantification through high-performance liquid chromatography (HPLC), and gas chromatography (GC). At the 20-day SRT operation, sludge and rumen fermenters showed different organic acid profiles predominated by propionate/acetate and butyrate/acetate, respectively. However, acetate was the primary organic acid at 15-day SRT operation, with washout occurring at 10-day SRT for swine wastewater fermentation with sludge inoculum. The rumen inoculum suffered a faster washout of the fermentative microbial communities starting at 15-day SRT due to either lower active biomass concentration or the sensitivity of fermentative rumen microbial communities to swine wastewater. During Trial 2, caproate was prevalent during all phases of sludge fermenter operation (20, 15 and 10-day SRT). The rumen inoculum showed lactate and caproate as the dominant VFAs in the 20-day and 15-day SRT, respectively, during Trial 2. The microbial biofilm anode with wastewater sludge and swine wastewater exhibited a different fermentation product profile than non-electrode fermenters, with

caproate predominance at 20-day SRT in both Trial 1 and 2. The anode area was observed as a rate-limiting step for kinetics and extent of swine wastewater fermentation. The recovery of VFAs from swine wastewater based on microbial reactions is becoming a promising research and development portfolio in the future due to its likely favorable life cycle and techno-economic footprints.

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Abbreviations

AD	Anaerobic Digestionz
AEM	Anion Exchange Membrane
ARB	Anode Respiring Bacteria
BES	2-Bromoethanesulfonate
CE	Circular Economy
CEM	Cation Exchange Membrane
Co-AD	Anaerobic Co-Digestion
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
EPS	Extra-Cellular
FW	Food Waste
GC-TCD	Gas-Chromatography - Thermal Conductivity Detector
GHG	Greenhouse Gas
HCl	Hydrochloric Acid
HPLC	High-Performance Liquid Chromatography
HRT	Hydraulic Retention Time
MDC	Microbial Desalination Cell
MEC	Microbial Electrolysis Cell
MES	Microbial Electrosynthesis Cell
MFC	Microbial Fuel Cell
MxC	Microbial Electrochemical Cell
NaOH	Sodium Hydroxide
OFMSW	Organic Fraction of Municipal Solid Waste
PP	Polypropylene
PS	Primary Sludge
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene
SRT	Solid Retention Time
TSS	Total Suspended Solids

UASB	Up-flow Anaerobic Sludge Blanket
VSS	Volatile Suspended Solids
WAS	Waste-Activated Sludge
WWTP	Wastewater Treatment Plant

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Chapter 1 - Introduction

Broad Concept of Resource Recovery from Wastewater and how it fits into a Circular Economy

According to a UN report in 2018, 700 million people around the world of the global population (7.63 billion) have water scarcity and it is predicted to increase to 1.8 billion people by 2025 (Jones, 2020; Roser, 2013). Food and energy requirements, droughts, groundwater depletion, and inadequate water infrastructure are the reasons for increasing water demand in the world (Jones, 2020). When water consumption increases, natural resources will start to dwell. Therefore, people should recycle and reuse resources as much as possible. Wastewater plays an important role in the water cycle and it should be properly managed because many wastewater treatment plants (WWTPs) treat wastewater only for disposal (Zarei, 2020). However, resource recovery from wastewater could avoid problems from water shortages in urban places and countries with water restrictions (Zarei, 2020). This paves way for WWTPsto progress towards resource recovery and energy production during the water treatment process because wastewater contains 50-100% of mispaced resources such as energy stored in Carbon, nutrients, and water (Puyol et al., 2017; Zarei, 2020).

The circular economy (CE) is to use a sustainable material or energy resource by minimizing the waste and impacts through recycling and reusing. The following **Figure 1** shows how wastewater can be applied to the concept of CE, due to their recovery potential for nutrients and energy while treating waste for reuse. Therefore, effluent from WWTPs can be sent for agricultural and industrial purposes instead of dumping to either sea or surface water stream.

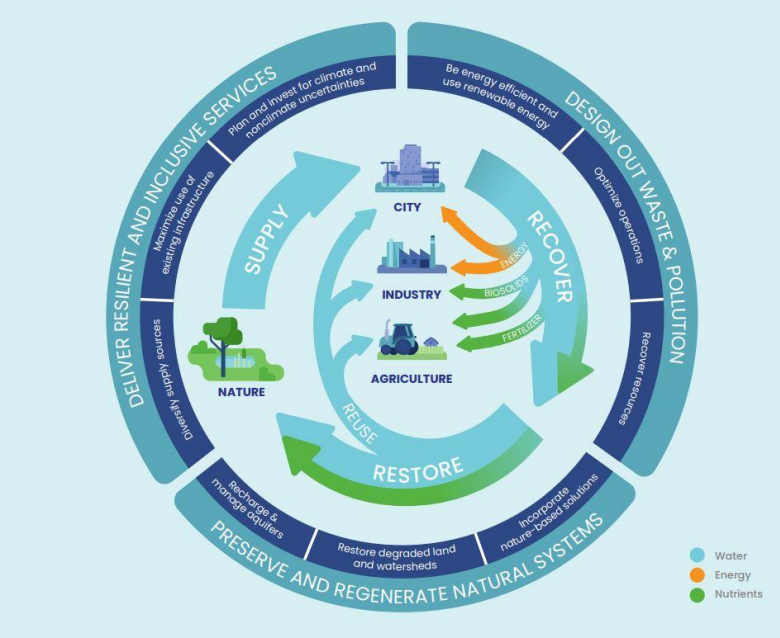


Figure 1: Circular Economy in water (*Water in Circular Economy and Resilience (WICER)*, 2021)

Nutrient Recovery

Nutrient recovery from WWTP has a great benefit to the environment since it decreases the dependence on fossil-based fertilizers and water consumption (Neczaj & Grosser, 2018). The oldest method of treating biosolids (sewage sludge) was to use filling lands as a fertilizer, which has been practiced in many countries, but it has many drawbacks: i) land requirements ii) health issues iii) odor issues and iv) public opposition (Neczaj & Grosser, 2018). Phosphorus from WWTPs is recovered through a struvite crystallization process, and it has been carried out on a large scale (Neczaj & Grosser, 2018). However, high chemical cost and clogging are major issues in the struvite crystallization of phosphorus (Neczaj & Grosser, 2018). Urine separation is another valuable option since it contains nitrogen and phosphorus (Neczaj & Grosser, 2018). Although developed countries collect urine for land applications, it has not been practiced yet due to public disapproval and technical issues (Neczaj & Grosser, 2018). Some researchers have shown that

aquatic species such as microalgae, duckweed, and wetland plants can capture nutrients (El-Shafai et al., 2007; Fernandez Pulido et al., 2021; K. Li et al., 2019; Neczaj & Grosser, 2018). After that, it can be applied as fertilizers since those aquatic species can be found in many wetlands naturally (Neczaj & Grosser, 2018). Even though this option does not require high energy and chemical demands, it is still not widely practiced due to issues associated with constructed wetlands where these species can be implemented.

Energy Recovery

Biogas, biodiesel, and bio-hydrogen can be utilized as renewable onsite energy sources at WWTPs. Methane and carbon dioxide are the main components of biogas. Biogas having a 65% v/v methane composition can be produced in a digester through anaerobic digestion (AD) and it can be a primary energy source in WWTPs to run equipment such as aeration blowers and pumps. Currently, large WWTPs use anaerobic digestors to produce biogas on a large scale, however, pretreatments are required to increase the biodegradability by breaking the solid structures of the sludge (Bachmann, 2015). Mechanical and thermal pretreatments are mainly involved in many WWTPs, while biochemical pretreatment is applied in high-strength industrial wastewaters (Bachmann, 2015). AD is widely used in different types of waste such as municipal wastewater sludge, animal manure, food waste, paper waste, energy crop residue, organic fraction of municipal solid waste (OFMSW), and slaughterhouse waste (Puyol et al., 2017). Some WWTPs conduct co-digestion (Co-AD) by mixing two different waste streams to increase methane production and organic loading rates and reduce toxicant concentrations in effluent (EPA, n.d.-b). It was reported that a WWTP in Germany has performed the Co-AD of sewage sludge with six substrates and the energy is higher than the requirement of the WWTP (Neczaj & Grosser, 2018). Biodiesel is another

product of capturing energy when sewage sludge is used as a substrate for oleaginous microbes like microalgae (Puyol et al., 2017). Therefore, some studies were conducted by growing microalgae in wastewater ponds since they can uptake nitrogen and phosphorus from sewage sludge when carbon dioxide is supplied. Then, after harvesting algal biomass, microbial lipids can be extracted to further purify into biodiesel production, while treated water can be sent to agricultural fields. Furthermore, biohydrogen can be produced from energy recovery by using wastewater as a substrate through dark fermentation (DF), but it is still in the pilot scale evaluation stage due to expensive cost of installation (Preethi et al., 2019; Puyol et al., 2017). Hydrogen is recognized as a clean fuel to use as an energy source due to the absence of carbon dioxide production during combustion (Puyol et al., 2017). Currently, hydrogen is mainly produced from petrochemical industry and required higher energy demand with releasing greenhouse gases (GHGs) (Puyol et al., 2017). Therefore, many studies were conducted to produce hydrogen through bio-photolysis, photo-fermentation, dark fermentation, and bio-catalyzed electrolysis (Manish & Banerjee, 2008). Only DF could be applicable with wastewater treatment by utilizing organic waste or wastewater (Puyol et al., 2017). However, it was reported that waste-activated sludge (WAS) and lignocellulosic waste would need pretreatment such as thermal, microwave, ultrasonic, chemical, and biological treatments to inhibit hydrogen consumers: methanogens and homoacetogens (Preethi et al., 2019; Puyol et al., 2017).

Water Reuse

Instead of dumping the treated wastewater from WWTP, they could be directed for agricultural and irrigation lands, industries, toilet flushing, groundwater replenishing, fire protection for car wash etc.(Neczaj & Grosser, 2018). Some places are already using the WWTP effluent for agricultural and irrigation lands to reduce the water stress while meeting agriculture

demand (Neczaj & Grosser, 2018). Florida state uses 44% of treated wastewater for landscape irrigation purposes (*General Facts and Statistics about Wastewater in Florida*, n.d.; Neczaj & Grosser, 2018). However, most of the water for reuse is obtained after conventional activated sludge processes with a sizeable energy and economic footprint for its production with incomplete resource recovery. This necessitates the need for alternate wastewater treatment platforms to couple water for reuse along with resource recovery.

Various Output Options for Carbon Capture

Methane

Methane is the primary component of natural gas, which is around 70-90% (Lacroix et al., 2020). In the United States, 32% of energy consumption is natural gas from oil refineries (*U.S. Energy Facts Explained*, n.d.) and an increasing fraction is also supplied from fracking of shale layers. More importantly, natural gas emissions and combustion contributes to do global warming. In 2020, 9% of methane emissions came from manure management in the US (EPA, n.d.-c). Due to rising livestock population, methane emission also is going to be increased, along with a commensurate increase of even more potent gases such as Nitrous Oxide. When animal manure is broken down to organic matter through the anaerobic digestion route according to the following **Figure 2**, methane and carbon dioxide mixture; biogas is produced.

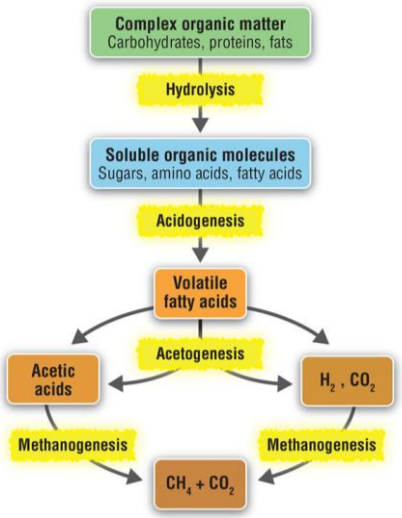


Figure 2: Metabolic pathway for the Anaerobic Digestion Route (Paul Greene, 2016)

Large WWTPs in many developed countries use anaerobic digestors to produce biogas as an energy source (Ramírez-Islas et al., 2020). Developing countries use the same technology at small and medium scales, installed in rural and semi-industrial areas, but, small-scale ADs are used for heat production only (Ramírez-Islas et al., 2020). Methane content in the biogas varies between 50 to 75% and carbon dioxide is the second highest component with other trace gases such as water vapor and hydrogen sulfide. Dissolved methane in the effluent of anaerobic process was a major challenge due it's higher concentration earlier, but now it can be captured through anaerobic membrane bioreactor (AnMBR) and degassing membrane bioreactor (K. Lim et al., 2019; Parthiba Karthikeyan et al., 2018). Another obvious benefit of anaerobic sludge digestion is the destruction of solids from the wastewater treatment process leading to a lower amount of biosolids to be sent to the landfill.

Volatile Fatty Acids (VFAs)

Acetate, Lactate, Propionate, iso-Butyrate, Butyrate, iso-Valerate, Valerate and Caproate are the short-chain VFAs, and used in diverse applications in chemical and manufacturing

industries. Although they can be recovered from the anerobic digestion route in the acidogenesis step as shown in Figure 2, most of them are derived from petrochemically due to meet global demand. Several technologies have been implemented to optimize the bio-based VFA recovery from different waste streams such as wastewater sludge, food waste, animal manure, etc. These include liquid-liquid extraction, vacuum distillation, resin-based adsorption, or membrane separation (Zhu et al., 2021). However, separation of VFAs from the fermented broth is still complicated due to low concentrations. Concentrations and compositions of VFAs may depend on the source of the original inoculum, substrate types, and process configurations.

Research Objectives

The primary goal of this thesis is to introduce a sustainable recovery of volatile fatty acids (VFAs) (ranging from C2 to C6), from swine lagoon wastewater. The following objectives were accomplished:

- To develop a modified microbial fermenter platform involving a Microbial Electrochemical Cell anode for generating VFAs from swine lagoon wastewater in a sufficient and sustainable way.
- To compare fermentation product profiles and concentrations between two different microbial inocula used for evaluations in this study from swine lagoon wastewater.

Chapter 2 - Literature Review

Conventional VFA production vs microbial fermentation routes

Although VFAs are produced naturally in the environment, synthetic VFAs have been manufactured in the petrochemical industry due to a high demand for applications of VFA in various industries (listed in **Table 1**) (Agnihotri et al., 2022). For example, acetic acid is produced through a catalytic reaction from methane and ethylene (Wainaina et al., 2019). Likewise, butyric acid is produced from crude oil through butyraldehyde oxidation in the chemical industry (Wainaina et al., 2019). Chemical synthesis routes for other VFAs are mentioned in the following **Table 1**. According to a techno-economic study of VFA production, the projected VFA market demand was 10 million MT in the global (Veluswamy et al., 2021). Market values (in USD) for each VFA for 2020 are reported in **Table 2**. The alternative routes can cover only 10% of market demand due to lower production rates and higher economic cost than petrochemical methods (Agnihotri et al., 2022). Due to depletion of fossil fuels, higher chemical and energy requirements, greenhouse gas emissions, and generation of industrial waste from petrochemical methods, many researchers have focused on bio-based routes such as anaerobic fermentation and oxidative fermentation (Agnihotri et al., 2022).

Table 1: VFA Characteristics, Synthesis and Applications

VFA Name *(IUPAC Name)*	**Molecular Formula**	**Molecular Weight (g/mol)**	**pKa Values**	**Chemical Synthesis**	**Applications**	**Odor**	**References**
Acetic (C2) *(Ethanoic acid)*	CH_3COOH	60.1	4.75	Methanol carbonylation Acetaldehyde oxidation Ethylene oxidation	Textile, preservatives, dyes, perfumes, polymers, solvent in food industry, plasticizers	Intensive vinegar smell	(Agnihotri et al., 2022; Banel & Zygmunt, 2011; Wainaina et al., 2019; Zacharof & Lovitt, 2013)
Lactic (C3) *(2-Hydroxypropanoic acid)*	$CH_3CHCOOH$	90.08	3.86	Chemical synthesis fermentation	Cosmetic industry, food-beverage addictive, buffering agent, polymers		(Zacharof & Lovitt, 2013)
Propionic (C3) *(Propanoic acid)*	CH_3CH_2COOH	74.1	4.87	Hydro carboxylation of ethylene Aerobic oxidation of propionaldehyde	Food and animal feed preservative, paints, plastics, pharmaceuticals,	Sharp, rancid, irritant	(Agnihotri et al., 2022; Banel & Zygmunt, 2011; Wainaina et al., 2019; Zacharof & Lovitt, 2013)
Iso-Butyric (C4) *(2-methylpropanoic acid)*	$(CH_3)_2CHCOOH$	88.1	4.85		Food flavoring agent, pesticide, paint, disinfecting and tanning agent	Rancid butter	(Agnihotri et al., 2022; Wainaina et al., 2019)
Butyric (C4) *(Butanoic acid)*	$CH_3(CH_2)_2COOH$	88.1	4.81	Chemical oxidation of butyraldehyde	Biofuels, animal feed supplement, pharmaceuticals, cosmetic industry, food flavoring,	Fusty smell	(Agnihotri et al., 2022; Banel & Zygmunt, 2011; Wainaina et al.,

					polymer, perfumes, textiles, plastics		2019; Zacharof & Lovitt, 2013)
Iso-Valeric (C5) *(3-methylbutanoic acid)*	$(CH_3)_2CHCH_2COOH$	102.1	4.78		Pharmaceuticals, perfumes, spices, fungicides, condiments, sedative and hypnotic agent	Sweat, body odor	(Agnihotri et al., 2022; Banel & Zygmunt, 2011; Wainaina et al., 2019; Zacharof & Lovitt, 2013)
Valeric (C5) *(Pentanoic acid)*	$CH_3(CH_2)_3COOH$	102.1	4.82	Chemical oxidation of 1-butene	Perfumes, polymers plasticizers, viscous agent, lubricating oils, surfactant, food and daily flavors, pharmaceuticals	Mold cheese	(Agnihotri et al., 2022; Wainaina et al., 2019)
Caproic (C6) *(Hexanoic acid)*	$CH_3(CH_2)_4COOH$	116.2	4.88	Ethylene oxidation	Rubber, grease, tobacco flavor, biofuel precursor, pharmaceuticals, paint and feed additive, antimicrobial agent	Sharp, stringent	(Agnihotri et al., 2022; Wainaina et al., 2019; Zacharof & Lovitt, 2013)

Table 2: Market Values for VFAs

VFA	Market Value in USD for 2020	Reference
Acetic Acid	13.51 billion	(Fernández, 2022a)
Lactic Acid	1.1 billion	(Fernández, 2022b)
Propionic Acid	1.64 billion	(Fernández, 2022c)
Butyric Acid	245.1 million	(*Butyric Acid - Global Market Trajectory & Analytics*, 2021)
Valeric Acid	15.06 million	(Agnihotri et al., 2022)
Caproic Acid	172.5 million	(*Caproic Acid - Global Market Trajectory & Analytics*, 2021)

Many scientists approached alternative routes to produce VFAs through natural and genetically-modified microbes. Through fermentation routes, acetic acid could be produced from different microbes such as *Thermoanaerobcter*, *Acetomicrobium,* and *Clostridrium* (Wainaina et al., 2019). It is reported that *Acetobacter* and *Gluconobacter* microbes are commercially used in the industry to produce acetic acid (Nayak & Pal, 2013; Pal & Nayak, 2017; Wainaina et al., 2019). Also, sugar additions such as glucose, melibiose and arabinose were optimized to enhance acetate production (Kadere et al., 2008; Wainaina et al., 2019). Different microbial strains are genetically engineered to reach the maximum propionate production rates by using commercial sugars like hexoses and pentoses (Agnihotri et al., 2022; Coral et al., 2008; Du et al., 2015; Wainaina et al., 2019). Glycerol, sugarcane molasses, cheese whey, and hemicellulose corn meals were found as cost-efficient carbon sources for propionate production compared to commercial sugars (Agnihotri et al., 2022; Goswami & Srivastava, 2001; Gupta & Srivastava, 2001; Ramsay et al., 1998; A. Zhang & Yang, 2009) while *Clostridium*, *Dysgonomonas* and *Streptococcus* are bacterial strains used to synthesize lactic acid (Luna-Flores et al., 2017; Wainaina et al., 2019). *Clostridium, Butyribacterium, Fusobacterium, Butyrivibrio, Sarcina, Megasphera* and *Eubacterium* were investigated to produce butyric acid (Agnihotri et al., 2022; Atasoy et al., 2018; Bhatia & Yang,

2017; Wainaina et al., 2019). Out of all, *Clostridium* strains are used to do industrial scale productions due to higher metabolic production rates and capability to utilize various carbon sources (Agnihotri et al., 2022). However, some *Clostridium* strains can consume only certain types of sugars (Agnihotri et al., 2022). For instance, glucose, xylose, fructose and lactate are the only sugars that can be utilized by *Clostridium tyrobutyricum* while *Clostridium butyricum* can have other alternatives such as glycerol, cheese whey permeates, ligonocelluloses and molasses (Agnihotri et al., 2022; Guo-qing et al., 2005). Iso-valerate and iso-butyrate productions were conducted biologically from bacterial strains of *Propionibacterium freudenreichii* and *Escherichia coli*, respectively (Atasoy et al., 2018; Wainaina et al., 2019; K. Zhang et al., 2011). A recent study showed that caproic acid could be synthesized from isolating *Caproiciproducens* sp. 7D4C2 at pH 5.2 and 30 ^{O}C (Esquivel-Elizondo et al., 2021). Besides, another study demonstrated caproate production from accumulating lactate and compared production rates with other substrates such as glucose and ethanol (Zhu et al., 2015). However, there are some drawbacks of individual VFA productions from certain bacterial strains, namely: i) some strains can utilize limited carbon sources ii) sterile operating conditions iii) high quality raw material and iv) expensive cost of commercial sugar types (Wainaina et al., 2019). Instead of optimizing operating conditions for pure microbial cultures, many researchers have been exploring the various routes to generate VFAs at low cost (Wainaina et al., 2019). Mixed culture fermentation is a better choice for VFA production since it has a broader range of different substrates for various microbes (Wainaina et al., 2019). Therefore, organic wastes such as agricultural and municipal wastes have been investigated to produce VFAs under different operating conditions to maximize the yields.

Different fermentation platforms based on animal waste vs municipal wastewater

When choosing a complex and mix-cultured source for VFA production, it should not compete with food insecurity like biofuel production from corn and soybeans (Sukphun et al., 2021). Animal manure, agricultural residues (straw waste, corn stalk, etc.), food waste, microalgae biomass, OFMSW, municipal wastewater, and industrial wastewater (winery, cheese whey, palm oil mill effluent) are abundant and cheap organic-rich wastes could be used substrates to produce VFAs under acidogenic fermentation conditions (Ramos-Suarez et al., 2021; Sukphun et al., 2021). However, each organic waste has pros and cons, which could affect acidogenic fermentation. Therefore, pretreatment is required to reduce impacts before beginning the fermentation process. **Table 3** summarizes different operational conditions and main VFAs coming from each waste and inoculum. Municipal wastewater and animal manure are the primary organic wastes and have been thoroughly analyzed in this review for their potentials to optimize VFA productions efficiently under different platforms.

Sewage sludge or municipal wastewater sludge has been intensively used in many studies for VFA productions at different operational conditions since it is an abundant organic waste in many places. According to an EPA report in 2019, around 4.75 million dry metric tons of sewage sludge were produced in the U.S., with 51% and 22% of them goingfor land application (agriculture, reclamation sites, forestry, lawns, and home gardens), and landfilling, respectively (EPA, n.d.-a). Municipal wastewater sludge has been intensively used in many studies as a substrate as well as an inoculum (Chen et al., 2007; Q. Yuan et al., 2011; P. Zhang, Chen, & Zhou, 2009). Wastewater sludge contains a diverse range of microbes, extracellular polymeric substances (EPS), and organic compounds (50-70%) such as carbohydrates, proteins, and lipids (Fu et al.,

2021; Sukphun et al., 2021). Because soluble chemical oxygen demand (COD) levels are 10-100 times lower than total COD in the sludge, one study stated that the rate of hydrolysis could be hindered (Sukphun et al., 2021). Therefore, pretreatment such as hydrothermal and acid-alkaline is required for the sludge to boost VFA production (Sukphun et al., 2021). Some WAS fermentations were performed without adding an inoculum due to the presence of fermentative microbes in the WAS (Chen et al., 2007; Q. Yuan et al., 2011; P. Zhang, Chen, & Zhou, 2009). Acetate was the predominant VFA from WAS fermentation, mostly at different pH and temperature conditions, even though its percentages and other VFA order positions were different due to different conditions (Chen et al., 2007; Q. Yuan et al., 2011; P. Zhang, Chen, & Zhou, 2009).

Animal manure is a richer-organic waste than municipal sludge and is widely used in fermenting investigations. Cow manure, swine wastewater (pig manure), and chicken manure have been researched to produce VFAs under different operational conditions. 1.4 billion tons of manure are produced annually in the United States (Pagliari et al., 20202). However, fermentation studies on animal manure are still limited compared to other substrates due to several reasons including accessibility to the waste streams, co-occurrence of high ammonia concentrations amongst others (Sukphun et al., 2021). The main benefits of using animal manure are: i) higher organic content than wastewater sludge ii) avoiding strong pH drops due to higher buffer capacity iii) higher biodegradability, and iv) low C/N ratio (9-19) (Sukphun et al., 2021). Since animal manure composition depends on feed, more lignocellulosic materials are digested through the animal's digestive tract (Sukphun et al., 2021). Therefore, thermal or alkaline pretreatment, is often required to break down lignocellulose structures into soluble carbohydrates during the hydrolysis phase (Sukphun et al., 2021).

One study was conducted to extract VFAs from real wastewater samples from municipal wastewater (from grate, primary settling tank and pumping station) and animal wastewater (Cattle and pig) (Banel & Zygmunt, 2011). It was confirmed that animal waste has higher VFA concentrations than municipal wastewater, and acetate was the most abundant followed by propionate (Banel & Zygmunt, 2011).

A livestock fermentation comparison was conducted between cattle manure and poultry litter under pH 5.5 with/without a thermal pretreatment (Kuruti et al., 2017). It has been reported that poultry litter has more VFA yield than cattle manure due to having higher VS content (Kuruti et al., 2017). However, the VFA composition of each manure was not presented (Kuruti et al., 2017). According to that study, acidic thermal pretreatment was required to carry out the acidogenic fermentation from cattle manure and poultry litter to optimize the VFA productions under pH 5.5 (Kuruti et al., 2017).

A study on chicken manure fermentation of granular sludge was demonstrated under different initial pH conditions: 5, 6, 7, 9, and uncontrolled. The highest net-VFA yield was obtained from heat-shocked chicken manure and inoculum under no pH control (D. min Yin et al., 2021). The leading VFAs were acetate, propionate, and butyrate (D. min Yin et al., 2021). It was reported that the low pH condition was not suitable for chicken manure fermentation due to two reasons: i) reduction of bacterial enzyme activity – *proteases* from *Coprothermobacterota* (D. M. Yin et al., 2019; D. min Yin et al., 2021) and ii) inhibition of microbial activity (D. min Yin et al., 2021). 10 mM BES addition was used in this study to improve the VFA production. But it was not promoted since heat-shock pretreatment could inhibit the acetoclastic methanogenesis activities (Hernández et al., 2019; D. min Yin et al., 2021).

Cow manure is a suitable substrate for VFA production due to the presence of inorganic and organic bulking agents (Jomnonkhaow et al., 2021). Cow manure fermentation of food waste (FW) and anaerobic sludge (AS) was conducted to generate VFAs from a long-term semi-continuous system (Jomnonkhaow et al., 2021). Unlike other investigations, pH was not controlled in the study due to two reasons: i) Prevention of intense pH decrease due to higher ammonia in the cow manure and ii) Avoiding use of using chemicals for pH adjustments (Jomnonkhaow et al., 2021). The prevailing VFA from cow manure fermentation was acetate, followed by propionate and butyrate (Jomnonkhaow et al., 2021). The same VFA distribution was observed in other studies, such as cow slurry fermentation (Tampio et al., 2018) and co-fermentation of cow manure and maize silage (Cavinato et al., 2017). According to the study, the highest VFA concentration was obtained from thermal-treated cow manure fermentation, but without keeping an external inoculum source, possibly due to the discrepancy in microbes between inoculum and cow manure (Jomnonkhaow et al., 2021).

Swine wastewater or pig manure contains more VFA concentrations than cow manure (Cooper & Cornforth, 1978; Møller et al., 2004). An anaerobic co-digestion study presented that swine manure was extremely rich with VFA concentrations of acetate, propionate and butyrate (Baek et al., 2020). A study of swine manure fermentation of anaerobic sludge was reported that initial alkaline pH condition enhanced to produce VFAs with an acetate dominance and followed by propionate (W. Zhang et al., 2020). Previous studies showed that initial pH conditions (10 and 11) could enhance the VFA concentration compared to acidic (Lin et al., 2013) and neutral (W. Huang et al., 2016) conditions from swine manure fermentations since initial alkaline pH could affect the microbes in the swine manure and boosts the hydrolysis (Lin et al., 2013). Although an inoculum addition was not required to have in chicken and cow manure fermentations to achieve

VFA yield, a pig manure fermentation was used an anaerobic digester sludge as an inoculum and achieved higher VFA concentrations at pH 10 (W. Zhang et al., 2020).

Packed bed reactor, fluidized bed reactor, up-flow anaerobic sludge blanket reactor (UASB) and continuous stirred-tank reactor (CSTR) are primary reactor configurations used in anaerobic VFA production (W. S. Lee et al., 2014). Attached growth and suspended growth processes are the technologies used in the reactors. Packed bed and fluidized bed reactor are made up to operate with attached growth process, while UASB and CSTR are based on suspended growth process. Fluidized bed reactor is better than packed bed reactor due to clogging from higher concentrations of suspended growth (W. S. Lee et al., 2014). Overall, CSTR is the best reactor configuration due to complete mixing, however high speed could cause shear stress to the suspended microbes (W. S. Lee et al., 2014). Requiring a longer time for granulating the inoculum is the main drawback of UASB (W. S. Lee et al., 2014).

Also, membrane-based reactors such as membrane contractor, electrodialysis, AnMBR, and membrane pervaporation are applied due to reduction of recovery steps under a shorter residence time and economical process (Sukphun et al., 2021). A hydrophobic porous membrane such as polypropylene (PP), polyvinylidene fluoride (PVDF) and polytetrafluoroethylene (PTFE) is used to separate two aqueous phases (Sukphun et al., 2021). The pH gradient between two phases is the driving force in the membrane contractors, while either partial pressure or concentration difference can use to measure the transmembrane pressure (Sukphun et al., 2021). Electrodialysis uses either cation-exchange membrane (CEM) or anion-exchange membrane (AEM) to pass the certain ions towards the concentrate chamber while retaining other ions. Many researchers have used electrolysis due to its higher VFA recovery rate, but higher energy demand, membrane cost and back diffusion are the main drawbacks (Sukphun et al., 2021). AnMBR is one of the promising

VFA recovery technologies due its benefits such as low energy demand and sludge generation (Sukphun et al., 2021). It employs a unique combination of membrane separation coupled with anaerobic microbiology to achieve superior treatment of the wastewater. This reactor can be operated at higher total suspended solid (TSS) concentrations and organic loading rates from a complex stream, however membrane fouling and biofilm formation are main issues to deal (Sukphun et al., 2021). Furthermore, superior effluent quality without solids and pathogens, increase in biogas production with a higher methane content, and reduction of carbon footprint on AD operations are the additional advantages of the AnMBR platform (Valentino et al., 2021). Membrane pervaporation is based on the ability of a non-porous membrane to transfer VFAs into a vapor-permeate phase from a feed solution by difference of its chemical potential gradient (Sukphun et al., 2021). Higher concentrations of VFA recovery and low capital costs are the benefits while membrane fouling, feed pH control, and impurities in the permeate are the drawbacks of the membrane pervaporation (Sukphun et al., 2021).

Table 3: Operating conditions for VFA production from different waste streams

Inoculum Type	Substrate Type	Operational Conditions	Main VFAs	Reference
Anaerobic digestated sludge	Algae Chlorella residues	Reactor Configuration: Batch HRT: 0-5 days Temperature: 35 OC pH: 4, 6, 8 and 10 S/I Ratio: 3:1	Acetate>propionate>butyrate (pH 4, 8, 10) Acetate>butyrate>propionate>iso-valerate (pH 6)	(Y. Li et al., 2013)
Anaerobic digestate	Olive oil mill wastewater	Reactor Configuration: Batch HRT: 45 days Temperature: 25 ^{O}C pH: Initial pH 6.5 S/I Ratio: N/A Added calcium oxide to increase pH initially.	Acetate>butyrate, propionate>valerate	(Dionisi et al., 2005)
Anaerobic digestate	Municipal Solid Waste	Reactor Configuration: PFR HRT: 1.3 days Temperature: 55 ^{O}C pH: 6.6 – 7.2	Butyrate>Acetate>Propionate	(Sans et al., 1995)
Anaerobic sludge	Wasted Potato	Reactor Configuration: Batch HRT: 1 - 6 days Temperature: 35 ^{O}C pH: 6, 7 and 8 S/I Ratio: 10:1	Butyrate>acetate>propionate (pH 6 & 7) Acetate>butyrate>propionate (pH 8)	(Y. Li et al., 2019)
Anaerobic sludge	Dairy wastewater	Reactor Configuration: CSTR HRT: 0.5 - 1 days Temperature: 35 ^{O}C	Acetate, propionate, butyrate, valerate	(Demirel & Yenigun, 2004)

		pH: 6 - 8		
Food waste sludge Anaerobic sludge	Cow Manure	Reactor Configuration: Batch and an immersed membrane reactor (iMBR) HRT: 22 days Temperature: 37 ^{O}C S/I Ratio: 1:1 Compared with the thermally-treated substrate as well as inoculum.	Acetate, propionate, butyrate	(Jomnonkhaow et al., 2021)
Anaerobic sludge from a lab-scale anaerobic bioreactor	Waste activated sludge	Reactor Configuration: Semi-continuous reactor HRT: 12 days Temperature: 35 ^{O}C pH: 6.9 S/I Ratio: 2:1 Added calcium oxide as a pretreatment in different dosages.	Acetate>butyrate>iso-valerate>propionate	(Xin et al., 2021)
Anaerobic digested sludge	Urban sewage sludge	Reactor Configuration: Batch HRT: 10 days Temperature: 35 ^{O}C and 55 ^{O}C pH: 5.5 and 10 S/I Ratio: 1:1 Compared VFA generation under two different operational conditions: thermophilic vs mesophilic, acidic pH vs alkaline pH	Propionate>iso-Valerate (pH 5.5 and 35 ^{O}C) Acetate>iso-Valerate (pH 5.5 and 35 ^{O}C) Acetate>propionate (pH 10 and 35 ^{O}C) Acetate>iso-valerate (pH 10 and 55 ^{O}C)	(Esteban-Gutiérrez et al., 2018)
	Winery wastewater		Propionate, acetate (pH 5.5 and 35 ^{O}C) Butyrate>acetate (pH 5.5 and 35 ^{O}C) Acetate>propionate (pH 10 and 35 ^{O}C) Acetate>propionate (pH 10 and 55 ^{O}C)	
	Meat wastewater		Acetate>valerate (pH 5.5 and 35 ^{O}C) Acetate>butyrate (pH 5.5 and 35 ^{O}C) Acetate> iso-valerate (pH 10 and 35 ^{O}C) Acetate> iso-valerate (pH 10 and 55 ^{O}C)	

Primary sludge	Industrial wastewater	Reactor Configuration: CSTR HRT: 60 hours T (^{O}C): 25 pH: 6.9 S/I Ratio: 1:1	Acetate	(Maharaj & Elefsiniotis, 2001)
Rumen fluid	Rice Straw	Reactor Configuration: Batch HRT: 72 hours T (^{O}C): 39 pH: 5-7 range S/I Ratio: N/A Substrate load %: 1.0, 2.5, 5,0, and 10.0	Acetate>propionate>butyrate (Acetate decreased and propionate increased when rice straw load was increased)	(J. Liang et al., 2021)
Rumen fluid Swine liquid manure Sewage sludge	Swine wastewater	Reactor Configuration: Batch HRT: 140 days Temperature: 35 ^{O}C pH: 6 - 8 S/I Ratio: 9:1 Targeted for methane production only.	N/A	(Córdoba et al., 2016)
Rumen fluid	Cattail	Reactor Configuration: Batch HRT: 5 days Temperature: 40 ^{O}C pH: 5.8 – 7.6	Acetate>propionate>butyrate	(Hu et al., 2006)
Rumen fluid	80% Pig manure and 20% corn stover	Reactor Configuration: Continuous HRT: 8 days Temperature: 40 ^{O}C pH: 6.3	Acetate>butyrate>propionate>valerate, caproate	(Thanakoses et al., 2003)
Rumen fluid	Corn silage Wheat Straw	Reactor Configuration: Batch HRT: 7 days	N/A	(Nguyen et al., 2019)

		Temperature: 39 ^{O}C pH: 5.3 – 7.0 and 5.6 – 7.0 Compared with anaerobic sludge inoculum and got 4-times higher VFA generation than anaerobic sludge.		
Food waste sludge Anaerobic sludge	Cow Manure	Reactor Configuration: Batch in an immersed membrane reactor (iMBR) HRT: 22 days Temperature: 37 ^{O}C S/I Ratio: 1:1 Compared with the thermally-treated substrate as well as inoculum.	Acetate>propionate>butyrate	(Jomnonkhaow et al., 2021)
Granular sludge from up-flow anaerobic sludge blanket (USAB)	Chicken Manure	Reactor Configuration: Batch Temperature: 37 ^{O}C pH: Initial pH 5, 6, 7, 8, 9 and uncontrolled pH S/I Ratio: 6:1 Compared with heat-shocked cow manure and inoculum.	Acetate>Propionate>Butyrate>iso-valerate (all pH conditions)	(Yin et al., 2021)

Caproate and Medium-Chain VFA Synthesis from waste streams

Caproate, heptanoate, and caprylate synthesizes from swine wastewater fermentation were implemented from a mixture of short-chain VFA mixture under the two-stage process (W. Zhang et al., 2020). In the first stage, swine wastewater fermentation was conducted under three initial pH values: 6.0, 8.0, and 10.0. Acetate was the dominant short-chain VFA, followed by propionate in the first stage (W. Zhang et al., 2020). The initial pH 10 reactor was used for the second stage since it had the highest short-chain VFA concentration among other pH conditions (W. Zhang et al., 2020). Ethanol addition (15 g/L) was used as an electron donor to convert short-chain VFAs to medium-chain VFAs based on one reverse β-oxidation pathway. (W. Zhang et al., 2020) It was reported that caproate and caprylate productions were mediated through the chain elongation reactions using acetate and butyrate, respectively (W. Zhang et al., 2020). The exact mechanism was applied to the heptanoate synthesis from propionate with ethanol as a substrate, with valerate being the intermediate VFAbetween propionate and heptanoate (Reddy et al., 2018; Roghair et al., 2018; W. Zhang et al., 2020). *Caldicoprobacter*, *Ruminofilibacter*, and *Proteiniphilum* were the most abundant bacterial species at the initial pH of 6, 8, and 10, respectively, in the first stage (W. Zhang et al., 2020). Notably, the dominant microbial species was changed to *Clostridium* in the second stage (W. Zhang et al., 2020), and *Clostridium kluyveri* was responsible for the caproate synthesis (Steinbusch et al., 2011; Y. Yin et al., 2017).

A study on co-fermentation of swine manure and corn stalk silage was conducted under two stages to produce caproate without adding an external electron donor (W. Zhang et al., 2022). The inoculum of the first stage reactor was obtained from a lactate fermentation reactor and short-chain VFAs were produced under mesophilic conditions under no pH control (W. Zhang et al., 2022). Acetate and butyrate were the leading VFAs in the 1st stage since *Clostridum sensu stricto*

1 was the most abundant and they can produce acetate and butyrate by degrading lignocellulose (W. Zhang et al., 2022). When the reactor temperature was changed from mesophilic to thermophilic, the lactate production increased while other VFAs reduced (W. Zhang et al., 2022). It was reported that the lactate producing microbe, *Bifidobacterium thermophilum* overtook the abundance (W. Zhang et al., 2022). Then sheep rumen fluid was introduced as a new inoculum to the high-lactate mixture, but butyrate became dominant, without increasing caproate at pH 6 (W. Zhang et al., 2022). After lactate addition was increased, then productions of caproate, heptanoate and caprylate increased while butyrate declined (W. Zhang et al., 2022). *Pseudoramibacter* was abundant due to the impact of lactate addition to microbial community (W. Zhang et al., 2022). However, that microbe vanished after changing the reactor pH from 6 to 5 and *Capriociproducens* became dominant to produce higher concentrations of caproate (W. Zhang et al., 2022). Therefore, higher lactate concentration could convert the short-chain VFAs (especially butyrate) into caproate and other medium-chain VFAs through chain-elongation microbes without using an external electron donor like ethanol in co-fermentation of swine manure and corn stalk silage.

Microbial Electrochemical Systems (MxCs) as an integrated platform for VFA production

Microbial electrochemical system (MxCs) is an emerging field in anaerobic biotechnology for capturing electrons from an organic matter to produce the energy under different aspects: electric power, electric fuels (hydrogen and methane), manufacturing valuable chemicals (hydrogen peroxide, caproate and formic acid), nutrient recovery (ammonia, heavy metals), agricultural wastewater treatment, pollutant removal and water desalination. There are several MxC configurations under study, namely are Microbial fuel cell (MFC), microbial electrolysis cell (MEC), microbial electrosynthesis cell (MES), and microbial desalination cell (MDC). They are

differentiated by the cathode configurations in each system according to Figure 1 while all anode configurations comprise of oxidation of waste materials under anaerobic conditions. The cathodic reactions produce electric current (in MFC mode), hydrogen gas and other chemicals (in MECs), synthesis of organic compounds (MES) and drive desalination (MDS) (H. Wang & Ren, 2013). Most studies have been investigated on MFC out of all MxC systems yet (H. Wang & Ren, 2013).

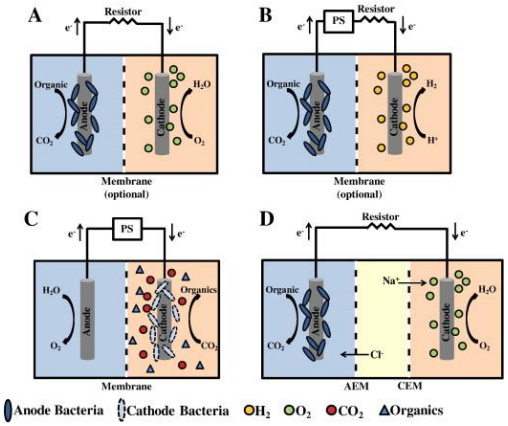


Figure 3: Types of MxCs [(A): MFC, (B): MEC, (C): MES and (D): MDS] (H. Wang & Ren, 2013)

Either anion exchange membrane (AEM) to cation exchange membrane (CEM) act to separate the anode and cathode compartments as a barrier to transport certain types of ions like H^+ ions transport from anode to cathode in MEC and O_2 from cathode to anode in MFC (Rittmann & McCarty, 2020). In the MEC, exo-electrogenic microbes or anode respiring bacteria (ARB) such as *Geobacter* and *Shewanella* convert the organic matter (especially acetate) in the anode compartment and release H^+ ions. Those H^+ ions will transfer through a CEM to the cathode and release as hydrogen gas (Lu & Ren, 2016; Rittmann & McCarty, 2020). However, external power supply is required to facilitate the endothermic reaction to produce hydrogen (Lu & Ren, 2016). The primary purpose of the MEC is to produce hydrogen from the cathode compartment. Although acetate was the best substrate in the anode compartment, many researches have investigated the

applicability of municipal wastewater and biorefinery effluent to produce hydrogen through MEC systems (Lu & Ren, 2016).

Hydrogen production and VFA removal were studied at different concentrations of ultra-pretreated WAS fermentations in MECs (W. Liu et al., 2012). It was reported that ultra-pretreated WAS had better VFA productions by 1-2 times higher than alkaline WAS fermentation (W. Liu et al., 2012). Acetate was the prevalent VFA, followed by propionate and butyrate in WAS fermentation MEC studies (W. Liu et al., 2012). Carbon degradability varied with different VFA compositions in the MEC systems and the highest removal system was achieved based on the simplest VFA composition: Acetate (W. Liu et al., 2012). More than 90% of acetate and propionate were converted to hydrogen (W. Liu et al., 2012).

MEC can be used to i) produce hydrogen as an energy product, ii) reduce dry-solid production, and iii) limit odor release at WWTPs. (Logan et al., 2008). But a sufficient hydrogen production is required in a cost-effective manner since energy input is required to produce hydrogen (Logan et al., 2008). Slow degradation of complex substrates and poor conductivity are the main drawbacks of using wastewater as a substrate in a MEC (Logan et al., 2008).

Instead of using two-compartments with a membrane, researchers found that a single-chamber could increase the hydrogen production rate while reducing high operational cost, and no membrane fouling (H. Liu et al., 2010; H. Wang & Ren, 2013). It was reported that single-chamber MEC could produce the current density ranging from 4.2 to 12 A/m^2 while 0.4-3.3 A/m^2 from a two-compartment MEC while oxidizing acetate. However, the biggest challenge of single-chamber is hydrogen consumption by other microbes especially hydrogenotrophic methanogens to produce methane (H. Liu et al., 2010; H. Wang & Ren, 2013). Adding methanogen inhibitors such as 2-Bromoethanesulfonate (BES), exposing the cathode to air periodically, reduce the pH

and thermal treatments of anode and inocula are the suggested ways to control methanogenesis (H. Liu et al., 2010; H. Wang & Ren, 2013; Parameswaran et al, 2010). Hydrogen gas separation from the mixed-gas headspace of the single-chamber MEC would be another issue since two-compartment MEC produces hydrogen separately from the cathode compartment.

In summary, there are several anaerobic environmental biotechnologies that favor the production of volatile organic acids such as selective fermentation, microbial electrochemical cells and others. However, a central challenge is to simultaneously produce and separate the organic acids at superior fermentation efficiency while also producing water of reuse quality, which may be possible through Anaerobic Membrane Bioreactor platform potential aided by microbial electrochemical cells.

Chapter 3 - Selective Swine Wastewater Fermentation with different inocula and resultant volatile fatty acids profile

Introduction

The type of inoculum could influence the VFA generation during fermentation due to microbial diversity and their distinctive ability to metabolize specific substrates. Anaerobic digested sludge, food waste (FW), primary sludge (PS), waste-activated sludge (WAS), rumen fluid, sewage sludge, swine manure, compost, raw dust and slaughter-house sludge are some of the inocula used in previous fermentation studies.

Anaerobic digested sludge is one of the most common inocula type to produce VFAs under acidogenic fermentation (Sukphun et al., 2021). Previous research has demonstrated anaerobic activated sludge could produce more VFAs than aerobic activated sludge due to an abundance of acidogenic bacteria (K. Wang et al., 2014). A possible drawback of using the anaerobic sludge is the co-occurrence of methanogens which can stimulate secondary VFA degradation and conversion to methane.

Activated sludge is the one of the most accessible biomass sources from wastewater treatment for generating VFAs under low-cost conditions for carbon conversion (Q. Yuan et al., 2011). It is widely used as an inoculum as well as a substrate under different variations such as acidic, neutral and alkaline pH conditions, mesophilic and thermophilic temperatures, batch and other reactor configurations and retention times (Q. Yuan et al., 2011; P. Zhang, Chen, & Zhou, 2009). Acetate and propionate were the dominant VFAs from WAS fermentation under different temperatures: 4, 14, and 24.6 ^{O}C (Q. Yuan et al., 2011). Another WAS fermentation study showed that acetate was the predominant acid in the pH 4-10 range, under both mesophilic and thermophilic temperatures (P. Zhang, Chen, Huang, et al., 2009). However, propionic acid was the

dominant VFA at uncontrolled pH and mesophilic conditions (P. Zhang, Chen, Huang, et al., 2009). Yet another study of WAS fermentation demonstrated that acetate percentage increased and iso-valerate percentage decreased when pH was increased from 4 to 11 (Chen et al., 2007).

Rumen fluid is another suitable inoculum type used in fermentation studies since it could convert lignocellulosic biomass easily due to its microbial population and cellulase system in the microbes (J. Liang et al., 2021). Therefore, rumen fluid was used an inoculum with lignocellulosic substrates such as cattail, rice straw, wheat straw, oaten hay, lurence hay and corn silage(Hu et al., 2006; J. Liang et al., 2021; Nguyen et al., 2019). Based on a study on rumen fermentation of cattail, optimal pH was 6.9 to obtain the highest VFA yield (Hu et al., 2006). Acetate was the dominant VFA followed by propionate and butyrate (Hu et al., 2006). They mentioned that VFA composition was influenced by pH, mainly acetate at low pH and butyrate at high pH (Hu et al., 2006). According to the same study, propionate concentrations depended on substrate concentration and not on pH (Hu et al., 2006). Another study compared the VFA levels from rumen microbes with anaerobic sludge microbes and proved that rumen fluid could produce four times higher VFAs from lignocellulosic biomass (Nguyen et al., 2019). According to their microbial community studies between rumen fluid and anaerobic sludge inocula, the presence of *Fibrobacter* and *Prevotellaceae* phyla were significantly higher in rumen fluid (Nguyen et al., 2019). *Firbrobacter* degrades plant-based cellulose in ruminant animals, while *Prevotella* supports ruminal fibrolytic activity, which could bring significantly higher VFA and soluble COD concentration levels (Nguyen et al., 2019). They obtained low cumulative biogas from rumen fluid compared to anaerobically digested sludge, due to the low abundance of methanogenic communities such as *Methanobacterim*, *Methanobrevibacter,* and *Methanomicrobium*, in the rumen fluid (Nguyen

et al., 2019). Therefore, a higher VFA yield with lower biogas production could be obtained from rumen fluid with significant degradation of lignocellulosic biomass (Nguyen et al., 2019).

Some studies investigated using glucose as a model substrate to observe how pH influences the hydrolysis step from several different inocula and resultant VFA generation. Acetate was the prevalent VFA from a 10 g/L glucose fermentation under at acidic pH levels: 4.5, 5.0 and 5.5 (Tamis et al., 2015). According to previous studies on glucose fermentation of dewatered sludge inoculum, the prevalent VFA acetate was reduced when the pH was reduced from 6.5 to 6.2 (Oh et al., 2003).

Another study showed a 1% glucose solution as a model substrate to a fermentation of anaerobic sludge at two initial levels (5 and 7) and unadjusted pH with BES addition (Jankowska et al., 2017). Acetate was the prevalent VFA at pH 5 and 7, and unadjusted pH conditions (Jankowska et al., 2017). Butyrate and propionate were second leading VFAs at pH 5 and 7, respectively (Jankowska et al., 2017). Besides the VFA composition, gas production is another important during the acidogenic fermentation. According to a previous study, hydrogen gas the main gaseous product at acidic and neutral pH conditions from 1% glucose fermentation of anaerobic sludge (Jankowska et al., 2017). However, under alkaline conditions the profile changed with a decrease in the hydrogen amount and a corresponding increase in the methane content (Jankowska et al., 2017). Another glucose fermentation with three different pH conditions (5.8, 7.3 and 11) with/without adding a buffer was conducted to evaluate hydrogen production (H.-S. Lee et al., 2008). Butyrate was the prevalent VFA at 7.3 pH with the buffer addition and at 5.8 pH without the buffer addition (H.-S. Lee et al., 2008). Around 200 mL hydrogen was produced at pH 11 without the buffer addition and at pH 5.8 with the buffer addition (H.-S. Lee et al., 2008).

Instead of using pristine inocula, some authors noted inoculum pretreatments such as pH, heat, freeze-thaw, and chemical treatment, either-individually, or in combination served to inhibit methanogenetic activity and enrich fermentative bacteria (Sukphun et al., 2021; Venkata Mohan et al., 2008). Thermal pretreatment could form spores in acidogenic microbes such as *Clostridium* and *Bacillus* under 100 ^{O}C for 15-120 minutes (Valdez-Vazquez & Poggi-Varaldo, 2009; Wainaina et al., 2019). Although they stated that the method could deactivate the methanogenic bacteria simultaneously, a few methane-forming archaea could survive under extreme heat conditions (Mei et al., 2016; Wainaina et al., 2019). Therefore, heat shock pretreatment depends on the temperature and duration of treatment for each inoculum (Wainaina et al., 2019). Overall, few studies have already claimed that thermal inoculum pretreatment is the most cost-effective method for anaerobically digested sludge and food waste with different feedstocks, but there are not enough studies to prove it for other inoculum types (Jomnonkhaow et al., 2021; Sukphun et al., 2021; Venkata Mohan et al., 2008; Wainaina et al., 2019; D. min Yin et al., 2021).

Freeze-thaw is another physical inoculum pretreatment without chemicals and a low energy input. One study mentioned that their anaerobic digested sludge inoculum was stored in a -20 ℃ freezer and thawed in a fridge at 4 ℃ to check the effects of inoculum pretreatments while using urban biowaste as the substrate (Blasco et al., 2020). They observed that thermal pretreatment increased VFA yield, such as more acetate and propionate production, along with methane production compared the freeze-thaw method in a -20 ℃ freezer and thawed in a fridge at 4 ℃ to check the effects of inoculum pretreatments while using urban biowaste as the substrate.

Adjusting the initial pH of inoculum with a substrate is another easiest pretreatment method, since higher VFA productions occur during acidogenic fermentation. However, chemicals such as 1 M HCl acid or NaOH base are still required to control the pH to a particular value.

Acidogenic and alkali treatments break down hemicellulose and lignocellulose, respectively, thereby aiding its degradation to VFAs (Sukphun et al., 2021). Studies on VFA production from alkali pretreatment are still limited because acid treatments are the most preferred and effective option to increase VFA yield and hydrogen production (Sukphun et al., 2021).

Some studies use BES as an effective methanogenic inhibitor, but others suggest that it could affect the abundance of acidogenic microbes (Ramos-Suarez et al., 2021; J. Yin et al., 2016a). One research mentioned that 10 mM BES is enough to stop the methanogenesis with cow manure and municipal wastewater (Webster et al., 2016). According to another study, the presence of BES impacted the microbial community by decreasing *Bacteriodes* and increasing *Gammaproteobacteria* (J. Yin et al., 2016a). However, no comparable effect occurred in the VFA composition with anaerobic activated sludge inoculum. Also, some studies found that long-term exposure to BES could eliminate *Archaea* and change the microbial community preference for electron acceptors (Chiu & Lee, 2001; Ye et al., 1999; J. Yin et al., 2016b). Chemical costs associated with BES addition and pH adjustment is one of the significant economic drawbacks, while physical pretreatments (thermal and freeze-thaw) do not require chemicals (Blasco et al., 2020).

Methods

Inoculum Preparation

We collected around 2L of activated sludge from the anoxic selector basin at the WWTP in Manhattan, Kansas. Then sludge was settled for 24 hours, and we collected 600 mL of the thickened settled sludge and added it to a 1L glass bottle that served as the anoxic sludge fermenter. The solids concentration of the sludge used as inoculum was analyzed.

(a) (b) (c)

Figure 4: Sludge Inoculum [(a): Anoxic Selector Basin in Manhattan WWTP, (b): Settled Sludge, and (c): Sludge Fermenter]

We obtained 150 mL of cattle rumen fluid from the K-State Veterinary Medical Laboratory. Due to limited availability of the inoculum, 350 mL of synthetic swine permeate was mixed with the cattle rumen fluid to get 500 mL as the volume of the rumen fermenter. The 1L of synthetic swine permeate (without COD and sulfide) was prepared from 3.0915 g of boric acid, 1.443 g of ammonium chloride, 0.24 g of sodium hydrogen phosphate, 0.0026 of ferric chloride, 0.0823 g of magnesium chloride, 0.108 g of calcium chloride and 0.652 g of potassium chloride (Damodara Kannan & Parameswaran, 2021; H. Huang et al., 2015; Raby et al., 2013). The rumen fluid has a light yellowish-brown color compared with anoxic sludge. Usually, the rumen fluid has an olive-green to greenish-brown color with a strong odor (Petrovski, 2017). Its color varies depending on the diet composition of the cattle, such as yellowish-brown in maize silage or straw-fed cattle and greenish-brown in pasture-fed cattle (Petrovski, 2017).

(a) (b)

Figure 5: Rumen Inoculum [(a): Rumen fluid and (b): Rumen Fermenter]

Experimental Procedure

Initially, 25 mM glucose was added to the respective inoculum bottles to enrich fermenters since glucose is the most accessible form of food for the fermentative microbes and serves to stimulate fermentative metabolism. pH values of both fermenters were adjusted to be around 5.00 through acidification by adding 1 M HCl to both fermenters since a low pH range between 4 and 6 is preferred for efficient fermentation processes. Sampling gas bags were attached to the gas line of a rubber stopper for collecting any gaseous products through the batch fermentation process. Both fermenters were kept at 30 ^{O}C temperature on a stirring plate (Thermo scientific Super NQVA multi-place, USA). The stirring speeds ranged between 500 and 800 rpm. After adding 25 mM glucose spikes to each reactor, VFA and glucose were measured through a HPLC, as described below. The spikes were intended to further enrich the carbohydrate fermentative microbes in the respective inoculum.

Analytical Methods

pH was measured daily using a Fisherbrand Accumet AB150 pH benchtop meter. pH was adjusted regularly to keep the value under 6.00 throughout the batch process for improving acidogenic fermentation by using 1 M NaOH and/or 1 M HCl. We quantified the VFAs using the high-performance liquid chromatography (HPLC) technique (Shimadzu LC-20AT, USA) using an Aminex HPX-87H column (Bio-Rad Laboratories, USA) equipped with two detectors: photodiode array and refractive index detectors. Aminex HPX-87 H column is designed to separate organic acids as well as sugars. For VFA quantification, the HPLC technique was used at 0.65 mL/min pump flowrate and 50 ^{O}C oven temperature with 2.5 mM sulfuric acid as eluent. We targeted detecting eight VFAs (Acetate, Lactate, Propionate, iso-Butyrate, Butyrate, iso-Valerate, Valerate and Caproate) from the effluent samples of both fermenters through the HPLC technique with an external standard VFA method. A different HPLC method under the same pump flowrate and 35 ℃ oven temperature with DI water as eluent was used to detect glucose. All the samples were filtered through 0.22 µm PVDF syringe filters to remove particulates to avoid clogging the HPLC equipment's pump and column. TSS and VSS were conducted for the inoculum samples in the second trial according to the standard methods 2540D and 2540E, respectively (Eaton et al., 2005).

Results and Discussion

Glucose as a Substrate

We chose glucose as the substrate for both batch fermenters since glucose is a monosaccharide molecule and can be broken to VFAs efficiently. Then sludge and rumen microbes could survive and initiate acidogenic fermentation with glucose to generate VFAs effectively. In this study, we added 25 mM glucose (=4.5 g/L) in the fermenters at the beginning and throughout the experiment by detecting the glucose content from the HPLC method. We

maintained the pH below6 in both fermenters during both trials to facilitateacidogenic fermentation under acidic conditions.

Inoculum Characteristics

The characteristics of inoculum types from both trials are presented in **Table 4**. However, the second trial had lower characteristic values for all parameters.

Table 4: Inoculum Characteristics

Inoculum Type	Anoxic Sludge		Rumen Fluid	
	Trial 1	Trial 2	Trial 1	Trial 2
Physical Structure	Slurry		Slurry	
pH	6.80	7.10	7.13	5.72
SCOD (mg/L)	13,260	7,570	67,000	38,300
TSS (mg/L)	19,000.00	13,366.67	9,933.33	3,066.67
VSS (mg/L)	16,350.00	10,083.33	9,550.00	2,508.33
Ratio VSS/TSS	0.861	0.754	0.961	0.818

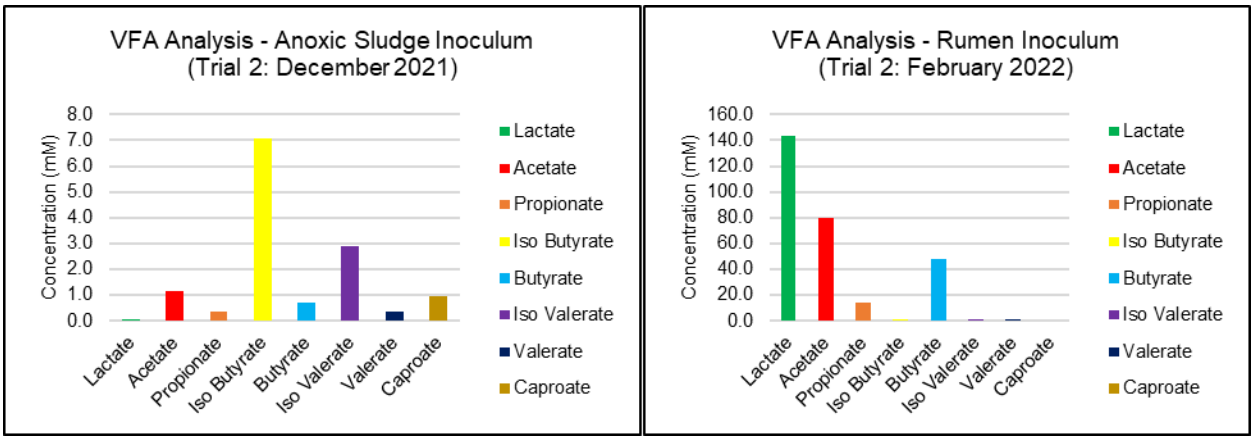


Figure 6: VFA Analysis on Inocula in Trial 2 [(a): Sludge Inoculum and (b): Rumen Inoculum]

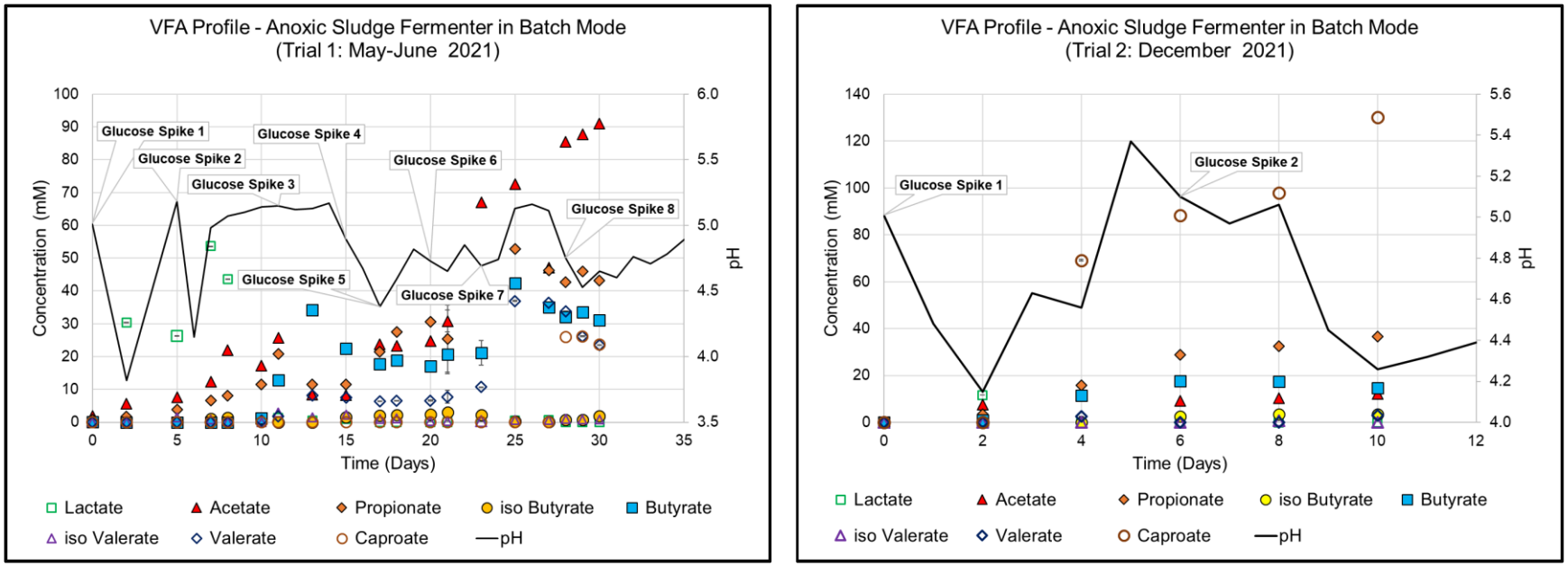
Figure 6 shows the VFA distributions from each inoculum to detect predominant VFA that could develop in the glucose-enriched batch fermentation. However, VFA analysis for both inocula was only conducted in the second trial, hence Trial 2 VFA data is presented for both inocula. Iso-butyrate (7 mM) was the main dominant VFA, followed by 3 mM valerate in the anoxic sludge inoculum. According to relevant studies on WAS fermentation, acetate and propionate are prevalent at any pH values (Q. Yuan et al., 2011; P. Zhang, Chen, & Zhou, 2009). Nevertheless, all other VFAs, including acetate and propionate, were detected in the anoxic sludge inoculum at less than 2 mM concentration levels. Alternately, rumen fluid had a wide range of VFA distributions due to the presence of 143 mM lactate, 79 mM acetate, and 48 mM butyrate. Anoxic sludge had 0.94 mM caproate while rumen fluid did not. Although lactate was detected as the prevalent VFA in the rumen fluid inoculum, having a higher amount of acetate could cause a low pH value (Hu et al., 2006). Therefore, more lactate production was anticipated from the rumen inoculum due to the presence of glucose as a substrate at a low pH range.

VFA Analysis

Sludge Fermenter

Figure 7 (a) shows the VFA distribution from the first trial of the anoxic sludge fermenter in glucose-enriched batch operation at 30 ℃. The pH was checked daily and adjusted regularly to maintain the acidic level (less than 5.5 pH). None of the VFAs were detected on day-0. Lactate was detected above 20 mM concentration only in the first few days of the batch operation due to glucose carryover. As predicted from the previous studies (Chen et al., 2007; Q. Yuan et al., 2011; P. Zhang, Chen, & Zhou, 2009), acetate production gradually increased in the entire batch mode when the pH was stable at around 5. More than 90 mM acetate concentration was detected on the 30^{th}-day in the effluent sample. After 10 days of batch operation, acetate, propionate, butyrate and

valerate production continued to increase before stabilizing. Although those VFA accumulation except acetate slightly decreased at the end of the batch operation, around 25 mM caproate formation was detected simultaneously towards the end. We repeated the batch experiment using 600 mL concentrated anoxic sludge from the Manhattan WWTP with a shorter batch period, which is referred to as Trial 2. Therefore, we added only two 25 mM glucose spikes to the fermenter. However, a different fermentation product profile was obtained, as shown in **Figure 7 (b)**, because caproate was the dominant VFA in the entire batch process, and the final concentration was detected as around 130 mM. Although less than 1 mM caproate was detected in the anoxic sludge inoculum prior to starting the second trial, the caproate buildup significantly increased from day-4. Also, iso-butyrate and iso-valerate were dominant VFAs from the inoculum, but they were detected at less than 5 mM concentration during batch operation. Not only caproate formation, but also propionate and butyrate were detected at lower than 40 mM. Unlike previous research studies (Q. Yuan et al., 2011; P. Zhang, Chen, & Zhou, 2009) and Trial 1, acetate was not detected as a dominant VFA even though the pH was lower than 6.0 during Trial 2. Therefore, the production of C4-C6 VFAs at acidic conditions from anoxic sludge was a novel and unique result which requires further investigations.



(a) (b)

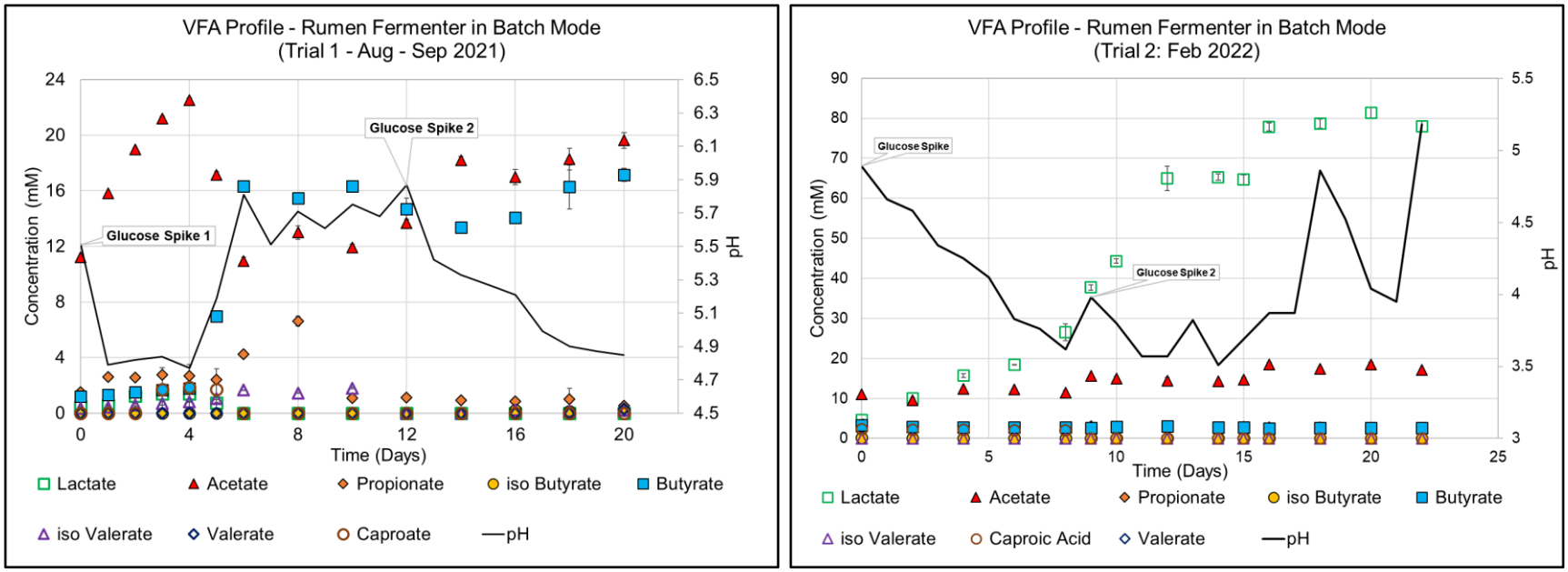
Figure 7: Sludge Batch Fermenter VFA profiles and pH range [(a): Trial 1 and (b): Trial 2]

Rumen Fermenter

Figure 8 (a) shows a distinct fermentation product profile during the first trial of the rumen fermenter due to butyrate formation in the middle of the process. Initially, acetate production gradually increased at a low pH range. When the pH was greater than 5.00, the butyrate production was boosted and shifted further to slightly lower the acetate concentration. After about 8 days of batch operation, acetate and butyrate were the predominant VFAs from glucose fermentation by the rumen inoculum. Previous studies mentioned that rumen pH influenced the VFA composition specifically more acetate and butyrate productions at low and high pH, respectively (Hu et al., 2006). Propionate exhibited a minor increase during the first 8 days, albeit at lower concentrations of around 2 mM, but stabilized at those low levels for the rest of the batch. Notably, around 1.76 mM caproate production and 1.64 mM iso-Valerate production were detected only between the 3rd and 5th days the and 6th and 10th day samples, respectively.

Trial 2 of the batch rumen inoculum with glucose addition demonstrated a sharp contrast in the VFA profile compared to Trial 1 (**Figure 8(b)**), as depicted by the significant lactate production (~80 mM) throughout the process. Lactate production gradually increased in the batch fermentation since lactate was detected as the dominant VFA from the rumen fluid inoculum. According to a previous study, that rumen pH would fall when lactate production increases due to metabolic acidosis (Hornbuckle et al., 2008). Nagaraja and Titgemeyer stated that lactate is responsible for getting low rumen pH due to D (-) lactate isomer in a racemic mixture of DL-lactate (Nagaraja & Titgemeyer, 2007). Although pH was adjusted through alkalization, it gradually decreased to 3.50 in the 1st half of the operation. While lactate rapidly increased, acetate production was stable between 10 mM and 20 mM concentration in the entire batch. Contrastingly, butyrate production in this second rumen trial did not exceed 5 mM concentration. In summary,

the second rumen trial was acclimated to lactate production instead of acetate and butyrate like the first rumen batch fermentation. According to a previous study on rumen culture, low pH played a role in less butyrate production in the Trial 2 (Hu et al., 2006). However, acetate production was not prevalent at low pH levels. Like the Trial 1, around 2.14 mM caproate was detected initially, although caproate was not detected in the rumen fluid inoculum in the Trial 2. The main logistical drawback of using cattle rumen fluid was the inability to obtain a larger quantity at once and frequently, unlike the anoxic sludge, coupled with a lower active biomass concentration.



(a) (b)

Figure 8: Rumen Batch Fermenter VFA profiles and pH trends [(a): Trial 1 and (b): Trial 2]

Chapter 4 - Selective Swine Wastewater Fermentation with different inocula as a function of HRT

Introduction

Many recent studies have shown that VFA generation occurs from different feedstocks such as food waste, municipal solid waste, dairy wastewater, and waste-activated sludge (Bolzonella et al., 2005; Demirel & Yenigun, 2004; Kuruti et al., 2017; S. J. Lim et al., 2008; Mengmeng et al., 2009). However, VFA generation from organic livestock waste such as animal manure (AM) is limited due to higher levels of ammonia content (Kuruti et al., 2017; Sukphun et al., 2021). Cow manure, chicken manure, poultry litter, and pig manure (swine wastewater) are the primary livestock organic wastes on many agricultural farms.

Chicken manure releases high concentrations of ammonia due to its higher protein content (D. min Yin et al., 2021). According to chicken manure fermentation of granular sludge, the highest net VFA yield was obtained with heat-shock under no initial pH condition, but alkaline pH condition is recommended to promote VFA production from chicken manure (D. min Yin et al., 2021). Cow manure contains more VFAs including little concentrations of medium-chain fatty acids (such as heptanoic- C7 and caprylic-C8) naturally, therefore it is an exceptional resource to produce VFAs as well as medium-chain VFAs. The maximum VFA yield was obtained from cow manure fermentation after thermal-heating, but eliminated an additional inoculum (Jomnonkhaow et al., 2021). Another study noticed the same observation from co-fermentation of cow manure with maze silage (Cavinato et al., 2017).

According to previous investigations, swine wastewater contains higher VFA concentrations than other animal manures and other wastes (Baek et al., 2020; Cooper & Cornforth, 1978; Møller et al., 2004). Previous investigations showed that swine wastewater

contains more VFA concentrations than cow manure (Baek et al., 2020; Cooper & Cornforth, 1978; Møller et al., 2004). Another research found a pig slurry contains more acetate and propionate naturally (Peu et al., 2004). Swine wastewater fermentation from a thermal-shocked anaerobic digested sludge was conducted to produce short-chain VFAs at three different initial pH conditions: 6, 8, and 10. It was reported that acetate was the dominant VFA, followed by propionate under all pH conditions, but the highest acetate concentration that was obtained was at initial pH 10 condition (W. Zhang et al., 2020). While acetate concentration increased with pH increase, propionate level was stable (W. Zhang et al., 2020). Notably, caproate production was detected swine manure fermentation at pH 10 (W. Zhang et al., 2020). It was reported that valerate and caproate productions could occur from protein-rich waste fermentation (Arslan et al., 2016). One study found that valerate and caproate were naturally found at less than 4% each of the total soluble organics in liquid swine manure and they could penetrate and kill the cells of microsclerotia fungus (Tenuta et al., 2002).

Other vital factors for acidogenic fermentation are HRT, SRT, pH, and temperature. HRT is a crucial variable in an anaerobic reactor since it is directly connectedto the initial capital investment (W. S. Lee et al., 2014). However, applying a longer or shorter HRT depends on the feedstock type as well as other operational conditions. Some studies mentioned that having a higher HRT is better since microbes can adapt to the substrate with time (Bengtsson et al., 2008; Ben et al., 2011; W. S. Lee et al., 2014; Sans et al., 1995; Wainaina et al., 2019), but it could promote to grow methanogens simultaneously which works against VFA generation (Sukphun et al., 2021). Consequently, HRT extension would not be helpful in the operation once the VFA production is stabilized (H. H. P. Fang & Yu, 2000; W. S. Lee et al., 2014; S. J. Lim et al., 2008). Few studies observed that an increase in HRT affected the VFA compositions by selecting for the

higher molecular VFAs such as valerate and caproate productions (Bolaji & Dionisi, 2017; Esteban-Gutiérrez et al., 2018b; Owusu-Agyeman et al., 2020; Shen et al., 2017; Wainaina et al., 2019).

SRT controls the predominant microbial populations within the anaerobic fermentation system (W. S. Lee et al., 2014). Many studies stated that a lower SRT is favorable for WAS and PS fermentation since it could inhibit the methanogenesis activities by washing out methanogens while increasing acidogens in an anaerobic reactor (Feng et al., 2009; W. S. Lee et al., 2014; Miron et al., 2000; Xiong et al., 2012). However, a sufficient SRT is required for the completionof hydrolysis activities in the WAS fermentation. Like other parameters, SRT also influences the VFA composition and depends on the substrate type due to the microbial population (W. S. Lee et al., 2014). However, the influence of SRT on VFA composition was contradictory due to contrasting influence from other operational conditions like pH and temperature (W. S. Lee et al., 2014).

pH is a critical parameter in acidogenic fermentation since it affects VFA production as well as composition (Ramos-Suarez et al., 2021; Sukphun et al., 2021). Acidogenic microbes have a broader pH range from 3 to 12, but an optimum pH is required to initiate the fermentation, depending on the feedstock type (Sukphun et al., 2021). Acidic pH (5-6) increased hydrolases while inhibiting methanogenesis, but low pH (<5) could inhibit acidogenic microbes (Ramos-Suarez et al., 2021). Some authors mentioned that alkaline pH (8 to 10) could improve substrate digestibility, especially for dissolving lignin in food waste, and inhibits methanogenesis (Feng et al., 2011; Ramos-Suarez et al., 2021; P. Zhang, Chen, & Zhou, 2009). However, when the pH increases to 11 or above, the acidogenesis activities reduce due to toxicity from strong alkaline conditions (Ramos-Suarez et al., 2021). Another study proved that alkaline and neutral pH

conditions favor degradation of soluble proteins and lipids more efficiently than under acidic conditions (Ramos-Suarez et al., 2021). According to previous studies, acidic pH produces propionate and butyrate, while alkaline pH contributes to acetate production (Garcia-Aguirre et al., 2017; Ramos-Suarez et al., 2021; Wu et al., 2009). Therefore, optimum pH mostly depends on the substrate type and is required to maintain in operation to obtain the highest VFA production since pH significantly impacts the hydrolysis process (Ramos-Suarez et al., 2021).

Like pH, temperature is essential in fermentation since it directly involves microbial growth, metabolism, and hydrolysis rates. Different temperature conditions could bring different VFA distributions from microbial fermentation involving other parameters such as inoculum, substrate, and pH (Hao & Wang, 2015). When a mesophilic temperature was raised to a thermophilic level, acetate and iso-valerate concentrations were increased while propionate and butyrate declined (Hao & Wang, 2015). However, there are drawbacks of using thermophilic conditions: i) temperature influence is minor for the VFA composition, unlike pH (W. S. Lee et al., 2014) ii) longer retention times are required due to slow kinetics (Ramos-Suarez et al., 2021) iii) higher operational cost is required (Ramos-Suarez et al., 2021), and iv) anaerobic microbes are sensitive to a sudden or minor environmental change at thermophilic conditions (W. Fang et al., 2020). When temperature changes from psychrophilic to mesophilic in the WAS fermentation, total VFA concentrations could enhance more than 2-folds due to soluble carbohydrate and protein concentrations at higher temperatures (W. S. Lee et al., 2014; Q. Yuan et al., 2011; P. Zhang, Chen, Huang, et al., 2009). From WAS fermentation, acetate was prevalent at any temperature and pH, but percentages of other VFAs did not change with temperature changes (W. S. Lee et al., 2014; Q. Yuan et al., 2011; P. Zhang, Chen, Huang, et al., 2009). In animal manure fermentations, the highest VFA concentrations were obtained at a mesophilic temperature level since hyper-

thermophilic temperature inhibited the growth of acidogenic microbes (Nozhevnikova et al., 1999). Like pH, temperature influences total VFA production, and VFA composition would be altered due to different waste streams and pH conditions.

Methods

Experimental Process

After completing the glucose-enriched fermentation experiments for both sludge and rumen fermenters in batch mode, swine lagoon wastewater was added as the only substrate (influent) to both fermenters in semi-continuous mode. We operated the fermenters at three different SRT (=HRT) scenarios: 20, 15, and 10-days. Similar to the glucose-enriched fermentations, we replicated trials for all SRT regimes and both inocula. We removed the specific amount of reactor content (solids + liquids) from both fermenters daily, corresponding to each SRT (=HRT). For instance, a volume of 60 mL was removed from the sludge reactor, and an equal amount of swine lagoon wastewater was added. **Table 5** shows the volumes of removal from the fermenter and swine wastewater addition for each HRT mode. The reactor volumes were maintained at 600 at mL and 500 mL for anoxic sludge and rumen fermenters, respectively.

Table 5: Daily Effluent Removal and Influent Volumes

SRT(=HRT) Mode (Days)	Daily Effluent and Influent Volume (mL)	
	Anoxic Sludge (AS) Fermenter	Rumen Fermenter
20	30	25
15	40	33
10	60	50

Due to the higher pH range in swine wastewater, we adjusted the influent pH to around 5 pH daily through acidification by adding 1 M HCl acid to continue the acidogenic fermentation. However, we added solid contents from the effluents back into the fermenters on certain days to

avoid washout of the inoculum entirely. We centrifuged the effluent samples and mixed the pellets (fermentative solid) with the swine wastewater influent in the anaerobic glove box (to avoid inactivation of the anaerobic microbes) and adjusted to 5 pH before adding them to the fermenters. Both fermenters were kept at the same temperature on the same stirring plate with the same stirring speeds as mentioned in the glucose-enriched fermentation studies (Chapter 3).

Analytical Methods

pH values of fermenter effluent and swine wastewater influent were measured daily using a Fisherbrand Accumet AB150 pH benchtop meter. Influent pH was adjusted daily to around 5 pH by adding 1 – 3 mL of 1 M HCl. If the effluent pH was lower than 4.50, then influent pH adjustment was not conducted since swine wastewater has a good buffering capacity range (Esquivel-Elizondo et al., 2016), which could increase the reactor pH. However, when the effluent pH dropped lower than 4, then 1 M sodium hydroxide (NaOH) was added to the fermenter directly to increase the pH in the reactor to be stable at around 5. The effluent pellets were mixed with influent swine wastewater. After that, influent pH values were measured using an Oakton pH 550 benchtop pH meter situated within the vinyl anaerobic chamber to avoid oxygen exposure to the anaerobic microbes. Figure 9 shows the centrifuged anoxic sludge and rumen effluents from the first trial in 20-day SRT/HRT mode.

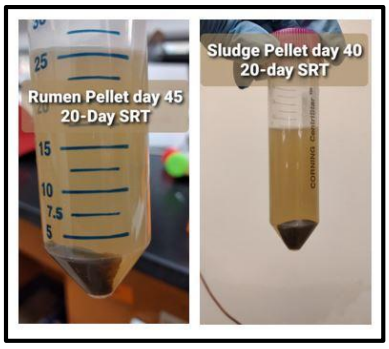


Figure 9: Fermenter Effluents in 20-Day SRT Mode

We used the same VFA quantification HPLC technique as mentioned in Chapter 3 to detect VFAs from the effluent samples, and influent swine wastewater samples during Trial 2 at various SRT modes for targeting any specific VFA presence in the swine wastewater. Chemical oxygen demand (COD) analysis was conducted by converting all VFA concentrations in each effluent sample to COD values (in mg/L) through electron balances as mentioned below in equations and Table 6.

Lactate: $2CO_2 + HCO_3^- + 12H^+ + 12e^- \rightarrow CH_3CHOHCOO^- + 4H_2O$ (Eq 1)

Acetate: $CO_2 + HCO_3^- + 8H^+ + 8e^- \rightarrow CH_3COO^- + 3H_2O$ (Eq 2)

Propionate: $2CO_2 + HCO_3^- + 14H^+ + 14e^- \rightarrow CH_3CHOHCOO^- + 5H_2O$ (Eq 3)

iso-Butyrate/Butyrate: $4CO_2 + HCO_3^- + 20H^+ + 20e^- \rightarrow CH_3(CH_2)_2COO^- + 6H_2O$ (Eq 4)

iso-Valerate/Valerate: $5CO_2 + HCO_3^- + 26H^+ + 26e^- \rightarrow CH_3(CH_2)_3COO^- + 8H_2O$ (Eq 5)

Caproate: $6CO_2 + HCO_3^- + 32H^+ + 32e^- \rightarrow CH_3(CH_2)_3COO^- + 10H_2O$ (Eq 6)

All electrons for each VFAs per mole were mentioned in following Table 6. They were multiplied by 8, because 8 g COD is equivalent for one electron.

Table 6: COD Conversions in VFAs

VFA Type	Number of electrons per mole	Conversion factor from mM to mg COD/L
Lactate	12	96
Acetate	8	64
Propionate	14	112
iso-Butyrate	20	160
Butyrate	20	160

iso-Valerate	26	208
Valerate	26	208
Caproate	32	256

For instance, when we assume 10 mM lactate was detected in the effluent sample and it was converted to COD values according to the following equation:

$$10\ \frac{\text{mmol}}{\text{L}} \times \frac{1\ \text{M}}{1000\ \text{mmol}} \times \frac{12\text{e}^-}{1\ \text{mol}} \times \frac{8\ \text{g COD}}{1\ \text{e}^-\text{eq}} \times \frac{1000\ \text{mg}}{\text{L}} = 960\ \frac{\text{mg COD}}{\text{L}} \quad \text{(Eq 7)}$$

The above calculations were performed to all VFAs in effluent samples from both Trials as well as influent samples in Trial 2.

Net VFA-COD ratios were calculated as mentioned in the following equations and example:

$$\text{Net VFA} - \text{COD Ratio} = \frac{\text{Net VFA Production}}{\text{Influent VFA content}} \quad \text{(Eq 8)}$$

$$\text{Net VFA Production} = \frac{\text{Effluent VFA content (mg)} - \text{Influent VFA content(mg)}}{\text{Reactor Volume (L)}} \quad \text{(Eq 9)}$$

$$\text{Effluent VFA content} = \text{Total VFA in Effluent}\ \left(\frac{\text{mg}}{\text{L}}\right) \times \text{Reactor Volume (L)} \quad \text{(Eq 10)}$$

$$\text{Influent VFA content} = \text{Total VFA in Influent}\ \left(\frac{\text{mg}}{\text{L}}\right) \times \text{Influent Volume (L)} \quad \text{(Eq 11)}$$

Example: The volume of the sludge fermenter is 0.6 L and influent volume is 30 mL. Then, VFA contents in both effluent and influent can be found by using the above equations. Then, new VFA production from a certain effluent sample could be determined (whether the fermentation occurred sufficiently or not).

COD analysis was performed for swine wastewater samples in the second trial to see if there was any VFA detection. Also, the soluble chemical oxygen demand (SCOD) values of the fermenter effluents and swine wastewater influents were measured using HACH 822 kits, ranging from 20-1500 mg/L concentration. HACH DR 3900 spectrophotometer was used to analyze the

HACH 822 kit vials. Effluent and influent samples were filtered through 0.45 µm PVDF syringe filters before doing the SCOD measurements. Gas-Chromatography (GC) with a thermal conductivity detector (TCD) was used to measure the methane gas production in the sampling gas bags of both fermenters. Argon was the carrier gas at a flow rate of 8.8 mL/min and pressure of 88.8 kPa. Temperatures of injection, column, and detector were 200, 80, and 150 ℃, respectively. TSS and VSS of the effluent samples were conducted according to the standard methods 2540D and 2540E, respectively.

Results and Discussion

Swine Wastewater as a Substrate

After completing glucose-enriched batch fermentation experiments for both inocula, we initiated the addition of swine wastewater as a substrate to both fermenters. Swine wastewater is one of the complex substrates since animal manure has more biodegradable complex organic matter and requires more time to break them into monomers for VFA generation (Kuruti et al., 2017). Also, swine wastewater has a better buffering capacity due to higher ammonia levels than other animal manure types (C. Zhang et al., 2013). Therefore, it could help to prevent strong pH drops due to accumulated VFAs in the fermentation broth. The pH of the swine wastewater samples was checked daily before adding to the reactor and adjusted at around pH 5.5 using 1 M HCl. pH and VFA analysis were demonstrated below. Also, COD analysis was done with influent wastewater soluble COD measurements as described below. We collected swine wastewater samples from a lagoon (**Figure 10**) near a swine farm and the AnMBR trailer at Manhattan, KS.

Figure 10: Swine Lagoon with the AnMBR trailer, Manhattan, KS 66502

Anoxic Sludge Fermenter

Effluent VFA Analysis – Anoxic Sludge Fermenter

20-Day HRT Mode

Figure 11 shows the VFA profiles of the sludge effluent samples during the 20-day HRT modes in both trials. According to **Figure 11 (a)**, effluent pH gradually increased from 5 to 7 with fluctuations in the entire 20-day HRT operation. When effluent pH exceeded 5.50, acid addition to the influent was raised to continue the VFA generation. Lactate detection exceeded 100 mM initially, and the same pattern was observed from the first trial of the glucose-enriched fermentation in the sludge fermenter. Therefore, lactate production from this sludge fermenter could likely have occurred from glucose carry-over along with the inoculum. Consequently, lactate was not detected after the 14^{th} day, likely due to gradual dilution of the carryover lactate, except on the 44^{th} day sample.

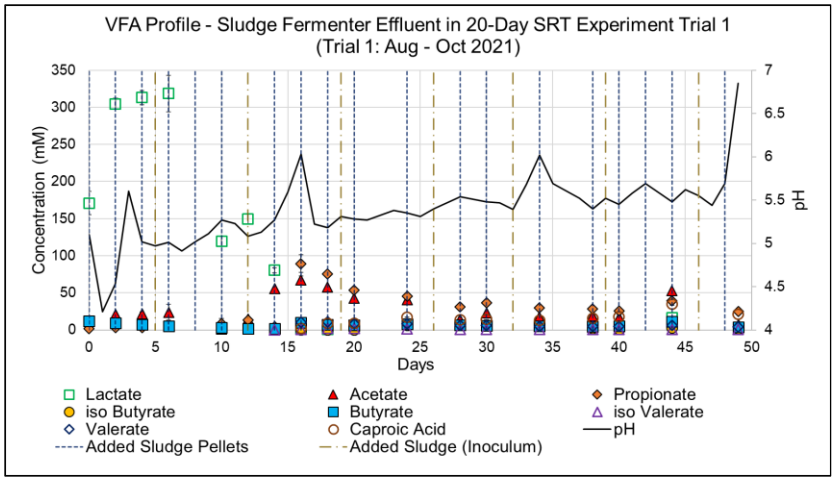
Figure 11 (b) shows the VFA profile without lactate presence in the sludge fermenter for better visualization of the other VFA profiles. Acetate and propionate were the leading VFAs in the whole 20-day SRT operation. The highest acetate and propionate concentrations were detected at 67 mM and 90 mM, respectively, on the 16^{th} day effluent sample, while the effluent pH was around 6.00. Butyrate production was stable throughout the process and did not exceed 11 mM.

Valerate was detected on the 16th-day and was stable at less than 10 mM until the end of the process. Also, caproate formation was detected on the same day and increased to 35 mM as the highest concentration on the 44th-day effluent sample.

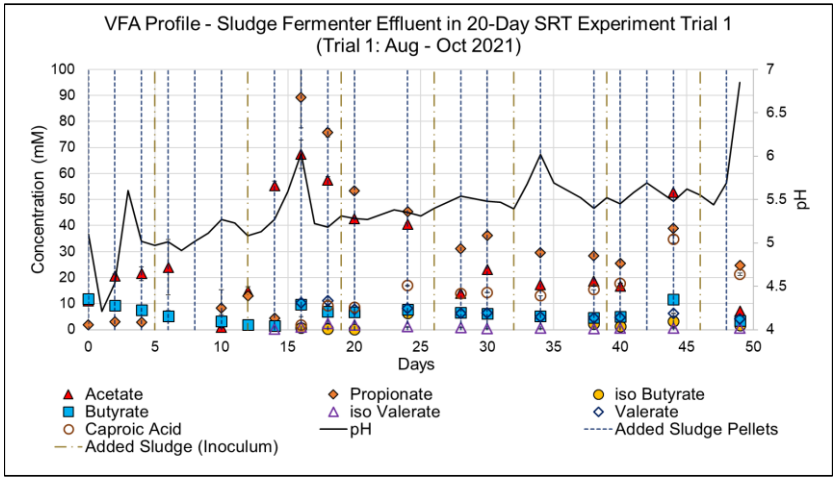
Figure 11 (c) shows the second trial for the sludge fermenter with swine wastewater in 20-day SRT mode. Unlike the first trial, the second trial had a pH decrease with minor fluctuations throughout the process. Moreover, a different VFA profile was obtained due to caproate presence throughout the operation. Caproate formation also occurred in the second glucose-enriched sludge batch operation. This could likely explain the initial detection of caproate in the second trial of the 20-day SRT mode, along with the detection of caproate in the influent swine wastewater, as explained later. Caproate production had a downward trend from 140 mM to 40 mM. However, other VFAs have been detected at lower than 20 mM throughout the process.

Figure 11 (d) shows the VFA distribution except for caproate in the second trial of 20-day SRT. Initially, propionate was the second dominant VFA in the second trial, but it shifted to acetate from the 6th-day effluent sample. After 10 days of operation, butyrate production was slightly increased, but it gradually declined like acetate and propionate until the end. Around 2 mM iso-butyrate and iso-valerate VFAs were detected, while around 5 mM butyrate production was detected between 5th and 10th days. Also, when butyrate production was increased to 4 mM between 24th and 27th days, valerate was detected at about 2 mM. Therefore, when butyrate production occurred considerably, other VFAs could also be detected from the sludge inoculum through swine wastewater fermentation in the 20-day SRT mode. Higher caproate concentrations from this study (Trial 2) could have occurred due to its presence in the swine wastewater influent (**Figure 16**). Caproate synthesis from swine wastewater of anoxic sludge under an acidic pH environment could be a unique feature, since a previous study stated that caproate synthesis was

performed from a short-chain VFA mixture at pH 10 in a two-stage process (W. Zhang et al., 2020).



(a)



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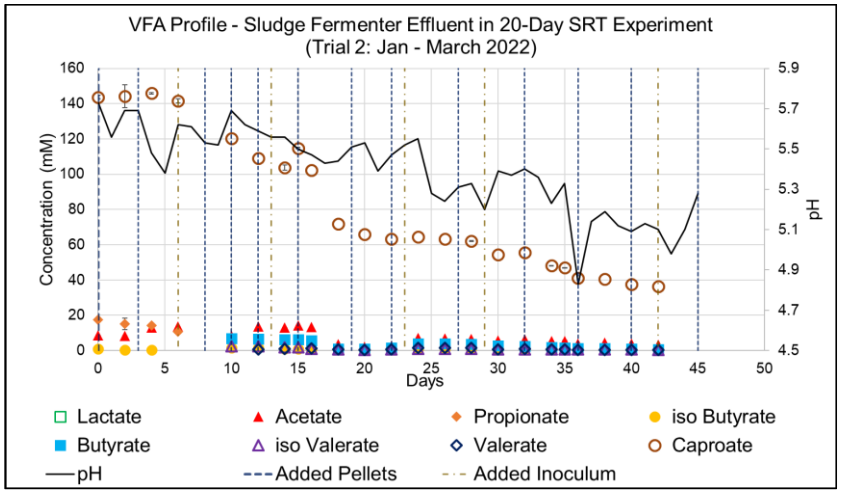
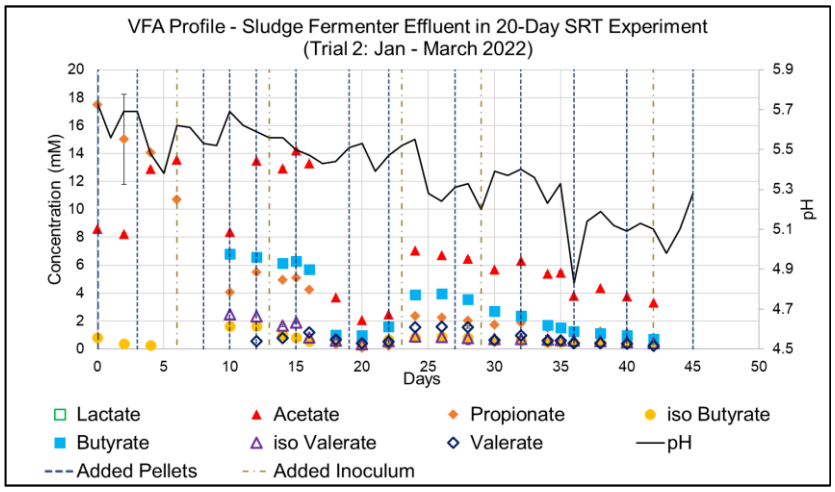


(c)



(d)

Figure 11: VFA Analysis for Sludge Fermenter in 20-Day HRT Mode for Trial 1[(a): all VFAs and (b): except lactate] and Trial 2 [(c): all VFAs and (d): except caproate

15-Day HRT Mode

Figure 12 shows a shift in the VFA profile in the sludge fermenter when the SRT mode was changed from 20 to 15-day. In the beginning, pH went beyond 6.0 and was stable around 6.0 for ten days, probably due to raised swine wastewater additions to the fermenter. During that period, propionate was the dominant VFA, followed by acetate. However, when the pH started to drop, caproate formation occurred in the middle of the operation, but it did not continue when the pH went lower than 5.0. Compared to other VFAs, caproate formation was a significant feature due to its higher concentrations. Propionate gradually decreased from 21 to 0.6 mM until the end. Acetate generation was stable at less than 10 mM in operation. Butyrate and valerate had almost similar downward trends throughout the process.

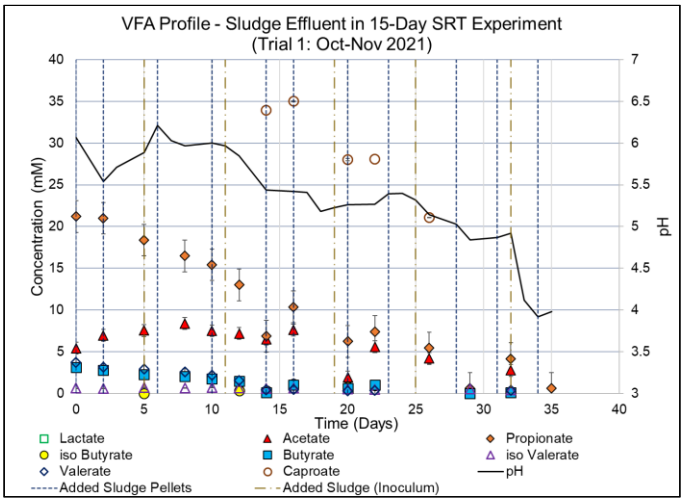
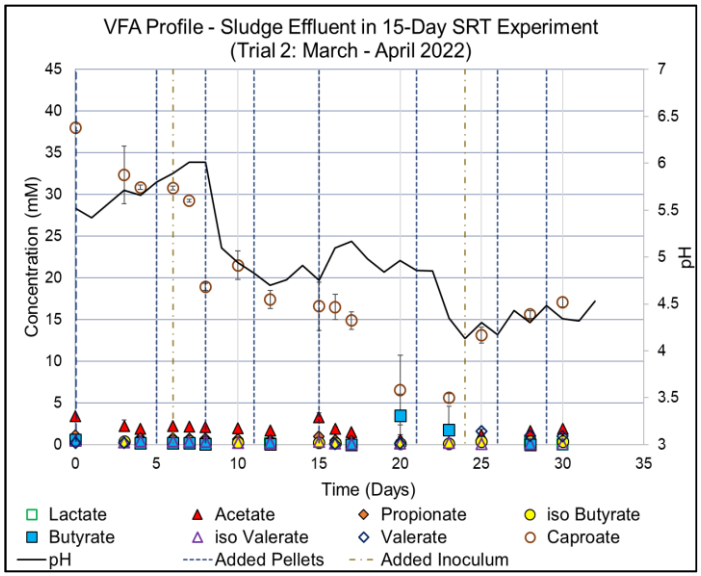


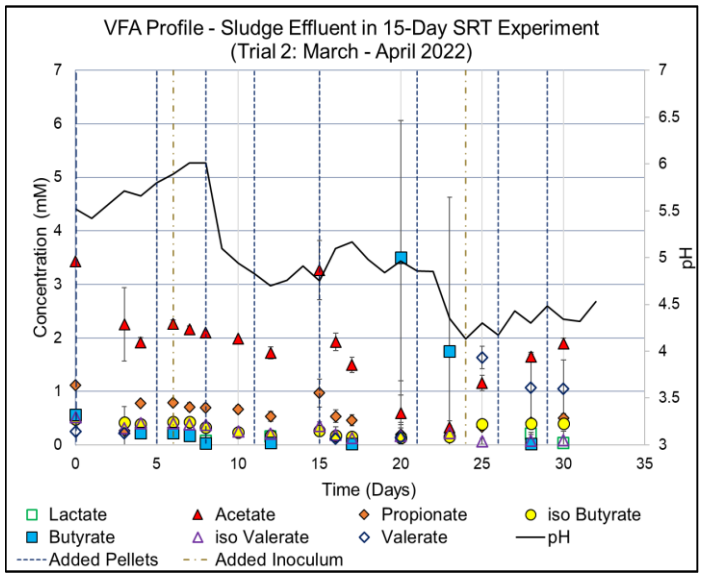
Figure 12: VFA Analysis for Sludge Fermenter in 20-Day HRT Mode for Trial 1

Figure 13 (a) shows the VFA profile of the sludge fermenter in the second trial of the 15-day SRT mode. Caproate was prevalent in the second trial of the 15-day SRT mode, like in the second trial of the 20-day SRT mode. Initially, pH was raised to 6.00 during the first trial of the 15-day SRT operation. After that, pH declined to 4.50 with few fluctuations. Figure 10 (b) shows the VFA distribution except for the caproate. Acetate and propionate productions were detected

at less than 4 mM and 2 mM, respectively. However, butyrate was detected at less than 1 mM in the first portion except for the 20th and 23rd-day effluent samples. Valerate production was detected at less than 2 mM at the end of the process.



(a)



(b)

Figure 13: VFA Analysis for Sludge Fermenter in 15-Day HRT Mode for Trial 1[(a): all VFAs] and Trial 2 [(b): except caproate

10-Day HRT Mode

Figure14 displays the VFA analysis from the sludge fermenter during the 10-day HRT operation in Trial 1. After completion of 20 and 15-day HRT modes, the sludge fermenter underwent the 10-day HRT mode with an increasing volume of effluent removal and swine wastewater addition daily. According to **Figure 14**, we observed a washout with minor VFA production in the operation's first half due to rapid pH increase above 6. When the pH went below 4, caproate and valerate concentrations gradually increased while the pH was stable below 5.5. Regardless, caproate was detected above 13 mM at the end of the process despite the pH rise to 6 in Trial 1, likely influenced to some extent by the presence of caproate in the influent wastewater.

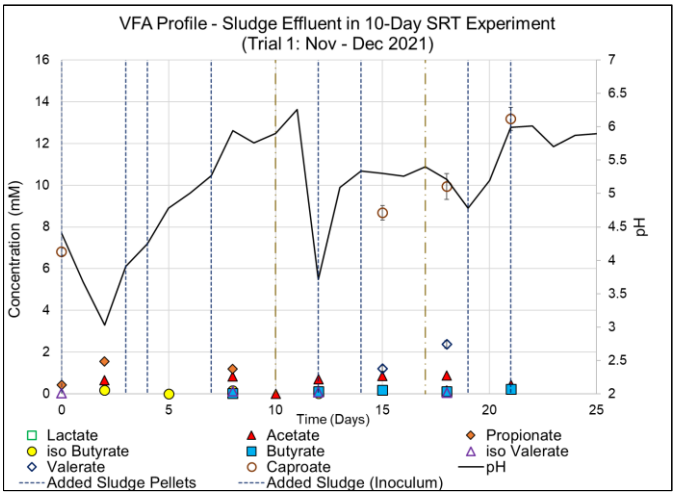
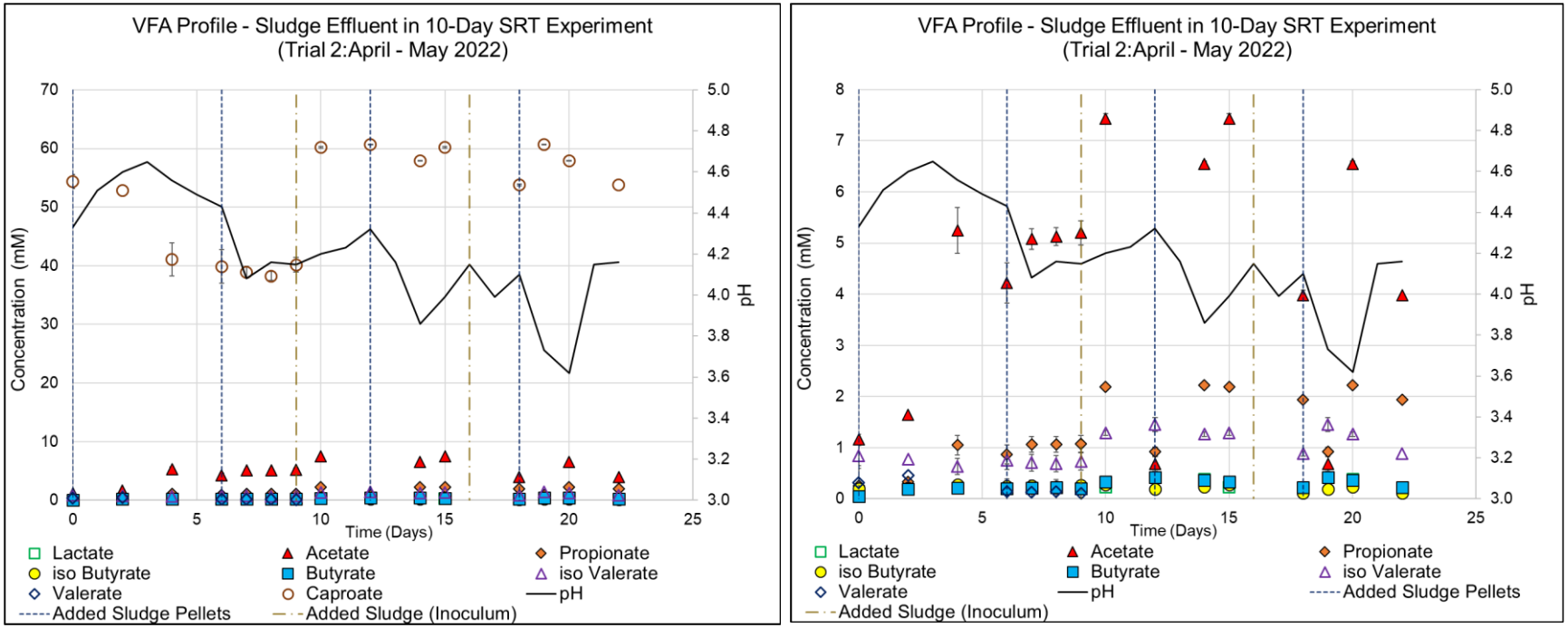


Figure 14: VFA Analysis for Sludge Fermenter in 10-Day HRT Mode for Trial 1

Similar to Trial 1, caproate was dominant in Trial 2, according to **Figure 15 (a)**. Although the volume of wastewater addition was increased, the fermenter effluent pH significantly dropped below 3.8 with some fluctuations. In the first half of Trial 2, caproate production declined from 55 mM to 40 mM, while it was stable at around 60 mM in the next half. **Figure 15 (b)** shows the VFA profile of Trial 2 without caproate presence for better visualizing other VFA distributions. Acetate

and propionate productions were generated in Trial 2, below 8 and 3 mM, respectively. Besides main VFAs, iso-valerate was detected below 2 mM in the entire process.

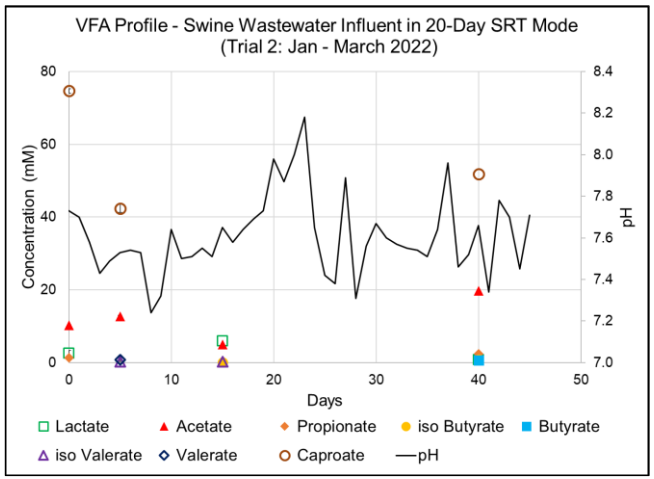


(a) (b)

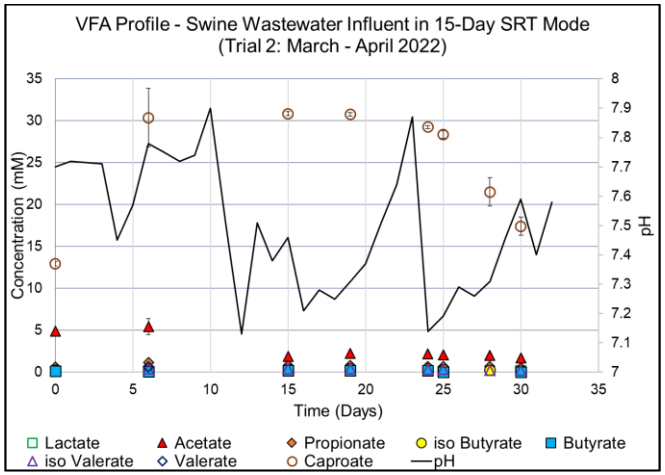
Figure 15: VFA Analysis for Sludge Fermenter in 10-Day HRT Mode for Trial 2[(a): all VFAs (b): except caproate]

Influent VFA Analysis – Anoxic Sludge Fermenter

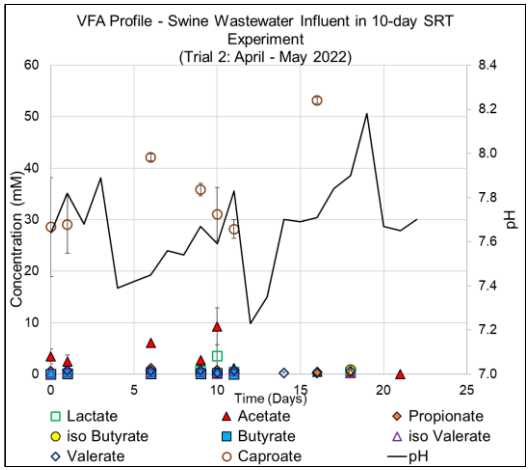
Figure 16 displayed the VFA distribution from the swine wastewater, the only influent for the sludge fermenter. In the second trial of all operations, we frequently analyzed influent swine wastewater samples to detect any VFA presence and whether they could influence the effluent VFA productions. Caproate was prevalent in the influent of all SRT operations. Therefore, caproate presence could be influenced to detect the higher caproate concentrations in the sludge effluents. Although caproate detection was not consistent in the 20-day mode, it was stable at 30 and 40 mM in the 15 and 10-day SRT modes, respectively. Though swine wastewater has a higher pH range, acetate was detected as the second leading VFA, but less than 20 mM in all SRT modes. Furthermore, none of the VFAs were detected on some days in the middle of 20 and 10-SRT modes. Influent was added to the sludge fermenter through acidification to 5 pH due to the influent pH variation between 7 and 8.5.



(a)



(b)



(c)

Figure 16: VFA Analysis in Influent Swine Wastewater in Trial 2 [(a): 20-day SRT Mode, (b): 15-day SRT Mode, and (c): 10-day SRT Mode]

Effluent VFA Analysis – Rumen Fermenter

20-Day HRT Mode

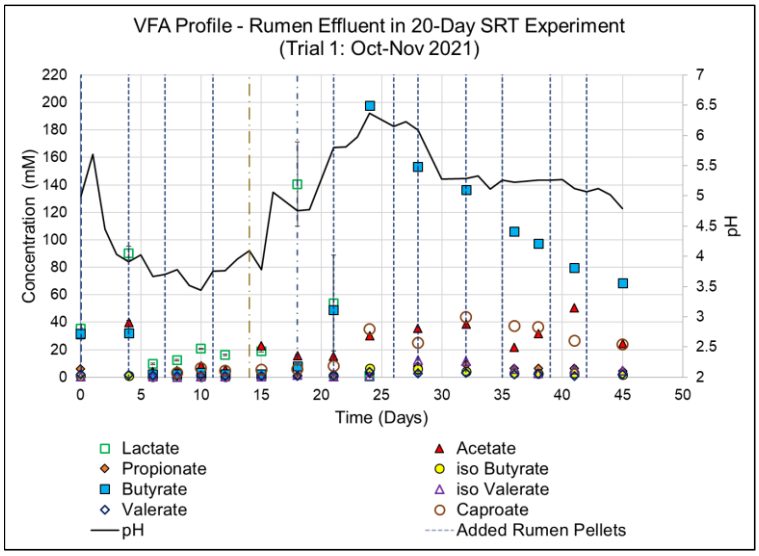
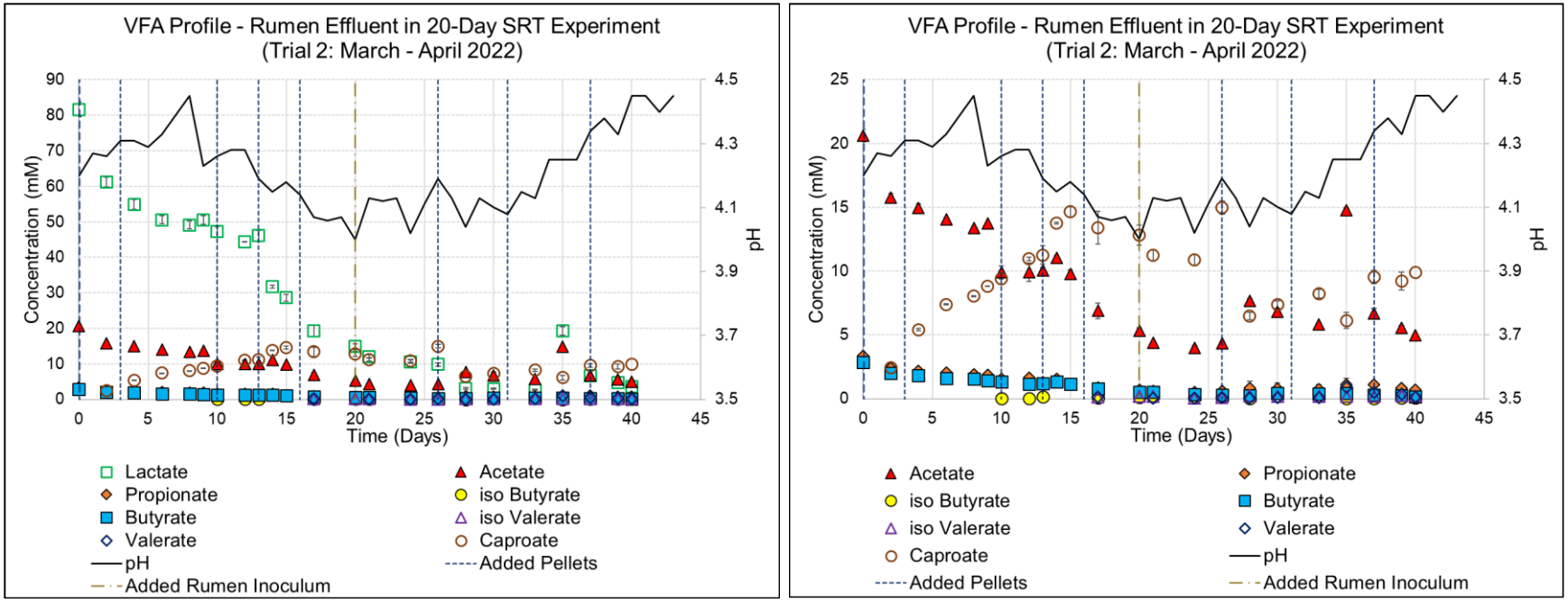


Figure 17: VFA Analysis for Rumen Fermenter in 20-Day HRT Mode for Trial 1

According to **Figure 17**, rumen effluent pH dropped quickly to 3.5 during the first 10-days in the 20-day SRT mode Trial 1. Therefore, the wastewater influent was adjusted with 1 M sodium hydroxide to bring the fermenter pH to around 5. Around 30 mM butyrate was initially detected with higher lactate concentrations. Around 10 mM lactate was the leading VFA during the low pH scope. A study mentioned that more lactate production caused to a fall in pH due to metabolic acidosis (Hornbuckle et al., 2008). Although lactate production gradually increased under a low pH (<4 pH) environment, acetate, butyrate, and caproate concentrations increased with the pH adjustment of the fermenter. Although pH went beyond 6 after finishing the first half of the operation, butyrate production increased by 4-folds and became dominant throughout the second half of the process. According to a study on fermentation from rumen fluid, higher pH influenced butyrate production, which is aligned with this study, even though a different substrate was used (Hu et al., 2006). Since fermenter pH was above the experimental condition (5.5. pH), wastewater

influent was acidified to maintain the fermenter pH below 5 to continue getting VFA productions. When the pH dropped and was stable at around 5, butyrate concentrations gradually decreased to 60 mM at the end. Besides the butyrate decrease, acetate and caproate concentrations were stable at around 30 mM until the end of the process.



(a) (b)

Figure 18: VFA Analysis for Rumen Fermenter in 15-Day HRT Mode for Trial 2[(a): all VFAs (b): except lactate

Figure 18 (a) shows the lactate dominant VFA profile from the second trial of swine wastewater fermentation with rumen fluid. Lactate production was initially detected at around 80 mM. During the glucose-enriched period, lactate production increased to 80 mM in Trial 2 and declined when the swine wastewater addition was started withpH fluctuations between 4.0 and 4.5. According to **Figure 18 (b)**, acetate production declined from 20 to 5 mM throughout the process, although low rumen pH influences more acetate production (Hu et al., 2006). Caproate production increased and stabilized at around 15 M in the middle of the operation, but it dropped with the pH rise. Therefore, controlling pH was a crucial step in the acidogenic fermentation of the rumen fluid since it directly affects the VFA composition. However, propionate and butyrate concentrations were detected below 5 mM and were diminished during the operation.

15-Day HRT Mode

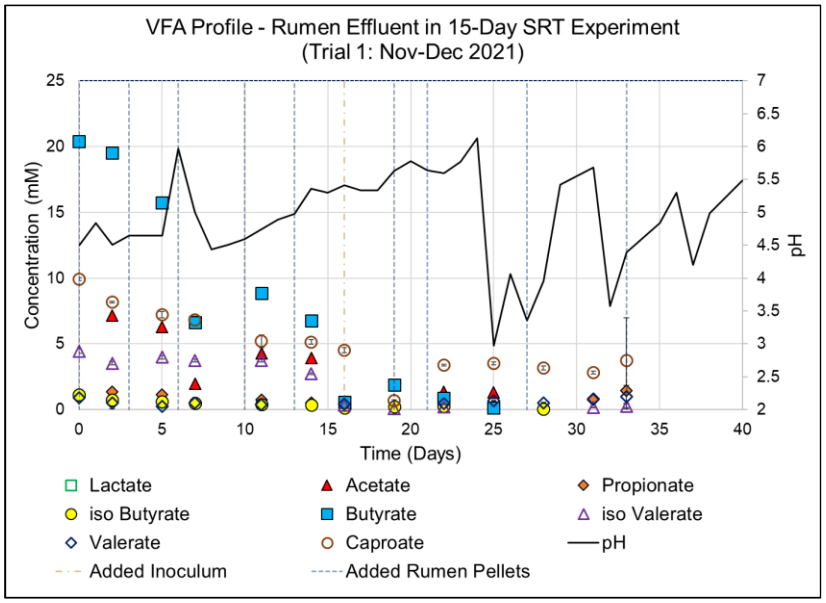
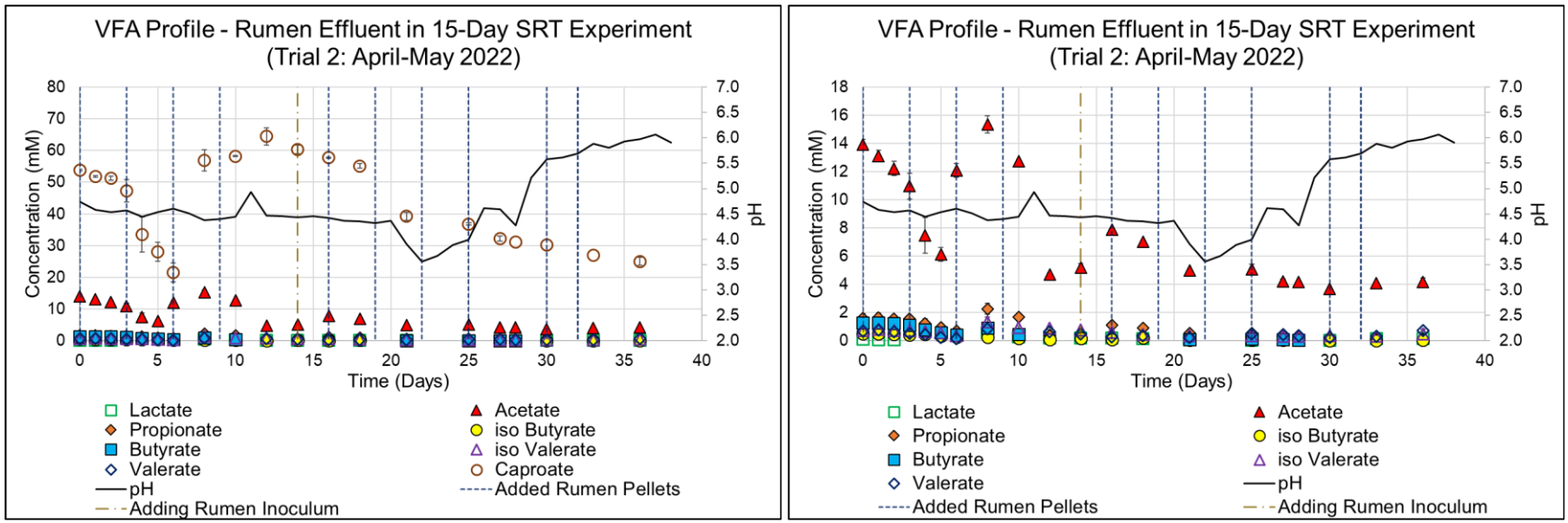


Figure 19: VFA Analysis for Rumen Fermenter in 15-Day HRT Mode for Trial 1

Figure 19 exhibits a drastic shift in VFA compositions in the rumen fermenter when SRT mode was switched to 15-day in the first trial. Fermenter pH increased rapidly to 6.0 due to

increased swine wastewater addition. Furthermore, butyrate buildup from the 20-day SRT mode declined entirely after 25 days of operation, even though higher pH was suitable for butyrate production from rumen culture. Therefore, the type and composition of substrate highly influenced the VFA composition in fermentation. Acetate, iso-valerate, and caproate concentrations declined in the first operation while pH was increased. Since daily acid addition to the influents did not work accurately, fermenter pH was adjusted directly to maintain a pH of around 5. Although pH fluctuated afterward, caproate production was stable around 3 mM while others were not. Since a washout occurred in the rumen fermenter, we wrapped the fermentation process without proceeding to 10-day SRT mode due to two possible reasons: i) ruminal bacterial communities are sensitive to the prolonged exposure to swine wastewater, and ii) lower biomass concentration compared to the sludge fermenter.

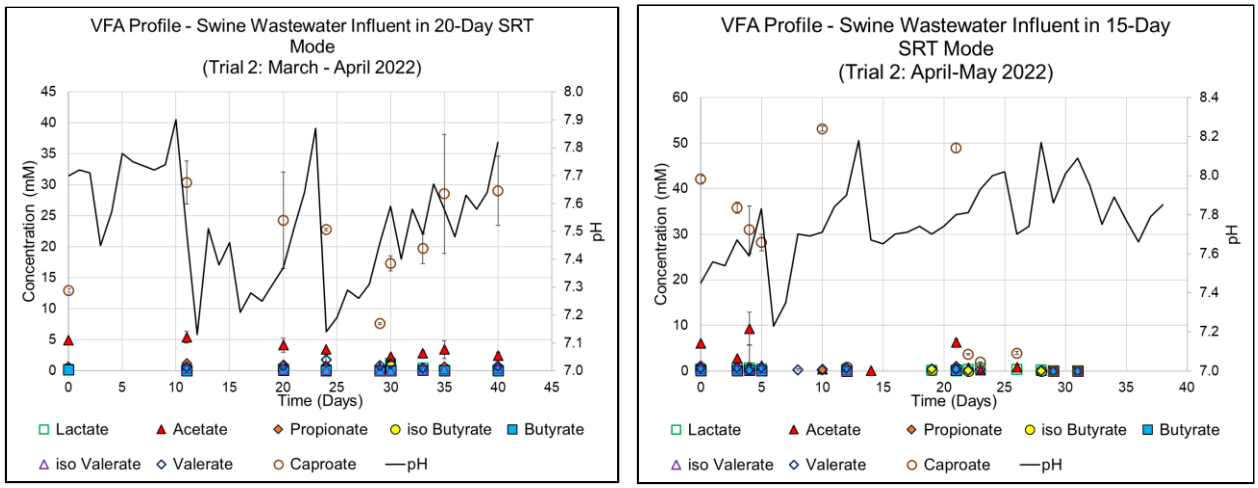


(a) (b)

Figure 20: VFA Analysis for Rumen Fermenter in 15-Day HRT Mode for Trial 2[(a): all VFAs and (b): except caproate

On the other hand, **Figure 20** displayed a distinct VFA profile with caproate prevalence in Trial 2 of the 15-day SRT operation from the rumen fermenter. Also, fermenter pH was controlled intensively and maintained not to exceed 6 in the first half of the process to avoid a washout like Trial 1. Initially, 50 mM caproate and 14 mM acetate were detected and dropped to half of that value for the latter period of operation. However, caproate production was stable at around 60 mM and dropped to 25 mM at the end of the process. According to previous studies on caproate and medium-chain VFA synthesis, caproate production could occur from swine wastewater fermentation, with and without addition electron donor. In 15-day SRT day Trial 2 from the rumen fermenter, caproate production could likely occur due to more lactate production. Acetate production was further dropped to 4 mM with fluctuations while the pH was raised to 6. Butyrate and propionate concentrations were initially detected at less than 2 mM and dropped entirely before the end.

Influent VFA Analysis – Rumen Fermenter



(a) (b)

Figure 21: VFA Analysis in Influent Swine Wastewater in Trial 2 [(a): 20-day SRT Mode, and (b): 15-day SRT Mode]

Figure 21 shows VFA patterns in the swine wastewater influent for the rumen fermenter in both 20 and 10-day SRT operations. Influent pH varied from 7.1 and 8.2 in both periods. Caproate prevalence in the second trial of the rumen fermenter was directly related to the presence of caproate in the influent. However, caproate presence was not uniform in the swine wastewater. Acetate was detected as the second leading VFA in the swine wastewater samples with less than 10 mM in both trials. Small amounts of other VFAs such as butyrate, propionate, and iso-butyrate were occasionally detected in the influent wastewater.

Caproate Peak Confirmation from HPLC

Detecting caproate in Trial 2 experimental rounds was a remarkable finding because it was prevalent in almost all SRT operations except for the 20-day SRT mode in the rumen fermenter. Therefore, we decided to verify the sample peaks by overlaying with the peaks of an external standard, 10 mM of 9 VFA standard mixture and 50 mM caproate. According to the Shimadzu HPLC program, peaks of unknown samples were detected and auto-assigned as a specific compound when they fell into the 5% window from the retention times of the standard. Also, unknown peaks were compared with standard peaks by overlaying the chromatograms. **Figures 22** and **23** show sludge and rumen samples have symmetrical and broadening peaks, whereas caproate standard peaks have left-tailing. Since the sample peak falls in the 5% retention time window area from the Shimadzu software, we decided to accept that the sample contains caproate. The same procedure was applied to confirm whether the last broader peak would appear in the water samples (**Figure 24**) and it was proved that caproate peak was not detected in the DI water samples.

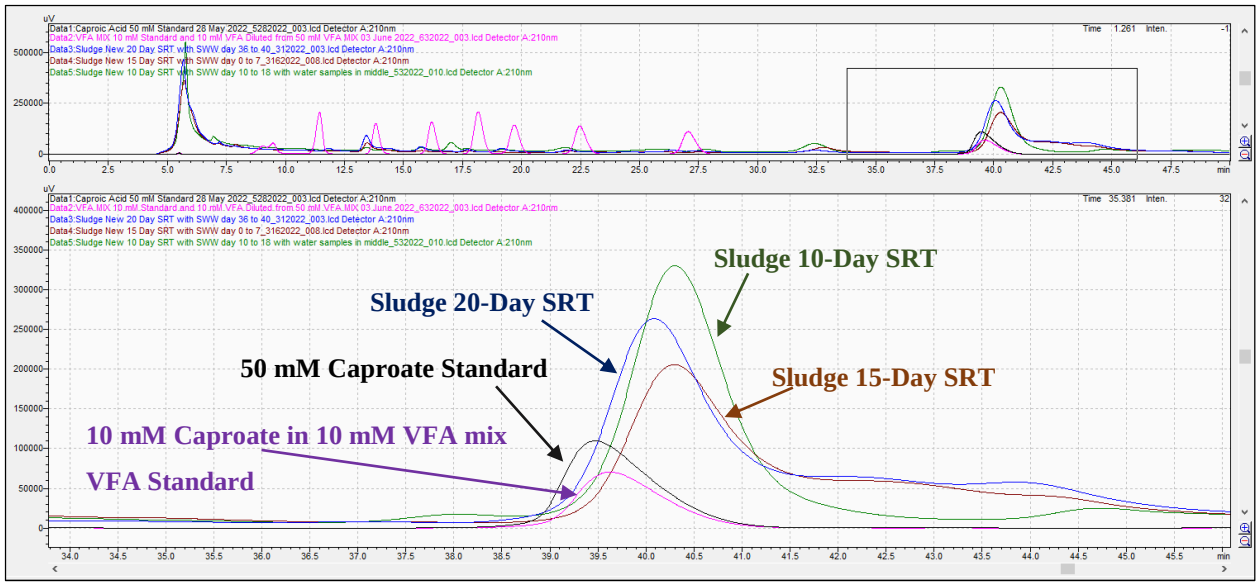


Figure 22: Figure 19: Caproate Peak Confirmation in Sludge Samples

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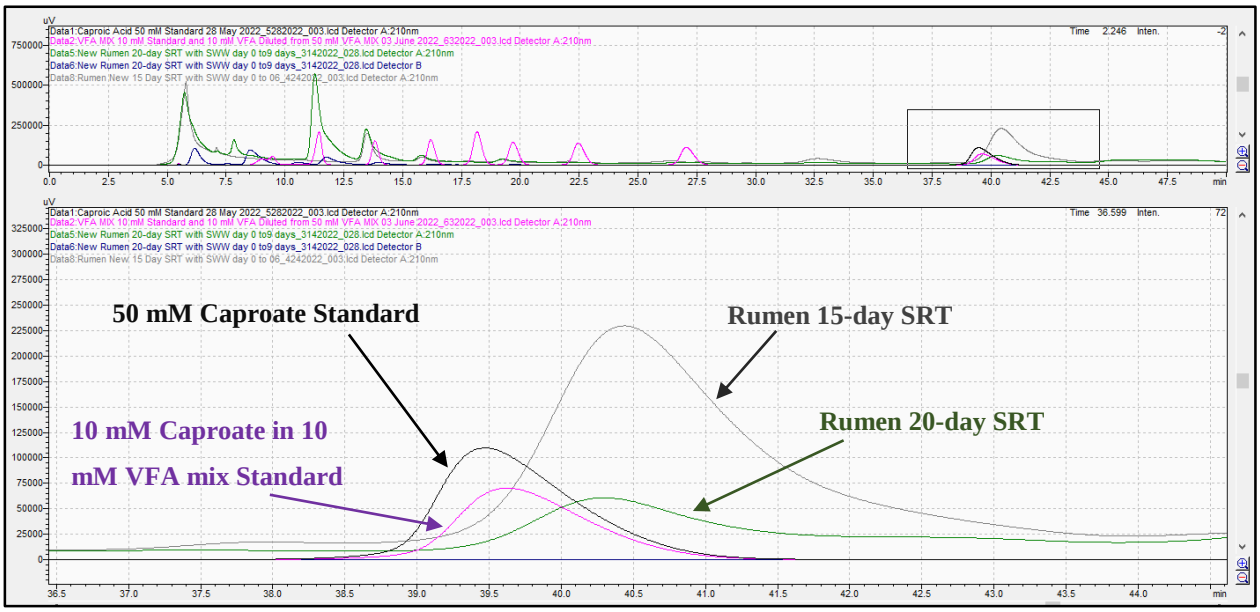


Figure 23: Caproate Peak Confirmation in Rumen Samples

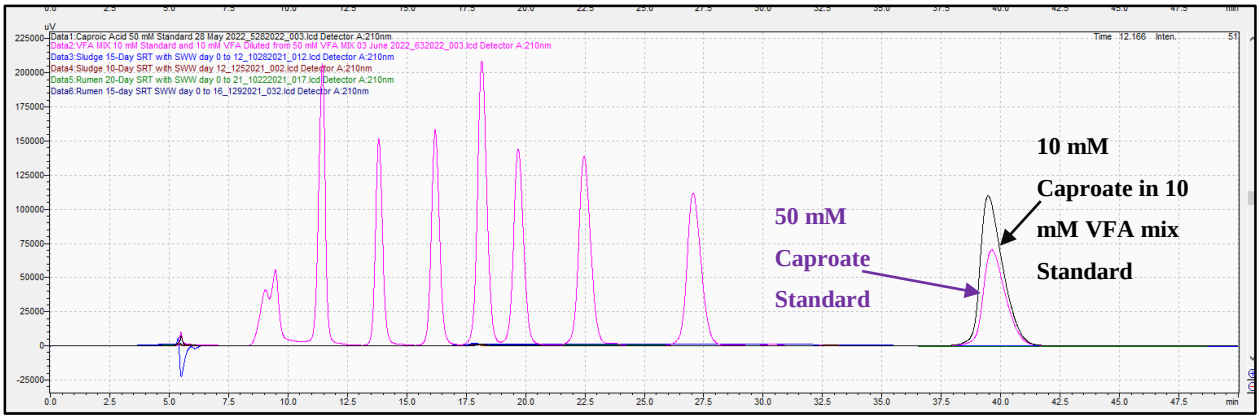


Figure 24: Caproate Peak verification in Water Samples which indicates an absence of the caproic acid peak in these samples, further confirming the validity of the unknown peaks identified as caproic acid.

COD Analysis

Effluent VFA concentrations were converted to COD values through electron balances, as mentioned in the method section. Also, SCOD values of fermenter effluent and wastewater influent were measured frequently through HACH 822 kits.

Ratios of Effluent VFA as COD/Influent Wastewater COD

The COD ratios of effluent VFA as COD and influent COD in sludge and rumen fermenters under all SRT operations were demonstrated in **Figures 25**, with/without caproate presence.

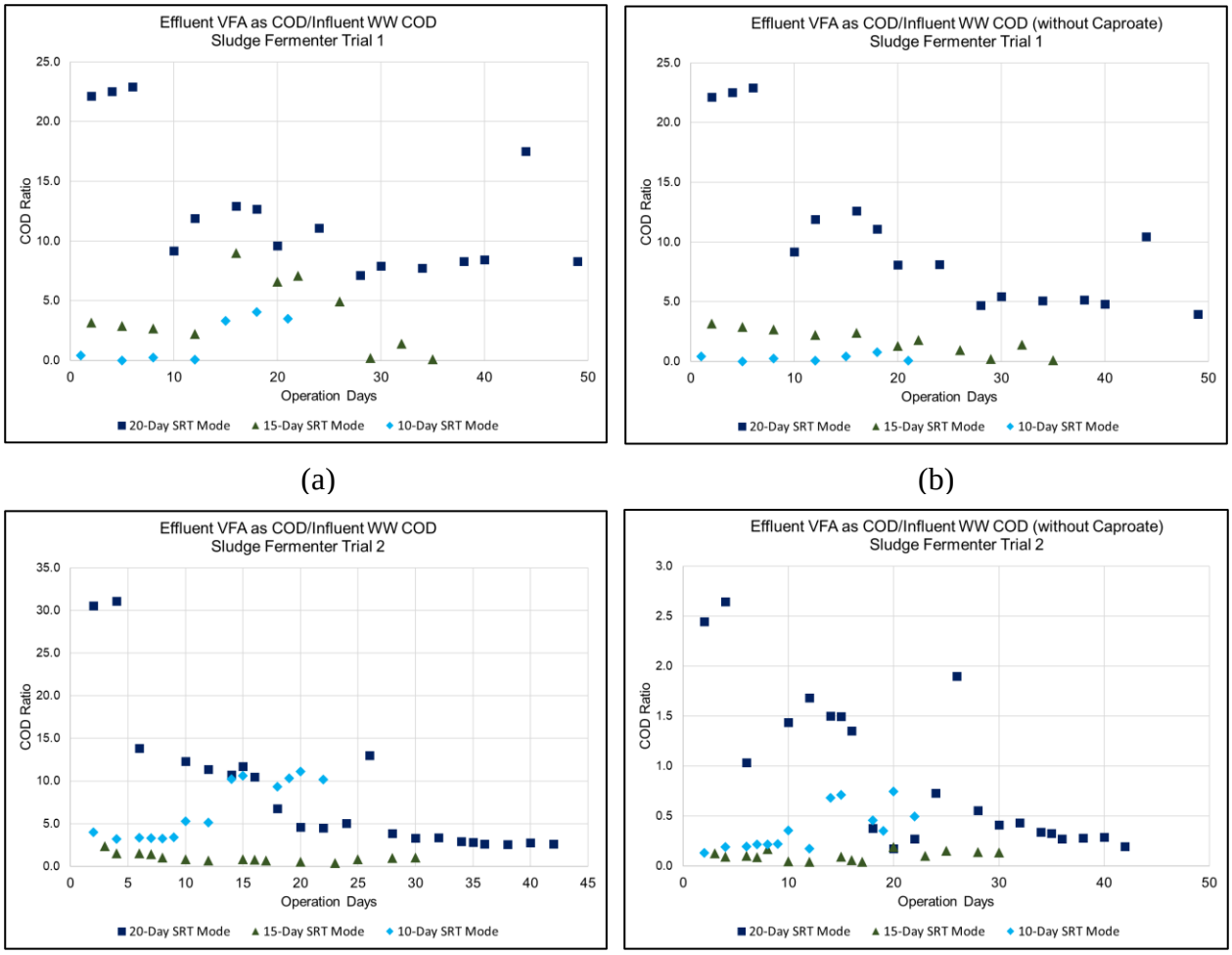


Figure 25: Effluent VFA as COD/Influent Wastewater COD in Sludge Fermenter [(a): Trial 1, (b): Trial 1 without Caproate, (c): Trial 2, and (d): Trial 2 without Caproate]

In Trial 1 (**Figure 25 (a)**), COD ratios from the 20-day SRT mode increased initially due to lactate production. Although acetate and propionate overtook the lactate in the middle of the 20-Day SRT Trial 1, the COD ratios declined towards the end due to the reduction of VFA generation over time. However, on the 44th-day of the 20-Day SRT Trial 1, a sudden COD ratio increment occurred due to the higher caproate detection. Although we removed the caproate presence from the graph (**Figure 25 (b)**), the downward trend was almost similar but a little lower in the second half due to caproate production at the end. When we switched the sludge fermenter to 15-day SRT

mode, the COD ratio decreased from three-fold initially due to less VFA production and increased swine wastewater addition. However, in the middle of the 15-day SRT Trial 1, COD ratios exceeded 5.0 due to caproate formations. When the caproate formation declined, the COD ratios were reduced at the end of the 15-day SRT Trial 1. COD ratios in the first half of the 10-day SRT Trial 1 were below 1.0 due to lower VFA production with increasing swine wastewater addition. From the 15^{th}-day, COD ratios increased due to caproate (above 8 mM) and valerate (above 1 mM) detections in the 10-day SRT Trial 1. However, there was a declining pattern in both 15 and 10-day SRT modes if there was no caproate (**Figure 25 (b)**).

All the SRT operations in Trial 2 (**Figure 25(c)**) had COD ratios > 1 due to higher caproate production in the fermenter. Except for the 10-day SRT mode, other SRT operations had a downward trend in the COD ratios since caproate production decreased with other VFAs. However, caproate concentrations in the 10-day SRT mode increased in the second half. According to **Figure 23 (d)**, the patterns of COD ratios were not changed, but the ratios went down by more than 10-times.

When SRT modes switched from 20 to 10-day, COD ratios dropped initially due to less VFA production from the fermenter and due to swine wastewater increment in the influent. When higher molecular VFA concentrations, mostly caproate in this study, were developed in the fermenter, COD ratios were expected to increase even in lower SRT operations like 10-Day SRT, due to the higher COD content per mole of the higher VFAs.

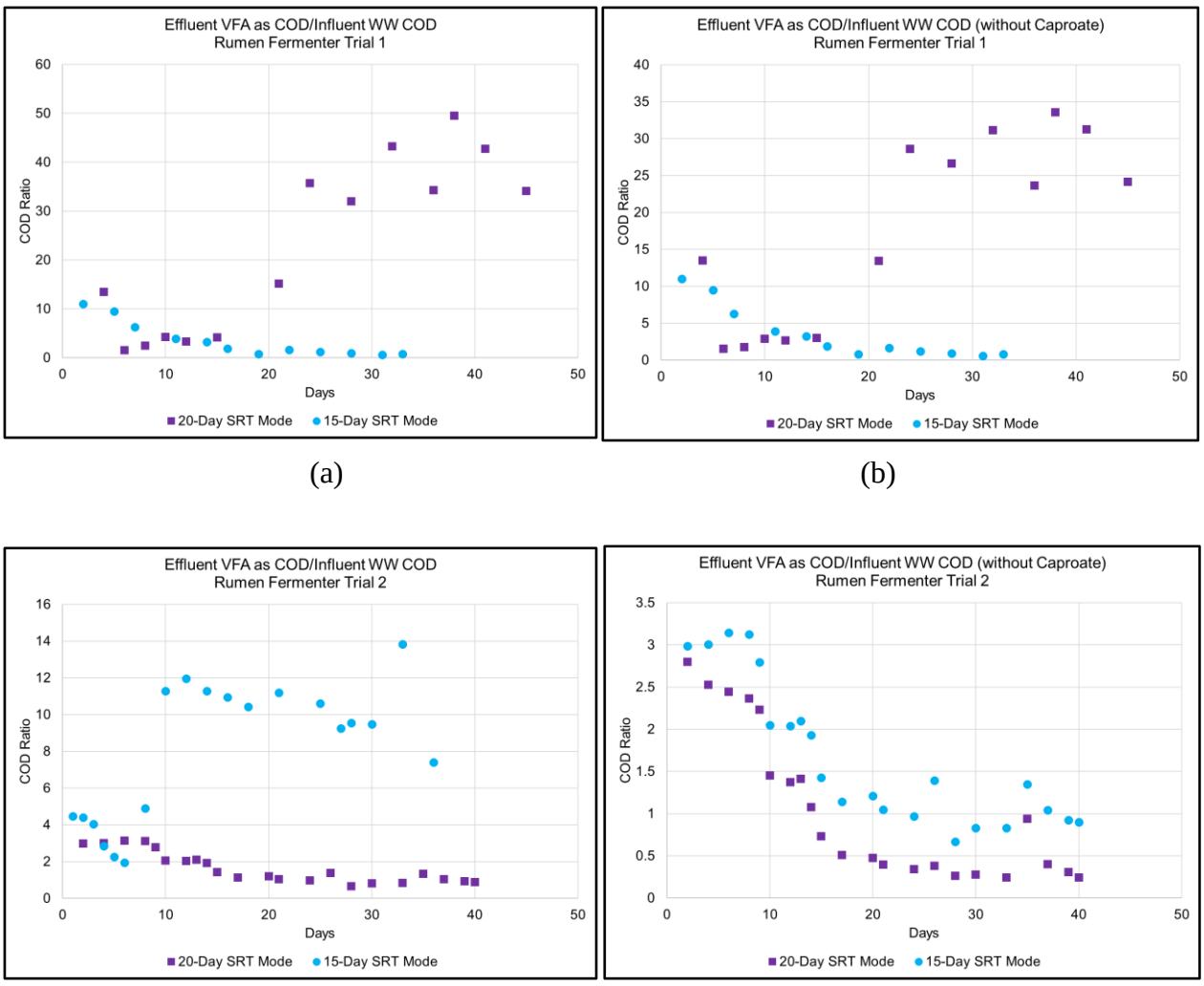


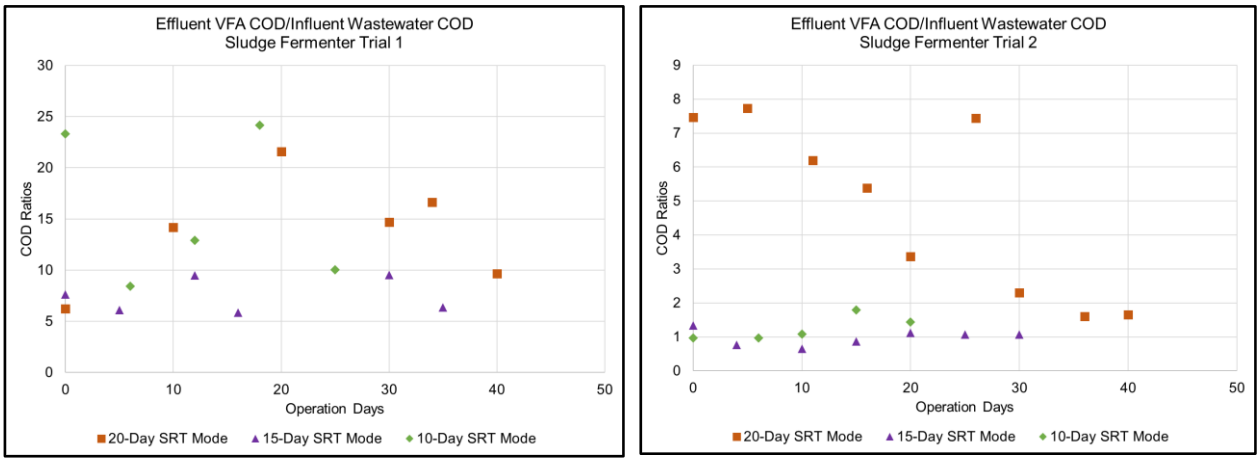
Figure 26: Effluent VFA as COD/Influent Wastewater COD in Rumen Fermenter [(a): Trial 1, (b): Trial 1 without Caproate, (c): Trial 2, and (d): Trial 2 without Caproate]

The 20-day SRT mode Trial 1 (**Figure 26 (a)**) had an increasing trend of COD ratios due to higher VFA productions, while washout in the 15-day SRT mode caused a decrease in COD ratios. **Figure 24 (b)** showed the same COD ratios without the presence of caproate in Trial 1. Caproate removal from the calculation steps did not affect COD ratios strongly for both modes in Trial 1 because butyrate was the dominant VFA in both SRT processes. According to **Figure 26 (c)**, 20-day SRT mode Trial 2 had lower COD ratios with a downward direction since lactate

production was prevalent and decreased throughout the process. However, COD ratios of 15-day SRT mode Trial 2 were raised due to increased caproate production. Therefore, as shown in **Figure 24(b)**, with the withdrawal of predominant VFA, the upward trend was reversed completely when the caproate was removed,

Ratios of Effluent COD/Influent Wastewater COD

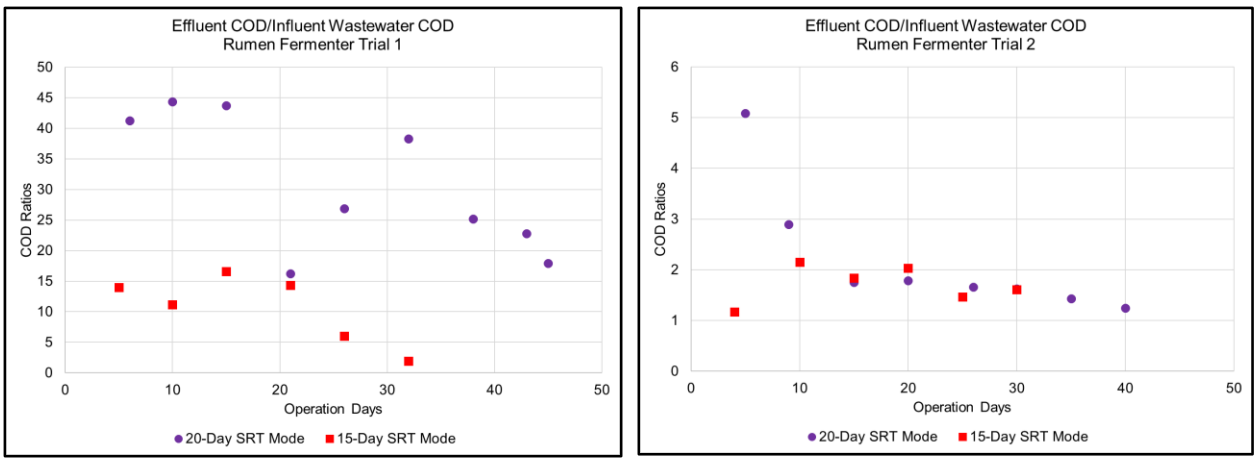
Soluble COD values of fermenter effluent and influent wastewater were measured in each trial from HACH 822 kits. The COD ratios of effluent and influent were shown in **Figures 27** and **28** for the sludge and rumen effluents, respectively.



(a) (b)

Figure 27: Effluent VFA COD/Influent Wastewater COD in Sludge Fermenter [(a): Trial 1 and (b): Trial 2]

The COD ratios of the first trial had an upward trend with fluctuations, while the second trial had a gradual decline in the entire process. Although the second trial had higher VFA production rates due to caproate buildups, the COD ratios were lower due to higher COD content from caproate in swine wastewater influent samples.



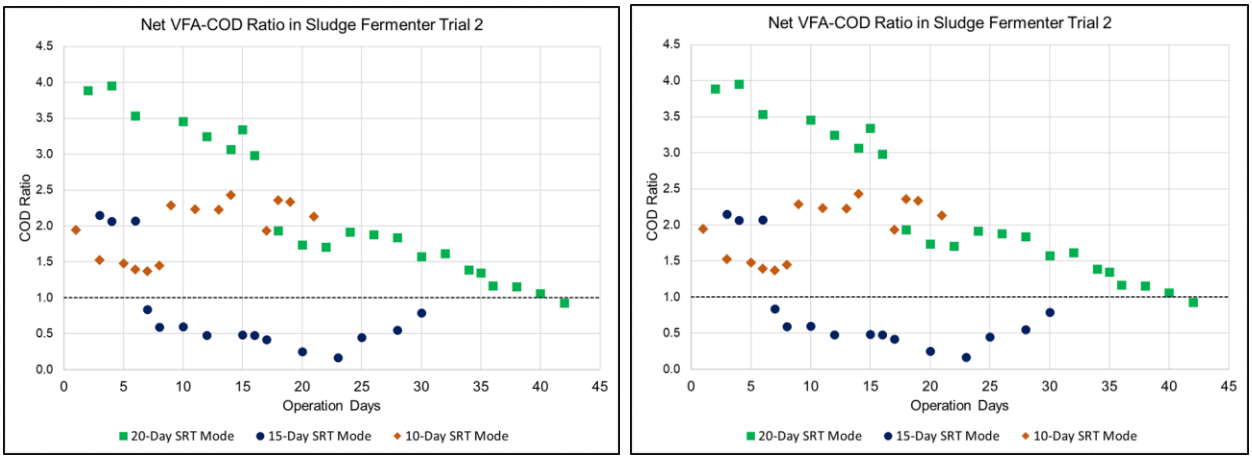
(a) (b)

Figure 28: Effluent COD/Influent Wastewater COD in Rumen Fermenter [(a): Trial 1 and (b): Trial 2]

Trial 1 had higher COD ratios for both SRT operations than Trial 2. The effluent and influent COD ratios were lower when the SRT operation was decreased to 15 due to lower VFA production. In Trial 2, the 20-day SRT mode gradually declined toward 1.0, and the 15-day SRT mode varied around 1.5.

Net VFA-COD Ratios

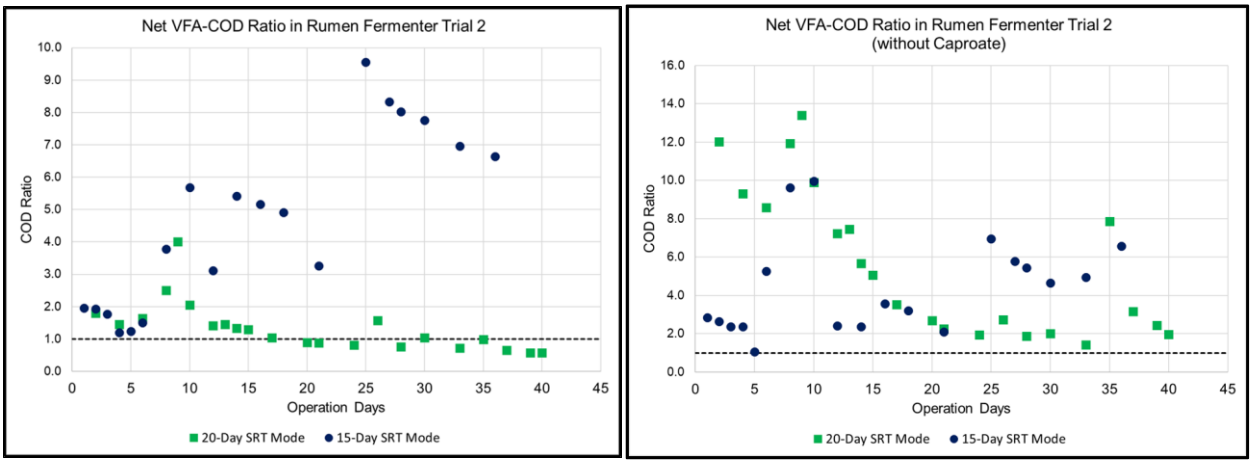
As mentioned in the analytical COD method section, net VFA-COD ratio calculations were performed for the sludge and rumen fermenters (**Figures 29** and **30**). However, net VFA productions were studied only in the second trial since we investigated the VFA analysis from the swine wastewater only in the second trial for both inocula.



(a) (b)

Figure 29: Net VFA-COD Ratios in Sludge Fermenter in Trial 2 [(a): All VFAs and (b): Without Caproate]

Figure 29 (a) showed that the 20-day SRT mode had the highest net VFA-COD ratios, although it had a downward trend. When the SRT mode was changed from 20 to 15-day, the net VFA productions dropped almost twice and went below 1.0 on most days (**Figure 29 (a)**). This scenario could be proved by obtaining less caproate production (>25 mM) between the 8th and 23rd operation days while detecting around 30 mM caproate concentrations from the swine wastewater samples. However, net VFA-COD ratios increased gradually after switching to the 10-day SRT operation due to stabilized 60 mM caproate in the second half of the operation and lower VFA productions from the influent. Even after removing caproate from the analysis, the ending COD ratios of the 10-day SRT mode went higher due to increased acetate, propionate, and iso-valerate productions and lowered VFA content in the influent. Therefore, it was confirmed that swine wastewater fermentation with anoxic sludge occurred adequately except in the 15-day SRT mode. When caproate production was neglected (**Figure 29 (b)**), then net VFA-COD ratios fluctuated from all three SRT modes, maintaining the trends.



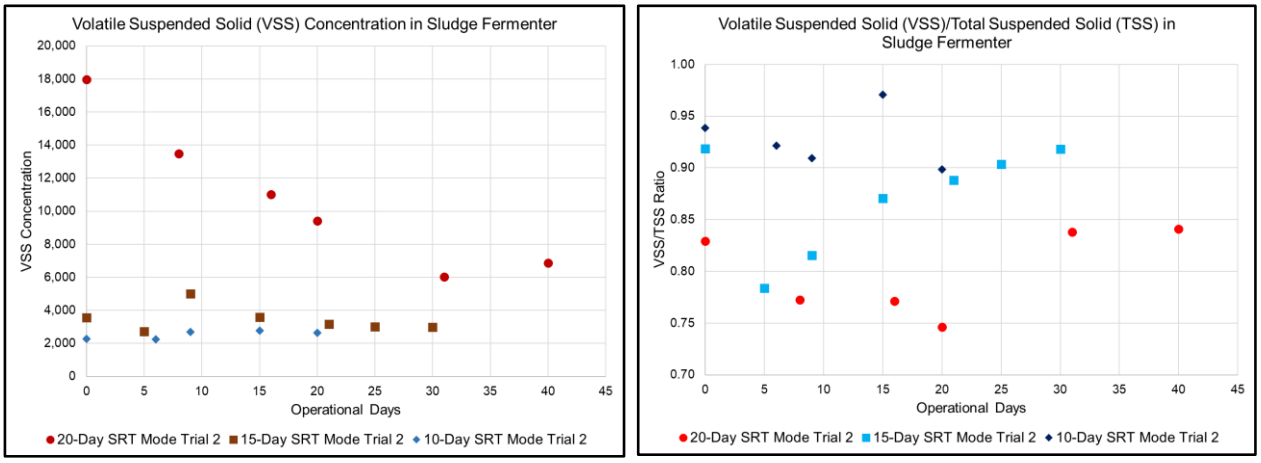
(a) (b)

Figure 30: Net VFA COD Ratio in Rumen Fermenter in Trial 2 [(a): all VFAs and (b): except caproate]

According to **Figure 30 (a)**, the 20-day SRT mode had a decreasing trend due to lactate being the predominant and higher VFA presence in the influent. However, when caproate presence was removed from the calculations, net VFA-COD ratios remained elevateduntil 20 days, according to **Figure 30 (b)**. Caproate was not the prevailing VFA in the effluent but in the influent. Therefore, net VFA-COD ratios were above 2.00. When the rumen fermenter was switched to 15-day SRT mode, net VFA-COD ratios increased significantly due to higher caproate production in the fermenter and less VFA content in the influent samples. Although caproate removal occurred in the calculations, the pattern of the 15-day SRT did not change since caproate was predominant in both effluent and influent samples. VFAs were produced from swine wastewater fermentation of rumen fluid successfully on most days due to COD ratios exceeding 1.0 except for some days from the second half of the 20-day SRT mode.

Solid Analysis

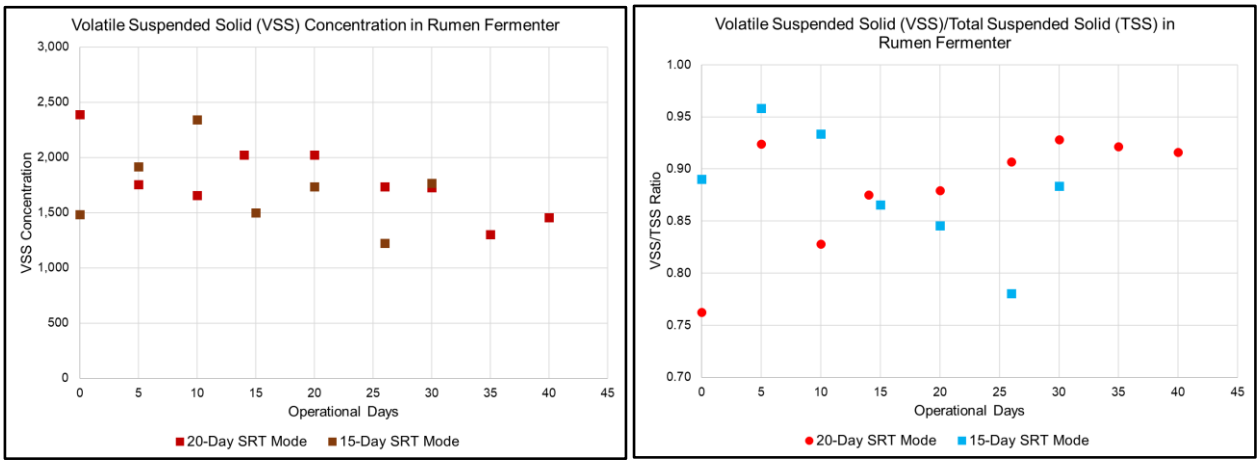
The VSS concentrations and ratios of VSS and TSS for the sludge and rumen fermenters in Trial 2 were shown in **Figure 31** and **Figure 32**, respectively.



(a) (b)

Figure 31: Solid Analysis in Sludge Fermenter Trial 2 [(a): VSS Concentration and (b): VSS/TSS Ratio]

The 20-day SRT mode gradually decreased VSS concentration from 18,000 to 6,000 mg/L, while 15-day SRT and 10-day SRT modes had stable trends around 4,000 mg/L and 3,000 mg/L, respectively. VSS concentrations were expected to decline during the process due to daily effluent removal from the fermenter with solid content. When the SRT was reduced from 20 to 15-day, the initial VSS was dropped by 4.5-fold in the sludge fermenter. We often centrifuged the effluents to avoid the washout from the fermenter due to less biomass content to add the pellets (solid content) in the wastewater influents. Unlike the 15-day SRT mode, VSS/TSS ratios of 20 and 10-day SRT modes fluctuated around 0.8 and 0.9, respectively. Unlike the 15-day SRT mode, VSS/TSS ratios of 20 and 10-day SRT modes fluctuated around 0.8 and 0.9, respectively, according to **Figure 31 (b)**.

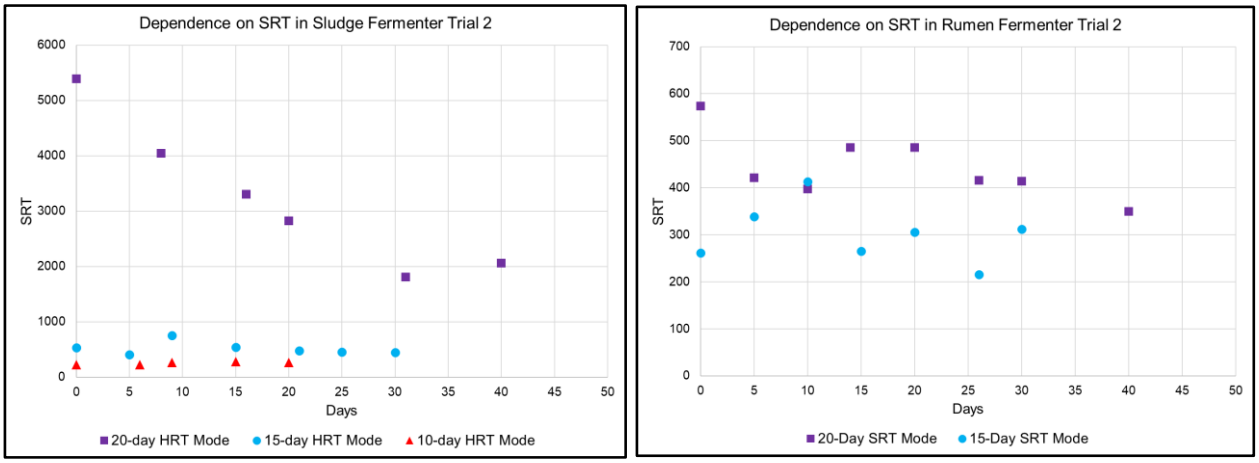


(a) (b)

Figure 32: Solid Analysis in Rumen Fermenter Trial 2 [(a): VSS Concentration and (b): VSS/TSS Ratio

The VSS of both 20 and 15-day SRT modes in the rumen fermenter varied between 1,000 and 2,500 mg/L and had a downward trend. The rumen fermenter had lower VSS concentrations than the sludge fermenter in both modes. Therefore, VFA washout could occur fast in the rumen fermenter due to biomass loss. To avoid that scenario, we frequently added the centrifuged pellets back to the reactor after mixing with the influent. **Figure 32 (b)** shows that the 20-day SRT mode increased VSS/TSS ratios. However, it was reduced when SRT mode was reduced to 15-day. Like the sludge fermenter, VSS/TSS ratios varied between 0.75 and 1.00.

SRT Calculation



(a) (b)

Figure 33: Dependence on SRT [(a): Sludge Fermenter and (b): Rumen Fermenter]

Since we added the effluent pellets (solid content) back to the fermenters after centrifuging, then SRT was not equal to HRT. Therefore, SRT values were obtained by using the following Equation 12 while assuming VSS of effluent was 250 mg/L for the both fermenters.

$$SRT = \frac{Fermenter\ Volume \times VSS\ of\ Fermenter}{(Effluent\ Flowrate \times VSS\ of\ Effluent + \cancel{(Waste\ Flowrate \times VSS\ of\ Waste)}} \quad \text{(Eq 12)}$$

Although these SRT values are much higher than usual values in **Figure 33**, 20-day HRT mode had the highest SRT values and it was reduced when HRT was reduced in the sludge fermenter. But rumen fermenter fluctuated SRT values for both 20 and 15-day SRT trials.

GC Analysis

Although sampling gas bags of both fermenters were not filled evidently, gas bags of the sludge fermenter at the end of the 10-day SRT operations were measured. The sludge fermenter produced methane around 7.68% and 6.86 % for Trial 1 and 2, respectively. The volume of the gas bag in Trial 2 was 394 mL.

Chapter 5 - Selective Swine Wastewater Fermentation from Microbial Electrochemical Cell (MXC)

Introduction

Microbial electrochemical cell (MxC) has been gaining attention in the environmental biotechnology field due to its wide applications in many industries including wastewater treatment, biorefinery from wastes, and energy production. This chapter is going to evaluate VFA production of swine wastewater by using a H-type microbial electrolysis cell (MEC): hydrogen production from cathode chamber by oxidizing waste through ARBs in anode compartment as shown in **Figure 34**.

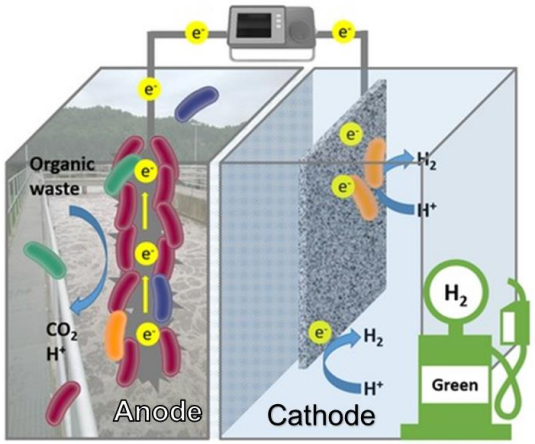


Figure 34: Schematic Diagram of MEC (H. Yuan & He, 2017)

Many MEC studies were conducted on acetate as a substrate due to its highest conversions compared to other substrates. Therefore, they had the best hydrogen production rates with the highest current densities (Cheng & Logan, 2007; D. W. Liang et al., 2011; Lu & Ren, 2016). When fermentable organics such as glucose, galactose, xylose, and maltose were used, lower hydrogen production rates were obtained (Lu et al., 2012; Lu & Ren, 2016). But, when the lignocellulosic

biorefinery products such as Furanic and phenolic wastewater, and De-oiled refinery wastewater were used as substrates, then higher hydrogen production rates and current densities were obtained (higher than fermentable organics and less than acetate) (Lewis et al., 2015; Lu & Ren, 2016).Lignocellulosic biorefinery products, are favorable to be consumed by ARBs due to the presence of large amount of monosaccharides, disaccharides, and alcohol in them. (Lu & Ren, 2016). It was reported that pure proteins had very low hydrogen production rates although they had higher current densities (Lu & Ren, 2016). The same scenario was observed in the domestic wastewater and WAS as substrates since they have higher protein contents (Lu & Ren, 2016; Nam et al., 2014). But ARBs prefer to consume simple substrates and they are not effective at oxidizing complex substrates (Lu & Ren, 2016). Therefore, they require fermentative microbes' collaboration to degrade the organics, cellulose, and proteins to simple substrates for ARB's consumption. (Parameswaran et al., 2009). Then, MEC coupling with fermentation was suggested to increase the hydrogen production from WAS or domestic wastewater (Lu & Ren, 2016).

It was reported that hydrogen recovery from acetate MECs accomplished more than 90%, but MECs of other waste streams obtained above 30% (Lu & Ren, 2016). When real wastewater is used as a substrate in the MEC, few challenges like low conductivity, low COD in domestic wastewater, alkalinity, slow degradation can appear due to various organic substrates and alternative electron acceptors than the anode (H. Liu & Logan, 2004; Rabaey et al., 2004; Xie et al., 2021). A single-chamber MEC study from swine wastewater with dewatered sludge inoculum was used to produce hydrogen gas at pH 5.8. However only methane and carbon dioxide were detected, without hydrogen (Wagner et al., 2009). When the reactor pH was lowered from 5.8 to 5, no hydrogen was recovered even though gas production was reduced (Wagner et al., 2009). The hydrogen consumption in the swine wastewater with sludge inoculum was controlled only at pH

5, while other pH conditions (6, 7 and 8) contributed to consume hydrogen quickly (Wagner et al., 2009). Therefore, hydrogen recovery from swine wastewater was a difficult task through a single-chamber MEC because it was proved that acetogenesis microbes consume hydrogen quickly (Wagner et al., 2009).

A research based on animal rendering wastewater which has long chain fatty acid concentrations fed to a MEC resulted in current densities above 1 A/m^2 for 80 days at ambient temperature (Xie et al., 2021). Acetate concentration was at around 2.45 mM for the first 48 days and it increased to 14.3 mM while other VFAs including propionate increased in the animal rendering wastewater fed MEC (Xie et al., 2021). The most common anode biofilm communities are *Geobacter* and *Shewanella*, but *Desulfuromonas* was majority in the rendering wastewater fed MEC's biofilm due to higher sulfate concentrations in the media (Xie et al., 2021).

In the lab scale experiments, phosphate buffer solutions are added to increase the buffer capacity and conductivity which is not practical for full scale applications (Lu & Ren, 2016). The main drawback of MECs is hydrogen loss due to methanogens in single-chamber MEC reactor overtime. It was found that hydrogenotrophic methanogens consume hydrogen to produce methane, but acetoclastic methanogens' acetate consumption to produce methane is lower than ARB's acetate consumption in the MEC (Lu & Ren, 2016). Although a pretreatment is required to inhibit methanogens in the MEC, adding chemical inhibitors such as BES is not recommended due to additional cost and contamination. Another disadvantage is most of the capital cost of MEC goes to the platinum-based cathode and catalyst (Lu & Ren, 2016). But later it was found that other materials such as nickel, carbon monotube, and graphite cathode showed better results than platinum-based ones (Lu & Ren, 2016). In summary, the MECs can be leveraged to channel more efficient VFA generation from wastewaters by either refining the mixed organic acids to few

compounds or by enabling chain elongation reactions with the additional H_2 from the cathode or other electron donors such as ethanol.

Methods

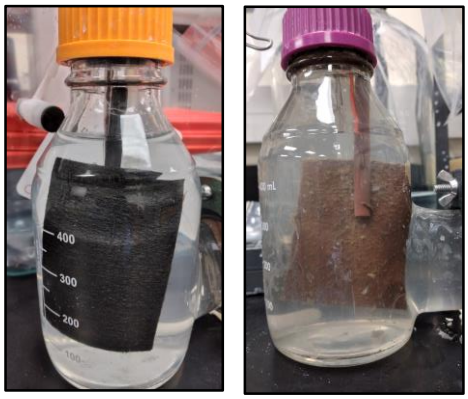
Experimental Procedure

A H-type MEC reactor was used and it contained two chambers: anode and cathode compartments were separated by an anion exchange membrane (AEM). Each compartment can hold around 700 mL of volume with the space of a bridge of membrane. The anode compartment had a rectangular carbon felt anode with a total surface area of 81.5 cm^2 and the cathode compartment had a rectangular 16cm^2 carbon electrode. An Ag/AgCl reference electrode was used to maintain the anode at -301 mV. The MEC reactor was kept at 30℃ temperature and the anode compartment was mixed at a rate of 1000 rpm on a stirring plate. A 1 L of phosphate buffer was prepared from 12.04 g of disodium hydrogen phosphate, 2.06 g of mono potassium phosphate and 0.41 g of ammonium chloride to fill both the anode and cathode compartments. Also, 10 mL of 1 L trace mineral media was added to the compartments. The 1 L trace mineral media was contained from 0.5 g of EDTA, 0.082 g of cobalt(II) chloride hexahydrate, 0.114 g of calcium(II) chloride dihydrate, 0.01 g of boric acid, 0.02 g of sodium molybdate dihydrate, 0.001 g of sodium selenite, 0.01 g of sodium tungstate dihydrate, 0.02 g nickel(II) chloride hexahydrate, 1.16 g of magnesium chloride, 0.59 g of manganese(II) chloride tetrahydrate, 0.05 g of zinc chloride, 0.01 g of copper(II) sulfate pentahydrate, and 0.01 of aluminum potassium sulfate (Parameswaran et al., 2009). 1 mL of 4 g/L ferric (II) chloride and 0.5 mL of 37.2g/l=L sodium sulfide nonahydrate stock solutions were added to the 1 L phosphate buffer (Parameswaran et al., 2009). pH of the cathode media was adjusted to 11.5 pH by using 1 M NaOH. 30 mM acetate was added to the anode compartment as an electron donor to grow biofilm. Also, pre-acclimatized acetate-fed biofilms were used as a

startup to grow biofilm around the carbon felt anode in the anode compartment. The finalized reactor setup is shown in **Figure 35 (a).** Sampling gas bags were attached to the gas line of a rubber stopper in the anode and cathode compartments for collecting any gaseous products. **Figure 35 (b)** shows how the black carbon felt anode was covered by dark red-color biofilm after few days.

(a) (b)

Figure 35: MEC Setup [(a): with 30 mM Acetate media in anode and (b): swine wastewater with synthetic permeate media in anode]



(a) (b)

Figure 36: Carbon Felt Anode [(a): Initial Carbon felt anode and (b): Coated biofilm (ARB) around the carbon felt anode]

Once the current density of the reactor reached above 5 A/m^2 and stabilized for few days, the anode media was replaced with a mixture of 30 mL of sludge effluent from the sludge fermenter (as an inoculum) 25 mM glucose (initial spiking substrate), 350 mL swine wastewater and 350 mL

synthetic swine permeate (mentioned in Chapter 3). **Figure 36 (b)** shows the anode compartment filled with swine wastewater. The anode pH was adjusted initially to be around 7since anode respiring bacteria (ARB) consume acetate and produce higher current density at neutral pH condition. The reactor was operated for a few days as a batch mode to adapt the ARB in a swine wastewater environment.

After the current density was dropped in the batch operation, the swine wastewater addition was started to the MEC reactor under 20-day HRT operation, like the non-electrode fermenters in Chapter 4. Similar to the non-electrode fermenters in Chapter 5, we replicated the 20-day HRT operation. We removed 35 mL from the reactor and added 35 mL of swine wastewater. Also, swine wastewater influent was adjusted to be around 6.95-7 pH since higher influent pH could deactivate the ARB. We added the solid contents from MEC effluents frequently to avoid washing out of the inoculum in the anode compartment. Like in the non-electrode fermenters in Chapter 4, we centrifuged the effluent samples and mixed the pellets with the swine wastewater influent in the anaerobic glove box to avoid the oxygen exposure to the ARBs and anaerobic microbes. Influent pH was adjusted to 7 before adding them to the MEC reactor.

Analytical Methods

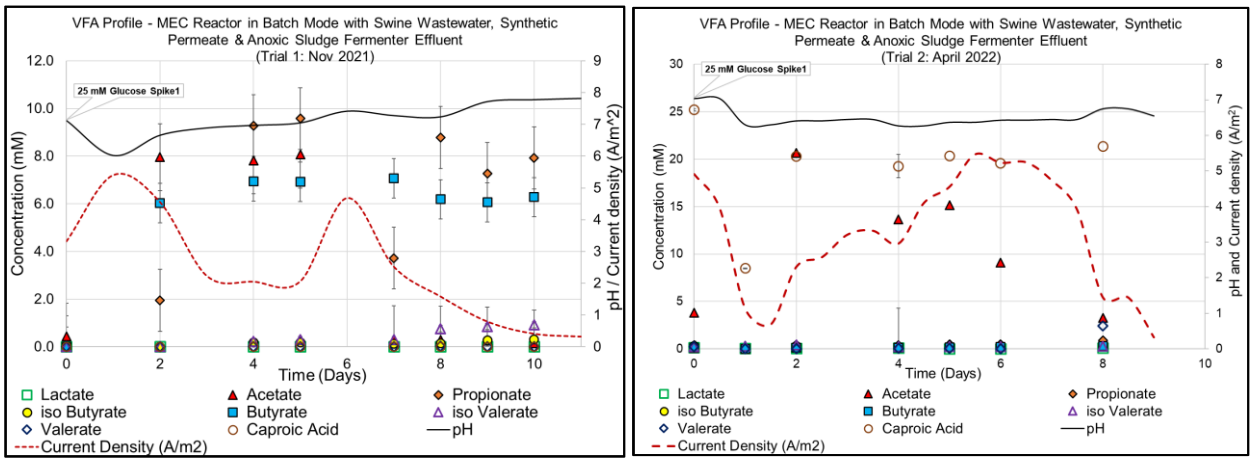
pH was measured daily using a Fisherbrand accumet AB150 pH benchtop meter and maintained regularly to keep the value around 7.00 throughout both batch and 20-day HRT mode. pH of the influent wastewater with MEC effluent pellets was measured from an Oakton pH 550 benchtop pH meter situated within the vinyl anaerobic chamber. We proceed with the same VFA quantification HPLC techniques as mentioned in Chapters 3 and 4 to detect eight VFAs from the MEC effluent samples (Trial 1 and 2) and influent wastewater samples during Trial 2. As mentioned in Chapter 4, we converted all VFA concentration values (in mM) to COD values (in

mg/L) in effluent and influent samples through electron balances in **Table 6** and equations (from **Eq 1** to **6**) for each VFA. Then net VFA-COD ratios were calculated according to the equations (from **Eq 8** to **11**) in **Chapter 4**. Also, soluble COD values of the MEC effluents and wastewater influents were measured using HACH 822 kits, ranging from 20-1500 mg/L concentration. Gas-Chromatography (GC-TCD) was used to measure the methane and hydrogen gas production in the sampling gas bags of the anode and cathode compartments in Trial 2. Argon was the carrier gas at a flow rate of 8.8 mL/min and pressure of 88.8 kPa. Temperatures of injection, column, and detector were 200, 80, and 150 ℃, respectively. Like the sludge and rumen fermenters in Chapter 5, TSS and VSS were conducted for the MEC effluent samples in the second trial according to the standard methods 2540D and 2540E, respectively (Eaton et al., 2005).

Results and Discussion

Effluent VFA Analysis

Glucose Initiated Batch Mode



(a) (b)

Figure 37: MEC in Batch Mode [(a): Trial 1 and (b): Trial 2]

Figure 37 shows the VFA distribution from the MEC in a batch mode with swine wastewater fermentation of anoxic sludge. Only one 25 mM glucose spike was added to the anode compartment of MEC, since it could activate the microbes in the sludge inoculum and degrade easily to VFAs initially. Therefore, the pH was checked daily and adjusted to be around 7. Although other fermenters (Chapter 3 and 4) were controlled at acidic conditions, MEC reactor was set to operate at neutral conditions since low pH impacts the ARB performance by reducing the current density. However, in Trial 1 (**Figure 37 (a)**), pH almost exceeded 8 even though it was adjusted. The current density initially increased to 5 A/m^2 and dropped. However, it increased again to 4.5 A/m^2 since the reactor had some acetate to consume (8 mM acetate). Since we used an acetate-fed biofilm into the reactor, ARBs are required to have acetate in the swine wastewater to consume and increase the current density. Also, other VFA concentrations could be stabilized since ARBs do not consume them.

Propionate was the dominant VFA, followed by butyrate in the first MEC batch operation. Around 1 mM iso-valerate production was observed at the end of the process. Although the pH increase impacted the current density drop in the end, VFA productions were stabilized.

We replicated the same experiment, but a different VFA profile was obtained (**Figure 37 (b)**). In Trial 2, caproate production was detected from the initial day and it was dominant with some fluctuations in the whole process. Unlike Trial 1, pH decreased to 6.3 from 7 within the 1^{st} day. Therefore, reactor pH was adjusted to 7 by adding 1M NaOH and then it was increased to 6.70 gradually. The current density dropped simultaneously due to the pH drop. Although pH did not reach7, current density was improved to go around 5 A/m^2. Caproate production was increased and stabilized around 20 mM, but other VFA productions were detected at less than 1 mM except the 2 mM valerate production on the 9^{th} day. Caproate production was expected from the second

trial since we used the sludge fermenter effluent which was in the 20-day HRT operation, and caproate was prevalent from the sludge fermenter in Trial 2. However, presence of acetate in the anode media was required to maintain the ARBs accurately.

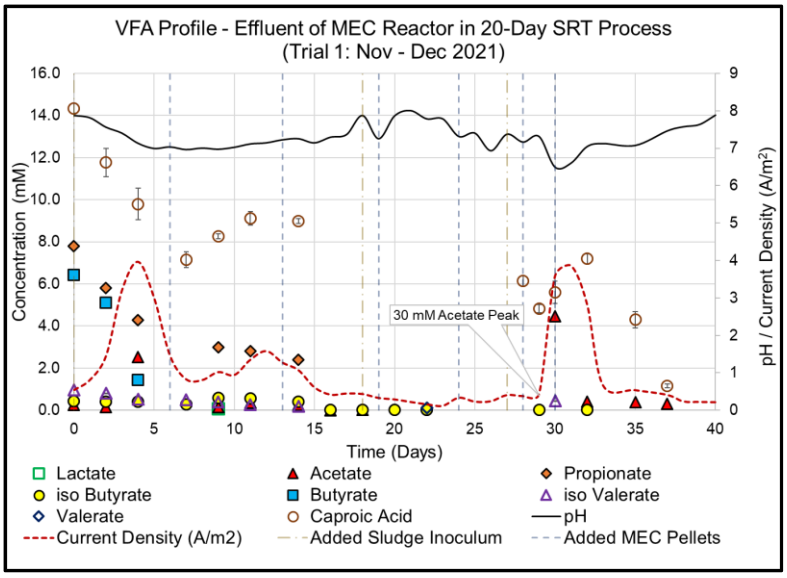
20-Day HRT Mode

Figure 37 (a) shows the VFA profile of the MEC anode effluent samples during the 20-day HRT mode. Initially, pH was 8 and it was reduced due to daily acidification of the influent wastewater samples. Therefore, VFA productions were detected in the first half of the operation. Caproate predominance was around 8 mM in the whole process, while propionate, and butyrate productions were detected until 14th day. When the pH was increasing back to 8, VFA productions reached washout levels except for small concentrations of iso-butyrate. The current density increased to 4 A/m^2 in the beginning when 2.5 mM acetate was detected. After that, current density gradually decreased until the 29th day due to higher pH range. To increase the current density, a 30 mM acetate spike was added to the reactor and it worked for a few days. Then caproate productions were observed at 8 mM when the pH was reduced to less than 7 and the current density was increased. When the pH increased to 8, then current density decreased and the caproate productions decreased. Therefore, maintaining a pH around 7 was critical to keep the current density at least above 1 A/m2 to generate VFAs simultaneously. Because, once the pH started to increase, it could rise rapidly due to buffering capacity from the swine wastewater. Due to less VFA productions and a quicker washout during the second half of the 20-day SRT operation, we decided not to continue for other SRT operations, since swine wastewater addition was increased.

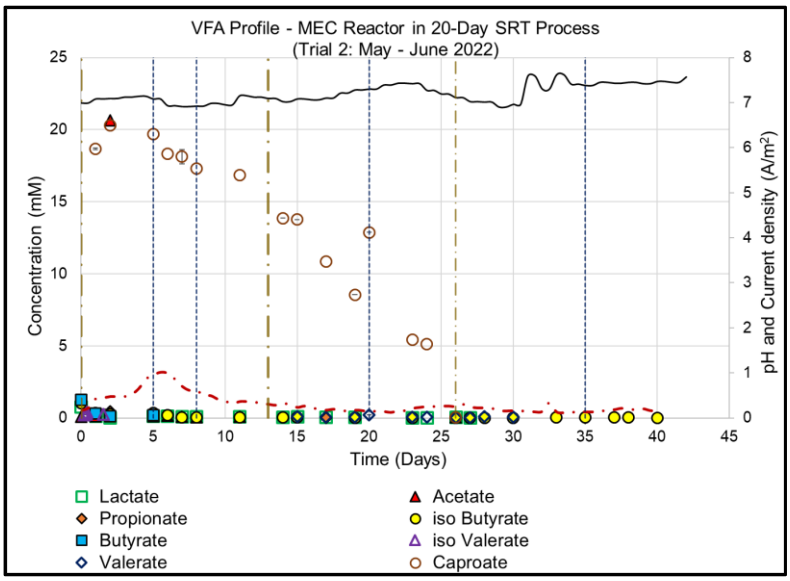
Figure 37 (b) shows Trial 2 of the 20-day SRT operation in MEC with caproate predominance, although it had a decreasing trend. Unlike Trial 1, this trial did not have proper VFA productions since current density did not go beyond 1 A/m^2. Due to the issue with pH rising

in Trial 1, we controlled the pH of Trial 2 to be around 7, but the current density did not respond correspondingly, indicating that acetate was limiting and not available from upstream fermentation. Therefore, we assumed that ARBs were deactivated due to oxygen exposure and that could lead to VFA washout in the second half of the process. Even though pellets (solids) from the sludge fermenter were added to increase VFA productions, it did not yield favorable outcome.

Therefore, acetate presence in the media is highly recommended to keep the biofilms activated and to produce other VFAs. If swine wastewater does not contain enough acetate, then acclimatized sludge inoculum from the sludge fermenter should be enriched with proper fermentative microbes to produce acetate through degradation of complex molecules in the swine wastewater. Then, other VFAs would be produced sufficiently and could be refined to a binary or tertiary VFA mixture while acetate was selectively consumed in the anode compartment.



(a)



(b)

Figure 38: VFA Analysis in MEC in 20-day SRT Process [(a): Trial 1 and (b): Trial 2]

Influent VFA Analysis

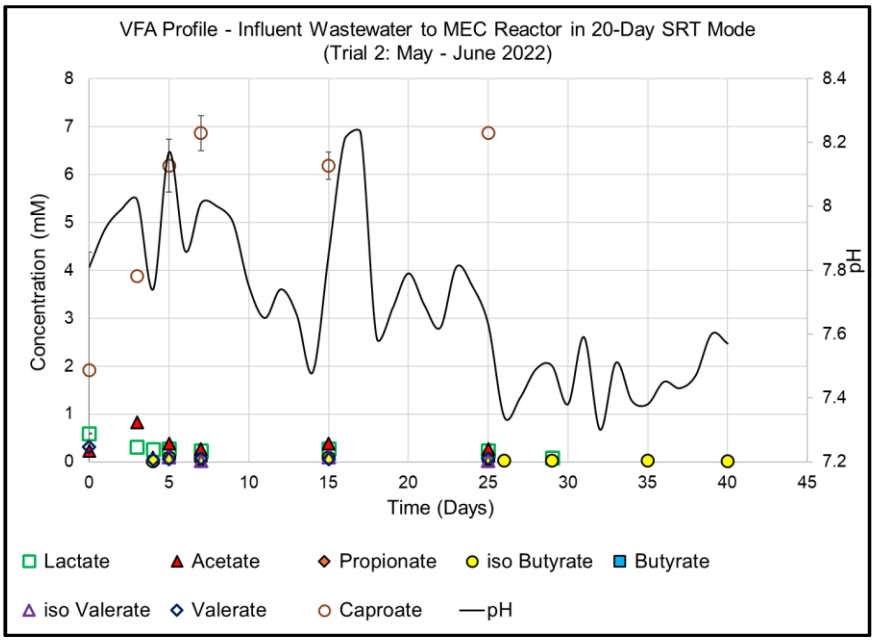
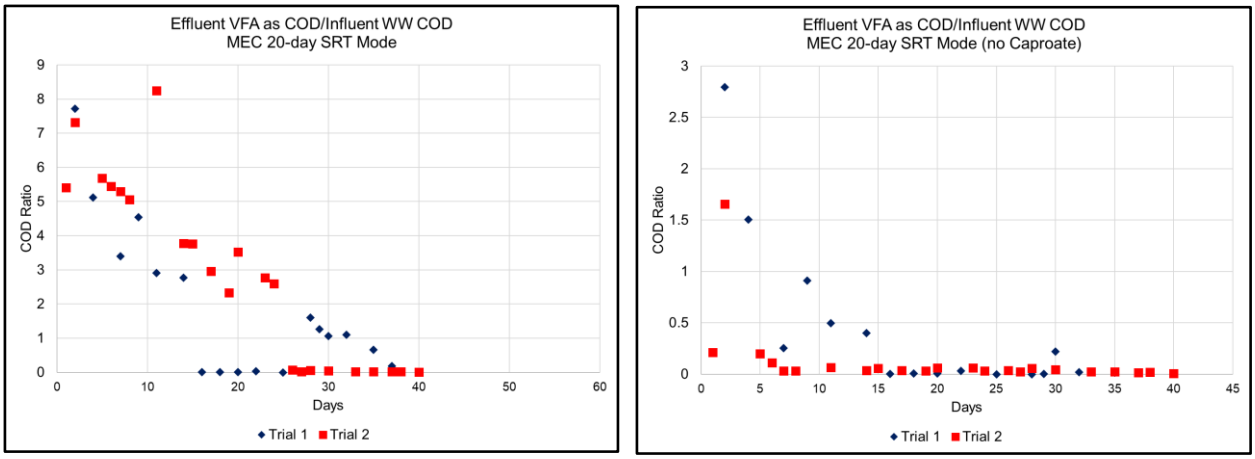


Figure 39: VFA Analysis in Influent to MEC

Above **Figure 38** displays the VFA distribution from the influent swine wastewater to the MEC Trial 2. Influent pH varied between 7.2 and 8.2 throughout the whole operation. In the influent samples, caproate was dominant and acetate detection was less than 1 mM. After the 25th operational day, low iso-butyrate concentrations were detected. The same pattern was observed in the effluent VFA analysis (**Figure 38 (b)**, thus, VFA production from MEC depends on the VFA presence (especially acetate) in the influent.

COD Analysis

Ratios of Effluent VFA as COD/Influent Wastewater COD



(a) (b)

Figure 40: Effluent VFA as COD/Influent Wastewater COD in MEC [(a): all VFAs (b): without Caproate]

The COD ratios of effluent VFA as COD and influent COD in MEC were displayed in **Figure 39 (a)**. When Effluent VFAs were calculated without the caproate presence, COD ratios decreased by more than one-fold. According to **Figure 39 (a)**, the COD ratios of the middle part of Trial 1 and second half of Trial 2 were almost zero due to VFA washout. When caproate presence was neglected as presented in **Figure 39 (b)**, the COD ratios decreased by more than one-fold. Trial 1 had propionate and butyrate productions except caproate in the beginning, then COD ratios were above 0.5 before the washout. Trial 2 did not have higher VFA productions in MEC beside caproate. Hence, low COD ratios were obtained except for the 3rd day due to 30 mM acetate detection.

Ratios of Effluent COD/Influent Wastewater COD

Like the non-electrode fermenter, soluble COD values of effluent and influent wastewater measured through HACH 822 kits. According to **Figure 41**, Trial 1 had higher COD ratios than Trial 2, although they were stable between 1.0 and 3.0 except for the 5th day ratio in trial 1 due to a lower influent SCOD measurement. Even though there were no VFA productions between 20th

and 25th days in Trial 1, COD ratios were above 2.0. Also, Trial 2 effluents had less SCOD measurements than Trial 1 by three-folds; that could be another reason for Trial 2 to be unsuccessful.

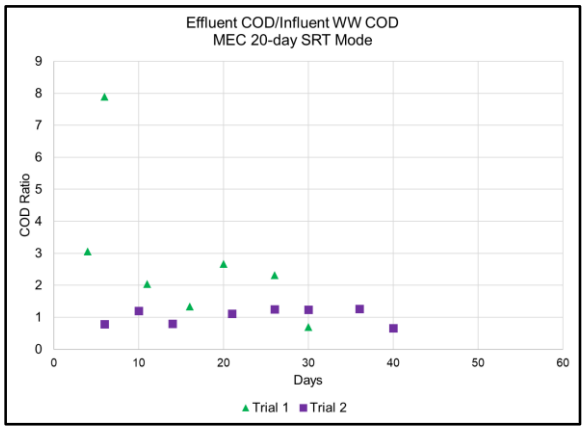


Figure 41: Effluent VFA COD/Influent Wastewater COD in MEC

Net VFA-COD Ratios

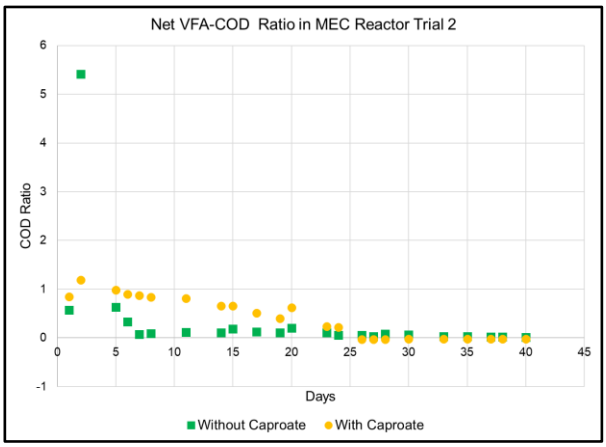


Figure 42: Net VFA-COD Ratios in MEC in Trial 2

The net VFA-COD ratios in 20-day SRT mode in MEC Trial 2 are displayed in **Figure 41** and it was lower than both sludge and rumen fermenters due to lower VFA productions. After 25 operational days, the net VFA-COD ratios were negative since net VFA from the fermenter was lower than the influent VFA. When caproate dominance was removed, it is clear that other VFAs

were not produced properly except for initial days. However, higher COD ratio was detected in the absence of caproate dominance on the 2nd day due to 20 mM acetate production as well as lower VFA content in the influent. It was proved that VFAs were not generated accurately in the MEC Trial 2 possibly due to less strength of swine wastewater or lower microbial activity from the inoculum supply, which was acclimated from the sludge fermenter in 20-day SRT mode after finishing the 10-day SRT mode. Caproate was the dominant VFA in Trial 2, therefore when it was removed, then net VFA-COD ratios were almost close to zero even before the 25th day.

Solid Analysis

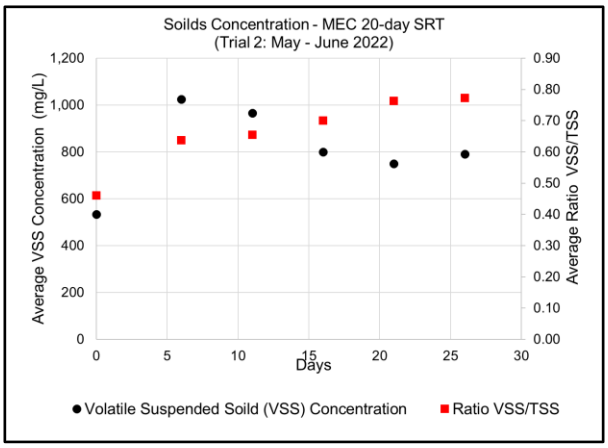


Figure 43: Solid Analysis in MEC Trial 2

According to the solid analysis in MEC as shown in **Figure 42**, initial VSS was lower than 1000 mg/L, therefore it could be another drawback of not producing VFAs properly. When the MEC effluent pellets were added on the 5th day, the VSS concentration on the 6th was increased a little above 1000 mg/L, but it decreased with effluent removal. It was reported that lower VSS/TSS ratio shows a less content of organic matter in the media. Therefore, it was confirmed to achieve less VFA concentrations from the beginning. Although the ratio was increasing due to VSS rise by adding the MEC effluent solids back to MEC, the VFA productions were not increased.

GC Analysis

The volume of the sampling gas bags of cathode compartments were 1234 mL and 1720 mL in Trial 1 and Trial 2, respectively. However, we checked the hydrogen content from Trial 2 bag only and it was 72.18% after finishing the 10-day glucose-enriched swine wastewater batch period. When the MEC was switched from batch to semi-continuous mode, then 730 mL with 63.88% of hydrogen was collected until the 8^{th} day of the operation. However, the gas bag was not filled visibly possibly due to decreasing VFA levels and less degradation in the anode compartment. From the sampling bag of the anode compartment, we collected 351 mL with 33.80 % of methane and 2321 mL with 13.02% methane in Trial 1 and 2, respectively, in the batch periods only.

Chapter 6 - Conclusion and Future Works

VFA recovery from swine wastewater fermentation is one approach to animal manure management to meet the requirements of the concept of the circular economy. Waste-derived VFA productions should be optimized to increase production cost-efficiently and sustainably from various sources, including wastewaters, to meet global VFA demand.

This study obtained unique fermentation product profiles separately from swine wastewater fermentation of anoxic sludge and rumen fluid. Sludge and rumen fermenters displayed acetate/propionate and acetate/butyrate VFA profiles, respectively, from glucose-enriched fermentations as well as 20-day SRT operation with swine wastewater, in Trial 1. Acetate was the dominant VFA at the 15-day SRT operation, and washout occurred at the 10-day operation. Unlike the sludge fermenter, rumen inoculum had a quicker washout at 15-day SRT mode. During Trial 2, caproate prevailed during all SRT phases of the sludge fermenter, including the glucose-enriched batch. During Trial 2, the rumen fermenter had a lactate predominance in glucose-enriched operation and 20-day SRT mode, but it was replaced with caproate at 15-day SRT mode.

Due to all the above summarized effluent VFA results observed at controlled acidic pH conditions, another semi-continuous operation (20-day SRT mode) at an initial alkaline pH condition could be performed to see progress in VFA production rates and whether it enhances concentrations of the higher range of VFAs from valerate (C4) to caprylate (C8). Since swine wastewater has its own buffering capacity, pH fluctuation due to accumulated VFAs should be well-controlled, while the inoculum should be thermal-shocked to inhibit methanogens. Importantly, 16s-DNA analysis sets for each fermenter during each operation are highly recommended to conduct frequently in the next round to observe what kind of microbes are most and least abundant.

Although we confirmed that a higher range of caproate concentrations was generated from sludge and rumen fermenters by overlaying the sample peaks with standard peaks, further investigations using another analytical instrument such as mass spectrometry are required.

During fermentation coupled with MEC, caproate predominance was observed from swine wastewater fermentation of anoxic sludge during Trial 1 and 2. However, anode media should be acclimatized with sufficient acetate concentration to enhance ARB's consumption and obtain an adequate current density in future experiments since VFA productions almost depend on the current density. Also, reactor pH should be maintained accurately under neutral conditions because a slight pH fluctuation impacts the ARB's performance, followed by a VFA washout. Also, a sufficient VSS concentration in the MEC is required to conduct acidogenic fermentation efficiently. The next round of fermentation based on MEC experiment will be optimal to be conducted in a flat-plate MEC with a much higher surface area of the anode to increase ARB formation.

Further studies on microbial composition, scale-up, in-situ recovery options, life-cycle analysis, and techno-economic analysis are required to transfer the lab-scale investigations to pilot-scale with the target of the commercial market.

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