Evaluation of arrested anaerobic digestion, co-digestion, and microbial chain elongation of swine wastewater for production of short and medium chain volatile fatty acids

by

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Abstract

Most short-chain fatty acids (SCFAs, C2–C6), are currently produced from petrochemicals, though biomanufacturing from waste streams shows strong potential. VFAs—both short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate and medium-chain fatty acids (MCFAs) such as caproate and caprylate —are intermediates of anaerobic digestion, which involves hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Suppressing methanogens via pH control (acidic or alkaline) promotes VFA accumulation, with production efficiency ranging from 15–25% at pH 5 to 35–50% at pH 9. Separation methods include membranes, ion exchange, liquid-liquid extraction, and distillation, though further development is needed. Mixed VFAs can serve as external carbon sources in nutrient removal and as feedstocks for bioplastics, animal feed supplements, and synthetic aviation fuels.

Although most municipal wastewater is unable to produce VFAs in large enough amounts to justify separation for downstream processing, high strength waste streams such as swine wastewater, fats, oils and greases (FOG), source separate organics (SSO) such as fruit and vegetable wastes have the potential to produces high concentrations of VFAs. Combining the two waste streams balances out the C:N ratio of the two substrates, making arrested anaerobic co-digestion of swine wastewater and FOG a viable candidate for high strength (high titer) VFA production. Additional processing of VFA-rich treated wastewater via microbial chain elongation can further enhance the final product by increasing the amount of longer-chain acids ($> C_7$). This study explores scaling up the VFA production process into a 114.7 L pilot-scale anaerobic membrane bioreactor (AnMBR), treating swine wastewater from a confined animal feeding operation (COD range 1,000 – 20,000 mg/L) via arrested anaerobic digestion at pH 9.0-9.5, producing both treated water and volatile fatty acids. The unique advantage of membrane

separation is that it not only enables separation of the medium and long chain acids from the waste slurry for efficient downstream valorization, but also minimizes feedback inhibition of the fermentative organisms due to the accumulation of these acids in the bioreactor. Semi-continuous operation of the AnMBR at hydraulic retention times (HRTs) ranging from 5-11 days yielded VFA concentrations of 1 – 9 g/L as COD, with the primary products being acetate, propionate, and iso-caproate (37 mM, 14 mM, and 9.2 mM respectively), and the highest fermentation efficiency (ratio of VFAs as COD to the input wastewater COD) being 90.92%. Batch-scale experiments on arrested co-digestion of the same swine wastewater and FOG at various blending ratios were also conducted, resulting in acetate concentrations as high as 40.9 mM and propionate concentrations as high as 19.2 mM at a (volumetric) blending ratio of 8:92 FOG and swine wastewater. However, the blended reactors were generally outperformed by the control reactor consisting of solely swine wastewater, indicating difficulties with long-chain fatty acid (LCFA) inhibition and mixing.

Treated permeate from the pilot-scale AnMBR has also been fed into a 2L bench-scale microbial chain elongation reactor inoculated with fermenter sludge from two existing reactors and bovine rumen fluid and maintained at pH 5. Chain elongating microbes present within the inoculum converted shorter-chain acids like acetate into medium-chain acids like butyrate or caproate via utilization of an electron donor. Introduction of ethanol as an electron donor significantly increased VFA production compared to control, primarily in the form of butyrate, with concentrations up to 71.9 mM.

With material demands for VFAs rising as more uses for them are developed, establishing alternative production sources is becoming increasingly critical. Resource recovery from wastewater and other waste streams enables production of VFAs as a valuable byproduct

alongside reusable water, promoting a circular economy and placing value onto otherwise discarded wastes.

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Dedication

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Chapter 1 - Background and Literature Review

Anaerobic Treatment and Digestion

Aerobic processes like the well-established activated sludge process account for 80% of municipal wastewater treatment in developed countries (McCarty et al., 2011). Aerobic treatment can be very energy intensive, with aeration accounting for 50-60% of a wastewater treatment plant's overall energy consumption if using activated sludge (Y. Chen et al., 2022). Because of this high energy demand in addition to high sludge production that must also be addressed, it is difficult to create an energy-neutral or energy-positive aerobic treatment process. Anaerobic treatment is an appealing alternative to aerobic treatment. It is much more feasible to create an energy-positive wastewater treatment process anaerobically due to both the lack of aeration needs and the production of methane-containing biogas that can be used for combined heat and power (CHP) or upgraded into renewable natural gas (RNG) (Crone et al., 2016; McCarty et al., 2011).

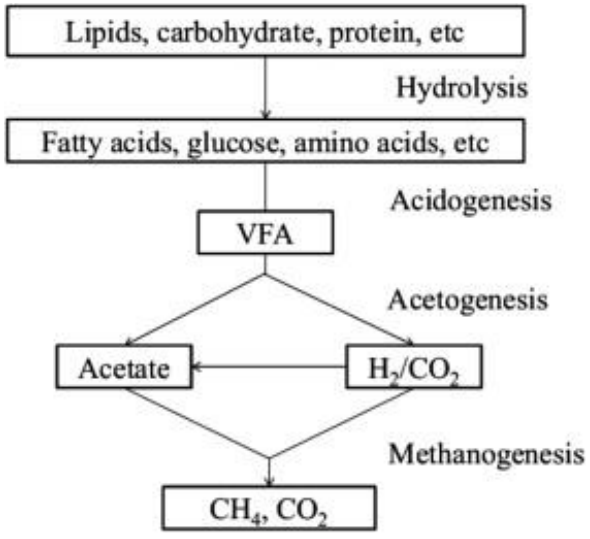


Figure 1-1. The four steps in the AD of organic substrates **(C. Zhang et al., 2014)**

The primary mechanism behind anaerobic treatment is anaerobic digestion. Anaerobic digestion is the process by which various anaerobic microbes work in tandem to break down complex organic matter such as lipids, carbohydrates, or proteins into the final product of methane and carbon dioxide (Shen et al., 2015). Anaerobic digestion has four distinct steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, as shown in Figure 1-1. Each product of the previous step is fed into the next step, creating a complex web that becomes the backbone of any anaerobic digester.

The first step, hydrolysis, is the primary rate limiting step where bacteria break down lipids, carbohydrates, proteins, and other complex organic matter into soluble and biodegradable products (Ventorino et al., 2018; Westerholm & Schnürer, 2019). Carbohydrates are converted to monosaccharides, proteins are converted to amino acids, and lipids are converted to long-chain fatty acids (LCFAs) (Pasalari et al., 2021). Hydrolytic bacteria include *Bacteriodetes, Firmicutes, Fibrobacter*, *Spirochaetes*, and *Thermotogae* (Li et al., 2018; Luo et al., 2018). Fermentative bacteria, who perform acidogenesis, metabolize the products of hydrolysis into short-chain fatty acids, such as butyric, propionic acid, acetic acid, and some alcohols (Rehman et al., 2019). Acidogenic fermentative bacteria include *Bacteroidetes, Firmicutes, Actinobacteria,* and *Proteobacteria* (De Vrieze et al., 2018). Acetogenesis, the third step, converts the short chain fatty acids and alcohols generated in acidogenesis into acetate, carbon dioxide, and hydrogen gas. (Rehman et al., 2019). Many acetogenic bacteria are from the *Syntrophomonas* genus (Di Marcoberardino et al., 2018). The final step of anaerobic digestion, methanogenesis, breaks down the remaining acetate, carbon dioxide, and hydrogen gas into methane via two different groups of archaea: acetoclastic methanogens and hydrogenotrphic methanogens (Martín-González et al., 2011). Acetoclastic methanogens break down acetate into

carbon dioxide and a methyl group, after which the methyl group is reduced down to methane (Pasalari et al., 2021). Hydrogenotrophic methanogens reduce carbon dioxide into methane, using hydrogen gas as an electron donor (Ventorino et al., 2018). Methanogenic archaea have been organized into five orders: *Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales,* and *Methanopyrales* (B. Wang et al., 2018; P. Wang et al., 2018). Anaerobic digestion is utilize to address many different organic waste streams and many factor can affect its efficacy, such as organic loading rate (OLR), pH, temperature, hydraulic retention time (HRT), ammonia content, alkalinity, and carbon to nitrogen (C/N) ratio (Fischer et al., 2019; S. Han et al., 2016).

Co-Digestion

One of the critical factors affecting anaerobic digestion, the C/N ratio, can be manipulated by introducing more than one substrate into a digester. During digestion, microbes generally consume carbon 25-30 times faster than nitrogen (Rehman et al., 2019). Because of this, a C/N ratio of 20:1 to 30:1 is ideal for maintaining the health of a digester. Low C/N ratios can cause accumulation of nitrogen and lead to ammonia toxicity. High C/N ratios can lead to insufficient Nitrogen to support anaerobic microbial metabolism, lowering biogas production. Blending together substrates with high and low C/N ratios can lead to more production when digested together than if they were digested separately. Swine wastewater tends to be higher in nitrogen content, leading to a C/N ratio of approximately 18:1 (Adelekan & Bamgboye, 2009). Fats, oils and greases (FOG), a nuisance waste in municipal wastewater systems, can have a C/N ratio of 45:1 to 147:1 (Gurd et al., 2020). This excessively high C/N ratio makes FOG difficult to treat. However, careful implementation of co-digestion with a lower C/N ratio substrate like

swine waste can help remediate and extract resources from FOG in the form of biogas or volatile fatty acids.

Anaerobic Membrane Bioreactors (AnMBRs)

An anaerobic membrane bioreactor is a developing wastewater treatment technology that pairs membrane filtration with anaerobic biological treatment (Kanafin et al., 2021; Menon & Kalyanraman, 2025). The implementation of a membrane enables retention of colloidal and suspended solids, completely decoupling the hydraulic retention time (HRT) and solids retention time (SRT). Maintaining solids within the reactor provides an amenable environment for slow-growing anaerobes, enabling enhanced treatment and biogas production. An anaerobic MBR has a significant advantage over an aerobic MBR, as it is able to produce higher quality effluent while keeping sludge production low, reducing the need for solids wasting (Mei et al., 2016). Being a completely anaerobic treatment process, the primary mechanism for treatment is anaerobic digestion. Because of this, AnMBRs need mesophilic or thermophilic temperatures, 35˚C or higher, to maintain treatment efficacy and high biogas production (Martin et al., 2011). Mesophilic conditions can improve treatment and potentially make the process energy positive (Y. Chen et al., 2013). When an AnMBR treating municipal wastewater is heated to mesophilic conditions, system efficiency can be from 78-92% higher than conventional activated sludge processes, while using 30-50% less energy and maintaining HRTs as low as 4-8 hours (Menon & Kalyanraman, 2025). High-strength wastewater streams such food and beverage wastewater or agricultural wastewater are even more complimentary to an AnMBR system, typically reaching up to 90-95% COD removal (Galib et al., 2016).

AnMBR Configurations

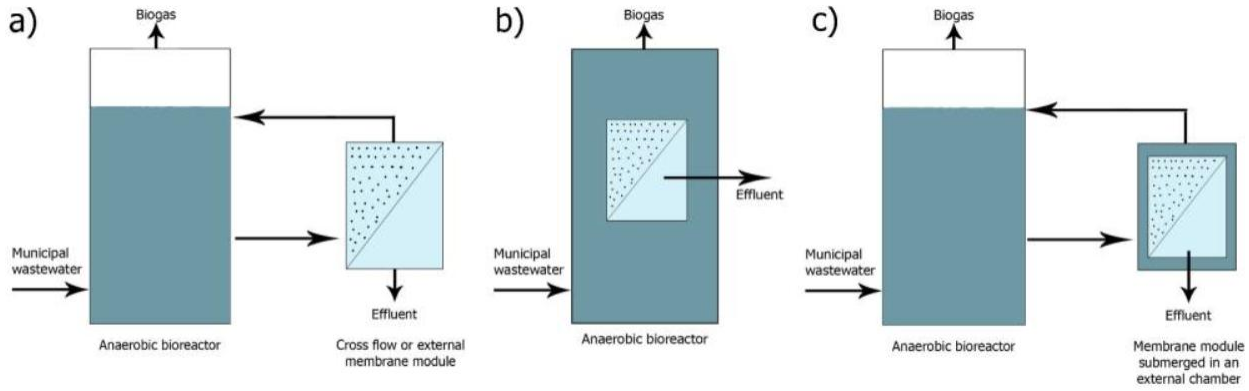


Figure 1-2. Simplified illustration of (a) an external/pressurized AnMBR; (b) a submerged AnMBR; and (c) externally submerged AnMBR **(Kanafin et al., 2021)**

Figure 1-2 above illustrates the three main configurations of AnMBR systems. In the side-stream configuration, depicted in Figure 1-2a, the membrane sits outside of the bioreactor. Under pressure, mixed liquor is fed from the bioreactor to the membrane module and pushed tangentially across the membrane surfaces. Permeate is extracted through the membrane and removed from the system, while any remaining liquor is fed back into the reactor. The submerged configuration, shown in Figure 1-2b, has the membrane module submerged directly within the bioreactor. One side of the membrane is in constant contact with the mixed liquor and the other side of the membrane is attached to a pump that applies negative pressure (or vacuum), causing the mixed liquor to filter through the membrane via a pressure differential. The externally submerged configuration, depicted in Figure 1-2c, features a secondary tank filled with mixed liquor that the membrane module is submerged in. This configuration features both the recirculation of a side-stream configuration, reducing cake fouling, and the negative pressure differential of a submerged configuration pulling permeate out of the system (Andrade et al., 2014). This configuration also has the benefit of easy replacement of the membrane module as the external tank can be completely disconnected from the main bioreactor, ensuring the

anaerobic environment is not disturbed. The most common AnMBR configuration is the submerged AnMBR, due to its smaller footprint, lower cost, and lower energy demand due to less pumping needed.

Membrane Configurations and Materials

In addition to overall AnMBR configurations, there are multiple configurations and materials for the membranes utilized in AnMBR systems. Hollow fiber, flat sheet, and tubular are the main configurations used in AnMBRs (Menon & Kalyanraman, 2025). Hollow fiber and flat sheet membranes are used in submerged configurations, while tubular membranes are used in side-stream configurations. Hollow fiber membranes are relatively inexpensive compared to the other configurations and typically have a much higher available surface area compared to other membrane configurations of the same size, enabling higher productivity (Baig et al., 2022; Stuckey, 2012). Flat sheet membranes trade performance for ease of use and integrity; they can handle a higher solids content in the mixed liquor and are easier to clean than hollow fiber membranes (Krzeminski et al., 2012). Tubular membranes also have a lower available surface area than the hollow fiber membrane but make up for it with even higher tolerances than flat sheet membranes. The shape of a tubular membrane, paired with the side-stream configuration, make them much less prone to fouling while also providing much higher capacity for solids contents (Lin et al., 2013). This comes at the cost of the larger footprint of a side-stream configuration AnMBR. The vast majority of membranes used in AnMBR systems are made of a polymer of some kind. Polymeric membranes are typically made out of synthetic organic polymers such as polyethersulphone (PES), polyvinylidene fluoride (PVDF), or polyacrylonitrile (PAN). Polymeric membranes have dominated wastewater treatment since the 1990s, with 90% of membrane manufacturers making PVDF and PES membranes (I. Ahmed et al., 2017; Kraume

& Drews, 2010). Polymeric membranes are easy to manufacture and the precursor materials for them are abundant. However, they have a high susceptibility to fouling. Ceramic membranes, the main competitor of polymeric membranes, are much less prone to fouling (Elmoutez et al., 2023). The pore size distribution on a ceramic membrane can be much lower than on a polymeric membrane, creating a higher quality membrane surface that can increase solids retention and therefore treatment efficacy (Z. Liu et al., 2019).

Volatile Fatty Acids

Uses and Conventional Production Methods

Although conventional anaerobic digestion focuses on methane as the final product of the process, the intermediate product of volatile fatty acids also has potential as an extractable product. Volatile fatty acids are used across a wide range of industries, such as pharmaceuticals, food, cosmetics, plastics, and even in production of synthetic aviation fuel (SAF) (Huq et al., 2021; Palar, 2024; H. Wu et al., 2024). Lactic acid is used as a pH neutralizer, as an ingredient in surgical sutures and preservatives, and is especially in demand for PLA bioplastics production (Grand View Research, 2026b; Yankov, 2022). It had a market size of USD 2.8 billion in 2025 and is expected to grow to USD 5 billion by 2033. Acetic acid had a market size of USD 15.8 billion in 2025 and is projected to grow to USD 28.9 billion by 2033 (Grand View Research, 2026a). It is a primary ingredient of vinegar and is used in plastic production, textiles, and chemicals. The primary market use of acetic acid is in the production of vinyl acetate monomers, which are used extensively in paints and coatings. Propionic acid has a market size of USD 1.1 billion and is used as a preservative specifically for breads and cheeses, as well as an intermediate in the production of herbicides, rubber compounds, and eco-friendly solvents (Grand View Research, 2026c). Propionic acid also has a use in wastewater treatment as an ideal

additional carbon source used for enhancement of biological nutrient removal (Y. Chen et al., 2013). Butyric acid has been paid special interest for its potential as a precursor to biofuel and to some experimental cancer drugs (Dwidar et al., 2012). It is also used in plastics and has some derivatives used as fragrances in cosmetics. It has a market size of USD 429.57 million (Fortune Business Insights, 2026). Valeric acid had a market size of USD 212.7 million in 2025 and is expected to double in the next 10 years (Johari, 2026). Valeric acid has similar uses to butyric acid in cosmetics as a fragrance and as a cancer drug that can suppress the growth of liver cancer (R. Han et al., 2020). Caproic acid is used as a lubricant, animal feed additive, and perfume base. It had a market size of USD 67.79 million in 2025 and is projected to grow to USD 109.1 million by 2034 (Swar, 2026).

Production of volatile fatty acids is incredibly resource intensive, typically involving high temperatures and pressures, multiple scrubbing steps, and use of valuable catalysts such as palladium or platinum. Table 1-1 below lists the various production processes for VFAs ranging from acetate (2 carbons) to caproate (6 carbons).

Table 1-1. VFAs Current Petroleum Feedstock Production **(Palar, 2024)**

VFA	Chemical Production Process	Reference
Acetate CH_3COOH	Nearly all acetate is produced through vapor-phase reaction using ethylene over a palladium catalyst. Carried out at 175-200 oC and 5-9 bar. Demand of 6000 kt/year.	(American Chemical Society, 2026)
Lactate $CH_3CHCOOH$	Ethene oxidation using palladium catalyst to form acetaldehyde, which under high pressure is converted to lactonitrile with an additional catalyst. Sulfuric acid is used on lactonitrile to produce lactic acid.	(Ojo & de Smidt, 2023)
Propionate CH_3CH_2COOH	Carboxylation of ethylene at 180 oC and 700 atm using a complex palladium catalyst. Also, can oxidize propionaldehyde where several separation steps occur after.	(Lapidus et al., 1979; Intratec, 2020)

Butyrate/Iso-Butyrate $CH_3(CH_2)_2COOH$	Oxidation, hydrogenation, and esterification of butyraldehyde, which is obtained from crude oil derived propylene, using aluminum complex catalysts, requiring lower temperatures at 0-20 oC.	(Varma & Madras, 2008)
Valerate/ Iso-Valerate $CH_3(CH_2)_3COOH$	Syngas and 1-butene implemented with platinum or palladium catalysts promotes hydroformylation to form valeraldehyde at high temperatures around 200 oC. This is then oxidized to form valeric acid.	(Kubitschke et al., 2014)
Caproate/Iso-Caproate $CH_3(CH_2)_4COOH$	Hexane undergoes oxidation with strong oxidizing agents carried out at 60 oC, where methyl groups present in hexane form to a carboxylic acid group which forms caproate as one resulting product.	(Kruse et al., 2006)

Biological VFA Production

Microbial pathways have attracted increasing attention as a route to scalable volatile fatty acid (VFA) production, since conventional synthesis is highly energy-intensive and carries significant environmental consequences. The scale of concern is considerable: acetic acid production for plastics manufacturing alone is estimated to account for nearly 3 million metric tons of emissions annually (Nicholson et al., 2021).

VFAs can be generated through two broad fermentation strategies: pure-culture systems, where a specific acid is the target, and mixed-culture systems, similar to conventional anaerobic digesters, where a mixed VFA profile is desired (Ramos-Suarez et al., 2021). Interest in the latter has grown substantially, particularly around acidogenic fermentation. Because acidogenesis corresponds to the intermediate stage preceding methanogenesis in anaerobic digestion — where VFAs appear as byproducts, as illustrated previously in Figure 1-1, the process offers a naturally grounded route to their production. Mixed-culture fermentation also holds an advantage over

pure cultures in flexibility in what feedstocks it can process, accommodating materials such as agricultural residues, food waste, and municipal wastewater solids (Ramos-Suarez et al., 2021).

When choosing a substrate for VFA production, the ideal candidates are sustainable and renewable sources that do not compete with food production, which is precisely why, along with a need for VFAs as a carbon source for biological nutrient removal, wastewater has become such a common fermentation substrate (Pandey et al., 2022). That said, municipal wastewater is not always well suited to anaerobic treatment since its organic loading is not consistently high enough for the process to operate efficiently. Concentration of influent waste and/or co-digestion should be considered when evaluating digestion of municipal wastewater.

Other waste streams high in carbohydrates and sugars, like agricultural wastes, food wastes, and FOG, have therefore been widely studied as VFA feedstocks (Pandit et al., 2021). Food waste requires more careful consideration, however, as it can contain up 11-23% lignin (Pandit et al., 2021). Lignin requires costly pre-treatment to be rendered digestible by AD microbes. Animal manure avoids this drawback, being rich in organic matter and carbon while requiring no pretreatment. Its appeal is amplified by a waste management problem: millions of tons are generated each year, much of it untreated or unmanaged, posing substantial environmental pollution risks (Guo et al., 2010).

Although animal manure remains understudied as a VFA feedstock, though some literature is available. One study, comparing anaerobic digestion of cow manure against municipal wastewater, measured a significantly greater cumulative biogas yield from the cow manure — an outcome attributed to the extra nutrients supplied to fermentative microbes, improving acidogenesis rates and subsequently raising biogas production (Macias-Corral et al., 2008). A study on chicken manure digested the waste in an anaerobic membrane bioreactor

(AnMBR), once while maintaining a pH of 6 and once while allowing the pH to remain uncontrolled, causing it to raise to 8. At the controlled pH of 6 VFA production reached up to 12 g/L-d, whereas the uncontrolled run reached up to 19 g/L-d (Yin et al., 2022). In a study on swine wastewater, heat-shock pretreatment (75 °C for 5 hr) was applied to inhibit methanogens, and batch reactors were run at initial pH values of 6, 8, and 10 over a 20-day operation. The pH 10 reactor achieved the highest carbon-to-VFA conversion at 71.2%, producing chiefly acetate and propionate (W. Zhang et al., 2020). Although there are some studies on alkaline arrested digestion, it remains largely unexplored in comparison to acidic fermentation.

Microbial Chain Elongation

As an emerging biotechnology, microbial chain elongation (MCE) has significant potential to alleviate resource constraints by converting organic waste into high-value medium-chain carboxylic acids (MCCAs) (Joshi et al., 2021). Electron donors such as ethanol, lactate, H_2, and CO provide carbon sources, metabolic energy, and reducing equivalents required to drive the reverse β-oxidation pathway; therefore, they play a crucial role in governing MCE performance (H. Wang et al., 2025). Reducing reliance on externally supplied electron donors can substantially improve the sustainability and economic feasibility of the process. Consequently, identifying sustainable and waste-derived electron donors is essential for enhancing the environmental performance of MCE systems.

In recent years, several strategies have been developed for MCCA production from organic wastes without external electron supplementation. For example, beer wastewater containing residual ethanol and acid whey rich in lactate can directly serve as endogenous electron donor sources for chain elongation (Andersen et al., 2017). Alternatively, organic wastes can be pre-fermented to generate ethanol or lactate in situ, which

are subsequently utilized during CE. Table 2 summarizes previously reported chain elongation studies for organic waste fermentation under different substrates, pH conditions, and electron-donor additions.

Table 1-2. Literature chain elongation production from anaerobic fermentation of organic wastes.

Substrate	Culture strains	E-donor addition	pH	Temp.(℃)	Volume	Primary products	Titers	References
OFMSW	Mixed	Ethanol	5.5	30	5L	Caproate, caprylate, heptanoate	2.8, 1.6, and 0.6 g/L	(Grootscholten et al., 2013)
OFMSW	Mixed	Ethanol	6.5-7.0	35	1L	Caproate	12 g/L	(Grootscholten et al., 2014)
Food waste	Mixed	Ethanol	5.5	30	1L	Caproate	23.4 g/L	(Roghair et al., 2018)
Food waste	Mixed	-	6	37	3L	Caproate	4.8 g/L	(Crognale et al., 2021)
Food waste	Mixed	-	6	35	1L	Caproate, caprylate	10.1 g/L, and 2.9 g/L	(De Groof et al., 2020)
Beer and stillage	Mixed, Clostridium and Lactobacillus	-	5.5	-	5L	Caproate, caprylate	8.1 g/L, 3.2 g/L	(Andersen et al., 2017)
Wine lees	Mixed, *Lactobacillus* and *Ruminococcus*	-	5.2	37	4.5L	Caproate, caprylate	4.14 g/L, 2.76 g/L	(Kucek, Xu, et al., 2016)
Ensiled potato peels	Mixed	-	5.5	32	0.125L	Acetate, butyrate, some caproate	-	(Undiandeye et al., 2023)

Syngas fermentation effluent	Mixed	Ethanol	5.2	30	0.7L	Caprylate	19.4 g/L	(Kucek, Spirito, et al., 2016)
Fixed film reactor media	Mixed, C. kluyveri significant	MEC cathode	6	30	0.56L	Caproate, butyrate	0.739 g/L, 0.263 g/L	(Van Eerten-Jansen et al., 2013)
Synthetic food waste	Mixed, *Clostridium* significant	Particle fluidized reactor cathode	6	37	N/M	Caproate	3 g/L	(Yang & Jack, 2026)
Chinese cabbage waste	*Caproiciproducens, Clostridium*	Ethanol	6	37	1L	Caproate	8.2 g/L	(Shen et al., 2026)
AD sludge, WAS, garden soil	*Pseudomonas*	Lactate, ethanol	6.5	37	2L	Caproate	13.719 g/L	(Meng et al., 2026)
Yeast extract and acetate solution	*E. limosum*	Methanol	6.5	30	1L	Butyric	103 mM	(W. S. Chen et al., 2016)
AD sludge and acetate solution	Mixed	Glycerol	7	37	0.035L	Butyric, caproic	49.9 mM, 9.7 mM	(Leng et al., 2017)
Paper mill AD sludge, biochar	Mixed	Ethanol	7	35	18.2	Caproate, caprylate	2.11 g/L, 0.230 g/L	(Y. Liu et al., 2017)
8% TS sewage sludge	*Mixed, Clostridium_sensu_stricto_12* significant	Ethanol	6.75	35	0.3L	Caproate	43.45 mM	(Q. Wang et al., 2020)
Food waste	*Mixed*	Ethanol	5	35-37	0.15L	Caproate	4.77 g/L	(L. Wu et al., 2023)
			7			Caproate	1.08 mg/L	

			10			No MCFAs observed	N/A	
WAS	*Mixed*	Ethanol	5	37	0.15L	Caproate	1.5 g/L	(Y. Wang et al., 2025)
	Mixed, *C. kluyveri* and *S. acetigenes* significant					Caprylate	10 g/L	
	Mixed					No MCFAs observed	N/A	

Research Objectives

The goal of this research was to demonstrate a resource recovery platform centered around VFA production from swine wastewater via arrested anaerobic digestion. The research goals to accomplish these goals are:

- Implement arrested anaerobic digestion into a pilot-scale anaerobic membrane bioreactor (AnMBR) and observe how the system responds to long-term performance with varying influent quality through fermentation efficiency analysis.
- Evaluate microbial chain elongation as a secondary step in VFA production by establishing MCE reactors with VFA-rich AnMBR permeate and introducing ethanol as an electron donor.
- Establish a baseline fats, oils and grease (FOG) to swine wastewater ratio for implementation and exploration of co-digestion into the pilot-scale AnMBR via batch-scale experiments with varying blending ratios.

Chapter 2 - Arrested Anaerobic Digestion of Swine Wastewater in an AnMBR and Microbial Chain Elongation of Treated Permeate

Introduction

As of 2024, there were 21,279 concentrated animal feed operations in the United States (NPDES CAFO Permitting Status Report, 2024). Almost all livestock, approximately 75% of cattle, 98.6% of swine, and 99.9% of meat chickens, in the country lives on one of the CAFOs (Anthis, 2024). Pork consumption in particular has increased by 77% since 1990, with 113 million tons consumed in 2022, and pork production has increased by 140% since 1961 (Kim et al., 2024). Pork now makes up 34% of meat consumed globally, as compared to other meat sources shown in Figure 2-1. 79 million pigs lived in the United States in 2022. With each pig producing 4-8 L of wastewater per day, this means that approximately 1.73×10^{11} L or 4.57×10^{10} gallons of swine wastewater needs to be treated and discharged every year.

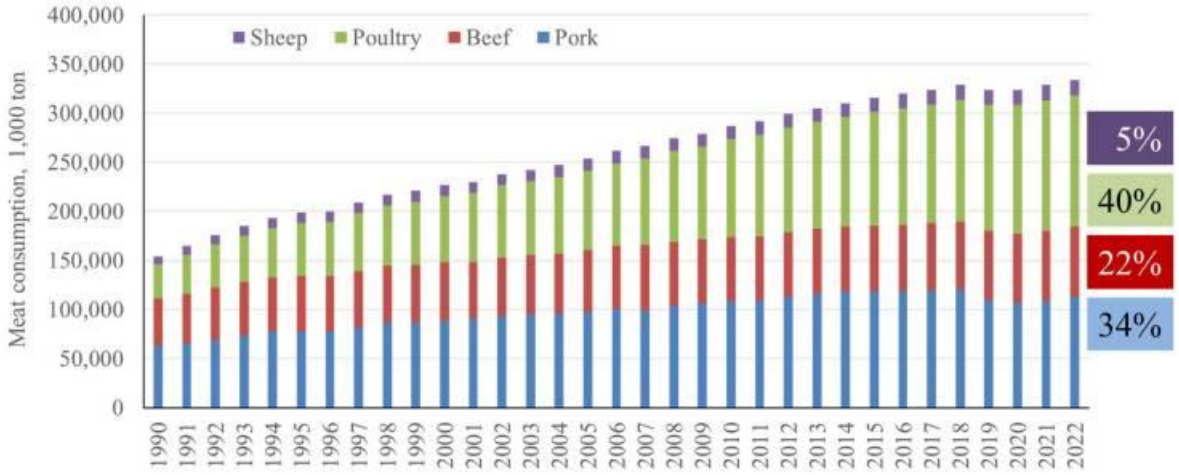


Figure 2-1. Consumption of various meats from 1990 to 2022 **(Kim et al., 2024)**

Most swine wastewater is treated in lagoons or simply discharged directly to local waterways (Kim et al., 2024). Some larger operations have implemented anaerobic digestion, but

the footprint required for conventional anaerobic digestion only makes it appealing to especially large animal operations. Because of this, most potential resources present in swine wastewater are lost, as methane produced in a lagoon is typically off gassed into the atmosphere and nutrients such as nitrogen and phosphorus are released into waterways, potentially contributing to eutrophication and harmful algal blooms. Pollutant concentration in swine wastewater can be quite high, with chemical oxygen demand (COD) ranging from 2,000-30,000 mg/L, ammonia nitrogen (NH_4-N) ranging from 110-1650 mg/L, and total phosphorus (TP) ranging from 100-620 mg/L (Cheng et al., 2019). The incentive for resource recovery of swine wastewater is significant and there is great potential in the development of a low-footprint, effective wastewater treatment and resource recovery method for agricultural wastewater.

Anaerobic treatment of swine wastewater provides an excellent platform for both water treatment and resource recovery. COD can be recovered as methane, but also as other products such as volatile fatty acids via arrested anaerobic digestion, which have broad uses across many different industries (Huq et al., 2021). Microbial chain elongation provides further refinement of VFAs, converting shorter-chain acids into more valuable medium-chain fatty acids (Joshi et al., 2021). This study evaluates arrested anaerobic digestion of swine wastewater in an anaerobic membrane bioreactor, producing volatile fatty acids. Further, the treated VFA-rich permeate from the AnMBR is implemented into microbial chain elongation reactors, evaluating that process as well.

Materials and Methods

Setup of PVDF membrane

Following the conclusion of previous experiments within the pilot-scale AnMBR, the reactor was drained and cleaned to prepare for transition into arrested digestion. Firstly, the

bioreactor was first subjected to a thorough cleaning protocol to remove residual biomass, biofilm, and inorganic deposits. The cleaning procedure consisted of sequential rinses with bleach solution and hydrogen peroxide (H_2O_2), followed by multiple washes with tap water to eliminate any remaining oxidants within 3 days. After cleaning, two new Zeeweed 2.5 polyvinylidene fluoride (PVDF – Veolia, USA) membrane module with a pore size of 0.04 μm was installed. Membrane integrity was verified through a pressure-hold test, and baseline hydraulic performance was assessed by measuring the net flux under by varying Q_{set} from 5 to 44 L/hr. The installation of two new PVDF membranes, preparation of the inoculum mixture, and pH adjustment to transition the system to fermentative mode are illustrated in Figure 2-2 and Figure 2-3. Aerobic digester sludge and raw swine wastewater were collected from the local wastewater treatment plant (WWTP) and Kansas State University's Swine Research and Teaching Unit, respectively. Raw swine wastewater was initially screened by mesh to remove coarse particles and mixed with anoxic sludge using a sump pump. These materials combined at a volumetric ratio of 50:50 (v/v) to prepare the feed mixture, achieving a target total solids (TS) concentration of 2% w/v. The mixture was adjusted to pH 9 using 5 M NaOH to evaluate VFA production in an alkaline environment. The mixture was thoroughly homogenized and transferred into the bioreactor to ensure uniform distribution of solids and microbial communities. The pH was monitored and adjusted to 9 as necessary and permeate was monitored for VFA production. Following 28 days of batch acclimation, the AnMBR was operated on a semi-continuous basis five days a week, maintaining an HRT of 9-11 days. Clean-in-place (CIP) procedures using 0.2 g/L bleach and 2 g/L citric acid solutions were performed 1-2 times a week, with frequency depending on membrane performance.



Figure 2-2. The new PVDF membrane modules were installed into the reactor following flux testing.



Figure 2-3. The process for preparing the inoculum for arrested digestion mode.

Setup of stainless-steel membrane

Following completion of setup with the PVDF membranes, a stainless-steel (SS) membrane module (Mott, USA) with a pore size of 0.5 µm was installed in a side-stream configuration outside the bioreactor for parallel performance evaluation (Figure 2-4). The hydraulic performance of the SS membrane was evaluated by adjusting the valve connecting the membrane unit to the bioreactor and operating the pump at different set pressures ranging from 15 to 70 PSI. Key operational parameters, including transmembrane pressure (TMP) and net flux (LMH), were monitored under varying valve opening conditions. Following installation and testing, operation of the SS membrane was implemented, with semi-continuous operation of the AnMBR alternating between the PVDF and SS membranes week by week. Occasional simultaneous operation of both membranes also occurred. The operation of the two different membranes was due to other work ongoing at the same time as evaluation of arrested digestion and VFA production was largely unimpacted by chosen membrane, as discussed later in this chapter.



Figure 2-4. Images showing the setup of the stainless-steel membrane in side-stream configuration.

Setup of microbial chain elongation reactors

To facilitate the microbial chain elongation process, VFA-rich permeate from the AnMBR was acidified to pH 5 and blended with three inoculums: pH 5 bench-scale fermentation sludge, commercial fermenter supernatant obtained from the City of Olathe, KS, and rumen fluid obtained from K-State Veterinary Medicine. This mixture of three inoculums aims to introduce as many chain elongating microbes as possible, giving the best chance of kickstarting chain elongation within these reactors. The inoculum-substrate mixture was then separated into two 2 L bench-scale reactors. One reactor was reserved as a control, the second was dosed with 150 mM/feed ethanol as a secondary electron donor to initiate chain elongation. Each reactor was maintained at pH 6, so as to remain within the ideal range for chain elongation and stirred at 350 RPM. The HRT was maintained at 10 days by feeding the reactors with fresh permeate from the AnMBR three times per week. The reactors were monitored for VFA and gas production throughout the duration of the experiment.

Analytical Methods

VFA content was quantified via HPLC (Shimadzu LC-20AT, USA) with a photodiode array detector and a refractive index detector. The HPLC was equipped with an Aminex HPX-87H column (300 mm × 7.8 mm, Bio Rad Corp., USA) and run at a pump flow rate of 0.65 mL/min and 50˚C oven temperature with 2.5 mM sulfuric acid as eluent. TCOD/SCOD, ammonia, total nitrogen, and total/reactive phosphorus contents were measured with HACH TNT 822, 832, 828, and 845 tests, respectively. All HACH tests were measured with a HACH DR3900 spectrophotometer. The pH of the influent, sludge from the middle and bottom of the reactor were monitored daily using an Oakton pH 550 benchtop pH meter. Total and volatile solids (TS and VS) of the bottom and middle of the reactor were measured twice weekly

according to the Standard Methods for Examination of Water and Wastewater 2540B and 2540E. Methane production was monitored via a gas chromatographer equipped with a thermal conductivity detector (GC-TCD). The carrier gas argon has a flow rate of 8.88 mL/min and pressure of 88.8 kPa. Injection, column, and detector temperatures were 200, 80, and 150 ˚C, respectively.

VFA Analysis

From the HPLC, VFA data initially recorded in mM was converted to COD to analyze conversion of influent COD into VFAs during arrested digestion. The conversion is done via an electron balance and COD conversion factor that represents 8 g COD as a standard equivalent to one electron, in accordance with previous work within the group (Palar, 2024).

Acetate:

$$CO_2 + HCO_3^- + 8H^+ + 8e^- \rightarrow CH_3COO^- + 3H_2O$$

$$\left(\frac{mmol\ \boldsymbol{acetate}}{L}\right)\left(\frac{8\ meq}{mmol}\right)\left(\frac{8\ mg\ COD}{meq}\right) = \frac{mg\ COD}{L}$$

Lactate:

$$2CO_2 + HCO_3^- + 12H^+ + 12e^- \rightarrow CH_3CHOHCOO^- + 4H_2O$$

$$\left(\frac{mmol\ \boldsymbol{lactate}}{L}\right)\left(\frac{12\ meq}{mmol}\right)\left(\frac{8\ mg\ COD}{meq}\right) = \frac{mg\ COD}{L}$$

Propionate:

$$2CO_2 + HCO_3^- + 14H^+ + 14e^- \rightarrow CH_3CHOHCOO^- + 5H_2O$$

$$\left(\frac{mmol\ \boldsymbol{propionate}}{L}\right)\left(\frac{14\ meq}{mmol}\right)\left(\frac{8\ mg\ COD}{meq}\right) = \frac{mg\ COD}{L}$$

Iso-Butyrate and Butyrate:

$$4CO_2 + HCO_3^- + 20H^+ + 20e^- \rightarrow CH_3(CH_2)_2COO^- + 6H_2O$$

$$\left(\frac{mmol\ (\boldsymbol{iso}-)\boldsymbol{butyrate}}{L}\right)\left(\frac{20\ meq}{mmol}\right)\left(\frac{8\ mg\ COD}{meq}\right)=\frac{mg\ COD}{L}$$

Iso-Valerate and Valerate:

$$5CO_2 + HCO_3^- + 26H^+ + 26e^- \rightarrow CH_3(CH_2)_3COO^- + 8H_2O$$

$$\left(\frac{mmol\ (\boldsymbol{iso}-)\boldsymbol{valerate}}{L}\right)\left(\frac{26\ meq}{mmol}\right)\left(\frac{8\ mg\ COD}{meq}\right)=\frac{mg\ COD}{L}$$

Iso-Caproate and Caproate:

$$6CO_2 + HCO_3^- + 32H^+ + 32e^- \rightarrow CH_3(CH_2)_4COO^- + 10H_2O$$

$$\left(\frac{mmol\ (\boldsymbol{iso}-)\boldsymbol{caproate}}{L}\right)\left(\frac{32\ meq}{mmol}\right)\left(\frac{8\ mg\ COD}{meq}\right)=\frac{mg\ COD}{L}$$

Fermentation Efficiency

Fermentation efficiency is a measure of how much influent COD is converted to VFAs as COD in the effluent.

$$Fermentation\ Efficiency = \frac{\Sigma VFAs\ as\ COD}{Influent\ COD} * 100\%$$

Results and Discussion

Following the pilot-scale AnMBR startup with blended aerobic digester sludge and raw swine wastewater, the mixture was allowed to acclimatize for 28 days in batch mode before semi-continuous operation began. Figure 2-5 below shows the VFA profile of the acclimatization phase, along with the fermentation efficiency and total VFAs as COD measured from days 1-35.

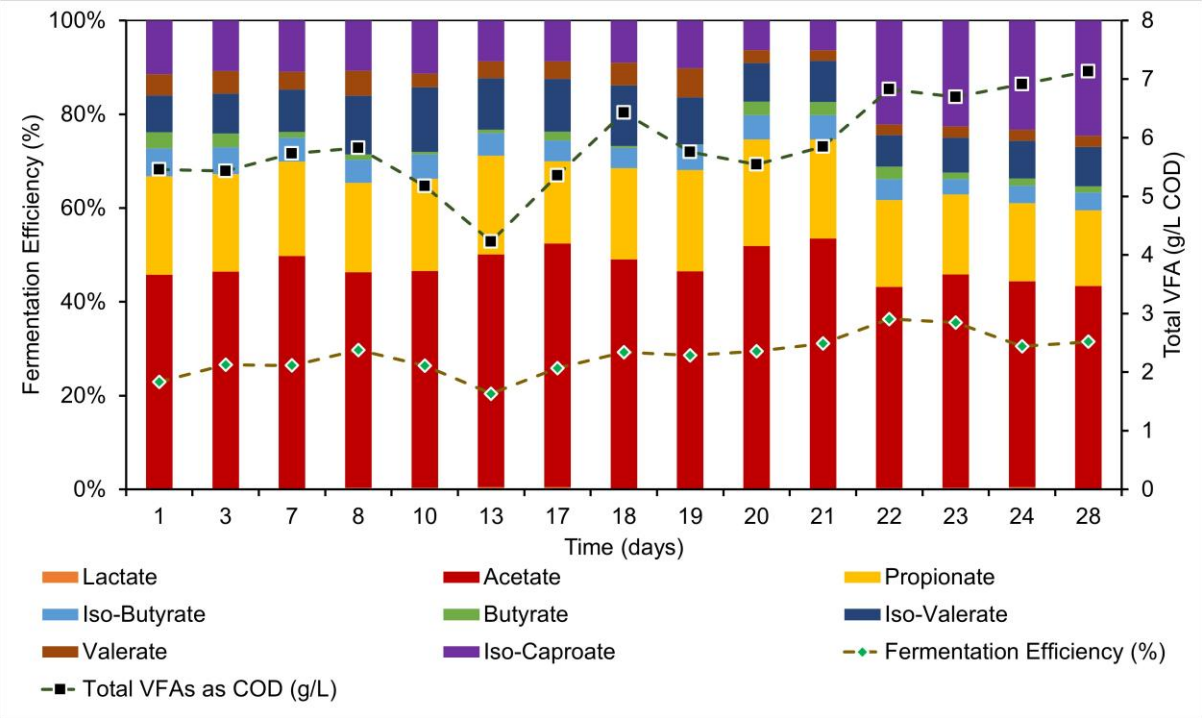


Figure 2-5. VFA profile and fermentation efficiency in the pilot-scale AnMBR at pH 9 during batch start-up phase.

VFA content was immediately observed at 5.5 g/L, indicating presence of VFAs in the initial mixture. Fermentation occurred immediately with little to no lag phase, eventually increasing total VFA concentrations up to 7.2 g/L at the end of the acclimatization phase. Acetate is the primary product, generally making up about 40-50% of the overall profile of VFAs produced, followed by propionate making up about 20% of the profile and iso-caproate making up 10-20% as well. The last five data points collected during acclimatization feature an increase in iso-caproate up closer to 20% of the profile, which is a trend that generally continued as semi-continuous operation began. Other medium-chain VFAs, (iso-)butyrate and (iso-)valerate, make up the rest of the remaining ~20% of the profile combined. This could indicate that initial chain elongation of acetate into longer chain acids is a rate-limiting step, allowing for accumulation of

acetate, while chain elongation of butyrate and valerate into longer chain caproate and caprylate occurs faster.

Semi-continuous operation

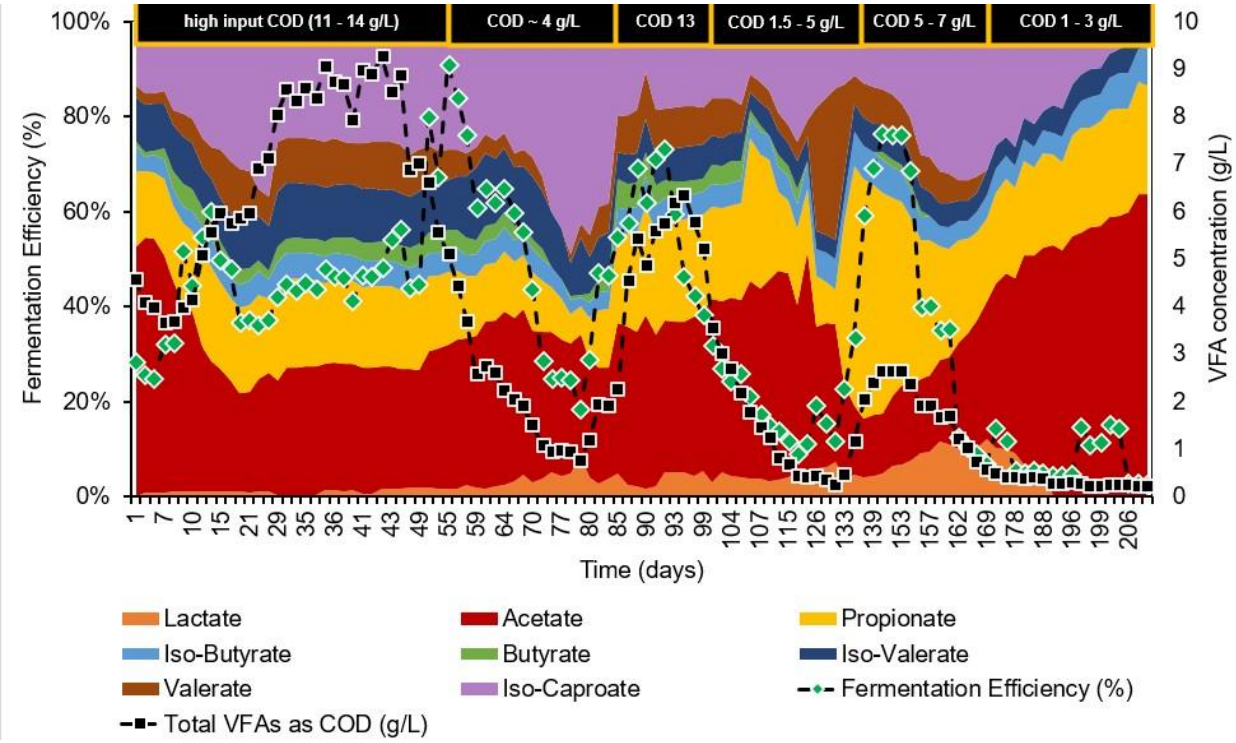


Figure 2-6. VFA profile and fermentation efficiency in the pilot-scale AnMBR at pH 9 during semi-continuous operation phase utilizing the PVDF and SS membranes.

Figure 2-6 above shows the VFA profile, overall fermentation efficiency, and total VFAs as COD in the pilot-scale AnMBR over 210 days of operation. During semi-continuous operation where pH adjusted wastewater was fed into the reactor and permeate was collected through the membrane, the AnMBR was generally operated for eight hours a day, five days a week, barring clean-in-place procedures as needed for the PVDF membranes. VFA production and fermentation efficiency were heavily dependent on influent COD throughout the entire period of operation, with higher influent COD resulting in higher VFA production overall as well as better conversion rates of influent COD into VFAs. The transfer to semi-continuous operation resulted

in a decrease in the proportion of acetate until much later in operation, lowering from the 40-50% seen during start-up to 20-30% of the overall VFA profile. This trend continued throughout the variations in influent swine wastewater COD until around day 133, where the acetate concentration dropped sharply in accordance with a smaller increase in COD, before steadily rising to above 50% during a period of extremely dilute influent (days 160 – 210). Propionate followed generally the same trend as start-up, making up about 20% of the overall profile, barring days 133 to 157, where it increases to 40% of the profile, corresponding with an increase in influent COD. Iso-caproate starts off making up ~30% of the profile before sharply increasing to 50% from days 77 to 85. The proportion of iso-caproate then remains consistent at about ~20% around day 157 where it increases to 30% before steadily declining to almost nothing towards the end of the observation period, corresponding to low influent wastewater COD.

The highest VFA concentrations occurred during the period with the highest influent COD between days 29-45, with the highest concentration of 9.28 g/L occurring on day 42. The drop in fermentation efficiency and total VFAs produced following day 115 corresponds to an extended period of low influent COD (700-3800 mg/L). Controlling COD input is a consistent challenge in arrested digestion, specifically for waste streams with dynamic fluctuations in organic C content, as the process generally requires higher influent COD to convert into VFAs. Fermentation efficiency varied along with influent COD, reaching as high as 90.92% on day 55 to as low as 2.51% on day 210. The average fermentation efficiency of the observation period was 37.1%. Changes in daily operation of a small-scale swine farm (cleaning regimes, chemicals used) can significantly concentrate or dilute wastewater, causing unreliable input into a wastewater treatment system. Figure 2-7 below shows the influent VFA content of the raw swine wastewater. The VFA content can vary anywhere from 0-7 g/L. Following day 100, little to no

VFA content has been observed in the influent wastewater. This lack of initial VFA content as well as consistently low influent COD has resulted in a period of especially low VFA production in the reactor. The next chapter will explore FOG addition as a method of creating a more consistent COD loading in an AnMBR, possibly improving and maintaining VFA production over time.

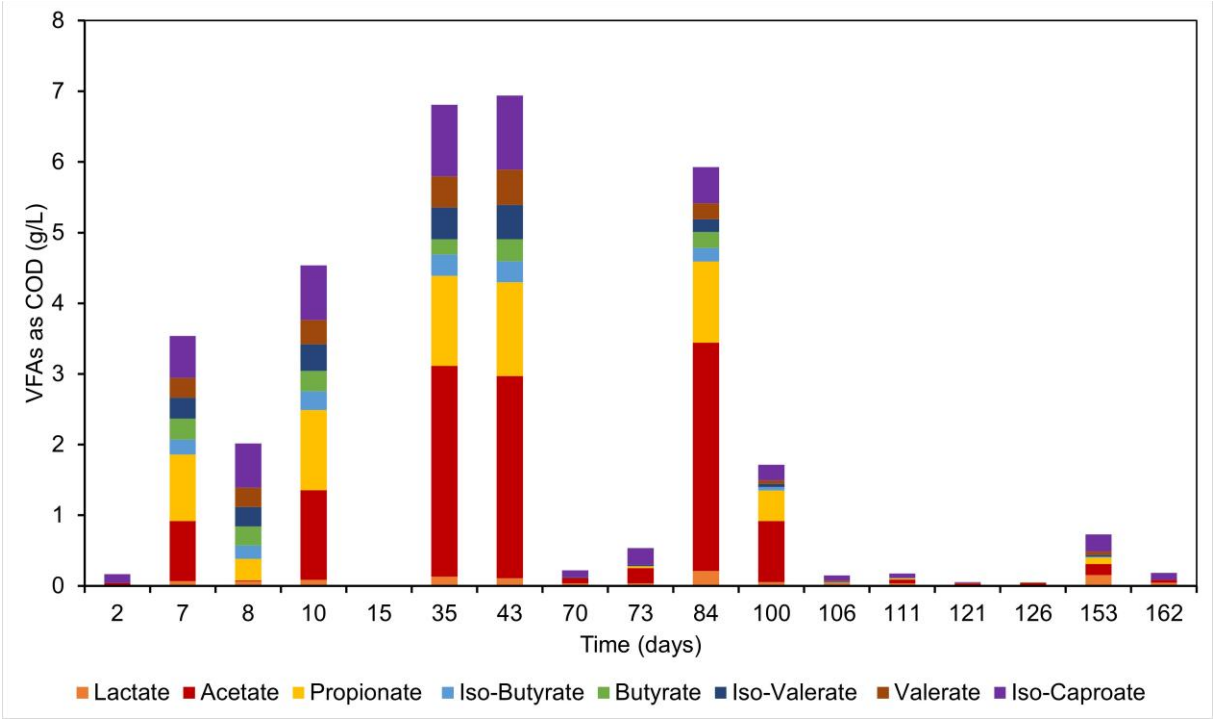


Figure 2-7. VFA data in the swine wastewater influent for the pilot-scale AnMBR during semi-continuous operation.

Figure 2-8 below shows a comparison of VFA production data between the PVDF and SS membranes over 4 days of operation. During this period, the AnMBR was run with the PVDF membrane for the first 4 hours of operation and the SS membrane was run during the last 4 hours of operation. VFA samples were taken during the operation of each membrane to evaluate any differences in VFA production caused by the choice of membrane. Fermentation efficiency and VFA content remained relatively consistent across each membrane, with only a 1-1.5% decrease in fermentation efficiency and 0.1-0.3 g/L COD decrease in the SS membrane observed.

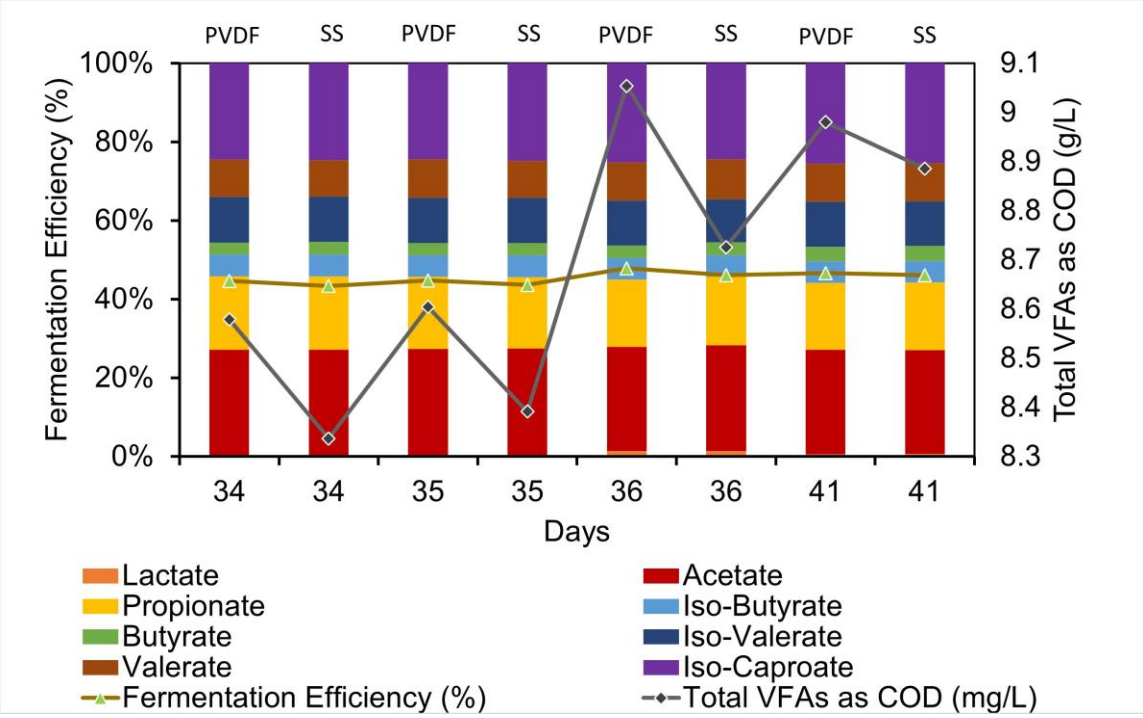


Figure 2-8. VFA profile and fermentation efficiency of both the PVDF and SS membranes.

Gas Production

Figure 2-9 below shows the biogas production and methane composition of biogas from days 1-210. Biogas composition generally remained below 4 L per day for the majority of operation, although methane concentration increased to ~30-40% of the total gas composition between days 30-70. In response to the increase in methane content, the operating pH of the AnMBR was increased to 9.5 for the remainder of the operation period to maximize inhibition of methanogenesis.

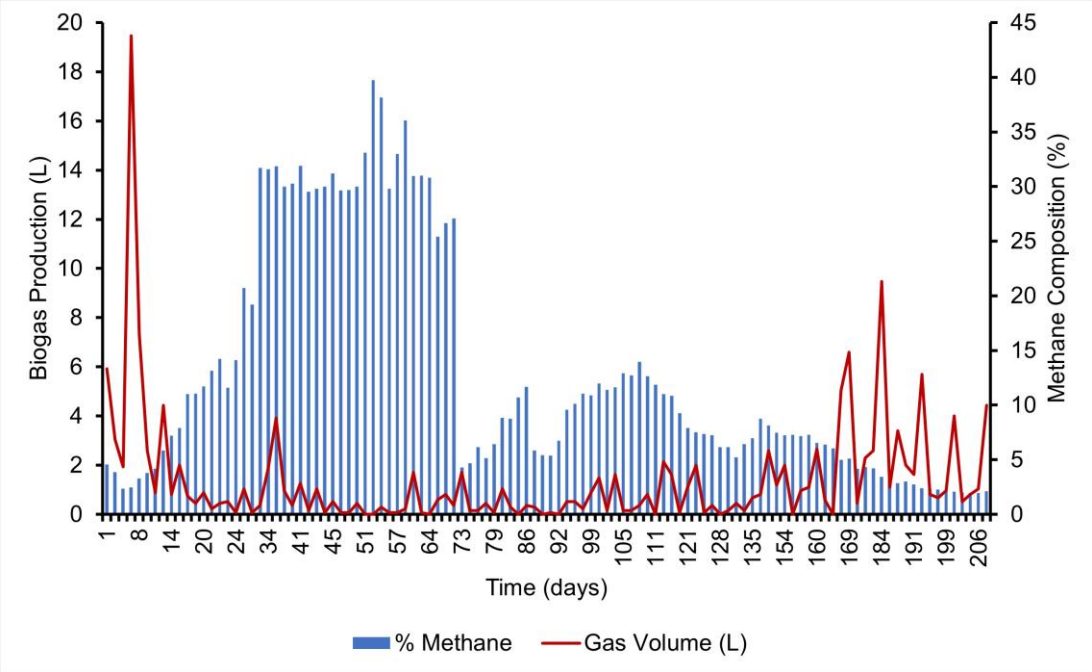
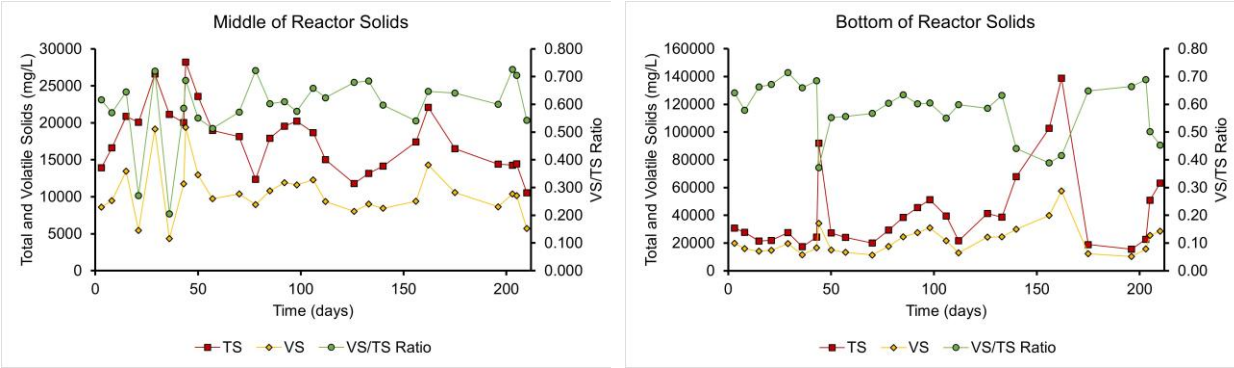


Figure 2-9. Gas volume and methane composition of the AnMBR system.

Solids Analysis



(a) (b)

Figure 2-10. TS and VS during AnMBR operation.

Figure 2-10 represents the total solids (TS), volatile solids (VS) and TS/VS ratio throughout the operating period of the AnMBR. The VS/TS ratio is used as an indicator of overall reactor health. A VS/TS ratio over 0.5 typically represents a healthy reactor. Volatile solids

represent the organic solids content, with TS representing both organic and inorganic solids. The VS/TS ratio of the AnMBR tended to stay within the range of 0.5 to 0.7, with occasional deviations above or below but no consistent streak of a low ratio. Note that the bottom solids have more variation, as well as a significantly higher maximum value of 138,826 mg/L TS. The period of high solids in the bottom of the reactor, days 140-162 corresponds to a switch to the PVDF membrane after a period of extended 3-month operation of the SS membrane. The sudden lack of the powerful mixing power of the SS membrane pump may have resulted in more solids suddenly settling to the bottom of the reactor as allowed by the PVDF recirculation pump. This accumulation led to the team deciding to waste solids from the reactor, the effects of which are immediately observed on day 175.

pH Adjustment Requirements

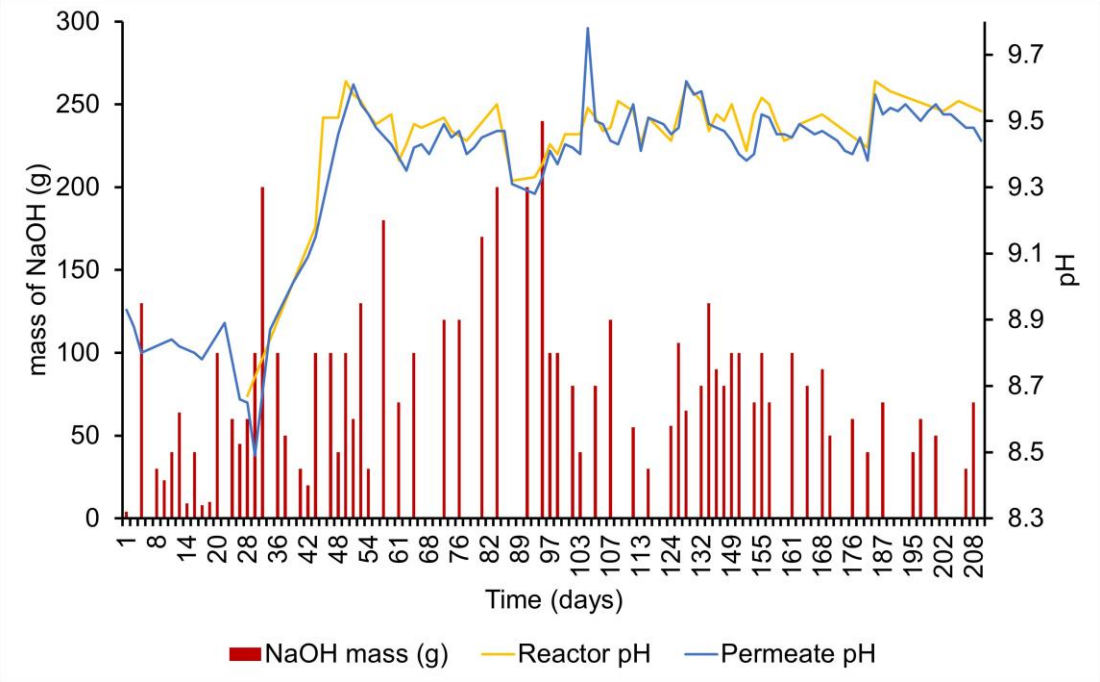


Figure 2-11. Base requirement for AnMBR system.

The pH of the influent and reactor were both measured on a daily basis and adjusted as needed. Figure 2-11 above shows the amount of NaOH added during the operation period, along with the observed reactor and permeate pH. On day 44, in response to elevated methane content in the biogas generated by the system, the operating pH was increased from 9.0 to 9.5 to further inhibit methanogenesis. As the system continued to acclimate, the amount of NaOH necessary to maintain reactor pH decreased, remaining relatively steady following day 98. Over the observation period, 26.87 L of 5M NaOH was added to the AnMBR, corresponding to 5.37 kg. With a cumulative permeate volume of 2458.12 L from days 1-210, this corresponds to 2.18 g NaOH added per liter of permeate produced.

Microbial Chain Elongation

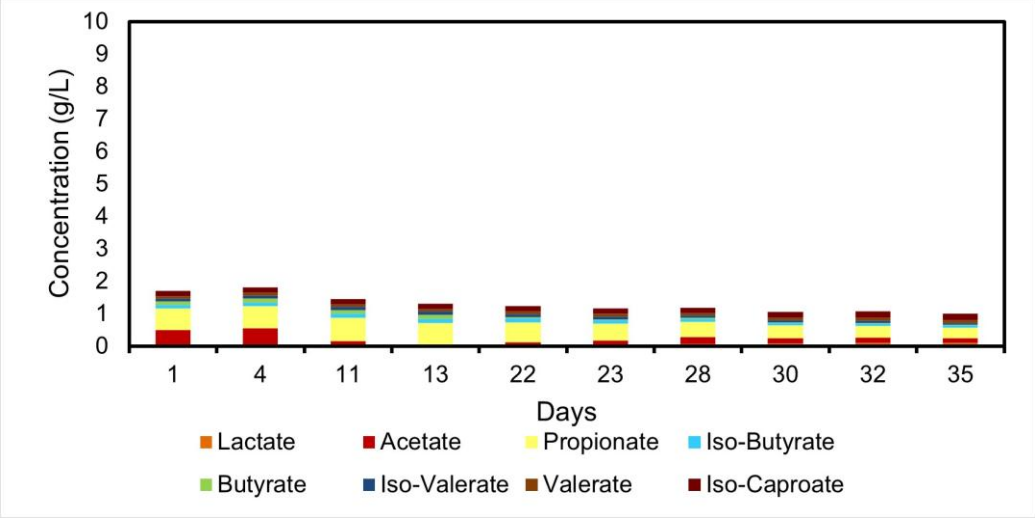


Figure 2-12. VFA data in the MCE control reactor with no electron donor addition.

Figure 2-12 above shows the VFA concentrations within the control reactor, with no electron donors added. Day 1 of MCE operation corresponds to day 140 of AnMBR operation. Given that the permeate fed into the reactor had little to no COD that had not already been converted to VFAs, there is only a steady decrease in VFAs as the HRT of 10 days was

maintained, in accordance with lower VFAs produced in the AnMBR. Notably, there is little to no lactate present in the MCE reactors despite lactate being present in the influent permeate during the same time. Lactate is used as an electron donor in chain elongation, though the amounts that were present, never increasing above 2 mM, did not contribute to any significant increase in VFA concentrations. The presence of chain elongating bacteria from the inoculum alone does not enable the chain elongation process without the presence of a substantial amount of a secondary electron donor, such as lactate or ethanol.

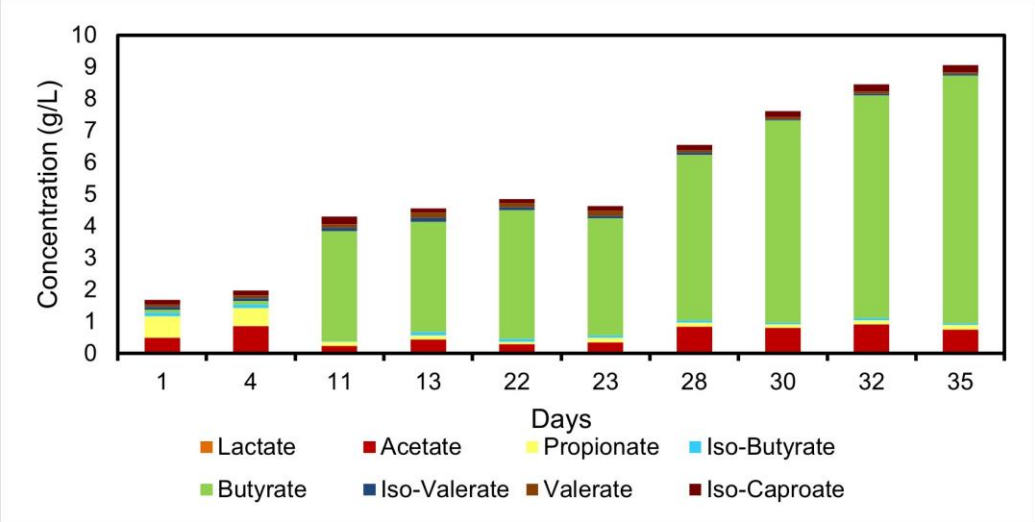


Figure 2-13. VFA data in the MCE reactor with ethanol added as an electron donor.

Figure 2-13 above shows the VFA data of the reactor with ethanol addition. Despite consistent removal of old permeate and addition of new permeate to maintain HRT, the concentration of butyrate had continued to increase since introducing ethanol addition on day 11. With the significant increase in butyrate concentrations, the total VFA concentration in the reactor increased up to 9.02 g/L by day 35. The majority of this was butyrate at a concentration of 7.76 =g/L, with acetate being the second highest at 0.75 g/L. There may also be accumulation of higher chain acids, however the HPLC used for VFA quantification is only able to reliably detect up to iso-caproate.

Conclusions

This study demonstrated production of VFAs via arrested anaerobic digestion of swine wastewater in an anaerobic membrane bioreactor and microbial chain elongation of the resulting permeate. Implementing pH control to maintain an alkaline environment of 9.0-9.5 enables fermentation efficiencies of up to 90.92% and total VFA concentration of up to 9.28 g/L. The primary products were acetate, propionate, and iso-caproate. VFA production and conversion efficiency was highly dependent on influent COD. The consistently low influent COD (<3 g/L) of the last month and a half of operation led to the lowest observed production during the study. Inhibition of methanogenesis was effective at an increased pH of 9.5, with methane proportions in biogas dropping from 39.73% of composition to below 12% following adjustment to 9.5. Microbial chain elongation of the AnMBR permeate during an extended period of low VFA production increased the total VFA concentrations from 2.6 g/L to up to 9.06 g/L via addition of ethanol. This study is the first to observe VFA production at this scale in an AnMBR setup and in additionally underexplored phenomena of alkaliphilic arrested digestion, as opposed to the typical acidophilic arrested digestion that is more common in literature. This study is the first demonstration of a stainless-steel membrane used in an AnMBR. This study also explores a theoretical two-step process for VFA production that demonstrates significantly increased concentrations and potential for conversion of shorter-chain acids into more valuable medium to longer-chain acids.

Chapter 3 - Arrested Anaerobic Co-Digestion of Swine Wastewater and Fats, Oils and Greases at the Batch Scale

Introduction

Fats, oils, and grease (FOG) is a nuisance waste in municipal wastewater systems. Although food service establishments are required to install grease interceptors in many parts of the world, they remain the primary source of FOG in municipal sewers (He & Yan, 2016). Approximately 30-40% of sewer clogs are due to FOG accumulation resulting in significant costs for repairs and maintenance of local sewers (N. Ahmed et al., 2025). In addition, it is estimated that 40-50% of all sanitary sewer overflows are caused by blockages from FOG deposits (Ducoste et al., 2009). In the United States, costs related to maintenance and repairs due to FOG accumulation is estimated to be USD 25 billion per year (He et al., 2017). Even if FOG travels through the entire sewer system and ends up at a wastewater treatment plant, it can cause problems there too, clogging pumps at the headworks and potentially throwing the wastewater plant's ecosystem out of balance, affecting effluent water quality.

It is difficult to remediate or treat FOG on its own, as its insolubility in water and extremely high carbon to nitrogen ratio make it unconducive to biological treatment (Collin et al., 2020). However, blending FOG with another substrate with a much lower carbon to nitrogen ratio can make it possible, and even beneficial to treat FOG biologically. Swine wastewater has a lower carbon to nitrogen ratio due to its generally elevated levels of nitrogen (Adelekan & Bamgboye, 2009). Blending together swine wastewater and FOG, balancing out the carbon to nitrogen ratio to the ideal range of 20-30, will likely end in higher production of beneficial resources like biogas or volatile fatty acids than what would be produced if swine wastewater and FOG were treated separately (Rehman et al., 2019). This study evaluates arrested anaerobic

co-digestion of swine wastewater and FOG at the batch scale to determine a blending ratio to be used in implementation of co-digestion in a pilot-scale anaerobic membrane bioreactor (AnMBR).

Materials and Methods

Three FOG samples were collected from various points in the wastewater treatment system in Manhattan, KS: a lift station, the grit building, and the biosolids farm. Stone Point is a lift station approximately 7 miles from the Manhattan WWTP. FOG was collected from the top layer of water within the station the day before the scheduled monthly cleaning of the lift station. In addition to FOG, the sample also contained other floating waste, such as hygiene products, flushable wipes, and hair, making it difficult to process and separate out the FOG. The grit building and biosolids farm are bot within the wastewater plant with both samples being collected directly after the monthly cleaning of the lift station. The sample from the grit building as solid, as the floating FOG is battered against the screens at the headworks of the plant, forming what the plant operators refer to as “grease balls” over time. The biosolids farm sample was stratified, with solids at the bottom, liquid at the top, and floating particles throughout the liquid layer. Three samples were taken from each location. Table 3-1 below shows the average characteristics of each FOG source. Figure 3-1 below shows images of each of the FOG samples after collection.

Table 3-1. Basic characteristics of fats, oils and grease samples taken from various points in the Manhattan, KS wastewater system.

	pH	Total COD (mg/L)	Total Nitrogen (mg/L)
Stone Point	4.81	58900	499.43
Grit Building	6.00	66250	418.54
Biosolids Farm	5.56	24735	253.58

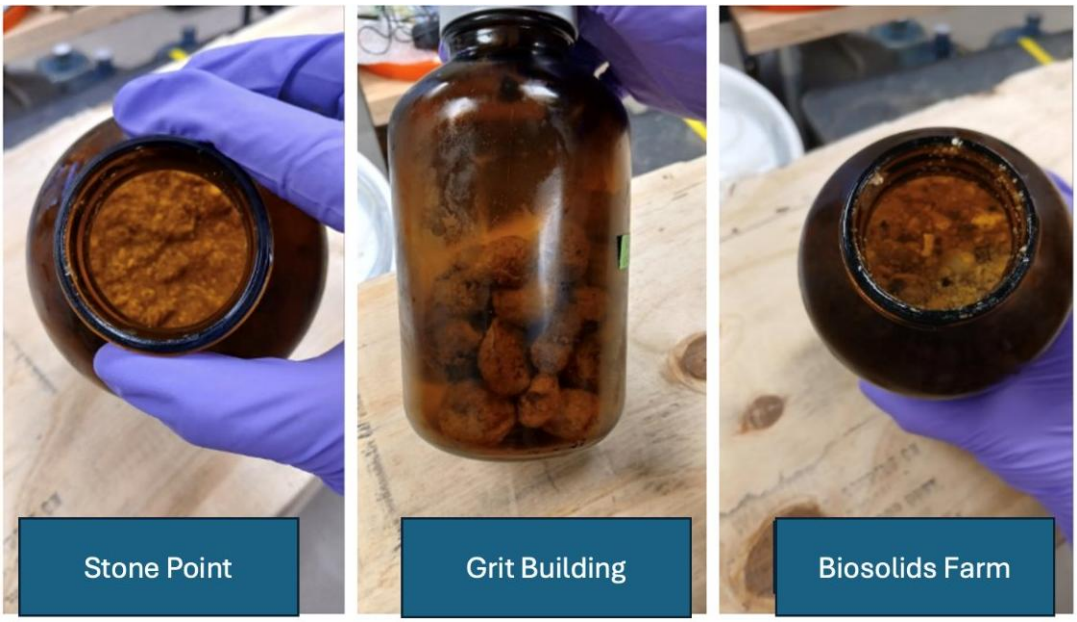


Figure 3-1. Images of the FOG samples collected from the Stone Point lift station, the Manhattan WWTP grit building, and the Manhattan WWTP biosolids farm.

Following initial handling and testing of the three samples, the samples collected from the grit building were selected for experimentation for their ease of use, homogeneity, high strength (~55,000-75,000 mg/L COD), and relative lack of extraneous debris that other samples contained, such as flushable wipes. Real swine wastewater was also collected from the K-State North Research Farm for use.

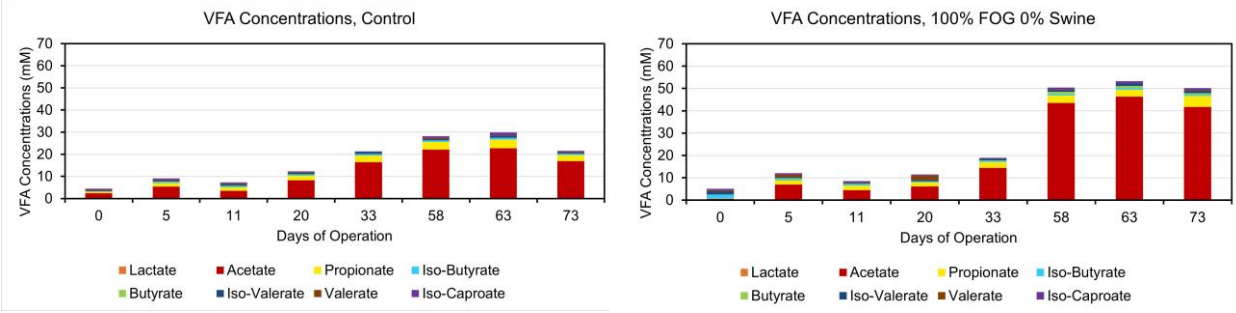
For the first round of experiments, both substrates were blended at various ratios: 100% FOG to 0% swine, 75% FOG to 25% swine, 50% FOG to 50% swine, 25% FOG to 75% swine, and 0% FOG to 100% swine. The chosen COD load was 80 mg/L. The blended mixtures were introduced to an inoculum mixture of anoxic sludge, anaerobic media, and solids from an existing pH 9 arrested digester, for a total volume of 200 mL. Each ratio was duplicated, for a total of 12 batch-scale reactors including two controls. Each reactor was maintained at pH 9.

The second round of experiments were prepared with the same method as the first round but with a different COD load and selection of ratios. The chosen ratios, on a COD basis, were: 25% FOG to 75% swine, 16% FOG to 84% swine, 8% FOG to 92% swine, and 0% FOG to 100% swine. The chosen COD load was 23.5 g/L. The desired COD load was 80 g/L, but the swine wastewater collected at the time only had a COD of 23.5 g/L, making it impossible to maintain each ratio at the desired loading. Each ratio was performed in triplicate for a total of 12 reactors.

Initial and final total chemical oxygen demand (TCOD) and total nitrogen (TN) analyses were performed using HACH 822 TNT and 826 TNT kits and a HACH DR 3900 spectrophotometer. Initial and final total solids analysis was performed according to standard methods (2540D; Standard Methods Water and Wastewater). pH was measured and readjusted twice a week using an Oakton pH 550 benchtop pH meter and 5M NaOH. VFAs were quantified using a high-performance liquid chromatograph (HPLC, Shimadzu LC-20AT, USA) with an Aminex HPX-87H column (300 mm × 7.8 mm, Bio Rad Corp., USA) equipped with two detectors: photodiode array and refractive index detectors at 0.65 mL/min pump flow rate and 50 ℃ oven temperature with 2.5 mM sulfuric acid as eluent.

Results and Discussion

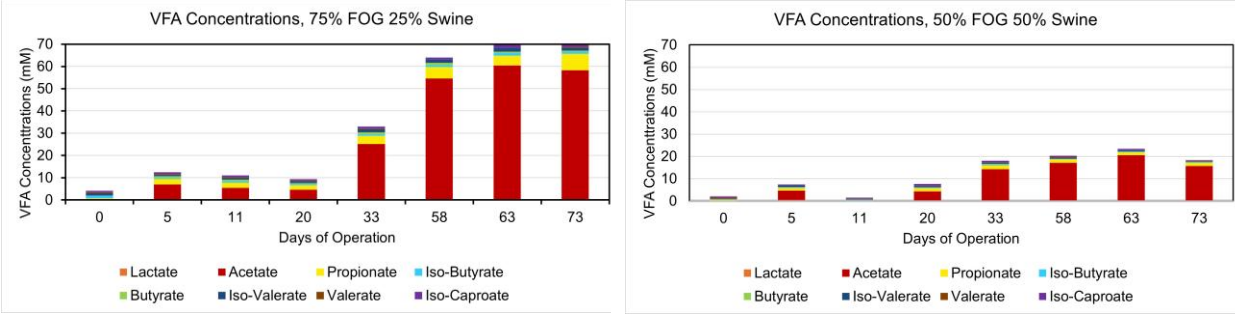
0-100% FOG Experiments



(a) (b)

Figure 3-2. (a) Average VFA concentrations in the control reactors. (b) Average VFA concentrations in the 100% FOG reactors.

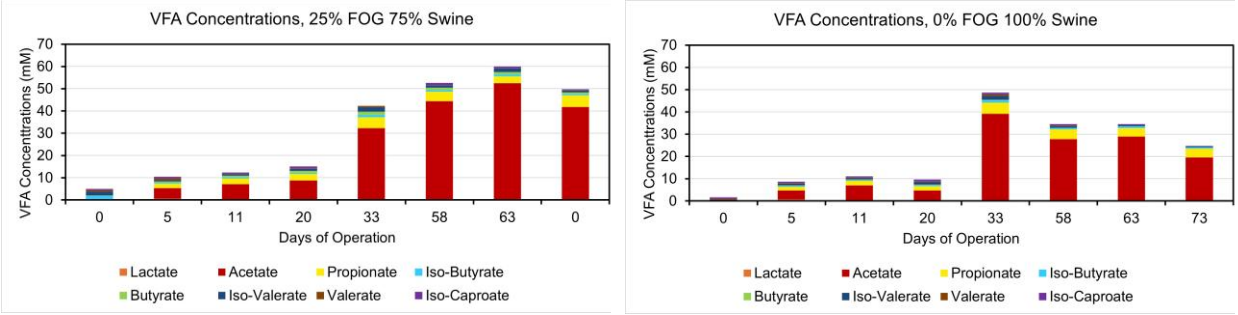
The arrested anaerobic co-digestion experiments were concluded after 73 days of operation. Figure 3-2a above shows the average VFA concentrations in the two control bottles (anoxic inoculum + anaerobic media alone). The primary product was acetate, followed by propionate. The VFA content increases steadily until the final observation on day 73, where the total VFA concentration has diminished to about half of what it was 10 days prior on day 63. This decrease in VFA content between days 63-73 remains consistent with most of the other ratios, indicating that the initial substrate added was completely consumed, leading to microbes beginning to consume the VFAs instead. Figure 3-2b shows the average VFA concentrations in the two 100% FOG bottles. The primary product was acetate, followed by propionate and relatively similar levels of butyrate, iso-valerate, and iso-caproate in the final sample. VFA concentrations were similar or lower than the control for the first 12 days, likely indicating inhibition from presence of long chain fatty acids (LCFAs). The sharp increase in VFAs between days 33-58 likely indicates when feedback inhibition was overcome.



(a) (b)

Figure 3-3. (a) Average VFA concentrations in the 75% FOG reactors. (b) Average VFA concentrations in the 50% FOG reactors.

Figure 3-3a shows the average VFA concentrations in the two 75% FOG bottles. The primary products were acetate, followed by propionate, iso-valerate, and iso-caproate in the final sample. The data were similar to the 100% FOG bottles, except that higher amounts of VFAs were generated once LCFA inhibition was overcome after day 33. The greater increase in VFAs between days 20-33 may also imply that feedback inhibition was overcome sooner than in the 100% reactor. The 75% FOG reactors exhibit the highest VFA concentrations of any ratio from days 33-73 but exhibits similar low VFAs up to day 20 as other reactors. Figure 3-3b shows the average VFA concentrations in the two 50% FOG bottles. The primary product was acetate followed by propionate in the final sample. The 50% reactors did not produce relevant data, and there was significant noise between the duplicated reactors. This suggests user error during initial preparation, likely incorrect mixing volumes of initial swine-FOG solution or presence of more extraneous debris than FOG in the substrate added to the 50% reactors.



(a) (b)

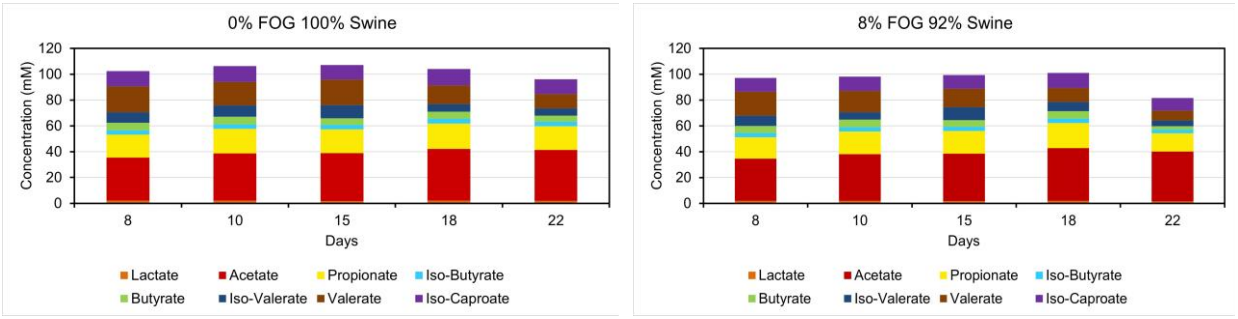
Figure 3-4. (a) Average VFA concentrations in the 25% FOG reactors. (b) Average VFA concentrations in the 0% FOG reactors.

Figure 3-4a shows the average VFA concentrations in the two 25% FOG bottles. The primary product was acetate, followed by propionate and iso-valerate in the final sample. The steady increase in acetate concentrations over time, as opposed to each of the other reactors which experience a decrease in VFAs on day 20, indicates muted LCFA inhibition. Figure 3-4b shows the average VFA concentrations in the two 0% FOG (100% swine) bottles. The primary products were acetate and propionate in the final sample. There was a sharp decrease in VFA concentrations after achieving the highest concentration by 33 days, which was not observed in any other reactors. Given the lack of FOG present in these reactors, this shows that the swine wastewater degrades much faster than the grit building FOG.

The data generated from the bench-scale experiments exhibited effects of the extended existence of FOG in anaerobic digesters, with the sharp increase in VFAs seen in the reactors with higher FOG content. The microbial communities may be overcoming feedback inhibition by breaking down long-chain fatty acids (LCFAs) in the FOG, resulting in an increase in measured VFAs as they are broken down into shorter-chain acids that can be measured reliably with the HPLC. The data generated in this initial round of experiments led to the conclusion that the next round should narrow down to the 0-25% FOG ratio range. This is primarily due to the

lack of initial inhibition that was present in every other FOG ratio within the first 20 days of observation. Given that these experiments are intended to inform implementation of FOG co-digestion into the anaerobic membrane bioreactor system discussed in the previous chapter, conditions observed within the first 20 days are the most consistent with the AnMBR system as its HRT ranges from 9-15 days. Implementing higher FOG ratios also increases the risk of inhibition within the system, which can pose a significant risk to the health of the reactor and effectiveness of treatment.

0-25% FOG Experiments

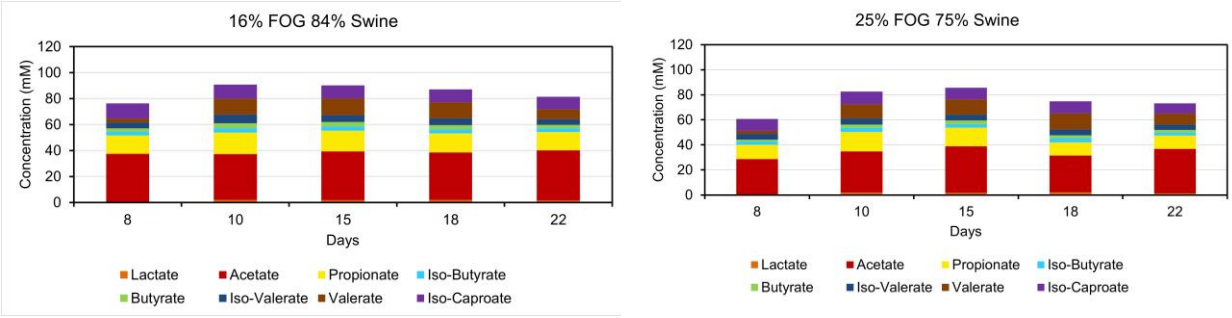


(a) (b)

Figure 3-5. (a) Average VFA concentrations in the 0% FOG reactors. (b) Average VFA concentrations in the 8% FOG reactors.

Figure 3-5a above shows the average VFA contents of the three 0% reactors. The primary products are acetate, ranging from 33-40 mM, valerate, ranging from 11-20 mM, and propionate, which remained relatively consistent at about 17-19 mM. The 0% reactors exhibit a minimal increase in total VFA concentrations from days 8-15 before dropping steadily from days 18-22. The decrease stems mostly from the valerate and iso-valerate concentrations, whereas the acetate concentration continues to increase slightly. Figure 3-5b shows the average VFA contents of the 8% FOG reactors. The primary products are acetate at 32-41 mM, propionate at 16-19 mM, and

valerate at 7-18 mM. The introduction of FOG actually decreased the VFA concentrations within the reactors and led to a sharper decrease between days 18-22. Given the higher target COD load compared to the previous round of experiments, LCFA inhibition may be more apparent within the reactors.



(a) (b)

Figure 3-6. (a) Average VFA concentrations in the 16% FOG reactors. (b) Average VFA concentrations in the 25% FOG reactors.

Figure 3-6a above shows the average VFA concentrations of the three 16% FOG reactors. The primary products are acetate at 35-38 mM, propionate at 14-16 mM, and valerate at 3-13 mM. The 16% reactors have a lower still total VFA concentration compared to the lower 8% FOG ratio. However, the increase in concentration between days 8-10 is much higher, and the decrease between days 18-22 is much lower. Figure 3-6b shows the average VFA concentrations of the three 25% reactors. The primary products are acetate at 28-37 mM, propionate at 10-15 mM, and valerate at 2-13 mM. The 25% reactors have the lowest VFA concentrations overall but exhibited the sharpest increase in total concentrations between days 8-10. VFA concentrations decreased past day 15 in both the 16% and 25% reactors, which may inform an initial HRT to maintain when evaluating implementation into the pilot-scale AnMBR system.

These data indicate that the higher concentrations of FOG in the 16% and 25% reactors encourage production of longer chain acids like valerate and iso-caproate, either via breakdown of LCFAs present in the FOG or elongation of shorter chain acids like acetate and propionate. Presence of FOG may also be inhibiting acetogenesis, allowing longer chain acids to accumulate while preventing their conversion into acetate. Conversely, a lack of adequate FOG leads to longer chain acids being broken down into acetate at a higher, observable rate. Based on the data collected so far, the 16% and 25% FOG ratios show the most promising results for further evaluation, particularly due to the increase in longer chain acids by day 10, which is the typical HRT in the AnMBR. One key factor that cannot be replicated at the batch scale is the decoupling of the HRT and SRT via membrane separation of permeate and solids. The exceptionally high SRT of the AnMBR will enable accumulation and extended presence of FOG within the bioreactor. The continuous removal of VFAs via the membrane will assist the system in overcoming feedback inhibition that is likely occurring in the batch-scale reactors; the effects of this remain to be seen and can only be evaluated by implementing FOG addition in the pilot-scale AnMBR system. Nevertheless, this is an important factor to keep in mind when evaluating the batch-scale data, as this is a critical component of the AnMBR system that cannot be replicated at this scale.

Conclusions

This study demonstrated production of VFAs at the batch-scale via arrested anaerobic co-digestion of swine wastewater and fats, oils and greases at pH 9. The first round of experiments exhibited the highest VFA concentrations at the 75% FOG ratio, but the elevated concentrations occurred after 58 days of observation. The short-term data up to day 20 showed inhibition via a decrease in total VFAs. The 25% ratio had a better initial 20 days, which lines up more with the

<20 day HRT used in an AnMBR system. This led to the second round of experiments exploring FOG ratios between 0-25%. The second round of experiments showed stronger initial production of VFAs in the 16% and 25% FOG reactors, although the 8% and 0% reactors has higher VFA concentrations throughout the entire experiment. The experiments also demonstrated a general decrease in VFA concentrations past day 15 of observation. This demonstrates an initial target HRT to stay below in order to avoid the bulk of possible feedback inhibition. Future work will utilize these data to inform implementation into a pilot-scale AnMBR system in order to increase VFA concentrations and maintain a more consistent COD loading rate via FOG addition at ratios between 16% and 25%.

Chapter 4 - Future Work and Conclusions

Future Work

Implementation of Arrested Co-Digestion into the Pilot-Scale AnMBR

Arrested co-digestion of swine wastewater and FOG at the batch-scale provided valuable data on what ratios may be worth starting off with when evaluating implementation of co-digestion in the pilot-scale AnMBR. VFA concentrations of up to 100 mM demonstrate excellent potential for scaling up the process. Although arrested digestion of FOG has not been studied extensively, conventional digestion of FOG and wastewater is a well-established pair of substrates. Blending FOG and municipal sewage sludge at 10-30% FOG by volume net a 30-80% increase in biogas production in two different full-scale digesters (Bailey, 2007; Muller et al., 2010). Increases in production this high often enable wastewater treatment plants to offset over 50% of their electricity demand through on-site combined heat and power (CHP) or renewable natural gas (RNG) generation (Davidsson et al., 2008; Luostarinen et al., 2009). However, great care needs to be taken in ensuring operational stability of the reactor. Co-digestion with FOG also brings a litany of problems, including digester foaming, long chain fatty acid (LCFA) inhibition, and pump blockages due to increase solids content (Jeganathan et al., 2006; Pereira et al., 2004). Dialing in the proper ratio that ensures maximum VFA production while minimizing any operational problems will be critical and should be reevaluated often as the conditions of influent swine wastewater vary over time.

Exploration of Alternative Electron Donors for Microbial Chain Elongation

Ethanol is the standard electron donor for study of microbial chain elongation (Y. Liu et al., 2024). Evaluation of MCE of a new product like the AnMBR permeate discussed in Chapter 3 is relevant, but more evaluation is always necessary when exploring alternative electron donors

for MCE. Addition of lactate in tandem with ethanol has demonstrated a strong synergy, increasing caproate yield by up to 58% vs. ethanol alone (Meng et al., 2026). In-situ generation of ethanol or lactate from complementary waste streams or from co-located waste sources could be economically viable. Methanol and glycerol have both demonstrated potential for butyric acid production in particular, generating concentrations up to 103 mM and 50 mM, respectively (W. S. Chen et al., 2016; Leng et al., 2017). Syngas, a mixture of carbon monoxide, hydrogen gas, and carbon dioxide has also been evaluated as it can itself be fermented into ethanol (Spirito et al., 2014). It has been able to produce up to 1.7 g/L-day caproate 20 g/L-day butyrate. Evaluation of all of these electron donors and more may be of interest, especially with more diverse substrates such as treated permeate, providing the framework for a two-step VFA production ecosystem.

Conclusions

In conclusion, this work demonstrates the successful scale-up of arrested anaerobic digestion for simultaneous wastewater treatment and volatile fatty acid (VFA) production. Operating a 114.7 L pilot-scale anaerobic membrane bioreactor (AnMBR) on swine wastewater (COD 1,000–20,000 mg/L) at pH 9.0–9.5 and hydraulic retention times of 5–11 days, semi-continuous operation achieved VFA concentrations of 1–9 g/L as COD, dominated by acetate, propionate, and iso-caproate (37, 14, and 9.2 mM, respectively), with a peak fermentation efficiency of 90.92%. Complementary batch experiments on arrested co-digestion of swine wastewater with FOG yielded acetate concentrations of up to 40.9 mM and propionate up to 19.2 mM at an 8:92 FOG-to-swine wastewater blending ratio; however, the blended reactors generally underperformed the swine wastewater-only control, suggesting that long-chain fatty acid (LCFA) inhibition and poor mixing constrained co-digestion performance. Downstream valorization was

also demonstrated, as treated AnMBR permeate supported microbial chain elongation in a 2 L bench-scale reactor, where ethanol addition as an electron donor markedly increased VFA production, primarily butyrate, reaching concentrations of up to 71.9 mM. Collectively, these findings establish an integrated process for converting an underutilized waste stream into both reusable water and VFAs of growing commercial value. As demand for VFAs continues to expand alongside emerging applications, recovering them from wastewater offers a practical route towards a circular economy approach that assigns value to materials otherwise discarded.

References

Adelekan, B., & Bamgboye, A. I. (2009). Comparison of biogas productivity of cassava peels mixed in selected ratios with major livestock waste types. *African Journal of Agricultural Research*, *4*, 571–577.

Ahmed, I., Balkhair, K. S., & Shaiban, M. H. A. and A. A. J. (2017). Importance and Significance of UF/MF Membrane Systems in Desalination Water Treatment. In *Desalination*. IntechOpen. https://doi.org/10.5772/intechopen.68694

Ahmed, N., Straub, A., & Ali, M. (2025). Fats, oil, and grease (FOG) in wastewater: Sources, mechanisms, control, and removal strategies. *International Journal of Environmental Science and Technology*, *23*(2), 161. https://doi.org/10.1007/s13762-025-06977-2

American Chemical Society. (2026). *Vinyl acetate synthesis*. American Chemical Society. https://www.acs.org/molecule-of-the-week/archive/v/vinyl-acetate/vinyl-acetate-synthesis.html

Andersen, S. J., De Groof, V., Khor, W. C., Roume, H., Props, R., Coma, M., & Rabaey, K. (2017). A Clostridium Group IV Species Dominates and Suppresses a Mixed Culture Fermentation by Tolerance to Medium Chain Fatty Acids Products. *Frontiers in Bioengineering and Biotechnology*, *5*. https://doi.org/10.3389/fbioe.2017.00008

Andrade, L. H. de, Mendes, F. D. dos S., Espindola, J. C., & Amaral, M. C. S. (2014). Internal versus external submerged membrane bioreactor configurations for dairy wastewater treatment. *Desalination and Water Treatment*, *52*(16), 2920–2932. https://doi.org/10.1080/19443994.2013.799048

Anthis, J. R. (2024). *US Factory Farming Estimates*. *Estimates*. https://sentienceinstitute.org/us-factory-farming-estimates

Baig, M. I., Pejman, M., Willott, J. D., Tiraferri, A., & de Vos, W. M. (2022). Polyelectrolyte Complex Hollow Fiber Membranes Prepared via Aqueous Phase Separation. *ACS Applied Polymer Materials*, *4*(2), 1010–1020. https://doi.org/10.1021/acsapm.1c01464

Bailey, R. (2007). Anaerobic Digestion of Restaurant Grease Wastewater to Improve Methane Gas Production and Electrical Power Generation Potential. *Proceedings of the Water Environment Federation*, *2007*, 6793–6805. https://doi.org/10.2175/193864707787223619

Chen, W. S., Ye, Y., Steinbusch, K. J. J., Strik, D. P. B. T. B., & Buisman, C. J. N. (2016). Methanol as an alternative electron donor in chain elongation for butyrate and caproate formation. *Biomass and Bioenergy*, *93*, 201–208. https://doi.org/10.1016/j.biombioe.2016.07.008

Chen, Y., Li, X., Zheng, X., & Wang, D. (2013). Enhancement of propionic acid fraction in volatile fatty acids produced from sludge fermentation by the use of food waste and

Propionibacterium acidipropionici. *Water Research*, *47*(2), 615–622. https://doi.org/10.1016/j.watres.2012.10.035

Chen, Y., Zhang, H., Yin, Y., Zeng, F., & Cui, Z. (2022). Smart energy savings for aeration control in wastewater treatment. *Energy Reports, 2021 International Conference on New Energy and Power Engineering*, *8*, 1711–1721. https://doi.org/10.1016/j.egyr.2022.02.038

Cheng, D. L., Ngo, H. H., Guo, W. S., Chang, S. W., Nguyen, D. D., & Kumar, S. M. (2019). Microalgae biomass from swine wastewater and its conversion to bioenergy. *Bioresource Technology*, *275*, 109–122. https://doi.org/10.1016/j.biortech.2018.12.019

Collin, T. D., Cunningham, R., Asghar, M. Q., Villa, R., MacAdam, J., & Jefferson, B. (2020). Assessing the potential of enhanced primary clarification to manage fats, oils and grease (FOG) at wastewater treatment works. *Science of The Total Environment*, *728*, 138415. https://doi.org/10.1016/j.scitotenv.2020.138415

Crognale, S., Braguglia, C. M., Gallipoli, A., Gianico, A., Rossetti, S., & Montecchio, D. (2021). Direct Conversion of Food Waste Extract into Caproate: Metagenomics Assessment of Chain Elongation Process. *Microorganisms*, *9*(2), 327. https://doi.org/10.3390/microorganisms9020327

Crone, B. C., Garland, J. L., Sorial, G. A., & Vane, L. M. (2016). Significance of dissolved methane in effluents of anaerobically treated low strength wastewater and potential for recovery as an energy product: A review. *Water Research*, *104*, 520–531. https://doi.org/10.1016/j.watres.2016.08.019

Davidsson, Å., Lövstedt, C., la Cour Jansen, J., Gruvberger, C., & Aspegren, H. (2008). Co-digestion of grease trap sludge and sewage sludge. *Waste Management*, *28*(6), 986–992. https://doi.org/10.1016/j.wasman.2007.03.024

De Groof, V., Coma, M., Arnot, T. C., Leak, D. J., & Lanham, A. B. (2020). Adjusting Organic Load as a Strategy to Direct Single-Stage Food Waste Fermentation from Anaerobic Digestion to Chain Elongation. *Processes*, *8*(11), 1487. https://doi.org/10.3390/pr8111487

De Vrieze, J., Pinto, A. J., Sloan, W. T., & Ijaz, U. Z. (2018). The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool. *Microbiome*, *6*, 63. https://doi.org/10.1186/s40168-018-0449-9

Di Marcoberardino, G., Foresti, S., Binotti, M., & Manzolini, G. (2018). Potentiality of a biogas membrane reformer for decentralized hydrogen production. *Chemical Engineering and Processing - Process Intensification*, *129*, 131–141. https://doi.org/10.1016/j.cep.2018.04.023

Ducoste, J. J., Keener, K. M., & Groninger, J. W. (2009). *Fats, Roots, Oils, and Grease (FROG) in Centralized and Decentralized Systems*. IWA Publishing. https://doi.org/10.2166/9781780403649

Dwidar, M., Park, J.-Y., Mitchell, R. J., & Sang, B.-I. (2012). The Future of Butyric Acid in Industry. *The Scientific World Journal*, *2012*(1), 471417. https://doi.org/10.1100/2012/471417

Elmoutez, S., Abushaban, A., Necibi, M. C., Sillanpää, M., Liu, J., Dhiba, D., Chehbouni, A., & Taky, M. (2023). Design and operational aspects of anaerobic membrane bioreactor for efficient wastewater treatment and biogas production. *Environmental Challenges*, *10*, 100671. https://doi.org/10.1016/j.envc.2022.100671

Fischer, M. A., Güllert, S., Refai, S., Künzel, S., Deppenmeier, U., Streit, W. R., & Schmitz, R. A. (2019). Long-term investigation of microbial community composition and transcription patterns in a biogas plant undergoing ammonia crisis. *Microbial Biotechnology*, *12*(2), 305–323. https://doi.org/10.1111/1751-7915.13313

Fortune Business Insights. (2026, October). *Butyric Acid Market Size, Share, Trends & Growth Forecast, 2034*. https://www.fortunebusinessinsights.com/butyric-acid-market-109660

Galib, M., Elbeshbishy, E., Reid, R., Hussain, A., & Lee, H.-S. (2016). Energy-positive food wastewater treatment using an anaerobic membrane bioreactor (AnMBR). *Journal of Environmental Management*, *182*, 477–485. https://doi.org/10.1016/j.jenvman.2016.07.098

Grand View Research. (2026a, June). *Acetic Acid Market Size And Share Report, 2026-2033*. https://www.grandviewresearch.com/industry-analysis/acetic-acid-market

Grand View Research. (2026b, June). *Lactic Acid Market Size, Share & Trends Report, 2026-2033*. https://www.grandviewresearch.com/industry-analysis/lactic-acid-and-poly-lactic-acid-market

Grand View Research. (2026c, July). *Propionic Acid Market Size And Share Report, 2024-2030*. https://www.grandviewresearch.com/industry-analysis/propionic-acid-market

Grootscholten, T. I. M., Kinsky dal Borgo, F., Hamelers, H. V. M., & Buisman, C. J. N. (2013). Promoting chain elongation in mixed culture acidification reactors by addition of ethanol. *Biomass and Bioenergy*, *48*, 10–16. https://doi.org/10.1016/j.biombioe.2012.11.019

Grootscholten, T. I. M., Strik, D. P. B. T. B., Steinbusch, K. J. J., Buisman, C. J. N., & Hamelers, H. V. M. (2014). Two-stage medium chain fatty acid (MCFA) production from municipal solid waste and ethanol. *Applied Energy*, *116*, 223–229. https://doi.org/10.1016/j.apenergy.2013.11.061

Guo, X. M., Trably, E., Latrille, E., Carrère, H., & Steyer, J.-P. (2010). Hydrogen production from agricultural waste by dark fermentation: A review. *International Journal of Hydrogen Energy, Indo-French Workshop on Biohydrogen: From Basic Concepts to Technology*, *35*(19), 10660–10673. https://doi.org/10.1016/j.ijhydene.2010.03.008

Gurd, C., Villa, R., & Jefferson, B. (2020). Understanding why fat, oil and grease (FOG) bioremediation can be unsuccessful. *Journal of Environmental Management*, *267*, 110647. https://doi.org/10.1016/j.jenvman.2020.110647

Han, R., Nusbaum, O., Chen, X., & Zhu, Y. (2020). Valeric Acid Suppresses Liver Cancer Development by Acting as a Novel HDAC Inhibitor. *Molecular Therapy Oncolytics*, *19*, 8–18. https://doi.org/10.1016/j.omto.2020.08.017

Han, S., Liu, Y., Zhang, S., & Luo, G. (2016). Reactor performances and microbial communities of biogas reactors: Effects of inoculum sources. *Applied Microbiology and Biotechnology*, *100*(2), 987–995. https://doi.org/10.1007/s00253-015-7062-7

He, X., de los Reyes III, F. L., & Ducoste, J. J. (2017). A critical review of fat, oil, and grease (FOG) in sewer collection systems: Challenges and control. *Critical Reviews in Environmental Science and Technology*, *47*(13), 1191–1217. https://doi.org/10.1080/10643389.2017.1382282

He, X., & Yan, T. (2016). Impact of microbial activities and hydraulic retention time on the production and profile of long chain fatty acids in grease interceptors: A laboratory study. *Environmental Science: Water Research & Technology*, *2*(3), 474–482. https://doi.org/10.1039/c6ew00013d

Huq, N. A., Hafenstine, G. R., Huo, X., Nguyen, H., Tifft, S. M., Conklin, D. R., Stück, D., Stunkel, J., Yang, Z., Heyne, J. S., Wiatrowski, M. R., Zhang, Y., Tao, L., Zhu, J., McEnally, C. S., Christensen, E. D., Hays, C., Van Allsburg, K. M., Unocic, K. A., … Vardon, D. R. (2021). Toward net-zero sustainable aviation fuel with wet waste–derived volatile fatty acids. *Proceedings of the National Academy of Sciences*, *118*(13), e2023008118. https://doi.org/10.1073/pnas.2023008118

Jeganathan, J., Nakhla, G., & Bassi, A. (2006). Long-Term Performance of High-Rate Anaerobic Reactors for the Treatment of Oily Wastewater. *Environmental Science & Technology*, *40*(20), 6466–6472. https://doi.org/10.1021/es061071m

Johari, A. (2026, September 18). *Valeric Acid Market Size, Share & Industry Growth | 2035*. Claight Corporation (Expert Market Research). https://www.expertmarketresearch.com/reports/valeric-acid-market

Joshi, S., Robles, A., Aguiar, S., & Delgado, A. G. (2021). The occurrence and ecology of microbial chain elongation of carboxylates in soils. *The ISME Journal*, *15*(7), 1907–1918. https://doi.org/10.1038/s41396-021-00893-2

Kanafin, Y. N., Kanafina, D., Malamis, S., Katsou, E., Inglezakis, V. J., Poulopoulos, S. G., & Arkhangelsky, E. (2021). Anaerobic Membrane Bioreactors for Municipal Wastewater Treatment: A Literature Review. *Membranes*, *11*(12), 967. https://doi.org/10.3390/membranes11120967

Kim, S. W., Gormley, A., Jang, K. B., & Duarte, M. E. (2024). Current status of global pig production: An overview and research trends. *Animal Bioscience*, *37*(4), 719–729. https://doi.org/10.5713/ab.23.0367

Kraume, M., & Drews, A. (2010). Membrane Bioreactors in Waste Water Treatment – Status and Trends. *Chemical Engineering & Technology*, *33*(8), 1251–1259. https://doi.org/10.1002/ceat.201000104

Kruse, A., Dahmen, N., Dinjus, E., & Ederer, H. (2006). Oxidation of hexanal to hexanoic acid in supercritical carbon dioxide: 1. Experiments in a tubular reactor and modeling. *The Journal of Supercritical Fluids, Special Issue in Memory of Professor E. Ulrich Franck*, *39*(2), 211–219. https://doi.org/10.1016/j.supflu.2006.04.001

Krzeminski, P., Gil, J. A., van Nieuwenhuijzen, A. F., van der Graaf, J. H. J. M., & van Lier, J. B. (2012). Flat sheet or hollow fibre—Comparison of full-scale membrane bio-reactor configurations. *Desalination and Water Treatment*, *42*(1), 100–106. https://doi.org/10.5004/dwt.2012.2465

Kubitschke, J., Lange, H., & Strutz, H. (2014). Carboxylic Acids, Aliphatic. In *Ullmann's Encyclopedia of Industrial Chemistry* (pp. 1–18). John Wiley & Sons, Ltd. https://doi.org/10.1002/14356007.a05_235.pub2

Kucek, L. A., Spirito, C. M., & Angenent, L. T. (2016). High n-caprylate productivities and specificities from dilute ethanol and acetate: Chain elongation with microbiomes to upgrade products from syngas fermentation. *Energy & Environmental Science*, *9*(11), 3482–3494. https://doi.org/10.1039/c6ee01487a

Kucek, L. A., Xu, J., Nguyen, M., & Angenent, L. T. (2016). Waste Conversion into n-Caprylate and n-Caproate: Resource Recovery from Wine Lees Using Anaerobic Reactor Microbiomes and In-line Extraction. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.01892

Lapidus, A. L., Pirozhkov, S. D., & Kopyakin, A. A. (1979). ChemInform Abstract: CATALYTIC SYNTHESIS OF PROPIONIC ACID BY THE CARBOXYLATION OF ETHYLENE BY CARBON DIOXIDE. *Chemischer Informationsdienst*. https://doi.org/10.1002/chin.197926178

Leng, L., Yang, P., Mao, Y., Wu, Z., Zhang, T., & Lee, P.-H. (2017). Thermodynamic and physiological study of caproate and 1,3-propanediol co-production through glycerol fermentation and fatty acids chain elongation. *Water Research*, *114*, 200–209. https://doi.org/10.1016/j.watres.2017.02.023

Li, L., He, J., Wang, M., Xin, X., Xu, J., & Zhang, J. (2018). Efficient Volatile Fatty Acids Production from Waste Activated Sludge after Ferrate Pretreatment with Alkaline Environment and the Responding Microbial Community Shift. *ACS Sustainable Chemistry & Engineering*, *6*(12), 16819–16827. https://doi.org/10.1021/acssuschemeng.8b04115

Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., & Zhang, Y. (2013). A review on anaerobic membrane bioreactors: Applications, membrane fouling and future perspectives. *Desalination*, *314*, 169–188. https://doi.org/10.1016/j.desal.2013.01.019

Liu, Y., Chen, L., Duan, Y., Li, R., Yang, Z., Liu, S., & Li, G. (2024). Recent progress and prospects for chain elongation of transforming biomass waste into medium-chain fatty acids. *Chemosphere*, *355*, 141823. https://doi.org/10.1016/j.chemosphere.2024.141823

Liu, Y., He, P., Shao, L., Zhang, H., & Lü, F. (2017). Significant enhancement by biochar of caproate production via chain elongation. *Water Research*, *119*, 150–159. https://doi.org/10.1016/j.watres.2017.04.050

Liu, Z., Zhu, X., Liang, P., Zhang, X., Kimura, K., & Huang, X. (2019). Distinction between polymeric and ceramic membrane in AnMBR treating municipal wastewater: In terms of irremovable fouling. *Journal of Membrane Science*, *588*, 117229. https://doi.org/10.1016/j.memsci.2019.117229

Luo, X., Yuan, X., Wang, S., Sun, F., Hou, Z., Hu, Q., Zhai, L., Cui, Z., & Zou, Y. (2018). Methane production and characteristics of the microbial community in the co-digestion of spent mushroom substrate with dairy manure. *Bioresource Technology*, *250*, 611–620. https://doi.org/10.1016/j.biortech.2017.11.088

Luostarinen, S., Luste, S., & Sillanpää, M. (2009). Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. *Bioresource Technology*, *100*(1), 79–85. https://doi.org/10.1016/j.biortech.2008.06.029

Macias-Corral, M., Samani, Z., Hanson, A., Smith, G., Funk, P., Yu, H., & Longworth, J. (2008). Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. *Bioresource Technology*, *99*(17), 8288–8293. https://doi.org/10.1016/j.biortech.2008.03.057

Martin, I., Pidou, M., Soares, A., Judd, S., & Jefferson, B. (2011). Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. *Environmental Technology*, *32*(9), 921–932. https://doi.org/10.1080/09593330.2011.565806

Martín-González, L., Castro, R., Pereira, M. A., Alves, M. M., Font, X., & Vicent, T. (2011). Thermophilic co-digestion of organic fraction of municipal solid wastes with FOG wastes from a sewage treatment plant: Reactor performance and microbial community monitoring. *Bioresource Technology*, *102*(7), 4734–4741. https://doi.org/10.1016/j.biortech.2011.01.060

McCarty, P. L., Bae, J., & Kim, J. (2011). Domestic Wastewater Treatment as a Net Energy Producer–Can This be Achieved? *Environmental Science & Technology*, *45*(17), 7100–7106. https://doi.org/10.1021/es2014264

Mei, X., Wang, Z., Miao, Y., & Wu, Z. (2016). Recover energy from domestic wastewater using anaerobic membrane bioreactor: Operating parameters optimization and energy balance analysis. *Energy*, *98*, 146–154. https://doi.org/10.1016/j.energy.2016.01.011

Meng, F., Yang, Q., Wang, L., Xu, J., Li, X., & Hao, J. (2026). Adaptive microbiome efficiently drives chain elongation to produce caproate via distinct lactate-assisting-ethanol electron donor cooperation strategy. *Journal of Environmental Chemical Engineering*, *14*(3), 122480. https://doi.org/10.1016/j.jece.2026.122480

Menon, S. S., & Kalyanraman, V. (2025). A comprehensive review of anaerobic membrane bioreactor (AnMBR) technology: Advancements and trends from 2010 to 2024. *Environmental Technology Reviews*, *14*(1), 17–39. https://doi.org/10.1080/21622515.2024.2447090

Muller, C., Lam, P., Lin, E., Chapman, T., Devin-Clark, D., Belknap-Williamson, J., & Krugel, S. (2010). *Co-digestion at Annacis Island WWTP: Metro Vancouver's Path to Renewable Energy and Greenhouse Gas Emissions Reductions*. 2706–2722. https://www.accesswater.org/publications/proceedings/-297343/co-digestion-at-annacis-island-wwtp--metro-vancouver-s-path-to-renewable-energy-and-greenhouse-gas-emissions-reductions

Nicholson, S. R., Rorrer, N. A., Carpenter, A. C., & Beckham, G. T. (2021). Manufacturing energy and greenhouse gas emissions associated with plastics consumption. *Joule*, *5*(3), 673–686. https://doi.org/10.1016/j.joule.2020.12.027

NPDES CAFO Permitting Status Report. (2024). Environmental Protection Agency. https://www.epa.gov/system/files/documents/2025-09/cafo-status-report-2024.pdf

Ojo, A. O., & de Smidt, O. (2023). Lactic Acid: A Comprehensive Review of Production to Purification. *Processes*, *11*(3), 688. https://doi.org/10.3390/pr11030688

Palar, S. (2024). *Comparison of conventional and electro-selective fermentations of swine manure to produce short and medium chain volatile fatty acids* [Kansas State University]. https://hdl.handle.net/2097/44299

Pandey, A. K., Pilli, S., Bhunia, P., Tyagi, R. D., Surampalli, R. Y., Zhang, T. C., Kim, S.-H., & Pandey, A. (2022). Dark fermentation: Production and utilization of volatile fatty acid from different wastes- A review. *Chemosphere*, *288*, 132444. https://doi.org/10.1016/j.chemosphere.2021.132444

Pandit, S., Savla, N., Sonawane, J. M., Sani, A. M., Gupta, P. K., Mathuriya, A. S., Rai, A. K., Jadhav, D. A., Jung, S. P., & Prasad, R. (2021). Agricultural Waste and Wastewater as Feedstock for Bioelectricity Generation Using Microbial Fuel Cells: Recent Advances. *Fermentation*, *7*(3), 169. https://doi.org/10.3390/fermentation7030169

Pasalari, H., Gholami, M., Rezaee, A., Esrafili, A., & Farzadkia, M. (2021). Perspectives on microbial community in anaerobic digestion with emphasis on environmental parameters:

A systematic review. *Chemosphere*, *270*, 128618. https://doi.org/10.1016/j.chemosphere.2020.128618

Pereira, M. a., Sousa, D. z., Mota, M., & Alves, M. m. (2004). Mineralization of LCFA associated with anaerobic sludge: Kinetics, enhancement of methanogenic activity, and effect of VFA. *Biotechnology and Bioengineering*, *88*(4), 502–511. https://doi.org/10.1002/bit.20278

Propionic acid production from propionaldehyde, Chemical Engineering. (2020). Intratec. https://cdn.intratec.us/docs/reports/previews/pa-e21a-b.pdf

Ramos-Suarez, M., Zhang, Y., & Outram, V. (2021). Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste. *Reviews in Environmental Science and Bio/Technology*, *20*(2), 439–478. https://doi.org/10.1007/s11157-021-09566-0

Rehman, M. L. U., Iqbal, A., Chang, C.-C., Li, W., & Ju, M. (2019). *Anaerobic digestion*. Wiley. https://doi.org/10.1002/wer.1219

Roghair, M., Liu, Y., Strik, D. P. B. T. B., Weusthuis, R. A., Bruins, M. E., & Buisman, C. J. N. (2018). Development of an Effective Chain Elongation Process From Acidified Food Waste and Ethanol Into n-Caproate. *Frontiers in Bioengineering and Biotechnology*, *6*. https://doi.org/10.3389/fbioe.2018.00050

Shen, Y., Linville, J. L., Urgun-Demirtas, M., Mintz, M. M., & Snyder, S. W. (2015). An overview of biogas production and utilization at full-scale wastewater treatment plants (WWTPs) in the United States: Challenges and opportunities towards energy-neutral WWTPs. *Renewable and Sustainable Energy Reviews*, *50*, 346–362. https://doi.org/10.1016/j.rser.2015.04.129

Shen, Y., Yang, S., Liu, L., Ji, X., Hou, L., & Zhu, J. (2026). Valorization of Chinese cabbage waste into caproic acid via anaerobic fermentation: Effects of cellulase and pre-fermentation. *Journal of Environmental Chemical Engineering*, *14*(2), 122052. https://doi.org/10.1016/j.jece.2026.122052

Spirito, C. M., Richter, H., Rabaey, K., Stams, A. J., & Angenent, L. T. (2014). Chain elongation in anaerobic reactor microbiomes to recover resources from waste. *Current Opinion in Biotechnology, Energy Biotechnology • Environmental Biotechnology*, *27*, 115–122. https://doi.org/10.1016/j.copbio.2014.01.003

Stuckey, D. C. (2012). Recent developments in anaerobic membrane reactors. *Bioresource Technology, Membrane Bioreactors (MBRs): State-of-Art and Future*, *122*, 137–148. https://doi.org/10.1016/j.biortech.2012.05.138

Swar, V. (2026, September 11). *Caproic Acid Market Companies, Size & Trends 2026-2035*. Precedence Research. https://www.precedenceresearch.com/caproic-acid-market

Undiandeye, J. A., Kiman, S., & Anaele, J. V. (2023). Production of medium chain fatty acids from ensiled potato peels; effect of inoculum type and kinetic study. *Nigerian Journal of Technological Development, 20*(3), 47–53. https://doi.org/10.4314/njtd.v20i3.1383

Van Eerten-Jansen, M. C. A. A., Ter Heijne, A., Grootscholten, T. I. M., Steinbusch, K. J. J., Sleutels, T. H. J. A., Hamelers, H. V. M., & Buisman, C. J. N. (2013). Bioelectrochemical Production of Caproate and Caprylate from Acetate by Mixed Cultures. *ACS Sustainable Chemistry & Engineering*, *1*(5), 513–518. https://doi.org/10.1021/sc300168z

Varma, M. N., & Madras, G. (2008). Kinetics of synthesis of butyl butyrate by esterification and transesterification in supercritical carbon dioxide. *Journal of Chemical Technology & Biotechnology*, *83*(8), 1135–1144. https://doi.org/10.1002/jctb.1897

Ventorino, V., Romano, I., Pagliano, G., Robertiello, A., & Pepe, O. (2018). Pre-treatment and inoculum affect the microbial community structure and enhance the biogas reactor performance in a pilot-scale biodigestion of municipal solid waste. *Waste Management*, *73*, 69–77. https://doi.org/10.1016/j.wasman.2017.12.005

Wang, B., Shen, X., Chen, S., Bai, Y., Yang, G., Zhu, J., Shu, J., & Xue, Z. (2018). Distribution characteristics, resource utilization and popularizing demonstration of crop straw in southwest China: A comprehensive evaluation. *Ecological Indicators*, *93*, 998–1004. https://doi.org/10.1016/j.ecolind.2018.05.081

Wang, H., Jeon, B. S., Ortiz-Ardila, A. E., & Angenent, L. T. (2025). Steering the Chain-Elongating Microbiome to C8 or C6 Production with Ethanol and Lactate as Coelectron Donors. *Environmental Science & Technology*, *59*(32), 16947–16958. https://doi.org/10.1021/acs.est.5c01461

Wang, P., Wang, H., Qiu, Y., Ren, L., & Jiang, B. (2018). Microbial characteristics in anaerobic digestion process of food waste for methane production–A review. *Bioresource Technology, Bioconversion of Food Wastes*, *248*, 29–36. https://doi.org/10.1016/j.biortech.2017.06.152

Wang, Q., Zhang, P., Bao, S., Liang, J., Wu, Y., Chen, N., Wang, S., & Cai, Y. (2020). Chain elongation performances with anaerobic fermentation liquid from sewage sludge with high total solid as electron acceptor. *Bioresource Technology*, *306*, 123188. https://doi.org/10.1016/j.biortech.2020.123188

Wang, Y., Wu, S.-L., Wei, W., Wu, L., Huang, S., Dai, X., & Ni, B.-J. (2025). pH-dependent medium-chain fatty acid synthesis in waste activated sludge fermentation: Metabolic pathway regulation. *Journal of Environmental Management*, *373*, 123722. https://doi.org/10.1016/j.jenvman.2024.123722

Westerholm, M., & Schnürer, A. (2019). Microbial Responses to Different Operating Practices for Biogas Production Systems. In *Anaerobic Digestion*. IntechOpen. https://doi.org/10.5772/intechopen.82815

Wu, H., Kim, T., Ferdous, S., Scheve, T., Lin, Y., Valentino, L., Holtzapple, M., Hawkins, T. R., Benavides, P. T., & Urgun-Demirtas, M. (2024). Sustainable Aviation Fuel from High-Strength Wastewater via Membrane-Assisted Volatile Fatty Acid Production: Experimental Evaluation, Techno-economic, and Life-Cycle Analyses. *ACS Sustainable Chemistry & Engineering*, *12*(18), 6990–7000. https://doi.org/10.1021/acssuschemeng.4c00167

Wu, L., Wei, W., Chen, Z., Shi, X., Wang, D., Chen, X., & Ni, B.-J. (2023). Medium chain fatty acids production from anaerobic fermentation of food wastes: The role of fermentation pH in metabolic pathways. *Chemical Engineering Journal*, *472*, 144824. https://doi.org/10.1016/j.cej.2023.144824

Yang, J., & Jack, J. (2026). Unlocking Chain-Elongating Microbiomes for Highly Selective Food Waste Conversion into Caproic Acid Using a Single-Stage Electro-Fermentation Technique. *ACS ES&T Engineering*, *6*(2), 802–815. https://doi.org/10.1021/acsestengg.5c00935

Yankov, D. (2022). Fermentative Lactic Acid Production From Lignocellulosic Feedstocks: From Source to Purified Product. *Frontiers in Chemistry*, *10*, 823005. https://doi.org/10.3389/fchem.2022.823005

Yin, D. M., Uwineza, C., Sapmaz, T., Mahboubi, A., De Wever, H., Qiao, W., & Taherzadeh, M. J. (2022). Volatile Fatty Acids (VFA) Production and Recovery from Chicken Manure Using a High-Solid Anaerobic Membrane Bioreactor (AnMBR). *Membranes*, *12*(11), 1133. https://doi.org/10.3390/membranes12111133

Zhang, C., Su, H., Baeyens, J., & Tan, T. (2014). Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews*, *38*, 383–392. https://doi.org/10.1016/j.rser.2014.05.038

Zhang, W., Yin, F., Dong, H., Cao, Q., Wang, S., Xu, J., & Zhu, Z. (2020). Bioconversion of swine manure into high-value products of medium chain fatty acids. *Waste Management*, *113*, 478–487. https://doi.org/10.1016/j.wasman.2020.06.021