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Microbial conversion of waste sludge into volatile fatty acids and polyhydroxyalkanoates

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ACADEMIC DISSERTATION

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“There is only one heroism in the world: to see the world as it is, and to love it.”

— Romain Rolland

Abstract

Microbial production of volatile fatty acids (VFAs) and polyhydroxyalkanoates (PHAs) from waste materials offers a potential approach for recovering organic carbon as value-added products. Wastewater sludge contains organic matter that can serve as a carbon source for microbial conversion, although the production of VFAs and PHAs does not by itself eliminate the remaining sludge or its potential contaminants. Therefore, resource recovery from sludge should be considered as a complementary approach to sludge management rather than as a complete sludge treatment method. Addressing environmental pollution and promoting resource recovery are of global importance.

The main objective of this doctoral dissertation was to investigate the potential for converting waste materials into VFAs and PHAs. Hydrolysis bioreactor experiments examined the effects of pH condition, volatile solids, and waste material type on VFA production and composition. Microbial communities and VFA production were analysed. In a separate PHA accumulation experiment, activated-sludge-derived microbial communities were supplied with acetate to evaluate PHA production with aeration conditions. Laboratory experiments, including VFA, PHA, and microbial analyses, were conducted in the Environmental Laboratory of Faculty of Biological and Environmental Sciences, University of Helsinki. Shotgun metagenomic sequencing was used to profile microbial populations at key process stages in VFA and PHA production.

This study shows that pH values of 7 and 9 can support relatively high VFA yields in both high volatile solid (BHVS) and high fixed solid (BHFS) systems. Among the waste sludges investigated, digester feed sludge was a suitable material for VFA production, likely due to its relatively higher organic matter content. In PHA accumulation bioreactors, aeration supported the efficient uptake of acetate and PHA accumulation by the activated-sludge-derived microbial community.

The bacterial genus *Tetrasphaera* of Actinomycetota was predominant during the initial stage of the enrichment process used to prepare the inoculum additive for the PHA accumulation bioreactor. *Tetrasphaera* spp. is known for their ability to ferment proteins and amino acids, releasing VFAs as metabolic products. During

the subsequent PHA accumulation stage, the bacterial genus *Rhizobacter* of Pseudomonadota became the most abundant microbial group. The PHA content reached 243.2 mg of 3-hydroxybutyrate per gram of dry weight, demonstrating the feasibility of using waste sludge as a feedstock for PHA production.

The results demonstrate the potential of waste sludge as a source of organic carbon for VFA production and show that acetate can support PHA accumulation by mixed microbial communities. This study contributes to understanding the yield of VFAs from varying volatile solids content substrates under different pH conditions, and the difference using various waste sludges and materials in VFA production. Additionally, this study replaces the commonly applied aerobic-anaerobic cycling approach with a fully aerobic process for PHA accumulation. However, the VFA experiments were conducted at laboratory scale, and the PHA accumulation experiment used acetate supplementation rather than sludge-derived VFAs directly. Further research is therefore needed to evaluate integrated VFA–PHA production, residual sludge management, and process performance at larger scales.

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ski and skate – experiences that will continue to enrich my life far beyond this academic journey.

List of abbreviations

Table 1 Terminology

Abbreviation	Full Term
DW	Dry weight
E-VFAs	Even-numbered carbon volatile fatty acids
FA	Fatty acid
FF	Feast and Famine
3HB	3-Hydroxybutyrate
3HV	3-Hydroxyvalerate
O-VFAs	Odd-numbered carbon volatile fatty acids
PHA	Polyhydroxyalkanoate
VFA	Volatile fatty acid
VS	Volatile solid
WWTP	Wastewater treatment plant

Table 2 Bioreactors

Abbreviation	Full Term
BD	Bioreactor with 42.4 g (DW) digested sludge
BDS	Bioreactor with 42.4 g (DW) digested sludge and 16 g (DW) sawdust
BF	Bioreactor with 26.7 g (DW) digester feed sludge
BFS	Bioreactor with 26.7 g (DW) digester feed sludge and 16 g (DW) sawdust
BLFS	Bioreactor with 9.2 g (DW) digester feed sludge and 16 g (DW) sawdust
BN	Bioreactor with 28.2 g (DW) ammonia removal sludge
BNS	Bioreactor with 28.2 g (DW) ammonia removal sludge and 16 g (DW) sawdust
BLNS	Bioreactor with 10.1 g (DW) ammonia removal sludge and 16 g (DW) sawdust
BHFS	Bioreactor with high fixed solid content
BHVS	Bioreactor with high volatile solid content

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List of articles and manuscripts

This thesis is based on the following research articles and manuscripts, which are referred to in the text by their Roman numerals:

I **Liu, L.**, Kontro, M.H. Microbial hydrolysis of cellulose, hemicellulose and lignin in high volatile or fixed solid bioreactors at different pH values. Manuscript.

II **Liu, L.**, Salminen, J., Lundell, T., Romantschuk, M., Kontro, M.H., 2026. Production of volatile fatty acids through microbial conversion of waste sludges: influence of substrates. *Bioresources and Bioprocessing* 13, 67. <https://doi.org/10.1186/s40643-026-01049-w>

III **Liu, L.**, Lundell, T., Romantschuk, M., Kontro, M.H., 2026. Activated sludge for aerobic polyhydroxyalkanoate (PHA) production with acetate supplement. *Waste and Biomass Valorization*. <https://doi.org/10.1007/s12649-026-03592-1>

1 Introduction

Wastewater sludge management and treatment are critical challenges for wastewater treatment plants (WWTPs), often accounting for a substantial proportion of operational costs. Inadequate sludge treatment can lead to severe environmental pollution, adversely affecting water quality, soil, and ecological systems. Furthermore, wastewater sludge may contain harmful compounds, limiting its direct application as fertiliser in agricultural food production. One promising approach to sludge valorisation is the recovery and conversion of biodegradable organic carbon from wastewater sludge into value-added products through bioreactor-based biological processes. Such processes do not necessarily eliminate the sludge or remove all harmful compounds; rather, they provide an opportunity to recover value from its organic fraction while leaving a residual material that requires further treatment or appropriate management. This dissertation primarily focuses on the biological conversion of sludge-derived organic matter into volatile fatty acids (VFAs) and polyhydroxyalkanoates (PHAs). Thus, the research addresses sludge valorisation by investigating the conversion of part of the organic carbon in wastewater sludge into potentially valuable bioproducts, rather than complete sludge reduction or contaminant removal. The research focus is to investigate how sludge characteristics and operating conditions influence VFA production and composition and to evaluate PHA production.

1.1 Production and characteristics of volatile fatty acids

1.1.1 Definition and characteristics of VFAs

Volatile fatty acids (VFAs) are short-chain fatty acids containing two to six carbon atoms, including acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid (Agnihotri et al., 2022). Based on the number of carbon atoms in their molecular structure, VFAs can be classified into even-numbered carbon VFAs (E-VFAs) and odd-numbered carbon VFAs (O-VFAs), which often exhibit different metabolic pathways and microbial production preferences (Zhang et al., 2014). Due to their relatively low molecular weight, VFAs are volatile under atmospheric pressure and can be readily recovered through

distillation, facilitating their detection and separation in both laboratory and industrial processes. VFAs have also been described in literature as volatile organic acids or low-molecular-weight carboxylic acids (Varghese et al., 2022).

VFAs are essential intermediate metabolites in anaerobic digestion, where complex organic materials such as wastewater sludge, food waste, and other biodegradable substrates are converted into simpler compounds. During the acidogenesis stage, hydrolysed monomers, such as sugars, amino acids, and fatty acids, are fermented by specialised microbial communities to produce VFAs. These VFAs subsequently serve as substrates for methanogenic archaea in the later stages of anaerobic digestion (Atasoy et al., 2018; Du et al., 2021). The composition and concentration of VFAs generated in wastewater treatment systems are influenced by multiple factors, including substrate type, pH, temperature, hydraulic retention time, and microbial community composition.

Beyond their central role in anaerobic processes, VFAs have significant industrial and environmental relevance. They are regarded as valuable renewable carbon sources and platform chemicals for the production of a wide range of bio-based products, such as pharmaceuticals, food additives, and agricultural chemicals (Chen et al., 2022). In addition, VFAs serve as important precursors for sustainable biopolymers such as polyhydroxyalkanoates (PHAs), and for the generation of biofuels such as methane and hydrogen (Szacherska et al., 2021). Within wastewater treatment systems, VFAs also play a critical role in biological nutrient removal, particularly for nitrogen and phosphorus removal, by acting as carbon sources for denitrification and enhancing the activity of polyphosphate-accumulating organisms (Diaz et al., 2023).

The physicochemical properties, biodegradability, and versatility of VFAs as feedstocks make them highly important for advancing sustainable wastewater management and the circular bioeconomy. Therefore, understanding the formation, characteristics, and behaviour of VFAs in wastewater treatment systems is essential for optimising anaerobic processes, improving resource recovery, and developing innovative bio-based technologies. Moreover, the controlled regulation of VFA production offers opportunities to tailor downstream bioprocesses towards targeted product synthesis, thereby enhancing process efficiency and reducing operational costs in full-scale wastewater treatment facilities.

1.1.2 Hydrolysis of complex organic substrates in wastewater sludge

Wastewater sludge contains a complex mixture of carbohydrates, proteins, lipids, and lignocellulosic materials, which originate from domestic wastewater, industrial discharges, and treatment additives (Demirbas et al., 2017). Before these organic compounds can be converted into VFAs, they must first undergo hydrolysis, which

is considered the initial and often rate-limiting step of anaerobic fermentation (Menzel et al., 2020). During hydrolysis, complex organic matters are decomposed into soluble monomers that can be further utilised by fermentative microorganisms for acid production.

In sludge fermentation processes aimed at VFA production, easily degradable organic compounds are preferentially utilised during the early stages of hydrolysis, leaving behind considerable amounts of recalcitrant lignocellulosic material and inorganic solids. The enrichment of these difficult-to-degrade fractions may increase osmotic pressure and alter microbial community dynamics, thereby affecting the degradation of cellulose and lignin and influencing subsequent resource recovery processes (Kouzi et al., 2020; Morgan-Sagastume et al., 2015).

Among the organic fractions present in sludge, lignocellulosic materials are of particular interest due to their variable biodegradability. Lignocellulose is primarily composed of cellulose, hemicellulose, and lignin (Shrestha et al., 2017). Cellulose is a linear polysaccharide found in the cell walls of plants and algae and is considered the most abundant organic material on earth (Etale et al., 2023). Hemicellulose is a natural polysaccharide abundant in lignocellulosic biomass. In contrast, lignin is an aromatic heteropolymer that serves as a protective and water-repellent barrier surrounding structural polysaccharides in plant biomass, thereby limiting their microbial degradation (Schoenherr et al., 2018). The different structures and biodegradability of these three components make them relevant model substrates for investigating the hydrolysis of complex organic matter. Therefore, cellulose, hemicellulose, and lignin were selected as substrates in Article I to investigate their hydrolysis under different pH and solids conditions and their potential contribution to subsequent VFA production.

The hydrolysis of cellulose and other biodegradable carbohydrates generates soluble sugars that can subsequently be converted into VFAs by acidogenic microorganisms (Kandylis et al., 2016). Consequently, the efficiency of hydrolysis strongly influences the overall VFA yield. The degradation of lignocellulosic substrates during sludge fermentation is influenced by several operational parameters, including pH, temperature, and substrate composition (Fang et al., 2020). In wastewater sludge treatment systems, hydrolysis performance also depends on the origin of the sludge, as different treatment streams contain distinct organic compositions and microbial consortia. Therefore, understanding hydrolysis is essential for optimising VFA production from wastewater sludge, as hydrolysis determines the release of soluble carbon substrates that serve as precursors for downstream fermentation processes.

1.1.3 Biochemical pathways of VFA production

VFAs can be produced through both chemical and biological processes; however, biological production routes are generally considered more economical and environmentally sustainable. Although chemical synthesis of VFAs is technically feasible, it is often limited by high production costs associated with expensive raw materials and energy-intensive reaction conditions (Ramos-Suarez et al., 2021). In contrast, biological production utilises microbial metabolism to convert organic substrates into VFAs under controlled anaerobic conditions, providing a potentially sustainable alternative for waste valorisation (Harirchi et al., 2022).

Traditionally, industrial VFA production has been carried out in anaerobic fermenters using pure sugars as the primary carbon source (Yin et al., 2016). Although this method can achieve relatively high VFA yields, it raises ethical and economic concerns because food-grade sugars as feedstocks for chemical production may compete with resources intended for human consumption and animal feed. Consequently, increasing attention has been directed towards alternative carbon sources, such as wastewater-derived organics and waste sludge, which provide low-cost substrates while simultaneously supporting resource recovery within the framework of the circular bioeconomy (Puyol et al., 2017).

The formation of VFAs in wastewater systems occurs primarily through acidogenic fermentation, which is one of the key stages of anaerobic digestion. During this process, complex organic matter present in wastewater, including carbohydrates, proteins, and lipids, are initially hydrolysed into soluble monomers. These monomers are subsequently metabolised by fermentative microbes, primarily bacteria, resulting in the production of VFAs together with smaller amounts of gases, alcohols, and other intermediates. The composition and yield of VFAs are strongly influenced by operating parameters, particularly pH, temperature, substrate type, and retention time (Atasoy et al., 2018).

Overall, the biochemical pathways of VFA production in wastewater systems reflect a complex interplay among microbial metabolism, substrate availability, and environmental conditions. Optimising these pathways not only enhances VFA yield and selectivity but also supports the dual objectives of sustainable waste treatment and value-added biochemical production (Moretto et al., 2019). Therefore, a comprehensive understanding of VFA production pathways is critical for designing efficient anaerobic processes capable of converting wastewater-derived organics into renewable chemical intermediates while considering operational costs and environmental impacts.

1.1.4 Factors influencing VFA production from wastewater organics

The production of VFAs from wastewater organics is a multifactorial process influenced by a combination of biochemical, physicochemical, and operational parameters that regulate microbial activity and substrate conversion efficiency (Bruni et al., 2021). Among these factors, pH is one of the most critical determinants of acidogenic performance (Zhang et al., 2005). Mildly acidic conditions are generally favourable for VFA accumulation, as they inhibit methanogenic activity while promoting the metabolism of hydrolytic and fermentative microorganisms (Qiu et al., 2023; Sun et al., 2020).

Substrate composition and biodegradability also strongly influence both VFA yield and composition. In particular, the carbohydrate-to-protein ratio plays an important role in determining the dominant VFA profile (Abdullah, 1984). Carbohydrate-rich substrates tend to favour the production of acetic and butyric acids, whereas protein-rich substrates are more likely to enhance the formation of propionic and valeric acids (Ochoa et al., 2021). The inoculum source is another decisive parameter, as diverse microbial consortia exhibit varying capabilities for hydrolysis, fermentation, and acid tolerance (de Lima Brossi et al., 2016).

A comprehensive understanding and optimisation of these interrelated factors are essential for advancing the integration of acidogenic fermentation into circular wastewater biorefineries, where VFAs serve as key intermediates for bioenergy and bioproduct synthesis. Future research should focus on multi-parameter modelling and real-time process control to predict and maximise VFA yields under variable wastewater compositions and operating conditions.

1.1.5 Role of VFAs in wastewater treatment and resource recovery

VFAs play a crucial role in the transformation of WWTPs into water resource recovery facilities by serving as both intermediate metabolites and value-added chemical precursors in the anaerobic conversion of organic matter. As readily biodegradable carbon sources, VFAs such as acetate and propionate are essential for biological nutrient removal processes, particularly denitrification and enhanced biological phosphorus removal (Liu et al., 2018). Their bioavailability enhances the growth of polyphosphate-accumulating and denitrifying bacteria, thereby promoting more stable and energy-efficient nutrient removal pathways compared with conventional carbon sources (Liu et al., 2018).

Beyond their role in nutrient removal, VFAs are important intermediates in resource recovery systems and can serve as precursors for the synthesis of bioplastics, such as polyhydroxyalkanoates (PHAs), as well as biohydrogen and biomethane (Bahreini et al., 2020). This bioconversion of wastewater-derived

organics into VFAs supports the transition from conventional waste stabilisation towards carbon valorisation strategy, in which organic carbon is recovered and converted into value-added bioproducts. VFAs can also be recovered through downstream processes such as membrane filtration (Zacharof and Lovitt, 2014), electrodialysis (Jones et al., 2021), and solvent extraction (Plácido and Zhang, 2018), allowing for their reuse as industrial feedstocks or as carbon sources for microbial bioprocesses. Consequently, VFA-centred wastewater treatment strategies offer significant potential for enhancing resource recovery, reducing waste generation, and improving the overall sustainability and economic viability of wastewater treatment operations.

1.2 Production and characteristics of polyhydroxyalkanoates

1.2.1 Introduction to PHAs

PHAs are biodegradable polymers synthesised by various microorganisms as storage compounds for intracellular carbon and energy under nutrient-limited conditions with an excess of carbon sources (Dalton et al., 2022). PHA-producing microorganisms include both Gram-negative and Gram-positive bacteria, as well as numerous extremophilic Archaea (Koller, 2018). To date, PHA synthesis has been identified in more than 300 microorganisms in the environment (Deng et al., 2024). Due to their renewable origin, biodegradability, and biocompatibility, PHAs have attracted significant attention as sustainable alternatives to conventional petroleum-based plastics, supporting the transition towards carbon-neutral materials and circular bioeconomy objectives (Alcântara et al., 2020).

PHAs are typically accumulated under conditions of excess carbon source availability and limitation of essential nutrients (e.g., nitrogen and phosphorus), which induce metabolic shifts towards polymer storage rather than cell growth (Higuchi-Takeuchi and Numata, 2019). Among the various PHA monomers identified, 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) were the most frequent monomers (Eschenlauer et al., 1996). After first being discovered by Lemoigne in 1926 (Lemoigne, 1926), PHAs have obtained significant attention due to their biodegradability, biocompatibility, and thermoplasticity (Kovalcik, 2021; Meereboer et al., 2020). Consequently, PHAs hold potential as sustainable alternatives to conventional plastics which are produced from fossil sources like oil provided by the petroleum industry (Alcântara et al., 2020).

Due to these properties, PHAs are usually applied in material production. Their non-toxic and biocompatible nature makes them particularly suitable for medical applications (Ansari et al., 2021), such as surgical sutures, drug delivery systems, and tissue engineering scaffolds (Shishatskaya, 2008). Additionally, their

thermoplastic properties enable their processing through conventional plastic manufacturing techniques, such as extrusion, injection moulding, and film blowing (Muthuraj et al., 2021). Beyond medical application, PHAs have also been utilised in various fields, including industrial and agricultural aspects (Bugnicourt et al., 2014).

The biodegradable nature of PHAs helps mitigate the environmental issues associated with plastic waste. PHAs can be degraded by a variety of microorganisms in natural environments (Suzuki et al., 2021), thereby reducing plastic waste accumulation. Biodegradable packaging materials can also be made from PHAs, and they can be used in biodegradable mulch films, controlled-release fertilisers, and seed coatings (Verma et al., 2023).

Despite their environmental advantages, the large-scale production and commercialization of PHAs remain economically challenging. In industrial processing, PHAs are mainly produced by defined microbial cultures with the use of expensive substrates (Reis et al., 2003). Aseptic conditions and chemical feedstock increase the costs of PHA production. Therefore, the PHA price in the current market is high, which limits the wider utilisation of PHA-based materials (Gurieff and Lant, 2007).

To make PHAs more economically competitive with everyday plastics produced from fossil resources, it is essential to select suitable renewable resources as carbon feedstocks to significantly reduce costs. Carbon feedstock represents one of the major contributors to overall production costs and accounts for up to 50% of the total PHA production expenses (Choi and Lee, 1999). Nonetheless, utilising waste for PHA production enhances waste management and supports circular economy practices. Studies have shown that using mixed microbial cultures and cheaper substrates for PHA production can reduce the cost (Reis et al., 2003).

1.2.2 PHA production using mixed microbial cultures

Accumulation of PHAs by mixed microbial cultures typically occurs under aerobic conditions or alternating aerobic-anaerobic conditions (Luo et al., 2019). PHA production with mixed microbial cultures commonly requires the selection of PHA-accumulating microorganisms by using a feast and famine (FF) strategy. This strategy typically occurs during intermittent feeding of the carbon source, where external carbon is taken up and accumulated intracellularly as PHA (feast phase), which is alternated with phases lacking substrate addition that favour cell growth on storage products (famine phase). This alternating system creates a selection pressure for PHA-storing microorganisms (Reis et al., 2003).

VFA composition in fermentation liquid significantly influences PHA synthesis. Even-carbon acids or acids with fewer carbon atoms supported more PHA production, and the rate of PHA production is related to the ease of carbon source

utilisation (Jia et al., 2014). Acetic acid supports the highest PHA production rate, followed by butyric acid, propionic acid, and valeric acid (Jia et al., 2014). The type of VFAs determines the monomers of PHAs; O-VFAs promote the synthesis of HV (Jayakrishnan et al., 2021), and E-VFAs, especially the acetate-dominant feedstock, can produce HB monomer (Morgan-Sagastume et al., 2010).

It has also been noticed that PHA content is related to the feast-to-famine ratio (F/F) (Hao et al., 2018), and to pH (Chua et al., 2003). The suitable pH range for PHA-accumulating microorganisms was narrower (Serafim et al., 2004) than the range (pH 5.0 to 9.0) for most bacterial growth (Basu et al., 2015). A higher PHA content can be obtained when pH values are greater than 7.5 (Montiel-Jarillo et al., 2017), especially at pH levels of 8 or 9 (Chua et al., 2003). The F/F ratio represents the extent of selective pressure during the selection of a PHA-accumulating culture, and an optimal ratio can lead to culture enrichment (Hao et al., 2018). The F/F ratio between 0.3 and 0.4 can achieve a high PHA content potential when acetate is used as a carbon source (Oliveira et al., 2017).

1.2.3 Role of PHAs in reducing plastic pollution

With the vast production and use of plastics, many plastics have been released into the environment, causing global pollution. Plastic waste increased from 1.5 million tons to 390.7 million tons between 1950 and 2021 (Otitoju et al., 2023). It was estimated that about 66.6 million tons of plastic waste were generated in the EU in 2018 (Filho et al., 2021). The tendency for plastic waste production has increased. As evaluated, over 100 million metric tons of plastics per year will be generated by 2060 (Rhodes, 2019). The largest source of plastic waste in Europe is packaging (Lorang et al., 2022), which made more than 16 million tons in total mass in 2016 (Jang et al., 2020).

The diversity of plastic pollution is caused by the broad function of plastics and the global manufacture of plastic products (Millican and Agarwal, 2021). Thousands of plastic products are consumed in daily life. Plastics enter the environment through different pathways, resulting in the diversity of plastic pollution. Although plastic pollution has become widespread worldwide, only 9% of plastics have been dealt with efficiently (Kumar et al., 2021). Plastic pollution has become a significant issue in Europe, where many plastic products are consumed (Filho et al., 2021).

When released into the natural environment, plastics can be transported from land to river and eventually reach the ocean (Kershaw et al., 2011), where they accumulate as debris known as microplastics (Andrady, 2017). During the process of migration, plastics have adverse influences on land, wildlife, and even human health (Prata et al., 2021). Additionally, plastics would carry some organic pollutants, microorganisms, and heavy metals (Rajmohan et al., 2019).

Plastics are typically persistent in the environment and degrade very slowly through natural processes (Chamas et al., 2020). The complete degradation of plastics may take years to centuries under natural conditions (Ali et al., 2021). Consequently, large amounts of plastic waste have accumulated on land and in the ocean, contaminating them and causing a detrimental effect on living organisms (Laist, 1987). In addition, plastics may release harmful substances and transfer contaminants through the food chain, raising concerns for both environmental and human health.

To reduce traditional plastic pollution, it is worthwhile exploring the production of biodegradable plastic materials, like PHAs. Recent studies have demonstrated that PHA-based materials can maintain mechanical strength and barrier performance comparable to polypropylene and polyethylene while offering complete environmental assimilation, a duality crucial for circular economy transitions (Koller and Mukherjee, 2020). Despite higher production costs compared with petrochemical plastics, technological advances in mixed microbial culture systems and waste-based feedstocks are steadily improving economic feasibility (Samadhiya et al., 2022). Thus, PHAs hold substantial potential not only as replacements for persistent plastics but also as a key enabler of systemic plastic pollution reduction, linking biotechnology, resource recovery, and environmental protection in a unified framework.

1.3 Valorisation of waste sludge for VFA and PHA production

1.3.1 Overview of waste sludge treatment

There are several disposal and beneficial uses for sludge, including land application (Singh and Agrawal, 2008), as well as incineration and composting (Chen et al., 2012). Treated sludge, known as biosolids, can be applied to land as a soil conditioner or fertiliser (Kumar et al., 2017). Burning the sludge could reduce its volume, and it has the potential to generate energy. Additionally, composting involves mixing the sludge with bulking agents, such as wood chips, to produce a stable and humus-like material (Uçaroğlu and Alkan, 2016).

However, there are drawbacks to these uses, such as the potential presence of pollutants and microorganisms in sludge, which could pose health risks to humans and animals (Buta et al., 2021). In land application, these contaminants may accumulate in the soil and potentially enter the food chain. Incineration reduces sludge volume but can release pollutants, including dioxins, furans, and heavy metals, into the atmosphere (Sharma et al., 2013). Additionally, incineration is an energy-intensive activity, which requires fuel to maintain the high temperatures for combustion. Compost in turn could produce a strong odour if sludge is not managed

correctly (Argun et al., 2017). Large spaces are needed for composting operations, which may not be feasible in urban or densely populated areas. Composts may contain contaminants, like heavy metals and pharmaceuticals, that would limit their use.

These drawbacks highlight the importance of the careful consideration of local conditions, regulatory requirements, and environmental impacts when selecting a sludge disposal method. Due to the disadvantages of disposal and beneficial uses, more advanced treatment methods have been explored, such as anaerobic digestion (Das et al., 2024) and hydrothermal oxidation (Zhang et al., 2020).

Anaerobic digestion is a biological process that decomposes organic matter from sludge in the absence of oxygen (Lin et al., 2019). It has attracted a large amount of attention because this method has many advantages, including renewable energy production, volume reduction, pathogen reduction, and nutrient recovery (Puyol et al., 2017). However, it requires careful management and investment in infrastructure to ensure efficient and safe operation.

Hydrothermal oxidation is an advanced sludge treatment technology that uses high temperature and pressure to oxidise organic matter in the sludge (Yu et al., 2024). This process offers significant benefits in terms of high efficiency reduction and fast processing speed. (Yu et al., 2024). However, it also comes with high costs and operational complexities. This method is particularly suitable for applications where complete oxidation of organic pollutants is required and where the benefits justify the investment in the necessary infrastructure.

1.3.2 Environmental and economic benefits

The treatment and management of waste sludge are critical challenges nowadays (Qian et al., 2016) because it has a close relationship with environmental sustainability, economic development, and public health. Over the past few years, there has been a significant increase in the production of waste sludge (Tyagi and Lo, 2013). This trend is expected to continue, driven by the increasing production of wastewater and the higher efficiency of wastewater treatment. There are about 13 million tons of dry sludge produced in Europe (Rorat et al., 2019), 12.56 million tons in the United States (Seiple et al., 2017), 0.67 million tons in Canada (Eskicioglu et al., 2008), and 4.54 million tons in China every year (Chi et al., 2009). The cost of sludge treatment occupies about 20 - 60% of the total operational cost of a wastewater treatment plant (WWTP) (Foladori et al., 2010). Faced with the large amount of sludge produced worldwide and the cost of sludge treatment, appropriate solutions should be used to reduce the adverse effects on the environment and economy (Islam, 2015).

1.3.3 Integration of VFA and PHA production into WWTPs in Finland

The necessity of investigating the integration of VFA and PHA production into WWTPs in Finland arises from the growing need to transform conventional wastewater management into a circular bioeconomy. While Finland's wastewater treatment sector is among the advanced in Europe in terms of nutrient removal efficiency and energy recovery, substantial potential remains for valorising organic carbon fractions that are currently oxidised or converted primarily to biogas (Ghimire et al., 2021). The production of VFAs through controlled fermentation of sludge offers a platform for generating carbon-rich intermediates that can be converted into PHAs with high market and environmental value. Integrating such biological conversion pathways into existing WWTP infrastructure could provide an additional route for carbon recovery alongside conventional sludge treatment and energy recovery. Moreover, the alignment of this research with European Union directives on waste valorisation and renewable carbon utilisation underscores its policy relevance and industrial applicability.

Despite notable advances in wastewater-based biopolymer production across Central and Southern Europe, the applicability of these technologies to Nordic conditions remains underexplored. Finnish WWTPs operate under unique climatic and operational constraints, such as low ambient temperatures, seasonal fluctuations in sludge composition, and stringent effluent standards, which may influence VFA production and PHA accumulation (Kruglova et al., 2020). Therefore, this study investigates the potential for converting municipal sludge-derived organic matter into VFAs and PHAs under controlled laboratory-scale conditions using sludge materials from several Finnish WWTPs.

2 Aims of the study

Although the production of VFAs and PHAs from waste-derived substrates has been widely reported, systematic understanding of the operational parameters, yield optimisation, and microbial mechanisms remain limited, particularly in Finland. Despite growing global interest in integrating VFA and PHA production within circular wastewater treatment frameworks, such processes are still underdeveloped and scarcely demonstrated in Finland, where local waste characteristics and temperature regimes may distinctly affect wastewater treatment plant management and microbial processes.

In this study, a series of laboratory-scale VFA-producing and PHA-accumulating bioreactors were constructed to investigate the potential of waste sludge as a feedstock for carbon valorisation. This thesis aimed to:

- 1) evaluate the VFA production with varying VS content (BHVS and BHFS) under various pH conditions (pH 9, pH 7, pH 5, and without pH adjustment) (Article I)
- 2) assess the difference of substrate types (digester feed sludge, digested sludge, and ammonia removal sludge with and without sawdust addition) on VFA yield and composition under pH 7 (Article II)
- 3) quantify PHA synthesis in an aerobic bioreactor fed with acetate (Article III)
- 4) elucidate microbial community dynamics at critical hydrolysis and accumulation stages to reveal functional relationships between microbial taxa and bioproduct formation (Articles I, II, & III)

The broader objectives of this work were to advance the scientific understanding of VFA and PHA production from waste sludges, develop optimised bioprocess conditions suitable for this purpose, and provide experimental evidence supporting the transition of Finnish WWTPs towards pilot-scale, resource-recovery-oriented systems. Ultimately, this work not only contributes to the global discourse on circular bioeconomy but also delivers a regionally novel framework for integrating biopolymer production and carbon recovery into the wastewater sector.

3 Materials and Methods

In this study, bioreactors and fermenters were built in the Environmental Laboratory of the University of Helsinki at the Lahti campus in Finland during the years 2019 – 2023 to evaluate VFA and PHA production. The bioreactor set-up tested the effects of substrates, pH, and VS content on VFA yield and composition. The fermentations assessed PHA production using the FF strategy or aerobic conditions. The findings provide a basis for further investigation of the scale-up of bioreactors and fermentation processes for pilot- and industrial-scale applications. An overview of the experimental design and analyses conducted in this research is presented below. More details about the materials and methods used in the experiments are described in Articles I, II, and III.

3.1 Waste materials and chemicals

All waste materials used in this study were collected from two wastewater plants in the city of Lahti, Finland, and one waste-treatment company in Espoo, Finland. Activated sludge and digested sludge were from Lahti Aqua Ltd, digester feed sludge and compost were collected from Labio Ltd, and ammonia removal sludge was obtained from Gasum Ltd. All sludge materials were stored at +4 °C (up to one week) before building the bioreactors (Articles I, II, & III).

Cellulose used in the experiments was purchased from Alfa Aesar GmbH (Karlsruhe, Germany), hemicellulose from Bolise Co., Ltd (Xiamen, China), and lignin from Stora Enso (Kotka, Finland). Hydrochloric acid and sodium hydroxide were purchased from Fisher Scientific Ltd (Loughborough, UK). Sulphuric acid (72%) was from Chem-Lab (Zedelgem, Belgium). Indium solution was obtained from Perkin Elmer (Waltham, MA, USA), and phosphorus standard solution from Hach Lange GmbH (Düsseldorf, Germany). Acetic, propionic, butyric, valeric, and caproic acids were obtained from VWR (West Chester, Pennsylvania, United States). All solvents (chloroform, methanol, acetone, hexane, and toluene), as well as nitric acid (65%) and Anaerotest™ strips, were of high-performance liquid chromatography (HPLC) grade from Merck (Darmstadt, Germany). Sawdust (S) was obtained from Salpakierto Ltd (Lahti, Finland). GF/C glass microfibre filters were purchased from Whatman (Maidstone, UK).

3.2 Experimental setup and operation

The aims of this study were achieved by constructing different series of laboratory-scale bioreactors for the production of VFAs (Articles I & II) and the generation of PHAs (Article III), all of which were operated at a room temperature of approximately 21 ± 2 °C. All hydrolysis bioreactor experiments were conducted in 500 mL volume glass flasks (Figure 1), and bioreactor supplements are presented in Tables 1 and 2, respectively. Optimised conditions and sludge material for VFA production were tested (Articles I & II). Five PHA-accumulating fermentations were conducted in Labfors 3 fermentors (Infors HT, Bottmingen, Switzerland) (Figure 2) with 5 L of activated sludge (Article III).

3.2.1 VFA production bioreactors

Bioreactor experiments in Article I were conducted in 500 mL flask bioreactors, classified into two main systems: high volatile solids bioreactors (BHVS) and high fixed solids bioreactors (BHFS) (Article I). Each system was operated at initial pH values of 9, 7, and 5, as well as under unadjusted pH conditions, with all treatments performed in triplicate (Table 1). BHVS produced VFAs with initial inoculation of activated sludge microorganisms, whereas the BHFS produced VFAs by spontaneously formed microbial community, mimicking digested sludge with elevated fixed solids (FS) content and recalcitrant organic matter.

To obtain a FS content of 60% (Table 1), 32.9 g of incinerated digested sludge was introduced into the BHFS. BHVS bioreactors were inoculated with 1.1 g DW (dry weight) of activated sludge, while BHFS received 0.3 g DW of a mixed inoculum composed of activated sludge, digester feed, digested sludge, and compost. The total water volumes were 299 mL and 244 mL for BHVS and BHFS, respectively.

Hydrolysis experiments in Article II were conducted in laboratory-scale bioreactors, each operated in triplicate, with digester feed sludge, digested sludge or ammonia removal sludge as the primary substrate and sawdust as an additive (Table 2) (Article II). Each bioreactor was inoculated with a composite microbial consortium comprising activated sludge, digester feed sludge, digested sludge, and compost, thereby introducing a diverse microbial inoculum for the degradation of sludge organic matter and initiation of VFA production. The total inoculum was equivalent to 0.3 g DW. Sludge VS content was standardised to 25 g in both the control bioreactors and sawdust-amended systems (Table 2).

In BLFS and BLNS, which employed digester feed sludge and ammonia removal sludge substrates, respectively, the sludge content was adjusted to 9.1 g (DW), and 15.9 g of sawdust was supplemented to maintain a constant total DW of 25 g (Table 2). The pH was maintained at 7.0 by daily adjustment with 4.0 M sodium hydroxide.

All VFA producing bioreactors (Articles I & II) were sealed with airtight caps and continuously agitated at 100 rpm using a shaking incubator (Edmund Bühler GmbH, Bodelshausen, Germany). Daily samples from liquid mixture (1.7 mL in Article I, and 3 mL in Article II) were withdrawn and immediately stored at -20°C for subsequent analyses. All experimental conditions were prepared and operated in triplicate to ensure reproducibility.



Figure 1 Laboratory-scale VFA production bioreactors (Articles I & II).

Table 1 Hydrolysis bioreactor supplements and substrate composition (reproduced from Article I). Quantities are given as DW mass and as % of DW. Bioreactor abbreviations are shown in Table 2 in the List of abbreviations.

Parameter\Bioreactor	BHVS	BHFS
Inoculum		
Activated sludge (g DW)	1.1	0.3
Digester feed sludge (g DW)	0	0.3
Digested sludge (g DW)	0	0.3
Compost (g DW)	0	0.3
Bioreactor supplements		
Cellulose (g)	13.1	7.9
Hemicellulose (g)	13.8	8.3
Lignin (g)	8.7	5.3
Ash from digested sludge (g)	0	32.9
Calculated values		
VS (% of DW)	99	40
FS (% of DW)	1	60
Total C (g)	16.4 $\pm$ 0.1	10.3 $\pm$ 0.1
Total N (mg)	138 $\pm$ 7	129 $\pm$ 6

Total P (mg)	24.0±4.3	111.6±38.1
C/N	119	80
Water (mL)	299	244
pH	9,7, 5, and no pH control	9,7, 5, and no pH control

Table 2 Hydrolysis bioreactor supplements and substrate composition (adapted from Article II). Quantities are given as DW mass and as % of DW. Bioreactor abbreviations are shown in Table 2 in the List of abbreviations.

Parameter\Bioreactor	BF	BFS	BLFS	BD	BDS	BN	BNS	BLNS
Bioreactor supplements								
Digester feed sludge (g DW)	26.7	26.7	9.2	0	0	0	0	0
Digested sludge (g DW)	0	0	0	42.4	42.4	0	0	0
Ammonia removal sludge (g DW)	0	0	0	0	0	28.2	28.2	10.1
Sawdust (g DW)	0	16	16	0	16	0	16	16
Calculated values								
VS (% of DW)	93	95	96	59	70	88	92	94
FS (% of DW)	7	5	4	41	30	12	8	6
Total C (g)	13.4	21	13	12	20	14	22	13
Total N (g)	0.8	0.8	0.3	2.0	2.0	2.3	2.3	0.9
Total P (g)	0.1	0.2	0.1	1.0	1.6	0.5	0.4	0.3
C/N	17	27	42	6	10	6	13	25
pH	7	7	7	7	7	7	7	7

3.2.2 PHA-accumulating fermentations

Five laboratory-scale fermentations were conducted in a 5 L working volume bioreactor (Labfors 3, Infors HT, Bottmingen, Switzerland). The pH was controlled using sodium hydroxide (2–6 M) and acetic acid (2–6 M), with acetic acid also serving as the primary carbon source. All fermentations were performed at room temperature (21±2 °C). Fermentations 1–3 were operated under a feast-and-famine (F/F) regime. The feast phase was carried out under aerobic conditions with air aeration and mechanical agitation at 150 rpm, while the famine phase proceeded without aeration. The F/F ratio was maintained at 1:3 for all three fermentations.

Fermentation 1 comprised alternating 6 h feast and 18 h famine phases and was operated for 34 days using sixfold-concentrated activated sludge collected on 21 April 2021. The pH was set at 6.5±0.5. Fermentation 2 was initiated with a 6 h feast phase, followed by repeated cycles of 1 h feast and 3 h famine phases over 29 days, with the pH maintained at 7.0±0.5. Fermentation 3 followed a 3 h feast and 9 h famine pattern for 19 days, with pH recorded between 5.4 and 5.9. Samples for polyhydroxyalkanoate (PHA) analysis were collected during the feast phase for

fermentations 1 and 2. For fermentation 3, samples were taken twice daily, once at the beginning and again at the end of each feast phase.

Fermentations 4 and 5 were conducted under fully aerobic conditions. Fermentation 4 was performed for 13 days to enrich PHA-accumulating bacteria. The pH was maintained between 5.0 and 7.6 until day 9 and subsequently between 5.2 and 6.9. On day 6, 250 mL of activated sludge (stored at $-4\text{ }^{\circ}\text{C}$ after collection) was introduced as a microbial supplement. After 13 days, the biomass was harvested by centrifugation ($3000 \times g$, 15 min; Biofuge Primo R, Heraeus, Hanau, Germany) and divided into five equal portions (wet weight 36.2–37.3 g), which were stored at $-20\text{ }^{\circ}\text{C}$ for use as inocula in fermentation 5. Fermentation 5 followed the same operational procedure as fermentation 4 and was operated for 64 days for PHA production. The five inocula were introduced sequentially on days 7, 12, 21, 30, and 41. The pH was maintained between 6.3 and 8.3 prior to the first inoculation and adjusted to 4.2–7.7 thereafter. Daily records included the consumption of acetic acid and sodium hydroxide in fermentations 4 and 5. The principal operating parameters for all fermentations are summarised in Table 3.

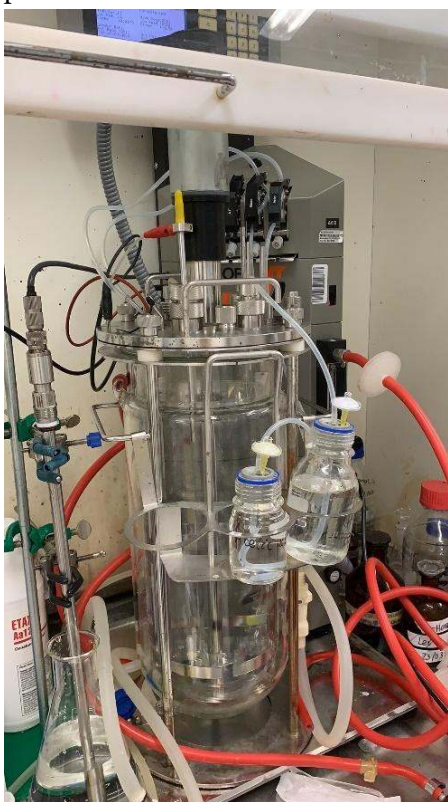


Figure 2 The Labfors 3 bioreactor (Infors HT, Bottmingen, Switzerland) used in PHA-accumulating fermentations (Article III).

Table 3 Operational parameters in the five PHA-accumulating bioreactor fermentations (reproduced from Article III).

Fermentation	1	2	3	4	5
Duration (d)	34	29	19	13	64
Activated sludge collection time	21 April 2021	15 February 2022	6 April 2022	4 May 2022	31 May 2022
Feast/Famine (h)	6/18	1/3	3/9	Aerobic	Aerobic
pH conditions	6.5±0.5	7.0±0.5	5.4 – 5.9	5.0 - 7.6 (day 1 - 9) 5.2 - 6.9 (day 10 - 13)	6.3 - 8.3 (day 1 - 6) 4.2 - 7.7 (day 7 - 64)

3.3 Analytical methods

Analytical methods in this study contained chemical and microbial analyses. Chemical analyses were conducted either at Lahti Campus or Viikki Campus laboratories, University of Helsinki, including DW, VS, FS, total VFAs, VFA composition, PHAs, total C, total N, total P, and cellulose and lignin content analyses. Microbial analyses were conducted at Lahti Campus laboratory and by Novogene (Cambridge, UK). Analyses of DW, VS, FS and microbial composition were used in Articles I, II, and III. Total VFAs, VFA composition, total C, total N, total P, cellulose and lignin analyses were determined in Articles I and II. The analysis of PHAs was included in Article III. Determination methods are presented in detail in Articles I, II, and III.

3.3.1 Chemical analysis

DW, VS and FS were determined using standard gravimetric methods involving sequential drying (105 °C) and combustion (550 °C), enabling the differentiation of moisture, organic, and inorganic fractions. Total C and total N were measured using a LECO CNS-200 elemental analyser (LECO Corporation, St. Joseph, MI, USA). Phosphorus (P) concentrations were determined following microwave-assisted acid digestion with nitric acid (standard SFS 3026-221214). The resulting extracts were analysed using an Elan DRC II ICP-Mass Spectrometer (PerkinElmer, Waltham, MA, USA) with external calibration and indium as an internal standard. Further methodological details are provided in Articles I and II.

3.3.2 Cellulose and lignin analysis

Cellulose and lignin contents were determined using a gravimetric fractionation method adapted from previously published protocols (Aldaeus et al., 2011; Bali et al., 2015; Gilarranz et al., 1998). Samples were subjected to alkaline treatment to separate cellulose from alkali-soluble lignin, followed by acid precipitation of lignin. Both fractions were quantified gravimetrically after drying (105 °C) for 16 h and combustion (450 °C) for 4 h. A detailed description of the procedure is provided in Articles I and II.

3.3.3 Volatile fatty acid analysis

VFAs, including acetic, propionic, butyric, valeric, and caproic acids, were analysed using gas chromatography with a flame ionization detector (GC-FID; Agilent 6890, Agilent Technologies, Santa Clara, CA, USA) equipped with a Zebron ZB-FFAP column (Phenomenex, Torrance, CA, USA). Samples were acidified, centrifuged, and analysed against external calibration standards. The chromatographic conditions and calibration procedures are described in detail in Articles I and II.

3.3.4 Polyhydroxyalkanoate analysis

PHAs were analysed using a gas chromatography–mass spectrometry (GC-MS) method based on established protocols (Braunegg et al., 1978; Comeau et al., 1988; Oehmen et al., 2005). Freeze-dried samples were subjected to acid-catalysed methanolysis, converting PHA polymers into their corresponding methyl esters. The resulting compounds were extracted into an organic phase and quantified using GC-MS (GCMS-QP-2010 Ultra, Shimadzu, Kyoto, Japan) equipped with a Zebron ZB-5MS column (Phenomenex, Torrance, CA, USA). Quantification was performed using external calibration based on 3-hydroxybutyrate (3HB) standards with different concentrations, with sodium benzoate as an internal reference standard. Further details of sample preparation, chromatographic conditions, and calibration procedures are provided in Article III.

3.3.5 Microbial community analysis

DNA was extracted from the bioreactor samples using a DNA extraction kit (DNeasy PowerSoil Pro Kit, Qiagen, Germany) following the manufacturer's protocol. DNA quality was analysed by agarose gel electrophoresis. The gel was stained with ethidium bromide, and DNA bands were visualised under UV light based on the fluorescence of the dye bound specifically to DNA. Shotgun metagenomic sequencing, sequence data analysis and microbial group profiling were performed

by an external service provider (Novogene, UK), and microbial community compositions based on relative abundance profiles were visualised using Krona plots. Further details on sequencing and bioinformatic analyses are provided in Articles I, II and III.

3.4 Data calculation and analyses

Data calculation and statistical analyses in this study were conducted using a combination of descriptive, inferential, and multivariate approaches. While detailed procedures are presented in the respective articles, the main analytical principles are summarised below.

Experimental results were generally obtained from biological replicate fermentations and replicate measurements ($n = 3$) and are reported as mean $\pm$ standard deviation (SD). In studies involving VFAs, maximum concentrations were estimated based on gas chromatography peak values observed across replicates.

Data analyses were conducted using IBM SPSS Statistics programme (Version 29.0.1.0, IBM Corporation, Armonk, New York, USA). Depending on data distribution and experimental design, both parametric and non-parametric methods were applied. Repeated measures analysis of variance (ANOVA) was used to assess temporal changes within bioreactor systems, followed by pairwise comparisons where appropriate.

When assumptions of normality and homogeneity of variance were not met, non-parametric tests, including the Kruskal–Wallis test and Mann–Whitney U test, were used to evaluate differences between experimental conditions such as sludge type and bioreactor composition. Statistical significance level of $p < 0.05$ was applied throughout.

Multivariate analysis was performed using principal component analysis (PCA) to explore relationships among process variables and system performance, enabling the identification of patterns and correlations within complex datasets.

Data processing and graphical visualization were conducted using Microsoft Excel. Further details of data calculation and statistical analyses are provided in Articles I, II, and III.

4 Results

The production of VFAs and their composition were evaluated under different pH conditions and substrate types to assess the performance of mixed microbial communities. Different bioreactor configurations were established to represent a range of operational scenarios relevant to wastewater and sludge treatment systems. The ability of activated sludge microbes to produce VFAs was assessed in BHVS. In contrast, BHFS modelled the situation after biogas production, where high concentrations of difficult-to-digest organic substances and inorganic compounds (digested sludge ash) could limit biogas production (Kouzi et al., 2020). To ensure a diverse microbial community in BHFS, a mixed inoculum of activated sludge, digester feed sludge, digested sludge and compost were applied, where the addition of digested sludge ash created a high FS content (Table 1).

To further differentiate substrate-specific effects, the ability of digester feed sludge, digested sludge, and ammonia removal sludge to produce VFAs was estimated in BF, BD, and BN, respectively. In addition, the influence of adding waste product (sawdust) was tested in BFS, BLFS, BDS, BNS, and BLNS, thereby introducing a high-carbon, structurally complex component into the substrate mixtures.

Following the evaluation of hydrolysis and VFA production, the potential for PHA accumulation by activated sludge was explored under different operational strategies. In Finland, ferrous sulphate is added to precipitate phosphorus in WWTPs. Precipitated phosphorus will be solubilised under anaerobic conditions, which supports availability of nutrients for growth of microbes instead of reaching PHA accumulation. Under this situation, it is necessary to test the feast and famine strategy, as presented in the elegant work by Morgan-Sagastume et al. in 2010 (Morgan-Sagastume et al. 2010), to produce PHAs using activated sludge. Based on the outcomes of fermentations 1 to 3, further process development was conducted through fermentations 4 and 5 to enhance PHA production.

Overall, these experiments enabled a systematic investigation of the conversion of waste substrates into VFAs and PHAs, while also allowing assessment of microbial communities.

4.1 VFA production with different substrates and operational conditions

4.1.1 Substrate degradation

The degradation of lignocellulosic substrates during sludge fermentations was evaluated by monitoring changes in cellulose and lignin contents in different bioreactors (Articles I & II). Overall, distinct differences were observed between readily biodegradable and recalcitrant fractions, as well as among substrate compositions.

Cellulose degradation in BHVS and BHFS occurred predominantly during the initial phase of bioreactor cultivations. In BHVS, cellulose content decreased from 27.1 g to 7.5–7.8 g within the first four days and after which no significant changes were observed. In BHFS, cellulose content decreased from 16.6 g to 10.8–11.7 g within the same period, followed by a gradual decline to 8.1–9.7 g by day 16. No significant effect of pH on cellulose degradation was observed in either system. These results indicate that more readily degradable cellulose fraction were rapidly consumed, whereas the remaining material was more resistant to further degradation.

A comparable degradation pattern was observed in waste sludge-based bioreactors, although the extent and rate of degradation varied with substrate composition. In particular, sawdust supplementation increased the initial cellulose content which was expected since wood may contain up to 50% of cellulose (Sjöström, 1993). Despite these differences, cellulose degradation occurred within the first two days in both digester feed sludge and ammonia removal sludge bioreactors supplemented with sawdust. Following this initial phase, cellulose concentrations remained relatively stable until the end of incubation, indicating limited degradation of the remaining cellulose fraction under the applied conditions.

Comparative analysis of degradation behaviour showed that more readily degradable cellulose fractions were rapidly consumed during the early hydrolysis stage. In BHVS, cellulose content decreased by approximately 19.3–19.6 g during the first four days, whereas the decrease in BHFS was smaller, at approximately 4.9–5.8 g during the same period. After this initial phase, cellulose concentrations remained relatively stable in all systems, suggesting that the remaining fraction was more resistant to microbial hydrolysis.

In contrast to cellulose, lignin exhibited significantly lower degradability. In BHVS, lignin content decreased from 8.8 g to 5.9–6.7 g during the first four days, with no further significant changes thereafter. In BHFS, lignin content remained largely unchanged throughout the experiment. These observations indicate that

only limited lignin degradation occurred during the early phase in BHVS, while lignin was largely recalcitrant in BHFS.

Overall, biodegradation of the added substrates across all bioreactors was characterised by rapid consumption of apparently available soluble carbohydrates, while recalcitrant cellulose and lignin persisted throughout the process. This consistent behaviour indicates that substrate accessibility, rather than total cellulose content, governed biodegradation efficiency, thereby influencing the availability of soluble intermediates for subsequent VFA production.

4.1.2 Effects of pH on VFA production

VFA concentrations in all bioreactors ranged from 2.7 to 3.6 mg g⁻¹ VS at the beginning of the hydrolysis stage, indicating a comparable starting point across experimental conditions. Distinct differences in VFA production were subsequently observed as a function of pH (Article I). In both BHVS and BHFS, enhanced VFA production occurred at pH 9 and 7, whereas significantly lower yields were obtained at pH 5 and under uncontrolled pH conditions (maximum pH 5.82).

In BHVS, VFA accumulation proceeded most rapidly at pH 9, increasing continuously until day 10, at which point the maximum yield of 41±6 mg g⁻¹ VS was reached. Thereafter, a slight decline in VFA concentration was observed. At pH 7, VFA production proceeded more gradually but reached a higher maximum value of 46±3 mg g⁻¹ VS on day 13. In contrast, VFA accumulation at pH 5 was limited, with a maximum of 29±7 mg g⁻¹ VS at the end of the experiment. Under uncontrolled pH conditions, VFA production remained negligible, reaching only 10±1 mg g⁻¹ VS (Fig. 3).

A similar situation occurred in BHFS, despite differences in volatile solids content and inoculum composition. The highest VFA yield was achieved at pH 9, reaching 51±3 mg g⁻¹ VS on day 11, followed by a decline to 40±3 mg g⁻¹ VS (Fig. 3). At pH 7, VFA production increased more gradually, reaching 39±9 mg g⁻¹ VS by day 14. In contrast, pH 5 and uncontrolled pH conditions resulted in low VFA production, with final VFA concentrations of 12±4 mg g⁻¹ VS and 1.0±0.2 mg g⁻¹ VS, respectively (Fig. 3).

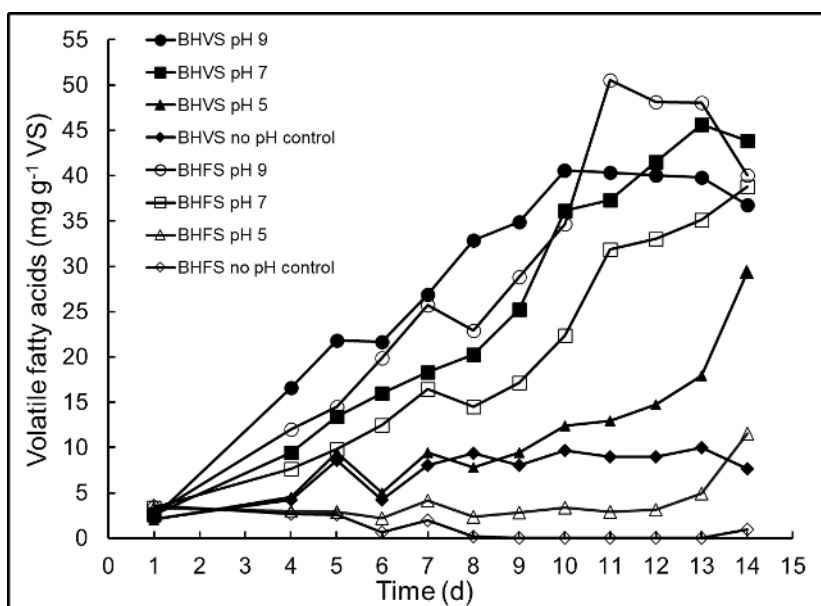


Figure 3 Volatile fatty acid profiles in high volatile solid (BHVS) and high fixed solid (BHFS) bioreactors at pH 9, 7, 5 and without pH control.

VFA composition was presented when the total VFA reached its maximum point, as the aim of the bioreactors was to produce VFAs. In BHVS, only acetic and butyric acids were detected, and the other VFAs were present at negligible levels. Acetic acid was the main individual VFA across all pH conditions, accounting for 99%, 73%, 88%, and 100% of total VFAs at pH 9, 7, 5 and without pH adjustment, respectively (Fig. 4). Similarly, in BHFS, only acetic and butyric acids existed under pH 9, pH 7, and pH 5, while VFA concentrations remained negligible without pH adjustment (Fig. 4). The proportions of VFAs at pH 9 and pH 7 were comparable, with acetic acid contributing 73% and 76% of total VFAs, respectively. Notably, the highest acetic acid proportion was observed at pH 9 in BHVS. In contrast, at pH 5 in BHFS, the VFA profile shifted markedly towards butyric acid, which accounted for 97% of total VFAs.

Overall, these results demonstrate pH not only influenced the overall VFA yield but also VFA composition. pH 9 and pH 7 favoured both VFA yield and acetic acid dominance, whereas pH 5 and uncontrolled pH conditions significantly limited VFA production and altered the metabolic distribution towards butyrate formation as the main individual VFA at pH 5 in BHFS.

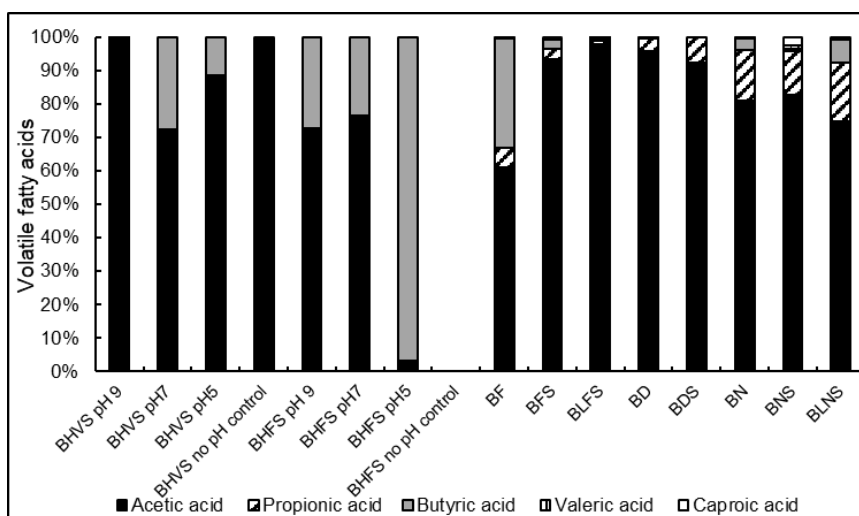


Figure 4 VFA composition profiles in all hydrolysis bioreactors.

4.1.3 VFA production potential across different waste sludge types

Based on the comparable VFA production observed at pH 9 and 7 (Chapter 4.1.2), and to minimise NaOH addition, pH 7 was used in subsequent hydrolysis bioreactors involving different waste sludge types (Article II). Distinct differences in VFA production were observed depending on the substrate source. Among all tested systems, VFA concentration increased significantly in the bioreactors using digester feed sludge. In particular, the highest VFA yield was observed in BF, reaching the maximum VFA yield of $171.6 \pm 6.3 \text{ mg g}^{-1} \text{ VS}$ after 11 days (Fig. 5). This result indicates that digester feed sludge contained readily biodegradable organic matter that could be converted into VFAs under the applied conditions. The addition of sawdust as an additive (BFS) did not enhance the VFA yield. Instead, the final VFA yield in BFS was reduced by approximately 50% compared to BF. A similar trend was observed in BLFS, suggesting that lignocellulosic additive such as sawdust limited VFA generation, likely due to their recalcitrant nature and slower hydrolysis rate. Bioreactors fed with digested sludge also produced VFAs, although at lower levels compared to digester feed sludge bioreactors. Within this group, the addition of sawdust (BDS) resulted in a higher VFA yield than BD, indicating a different response to co-substrate addition compared to digester feed sludge bioreactors (Fig. 5).

In contrast, bioreactors using ammonia removal sludge exhibited a distinct VFA production pattern. BN bioreactor showed a relatively high initial VFA content of $119.9 \pm 8.7 \text{ mg g}^{-1} \text{ VS}$, suggesting the presence of readily biodegradable organic matter at the start of the experiment. However, during the hydrolysis process, VFA

concentrations displayed a fluctuating trend, increasing until day 5 and then gradually decreasing thereafter (Fig. 5). This behaviour suggests that, although ammonia removal sludge initially contained measurable amounts of VFAs or readily fermentable intermediates, net VFA accumulation during incubation was limited. The limited accumulation may reflect differences in substrate conversion and microbial activity, although the specific mechanisms underlying this observation were not directly investigated.

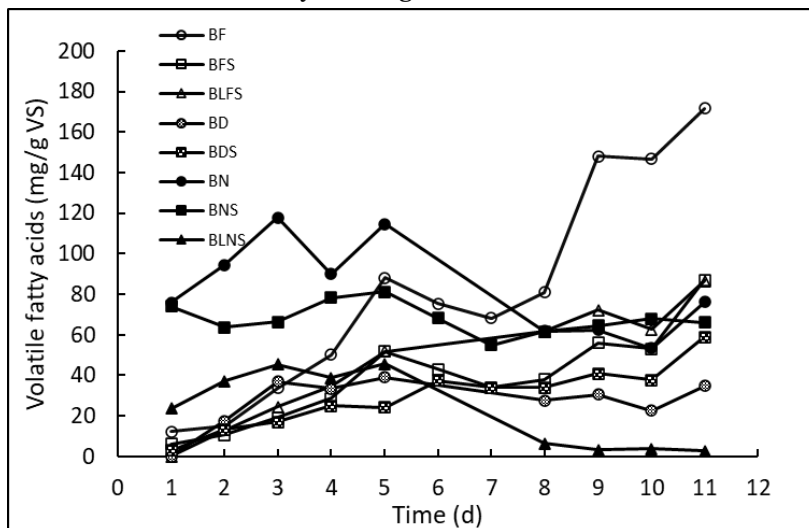


Figure 5 Volatile fatty acid profiles in bioreactors using ammonia removal sludge, or digester feed sludge, or digested sludge mixing with or without sawdust at pH 7.

Analysis of VFA composition revealed that acetic acid was the dominant individual VFA across all bioreactors, followed by propionic and butyric acids (Fig. 4). Together, these three acids accounted for about 96.6-100% of total VFAs. Minor amounts of valeric and caproic acids were also detected in all bioreactors, ranging from 0 to 0.9% and from 0 to 2.5%, respectively (Fig. 4). Overall, these results demonstrate that substrate types strongly influenced both VFA yield and composition, with digester feed sludge showing the highest potential, while lignocellulosic additives and more stabilised sludge types generally limited VFA generation.

4.1.4 Microbial communities in VFA-producing bioreactors

Microbial community composition in sludge VFA-producing bioreactors was analysed at the phylum to genus levels using shotgun metagenomic sequencing to investigate the microbial basis of VFA production under different substrate types and operational conditions (Articles I & II).

In the cellulose- and lignin-amended bioreactors (BHVS and BHFS), the microbial communities were dominated by four major phyla: Pseudomonadota (36.3-66.6%), Bacillota (3.4-30.0%), Bacteroidota (1.0-9.2%), and Actinomycetota (1.3-3.6%) (Article I). Composition of these communities was influenced by the presence of cellulose and lignin, as well as by operational conditions such as pH (7 and 9) and the addition of ash.

At the genus level, distinct differences were observed between BHVS and BHFS (Article I). In BHVS, *Klebsiella* and *Lactococcus* were the predominant genera under both pH conditions, and their relative abundances exceeded 1%. Notably, *Klebsiella* showed a strong dependence on pH, with a relative abundance of $33.2 \pm 0.8\%$ at pH 9 in BHVS, compared with $9.8 \pm 0.1\%$ at pH 7, suggesting the pH 9 condition favoured its relative abundance. In addition, BHVS also contained the genus *Enterococcus* ($1.2 \pm 0.3\%$) at pH 9.

In comparison, BHFS exhibited a broader distribution of dominant genera. At pH 7, four genera exceeded 1% relative abundance: *Lactococcus* ($13.9 \pm 0.6\%$), *Klebsiella* ($5.7 \pm 0.3\%$), *Clostridium* ($5.5 \pm 0.9\%$), and *Raoultella* ($2.6 \pm 0.1\%$). The greater diversity observed in BHFS may be associated with differences in substrate composition, including the presence of ash and lignocellulosic components, although the specific factors responsible for this community structure were not directly investigated.

In the waste sludge-based hydrolysis bioreactors, a broader range of microbial phyla was identified, including Pseudomonadota, Bacillota, Bacteroidota, Actinomycetota, Chloroflexota, and Synergistota (Article II). Bacterial microbial community composition varied significantly at the genus level depending on sludge type and substrate composition. BF and BFS shared a common abundant genus, which was *Lactobacillus*. The relative abundance was $59.9 \pm 2.1\%$ and $62.8 \pm 4.0\%$ for BF and BFS, respectively. However, *Lactobacillus* formed a much smaller proportion, only $3.5 \pm 1.5\%$ of the microbial population in BLFS bioreactors. Replacing *Lactobacillus*, *Citrobacter* ($9.0 \pm 2.1\%$) became the most dominant genus in BLFS.

Other waste sludge bioreactors exhibited distinct dominant genera. In BN, *Acetomicrobium* ($11.6 \pm 1.1\%$) was the most abundant, while *Corynebacterium* ($4.9 \pm 3.2\%$) dominated in BNS. In BLNS, *Acinetobacter* ($23.3 \pm 6.0\%$) was the predominant genus. These variations suggest that substrate type, sludge origin, and co-substrate addition may have influenced the microbial community structure.

Overall, the results indicate that microbial community composition in the hydrolysis bioreactors varied with substrate composition. Lignocellulosic addition was associated with the occurrence of genera such as *Klebsiella* and *Lactococcus*, while bioreactors using waste sludges showed a broader distribution of microbial genera, including *Lactobacillus*, *Citrobacter*, and *Acinetobacter*. These differences

in community structure may have contributed to the observed variations in VFA production among the bioreactors.

4.2 PHA accumulation under different operational strategies

4.2.1 PHA contents in fermentations 1 to 5

PHA accumulation varied markedly depending on operational strategy and microbial adaptation (Article III). PHA content, expressed as 3-hydroxybutyrate (3HB) per unit dry weight ($\text{mg 3HB g}^{-1} \text{ DW}$), reflected the effect of operational strategies.

Under feast–famine conditions (fermentations 1–3), PHA accumulation remained generally low and inconsistent throughout the experimental period, which may indicate limited enrichment of PHA-storing microorganisms under the applied conditions. In fermentation 1, PHA content ranged between approximately 1.2 and 3.5 $\text{mg 3HB g}^{-1} \text{ DW}$, suggesting limited storage activity under the applied conditions. In fermentation 2, an initial increase in PHA content to 102.4 $\text{mg 3HB g}^{-1} \text{ DW}$ was observed; however, this was followed by a continuous decline to 42.2 $\text{mg 3HB g}^{-1} \text{ DW}$, suggesting that stored PHA may have been consumed rather than further accumulated, possibly reflecting an imbalance between PHA storage and cellular growth. Temporal monitoring in fermentation 3 revealed only minor increases during feast phases, followed by reductions during famine periods, suggesting that the applied operational regime was not sufficient to promote stable PHA accumulation.

In contrast, fully aerobic conditions (fermentations 4 and 5) promoted sustained PHA accumulation and microbial enrichment. In fermentation 4, PHA content remained low during the early phase but began to increase after approximately 9 days, reaching values close to 70 $\text{mg 3HB g}^{-1} \text{ DW}$ by the end of operation. This increase coincided with stable process conditions and continuous substrate availability. A more pronounced accumulation was observed in fermentation 5, where biomass from fermentation 4 was used as inoculum, resulting in a clear enrichment effect. PHA content increased from approximately 6.1 $\text{mg 3HB g}^{-1} \text{ DW}$ to a maximum of 243.2 $\text{mg 3HB g}^{-1} \text{ DW}$, indicating successful enrichment of PHA-storing microorganisms under sustained aerobic conditions.

The relationship between VFA utilisation and PHA accumulation was apparent from the temporal trends. In aerobic systems, increases in PHA content were associated with the consumption of acetic acid, which served as a primary carbon source for PHA synthesis. In contrast, systems with limited VFA availability or unstable feeding conditions showed reduced or inconsistent PHA accumulation.

Overall, the results indicate that the operational strategy influenced PHA production in mixed microbial cultures. While the feast–famine conditions did not result in stable PHA accumulation, fully aerobic operation with adapted inoculum was associated with increased PHA accumulation, highlighting the potential importance of substrate availability and microbial selection in wastewater-derived PHA production systems.

4.2.2 Microbial communities in the course of PHA-accumulating fermentations

Shotgun sequencing analysis was utilised to characterise microbial populations during PHA-accumulating fermentations (Article III). Samples were collected from fermentation 4 on days 6 and 11, and from fermentation 5 on days 12, 21, 30, 41, and 64.

In fermentation 4, which served as an enrichment stage, the microbial community exhibited noticeable temporal shifts. On day 6, the genus *Tetrasphaera* was dominant, accounting for $12.0 \pm 0.6\%$ of the total community. On day 11, its relative abundance decreased substantially to $4.6 \pm 0.2\%$, making it the second most abundant genus. At this stage, *Rhizobacter* became the dominant genus, comprising $5.0 \pm 0.6\%$ of the microbial community. These changes indicate a transition in microbial structure during the enrichment phase, likely reflecting adaptation to the operational conditions.

In fermentation 5, which was operated for PHA accumulation, more pronounced shifts in dominant genera were observed over time. During the early stage (days 12 and 21), *Acinetobacter* was the dominant genus, accounting for $22.2 \pm 3.3\%$ and $7.2 \pm 0.4\%$ of the total community, respectively. However, as the fermentation progressed, the community structure shifted towards dominance by *Xanthobacter*. This genus exhibited a continuous increase in relative abundance on days 30, 41, and 64, reaching $10.4 \pm 1.1\%$, $35.5 \pm 1.8\%$, and $41.7 \pm 5.3\%$, respectively.

The enrichment of *Xanthobacter* coincided with the period of highest PHA accumulation observed in fermentation 5, suggesting that changes in microbial community structure contributed to the enhanced storage capacity of the mixed culture under prolonged aerobic operation.

Overall, the results indicate a temporal shift in microbial community composition during PHA-accumulating fermentations, with early-stage communities dominated by genera such as *Acinetobacter*, followed by a progressive enrichment of *Xanthobacter* during the later stages. This shift may reflect adaptation of the microbial community to the applied operational conditions and continuous availability of acetate-based substrates, with populations better adapted to the prevailing conditions becoming more abundant during prolonged cultivation.

5 Discussion

5.1 VFA production and PHA accumulation

5.1.1 pH condition influenced VFA production

pH was a key operational parameter affecting VFA production in the bioreactors. Across all bioreactors, differences in pH were associated with variation in VFA yields and, under some conditions, VFA compositions (Articles I). The results also suggest that pH conditions affected the hydrolysis processes and was associated with differences in microbial community composition.

The pH conditions were selected because stronger alkaline or acidic conditions may have a toxic influence on acidogenic bacteria (Yuan et al., 2006). The production of VFAs relies on the decomposition of complex organic compounds into soluble compounds through the hydrolytic and fermentative activities of mixed microbial cultures (Schink, 1997; Strazzera et al., 2021; Yuan et al., 2006). Alkaline conditions have been shown to improve the substrate hydrolysis rate and the VFA yield (Garcia-Aguirre et al., 2017), and to disrupt the waste sludge components and cellulose and lignin into smaller organic compounds, which improves the substrate concentration for hydrolysis (Chen et al., 2007).

In this study, both pH 7 and pH 9 supported relatively high VFA production, although the magnitude and temporal dynamics differed between BHVS and BHFS bioreactors. This is consistent with the previous research reporting alkaline pH optima for waste sludge-derived VFA production (Ma et al., 2016). The comparable VFA production observed at pH 7 in BHVS may indicate that the availability of readily biodegradable organic matter reduced the dependence on alkaline-enhanced solubilisation. In contrast, pH 9 resulted in the highest VFA yield in BHFS, which may indicate that an alkaline environment facilitates the release of more soluble substrates from the BHFS bioreactor. Under acidic conditions, the activities and functions of acidogenic bacteria could be inhibited, therefore, the digestion of sludge and the production of VFAs would be slower. More soluble substrates may be released for acidogenic microorganisms under alkaline conditions to produce VFAs (Chen et al., 2007). However, the present study did not directly measure changes in soluble substrate availability or microbial activity under the different pH conditions. Overall, the results indicate that pH 7 and pH 9

were favourable for VFA production under the tested conditions, but the optimal pH depended on the bioreactor and substrate characteristics.

pH influenced not only total VFA yields but also individual VFA composition under some conditions, particularly the relative proportions of acetic, propionic, and butyric acids (Bevilacqua et al., 2021). It is critical for downstream bioprocesses such as PHA production. Across most conditions, acetate was the dominant VFA product, particularly at pH 7 and 9. The predominance of acetate may be related to the metabolic characteristics of the microbial communities under the applied fermentation conditions (Lim et al., 2014; Sawatdeenarunat et al., 2017). This is relevant to PHA production because acetate can serve as a carbon precursor for 3HB formation (Morgan-Sagastume et al., 2010), while propionate and valerate contribute to 3HV incorporation in PHA copolymers (Jayakrishnan et al., 2021) (Article III).

Additionally, the predominance of acetate under alkaline conditions may be associated with pH-dependent microbial selection and the availability of soluble organic substrates derived from the substrates used in the bioreactors (Liu et al., 2014). Acetic acid is a key intermediate in anaerobic carbon conversion pathways and can be further consumed by methanogens, potentially reducing the overall VFA yield (Horn et al., 2003; Zhao et al., 2015). Therefore, higher VFA yields may be achieved by promoting hydrolytic degradation while limiting methanogenic activity. In this study, higher acetic acid production was observed in BHVS than in BHFS bioreactors at pH 5 and 9, whereas no clear difference observed at pH 7. The differences observed at pH 5 and 9 may be partly related to the higher VS concentration in BHVS; however, the absence of a similar difference at pH 7 suggests that VS concentration alone did not determine acetic acid production and that its effect may have depended on the pH conditions and substrate characteristics.

Under acidic conditions (pH 5), a higher relative contribution of butyrate was observed, particularly in BHFS, where butyrate became the dominant VFA. This change in VFA composition may be associated with differences in microbial community structure and fermentation pathways under lower pH conditions (Bevilacqua et al., 2021). However, overall VFA yields at pH 5 were significantly lower than those at pH 7 and 9, suggesting that the applied acidic conditions were less favourable for overall VFA production (Yuan et al., 2006).

From a process perspective, the results indicate that pH 7–9 provided favourable conditions for hydrolysis and VFA production under the tested experimental conditions. Although pH 9 generally resulted in faster VFA accumulation, pH 7 achieved a comparable final yield in BHVS while reducing chemical demand for pH control. Based on these results, pH 7 was selected for the subsequent experiments described in Article II.

Finally, the observed differences between BHVS and BHFS suggest an interaction between substrate characteristics and pH conditions. In BHVS, where the substrate contained a higher proportion of VS, relatively high VFA production was achieved at pH 7, whereas BHFS showed its highest VFA yield at pH 9. This suggests that the effect of pH on VFA production depended on the characteristics of the substrate and the bioreactor system. Previous studies have also reported that substrate characteristics can influence the response of sludge hydrolysis and VFA production to pH conditions (Kouzi et al., 2020). This interaction is further reflected in microbial community selection patterns discussed in Chapter 5.2.1.

Overall, these findings indicate that pH influenced VFA production and composition under the tested conditions, potentially through effects on substrate hydrolysis and microbial community composition. The resulting VFA composition is also relevant to downstream PHA production because the availability of different VFAs can influence the types of PHA monomers that can be formed (Article III).

5.1.2 Waste sludge influenced VFA production

While pH was identified as a key process parameter influencing VFA production and composition (Chapter 5.1.1), substrate composition and initial C/N ratio also influenced VFA production under the same pH and temperature conditions (Bevilacqua et al., 2021; Liu et al., 2008).

These differences may be related to variations in the amount and availability of biodegradable organic matter among the sludge types. VFAs can be produced from the main organic constituents of sludge, including proteins, carbohydrates, and lipids (Zehnder, 1988), and VFA production can be limited by inadequate solubilization of biodegradable substances from raw waste sludge (Wang et al., 2022). Digester feed sludge consistently exhibited the highest VFA yields. This may be associated with its relatively high content of readily biodegradable organic matter that is directly accessible to fermentative microorganisms (Li et al. 2018; Regueira et al. 2020). In contrast, digested sludge contained a higher proportion of stabilised or previously degraded organic matter, limiting hydrolysis efficiency and resulting in reduced or unstable VFA accumulation (Mottet et al., 2010; Quist-Jensen et al., 2018). Ammonia removal sludge, originating from nitrification–denitrification systems, is typically enriched in aerobic or facultative heterotrophic populations adapted for organic carbon oxidation, which may favour VFA consumption rather than net accumulation.

The influence of sludge type was also reflected in VFA composition. Bioreactors with higher proportions of protein-rich substrates tended to favour the formation of propionic and butyric acids (Atasoy and Cetecioglu, 2022), which may indicate a greater contribution of protein fermentation pathways in BF, BN, BNS, and BLNS. In contrast, acetic acid was the most abundant VFA across all sludge types, which

may be attributed to the presence of carbohydrate-rich substrates that favour acetate-producing fermentation pathways (Wang et al., 2014). Additionally, other VFAs can easily biodegrade to form acetic acid (Gerardi, 2003), resulting in a higher potential acetic acid concentration.

In addition to substrate composition, the initial C/N ratio contributed to observed differences in VFA performance. A low C/N ratio, particularly in BD, BDS, BN, and BNS bioreactors, may lead to elevated ammonia concentrations, which are known to inhibit the anaerobic fermentation process (Yen and Brune, 2007). Furthermore, a low C/N ratio was disadvantageous for the growth of acidogenic bacteria (Liu et al., 2008). Conversely, a high C/N ratio may result in insufficient nitrogen availability, which would limit the growth of the microbial population in the bioreactors (Yen and Brune, 2007). This may partially explain why VFA yields in BFS and BLFS were lower than those observed in BF, although substrate accessibility likely also contributed to the observed differences. A suitable C/N ratio, combined with a carbohydrate-rich substrate, benefited the butyric-producing bacteria, which may be associated with in the production of a certain amount of butyric acid in BF and BLNS (Bouallagui et al., 2004).

Importantly, the combined effects of waste sludge composition and C/N ratio determined overall fermentation efficiency rather than acting as isolated parameters. Whereas pH influenced the biochemical environment (Chapter 5.1.1), waste sludge types affected the biochemical potential of the system through differences in substrate availability and nutrient composition. As a result, even under favourable pH conditions, sludges with lower biodegradability exhibited limited VFA production, suggesting that substrate characteristics may be an important constraint on VFA production (Mottet et al., 2010; Wang et al., 2022).

Overall, these findings indicate that sludge characteristics affected the VFA production potential of the tested substrates, while pH influenced VFA production under the corresponding process conditions. This interaction between substrate characteristics and pH is relevant to the development and optimisation of sludge-to-VFA bioprocesses and may also contribute to differences in microbial community composition, as discussed in Chapter 5.2.1.

5.1.3 PHA production using activated sludge in Finland

In general, PHAs can be produced by the microbial consortia present in activated sludge, as reported in previous studies (Chua et al., 2003; Sakai et al., 2015). PHA content can be influenced by operational conditions and approaches (Chua et al., 2003). In the present study, for PHA production from acetate by the activated sludge microbes, a ratio of 1:3 was used for the feast-famine (FF) strategy, (Hao et al., 2018). However, FF strategy, which is commonly applied for PHA-accumulating mixed microbial culture fermentations, showed limited effectiveness. This differs

from earlier studies, where FF operation successfully selected PHA-storing microorganisms from activated sludge (Morgan-Sagastume et al., 2010), suggesting that local wastewater characteristics and treatment practices influenced the enrichment process.

A likely explanation for the low PHA accumulation under the FF strategy in this study is related to the operational characteristics of Finnish wastewater treatment plants. In Finland, ferrous sulphate is commonly applied for phosphorus precipitation (Toivakainen et al., 2013). Under anaerobic conditions, phosphorus associated with sludge can become solubilised, increasing nutrient availability in the reactor (Pokhrel et al., 2018). This may promote the growth of general heterotrophic microorganisms rather than selecting for specialised PHA-accumulating bacteria, thereby reducing storage PHA accumulation.

In contrast, fully aerobic operation resulted in substantially higher PHA accumulation (Bengtsson et al., 2008). In fermentations 4 and 5, continuous aeration combined with acetic acid feeding supported gradual enrichment of PHA-storing microbial communities and increased PHA accumulation (Article III). The final PHA content reached 243.2 mg 3HB g⁻¹ DW. This is comparable to the yield obtained using PHA-accumulating photosynthetic mixed cultures, which can achieve a polyhydroxybutyrate content of up to 390 mg HB g⁻¹ VS (Morgan-Sagastume et al., 2015). The content from this study indicated that aerobic conditions could obtain a considerable PHA content from activated sludge under Finnish wastewater treatment conditions.

5.2 Microbial communities

5.2.1 Microorganisms associated with VFA production

In both BHVS and BHFS operated at pH 7 and 9, VFA production was associated with the occurrence of fermentative and acidogenic bacteria, reflecting the potential influence of pH (Chapter 5.1.1) and substrate characteristics (Chapter 5.1.2) on microbial community composition (Articles I & II).

Klebsiella and *Clostridium* were detected in both systems. *Klebsiella* bacteria are facultative anaerobes that can produce VFAs from waste (den Boer et al., 2016). The growth of anaerobic bacteria, such as *Clostridium*, can accelerate the production of VFAs (Cao et al., 2022). The genus *Lactobacillus*, another genus detected in the bioreactors, comprises Gram-positive, facultatively anaerobic bacteria (Ibrahim and Ouwehand, 2019). Inoculum with the *Lactobacillus* genus is known to significantly increase the concentration of VFAs (Lim et al., 2020). In particular, *Lactobacillus buchneri*, as a heterofermentative lactic acid bacterium, is commonly used to produce acetic acid under anaerobic conditions (Rabelo et al., 2017). The occurrence of these genera in the present study is therefore consistent

with the potential for fermentative conversion of organic substrates to VFAs, although the individual contribution of each genus to VFA production was not directly determined.

In BF, a broader range of VFA-associated genera was observed, including *Citrobacter*, *Lactobacillus*, *Enterococcus*, *Lactococcus*, *Clostridium*, *Acidipropionibacterium*, *Propionibacterium*, *Bifidobacterium*, and *Olsenella* (Bücher et al., 2021; Dicks and Botes, 2009; Fang et al., 2022; Jin et al., 1998; Ma et al., 2019; McLoughlin et al., 2020; Pang et al., 2014; Yang et al., 2015). Among these, *Citrobacter* was the dominant genus within *Pseudomonadota* and has previously been associated with VFA production (Yang et al., 2015).

Lactobacillus was the most abundant genus in both BF and BFS, and previous studies have associated this genus with VFA production (Jin et al., 1998). The presence of *Enterococcus* has also been associated with increased VFA production in BF, BFS, and BLFS (Pang et al., 2014). *Lactococcus* can produce acetic, propionic, and butyric acids (Fang et al., 2022). *Acidipropionibacterium* and *Propionibacterium* have been reported to produce propionic acid (Bücher et al., 2021), while *Bifidobacterium* can produce acetic acid (Dicks and Botes, 2009) and butyric acid (Fernández-Navarro et al., 2018). *Olsenella* could produce acetic acid from fermented starch and glycogen substrates (McLoughlin et al., 2020). The occurrence of diverse VFA-producing microbial genera in the sludge bioreactors is consistent with the involvement of a diverse microbial community in the conversion of sludge-derived organic matter into VFAs.

Overall, the microbial community structure suggests that VFA production was associated with a diverse fermentative community rather than individual taxa. The higher abundance and broader distribution of VFA-associated genera in BF corresponded with its higher VFA yield (Article II), although the individual contribution of specific taxa to VFA production could not be determined from the metagenomic data.

These microbial patterns are consistent with the process conditions described in Chapters 5.1.1 and 5.1.2, where pH and substrate composition were associated with differences in microbial community composition. These observations may help explain the differences in VFA yields and compositions among the sludge substrates.

5.2.2 Microorganisms associated with PHA production

In fermentation 4, the genus *Tetrasphaera* of the phylum Actinomycetota dominated the microbial community during the initial stage of fermentation. Although its relative abundance was moderate, this genus is capable of using diverse organic compounds, such as amino acids and glucose, and producing fermentation products, including VFAs (Nguyen et al., 2023).

In fermentation 5, *Rhizobacter* of the phylum Pseudomonadota was present. This genus has been reported to accumulate PHAs as reserve polymers (Khamkong et al., 2022). *Rhodobacter*, the third most dominant genus, can also produce high concentrations of PHAs when a suitable carbon source is available (Hassan et al., 1996).

The genus *Acinetobacter* of the phylum Pseudomonadota further contributed to PHA accumulation, as many of its species are recognised PHA producers (Yang et al., 2013). In particular, *Acinetobacter nosocomialis* RR20 has been reported to produce PHB at a concentration of 7.82 g/L under optimised salt medium conditions (Yang et al., 2013). Other studies have also been observed to store PHB (RedCorn et al., 2019) and PHA (Cruz et al., 2022). The occurrence of these PHA-associated genera is consistent with the potential for PHA accumulation during the later stages of fermentation.

Overall, the changes in microbial community composition and the occurrence of genera previously associated with PHA production are consistent with the increased PHA accumulation observed during the later stages of fermentation. However, the individual contributions of these genera to PHA accumulation were not directly determined.

6 Conclusions and prospects

Given the growing global concern regarding plastic and waste pollution, biological processes for the recovery of valuable products from waste streams are of increasing interest. This thesis investigated the production of VFAs from different wastewater sludge substrates and the accumulation of PHAs using microbial communities derived from waste sludge.

VFA-producing bioreactor experiments demonstrated that the tested wastewater sludge substrates could be converted into VFAs under the applied laboratory conditions. In BHVS, the highest VFA yield was obtained at pH 7, whereas in BHFS, the highest yield was obtained at pH 9. Acetic acid remained the predominant VFA under most tested conditions, while a different VFA profile was observed in BHFS at pH 5. Thus, the effect of pH on VFA production and composition was dependent on the bioreactor conditions rather than being uniform across all treatments. Among the sludge types investigated, digester feed sludge produced the highest VFA yield, particularly in the absence of sawdust. This result indicates that the composition and characteristics of the sludge substrate influenced VFA production. In contrast, digested sludge and ammonia removal sludge produced lower VFA yields under the tested conditions. The differences among sludge types indicate that the suitability of wastewater sludge for VFA production depends on substrate characteristics and operating conditions.

PHA production was achieved using activated-sludge-derived microbial communities supplemented with acetate, reaching a PHA content of 243.2 mg 3HB g⁻¹ DW. This result demonstrates the potential of waste-derived microbial communities to convert acetate into PHA under the applied experimental conditions. However, comparison with previous studies indicates that PHA production performance can vary considerably depending on substrate, microbial community, and operating conditions.

The results showed that acetic acid was the predominant VFA under most of the tested conditions. Although valeric and caproic acids can be valuable fermentation products, their concentrations in the present study were generally lower than that of acetic acid. VFA composition may potentially be influenced by the selection of sludge substrates, co-substrates, and operating conditions. Further investigation of different waste material compositions and fermentation conditions could therefore be used to promote the production of specific VFAs when desired.

From a process perspective, this thesis highlights the possibility of laboratory-scale bioreactors using waste materials to produce VFAs and PHAs. Moreover, results from VFA production were based on laboratory-scale bioreactors (≤ 500 mL), which may not fully represent mass transfer and product recovery behaviour at larger scales. PHA accumulation was performed in a 5-litre fermenter using microbial communities derived from waste sludge supplemented with acetic acid. The conversion processes do not by themselves eliminate the residual sludge or potential contaminants, and appropriate management of the remaining material would therefore still be required. Further research is required to investigate the integration of VFA production and PHA accumulation, as well as differences in process performance between laboratory-scale and industrial-scale systems.

Overall, the results indicate that selected wastewater sludge streams have potential for VFA production and that waste-derived microbial communities can be used for PHA production under the conditions investigated. These findings support the potential of microbial conversion processes as a strategy for sludge valorisation. However, further studies involving process optimisation, scale-up, integration of VFA and PHA production, product recovery, and economic assessment are needed to evaluate their practical applicability.

References

- Abdullah, H., 1984. The effect of protein (as fish meal) on rumen VFA patterns of molasses-fed sheep. *Pertanika* 7 (3), 89–94. <https://www.semanticscholar.org/paper/6d650764e02899a0119cbb7a35748c726b2f7c9d>
- Agnihotri, S., Yin, D.M., Mahboubi, A., Sapmaz, T., Varjani, S., Qiao, W., Koseoglu-Imer D.Y., Taherzadeh, M.J., 2022. A glimpse of the world of volatile fatty acids production and application: a review. *Bioengineered* 13 (1), 1249–1275. <https://doi.org/10.1080/21655979.2021.1996044>
- Alcântara, J.M.G., Distante, F., Storti, G., Moscatelli, D., Morbidelli, M., Sponchioni, M., 2020. Current trends in the production of biodegradable bioplastics: The case of polyhydroxyalkanoates. *Biotechnol. Adv.* 42, 107582. <https://doi.org/10.1016/j.biotechadv.2020.107582>
- Aldaeus, F., Schweinebarth, H., Törngren, P., Jacobs, A., 2011. Simplified determination of total lignin content in kraft lignin samples and black liquors. *Holzforschung* 65 (4), 601–604. <https://doi.org/10.1515/hf.2011.102>
- Ali, S.S., Elsamahy, T., Koutra, E., Kornaros, M., El-Sheekh, M., Abdelkarim, E.A., Zhu, D., Sun, J., 2021. Degradation of conventional plastic wastes in the environment: A review on current status of knowledge and future perspectives of disposal. *Sci. Total Environ.* 771, 144719. <https://doi.org/10.1016/j.scitotenv.2020.144719>
- Andrady, A.L., 2017. The plastic in microplastics: A review. *Mar. Pollut. Bull.* 119 (1), 12–22. <https://doi.org/10.1016/j.marpolbul.2017.01.082>
- Ansari, S., Sami, N., Yasin, D., Ahmad, N., Fatma, T., 2021. Biomedical applications of environmental friendly poly-hydroxyalkanoates. *Int. J. Biol. Macromol.* 183, 549–563. <https://doi.org/10.1016/j.ijbiomac.2021.04.171>
- Argun, Y.A., Karacali, A., Calisir, U., Kilinc, N., 2017. Composting as a waste management method. *J. Int. Environ. Appl. Sci.* 12 (3), 244–255.
- Atasoy, M., Cetecioglu, Z., 2022. The effects of pH on the production of volatile fatty acids and microbial dynamics in long-term reactor operation. *J. Environ. Manage.* 319, 115700. <https://doi.org/10.1016/j.jenvman.2022.115700>
- Atasoy, M., Owusu-Agyeman, I., Plaza, E., Cetecioglu, Z., 2018. Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. *Bioresour. Technol.* 268, 773–786. <https://doi.org/10.1016/j.biortech.2018.07.042>
- Bahreini, G., Elbeshbishy, E., Jimenez, J., Santoro, D., Nakhla, G., 2020. Integrated fermentation and anaerobic digestion of primary sludges for simultaneous resource and energy recovery: impact of volatile fatty acids recovery. *Waste Manage.* 118, 341–349. <https://doi.org/10.1016/j.wasman.2020.08.051>

- Bali, G., Meng, X., Deneff, J.I., Sun, Q., Ragauskas, A.J., 2015. The effect of alkaline pretreatment methods on cellulose structure and accessibility. *ChemSusChem* 8 (2), 275–279. <https://doi.org/10.1002/cssc.201402752>
- Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Pal, M., Khurana, S., 2015. Evolution of bacterial and fungal growth media. *Bioinformation* 11 (4), 182–184. <https://doi.org/10.6026/97320630011182>
- Bengtsson, S., Werker, A., Christensson, M., Welander, T., 2008. Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. *Bioresour. Technol.* 99 (3), 509–516. <https://doi.org/10.1016/j.biortech.2007.01.020>
- Bevilacqua, R., Regueira, A., Mauricio-Iglesias, M., Lema, J.M., Carballa, M., 2021. Steering the conversion of protein residues to volatile fatty acids by adjusting pH. *Bioresour. Technol.* 320, 124315. <https://doi.org/10.1016/j.biortech.2020.124315>
- Bouallagui, H., Torrijos, M., Godon, J.J., Moletta, R., Cheikh, R.B., Touhami, Y., Delgenes, J.P., Hamdi, M., 2004. Two-phases anaerobic digestion of fruit and vegetable wastes: bioreactors performance. *Biochem. Eng. J.* 21 (2), 193–197. <https://doi.org/10.1016/j.bej.2004.05.001>
- Braunegg, G., Sonnleitner, B.Y., Lafferty, R.M., 1978. A rapid gas chromatographic method for the determination of poly- β -hydroxybutyric acid in microbial biomass. *Eur. J. Appl. Microbiol. Biotechnol.* 6 (1), 29–37. <https://doi.org/10.1007/BF00500854>
- Bruni, C., Foglia, A., Eusebi, A.L., Frison, N., Akyol, C., Fatone, F., 2021. Targeted bio-based volatile fatty acid production from waste streams through anaerobic fermentation: link between process parameters and operating scale. *ACS. Sustain. Chem. Eng.* 9 (30), 9970–9987. <https://doi.org/10.1021/acssuschemeng.1c02195>
- Bücher, C., Burtcher, J., Domig, K.J., 2021. Propionic acid bacteria in the food industry: An update on essential traits and detection methods. *Compr. Rev. Food Sci. Food Saf.* 20 (5), 4299–4323. <https://doi.org/10.1111/1541-4337.12804>
- Bugnicourt, E., Cinelli, P., Lazzeri, A., Alvarez, V.A., 2014. Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging. *eXPRESS Polym. Lett.* 8 (11), 791–808. <http://dx.doi.org/10.3144/expresspolymlett.2014.82>
- Buta, M., Hubeny, J., Zieliński, W., Harnisz, M., Korzeniewska, E., 2021. Sewage sludge in agriculture – the effects of selected chemical pollutants and emerging genetic resistance determinants on the quality of soil and crops – a review. *Ecotoxicol. Environ. Saf.* 214, 112070. <https://doi.org/10.1016/j.ecoenv.2021.112070>
- Cao, Q.T., Zhang, W.Q., Lian, T.J., Wang, S.L., Yin, F.B., Zhou, T.L., Zhang, H.Y., Zhu, J., Dong, H.M., 2022. Roles of micro-aeration on enhancing volatile fatty acids and lactic acid production from agricultural wastes. *Bioresour. Technol.* 347, 126656. <https://doi.org/10.1016/j.biortech.2021.126656>
- Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J.H., Abu-Omar, M., Scott, S.L., Suh, S., 2020. Degradation rates of plastics in the environment. *ACS Sustainable Chem. Eng.* 8 (9), 3494–3511. <https://doi.org/10.1021/acssuschemeng.9b06635>
- Chen, C., Zhang, X., Liu, C., Wu, Y., Zheng, G., Chen, Y., 2022. Advances in downstream processes and applications of biological carboxylic acids

- derived from organic wastes. *Bioresour. Technol.* 346, 126609. <https://doi.org/10.1016/j.biortech.2021.126609>
- Chen, H., Yan, S.H., Ye, Z.L., Meng, H.J., Zhu, Y.G., 2012. Utilization of urban sewage sludge: Chinese perspectives. *Environ. Sci. Pollut. Res.* 19 (5), 1454–1463. <https://doi.org/10.1007/s11356-012-0760-0>
- Chen, Y.G., Jiang, S., Yuan, H.Y., Zhou, Q., Gu, G.W., 2007. Hydrolysis and acidification of waste activated sludge at different pHs. *Water Res.* 41 (3), 683–689. <https://doi.org/10.1016/j.watres.2006.07.030>
- Chi, Y.Z., Zhang, S.T., & Li, Y.Y., 2009. Treatment and disposal of sewage sludge in China. *J. Environ. Conserv. Eng.* 38 (5), 45–50.
- Choi, J., Lee, S.Y., 1999. Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. *Appl. Microbiol. Biotechnol.* 51 (1), 13–21. <https://doi.org/10.1007/s002530051357>
- Chua, A.S.M., Takabatake, H., Satoh, H., Mino, T., 2003. Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent. *Water Res.* 37 (15), 3602–3611. [https://doi.org/10.1016/S0043-1354\(03\)00252-5](https://doi.org/10.1016/S0043-1354(03)00252-5)
- Comeau, Y., Hall, K.J., Oldham, W.K., 1988. Determination of poly- β -hydroxybutyrate and poly- β -hydroxyvalerate in activated sludge by gas-liquid chromatography. *Appl. Environ. Microbiol.* 54 (9), 2325–2327. <https://doi.org/10.1128/aem.54.9.2325-2327.1988>
- Cruz, R.A.P., Oehmen, A., Reis, M.A.M., 2022. The impact of biomass withdrawal strategy on the biomass selection and polyhydroxyalkanoates accumulation of mixed microbial cultures. *New Biotechnol.* 66, 8–15. <https://doi.org/10.1016/j.nbt.2021.08.004>
- Dalton, B., Bhagabati, P., De Micco, J., Padamati, R.B., O'Connor, K., 2022. A review on biological synthesis of the biodegradable polymers polyhydroxyalkanoates and the development of multiple applications. *Catalysts* 12 (3), 319. <https://doi.org/10.3390/catal12030319>
- Das, T., Al-Waili, I., Balasubramanian, V., Appleby, G., Kaparaju, P., Parthasarathy, R., Eshtiaghi, N., 2024. Process modelling and techno-economic analysis of anaerobic digestion of sewage sludge integrated with wet oxidation using a gravity pressure vessel and thermal hydrolysis. *Sci. Total Environ.* 912, 169024. <https://doi.org/10.1016/j.scitotenv.2023.169024>
- de Lima Brossi, M.J., Jiménez, D.J., Cortes-Tolalpa, L., van Elsas, J.D., 2016. Soil-derived microbial consortia enriched with different plant biomass reveal distinct players acting in lignocellulose degradation. *Microb. Ecol.* 71 (3), 616–627. <https://doi.org/10.1007/s00248-015-0683-7>
- Demirbas, A., Edris, G., Alalayah, W.M., 2017. Sludge production from municipal wastewater treatment in sewage treatment plant. *Energy Sources A: Recovery Util. Environ. Eff.* 39(10), 999–1006. <https://doi.org/10.1080/15567036.2017.1283551>
- den Boer, E., Łukaszewska, A., Kluczkiewicz, W., Lewandowska, D., King, K., Reijonen, T., Kuhmonen, T., Suhonen, A., Jääskeläinen, A., Heitto, A., Laatikainen, R., Hakalehto, E., 2016. Volatile fatty acids as an added value from biowaste. *Waste Manage.* 58, 62–69. <https://doi.org/10.1016/j.wasman.2016.08.006>
- Deng, B., Rao, L.F., Rodriguez-Freire, L., 2024. Evaluation and optimization of FTIR spectroscopy to quantify PHA production by municipal wastewater

- sludge. *Spectrochim. Acta*, Part A 312, 124012. <https://doi.org/10.1016/j.saa.2024.124012>
- Dias, J.M.L., Lemos, P.C., Serafim, L.S., Oliveira, C., Eiroa, M., Albuquerque, M.G.E., Ramos, A.M., Oliveira, R., Reis, M.A.M., 2006. Recent advances in polyhydroxyalkanoate production by mixed aerobic cultures: from the substrate to the final product. *Macromol. Biosci.* 6 (11), 885–906. <https://doi.org/10.1002/mabi.200600112>
- Diaz, R., Hong, S., Goel, R., 2023. Effect of different types of volatile fatty acids on the performance and bacterial population in a batch reactor performing biological nutrient removal. *Bioresour. Technol.* 388, 129675. <https://doi.org/10.1016/j.biortech.2023.129675>
- Dicks, L., Botes, M., 2009. Probiotic lactic acid bacteria in the gastro-intestinal tract: health benefits, safety and mode of action. *Benefic. Microbes* 1 (1), 11–29. <https://doi.org/10.3920/BM2009.0012>
- Du, J., Yin, Q., Gu, M., Wu, G., 2021. New insights into the effect of ethanol and volatile fatty acids proportions on methanogenic activities and pathways. *Environ. Res.* 194, 110644. <https://doi.org/10.1016/j.envres.2020.110644>
- Eschenlauer, A.C., Stoup, S.K., Srien, F., Somers, D.A., 1996. Production of heteropolymeric polyhydroxyalkanoate in *Escherichia coli* from a single carbon source. *Int. J. Biol. Macromol.* 19 (2), 121–130. [https://doi.org/10.1016/0141-8130\(96\)01114-2](https://doi.org/10.1016/0141-8130(96)01114-2)
- Eskicioglu, C., Prorot, A., Marin, J., Droste, R.L., Kennedy, K.J., 2008. Synergetic pretreatment of sewage sludge by microwave irradiation in presence of H₂O₂ for enhanced anaerobic digestion. *Water Res.* 42 (18), 4674–4682. <https://doi.org/10.1016/j.watres.2008.08.010>
- Etale, A., Onyanta, A.J., Turner, S.R., Eichhorn, S.J., 2023. Cellulose: a review of water interactions, applications in composites, and water treatment. *Chem. Rev.* 123 (5), 2016–2048. <https://doi.org/10.1021/acs.chemrev.2c00477>
- Fang, S.T., Qin, T., Yu, T., Zhang, G.X., 2022. Improvement of the gut microbiota in vivo by a short-chain fatty acids-producing strain *Lactococcus garvieae* CF11. *Processes* 10 (3), 604. <https://doi.org/10.3390/pr10030604>
- Fang, W., Zhang, X., Zhang, P., Wan, J., Guo, H., Ghasimi, D.S., Morera, X.C., Zhang, T., 2020. Overview of key operation factors and strategies for improving fermentative volatile fatty acid production and product regulation from sewage sludge. *J. Environ. Sci.* 87, 93–111. <https://doi.org/10.1016/j.jes.2019.05.027>
- Fernández-Navarro, T., Salazar, N., Gutiérrez-Díaz, I., Sánchez, B., Rúas-Madiedo, P., de Los Reyes-Gavilan, C.G., Margolles, A., Gueimonde, M., González, S., 2018. Bioactive compounds from regular diet and faecal microbial metabolites. *Eur. J. Nutr.* 57 (2), 487–497. <https://doi.org/10.1007/s00394-016-1332-8>
- Filho, W.L., Salvia, A.L., Bonoli, A., Saari, U.A., Voronova, V., Klōga, M., Kumbhar, S.S., Olszewski, K., De Quevedo, D.M., Barbir, J., 2021. An assessment of attitudes towards plastics and bioplastics in Europe. *Sci. Total Environ.* 755, 142732. <https://doi.org/10.1016/j.scitotenv.2020.142732>
- Foladori, P., Andreottola, G., Ziglio, G., 2010. *Sludge reduction technologies in wastewater treatment plants*. IWA Publishing.
- Garcia-Aguirre, J., Aymerich, E., González-Mtnez. de Goñi, J., Esteban-Gutiérrez, M., 2017. Selective VFA production potential from organic waste streams:

- assessing temperature and pH influence. *Bioresour. Technol.* 244, 1081–1088. <https://doi.org/10.1016/j.biortech.2017.07.187>
- Gerardi, M.H., 2003. *The microbiology of anaerobic digesters*. John Wiley & Sons, Inc.
- Ghimire, U., Sarpong, G., Gude, V.G., 2021. Transitioning wastewater treatment plants toward circular economy and energy sustainability. *ACS Omega* 6 (18), 11794–11803. <https://doi.org/10.1021/acsomega.0c05827>
- Gilarranz, M.A., Rodriguez, F., Olet, M., Revenga, J.A., 1998. Acid precipitation and purification of wheat straw lignin. *Sep Sci Technol* 33 (9), 1359–1377. <https://doi.org/10.1080/01496399808544988>
- Gurieff, N., Lant, P., 2007. Comparative life cycle assessment and financial analysis of mixed culture polyhydroxyalkanoate production. *Bioresour. Technol.* 98 (17), 3393–3403. <https://doi.org/10.1016/j.biortech.2006.10.046>
- Hao, J., Wang, H., Wang, X., 2018. Selecting optimal feast-to-famine ratio for a new polyhydroxyalkanoate (PHA) production system fed by valerate-dominant sludge hydrolysate. *Appl. Microbiol. Biotechnol.* 102 (7), 3133–3143. <https://doi.org/10.1007/s00253-018-8799-6>
- Harirchi, S., Wainaina, S., Sar, T., Nojoudi, S.A., Parchami, M., Parchami, M., Varjani, S., Khanal, S.K., Wong, J., Awasthi, M.K., 2022. Microbiological insights into anaerobic digestion for biogas, hydrogen or volatile fatty acids (VFAs): a review. *Bioengineered* 13 (3), 6521–6557. <https://doi.org/10.1080/21655979.2022.2035986>
- Hassan, M.A., Shirai, Y., Kusubayashi, N., Karim, M.I.A., Nakanishi, K., Hashimoto, K., 1996. Effect of organic acid profiles during anaerobic treatment of palm oil mill effluent on the production of polyhydroxyalkanoates by *Rhodobacter sphaeroides*. *J. Ferment. Bioeng.* 82 (2), 151–156. [https://doi.org/10.1016/0922-338X\(96\)85038-0](https://doi.org/10.1016/0922-338X(96)85038-0)
- Higuchi-Takeuchi, M., Numata, K., 2019. Acetate-inducing metabolic states enhance polyhydroxyalkanoate production in marine purple non-sulfur bacteria under aerobic conditions. *Front. Bioeng. Biotechnol.* 7, 118. <https://doi.org/10.3389/fbioe.2019.00118>
- Horn, M.A., Matthies, C., Küsel, K., Schramm, A., Drake, H.L., 2003. Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl. Environ. Microbiol.* 69 (1), 74–83. <https://doi.org/10.1128/AEM.69.1.74-83.2003>
- Ibrahim, F., Ouwehand, A.C., 2019. The genus *Lactobacillus*. In: *Lactic acid bacteria*. CRC Press, pp. 47–56.
- Islam, B., 2015. Petroleum sludge, its treatment and disposal: A review. *Int. J. Chem. Sci* 13 (4), 1584–1602.
- Jang, Y.C., Lee, G., Kwon, Y., Lim, J.h., Jeong, J.h., 2020. Recycling and management practices of plastic packaging waste towards a circular economy in South Korea. *Resour., Conserv. Recycl.* 158, 104798. <https://doi.org/10.1016/j.resconrec.2020.104798>
- Jayakrishnan, U., Deka, D., Das, G., 2021. Regulation of volatile fatty acid accumulation from waste: Effect of inoculum pretreatment. *Water Environ. Res.* 93 (7), 1019–1031. <https://doi.org/10.1002/wer.1490>
- Jia, Q.Q., Xiong, H.L., Wang, H., Shi, H.C., Sheng, X.Y., Sun, R., Chen, G.Q., 2014. Production of polyhydroxyalkanoates (PHA) by bacterial consortium from excess sludge fermentation liquid at laboratory and pilot scales.

- Bioresour. Technol. 171, 159–167.
<https://doi.org/10.1016/j.biortech.2014.08.059>
- Jin, L.Z., Ho, Y.W., Abdullah, N., Ali, M.A., Jalaludin, S., 1998. Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. *Anim. Feed Sci. Technol.* 70 (3), 197–209.
[https://doi.org/10.1016/S0377-8401\(97\)00080-1](https://doi.org/10.1016/S0377-8401(97)00080-1)
- Jones, R.J., Massanet-Nicolau, J., Fernandez-Feito, R., Dinsdale, R.M., Guwy, A.J., 2021. Fermentative volatile fatty acid production and recovery from grass using a novel combination of solids separation, pervaporation, and electrodialysis technologies. *Bioresour. Technol.* 342, 125926.
<https://doi.org/10.1016/j.biortech.2021.125926>
- Kandylis, P., Bekatorou, A., Pissaridi, K., Lappa, K., Dima, A., Kanellaki, M., Koutinas, A.A., 2016. Acidogenesis of cellulosic hydrolysates for new generation biofuels. *Biomass Bioenergy* 91, 210–216.
<http://dx.doi.org/10.1016/j.biombioe.2016.05.006>
- Kershaw, P., Katsuhiko, S., Lee, S., Woodring, D., 2011. Plastic debris in the ocean. United Nations Environment Programme.
- Khamkong, T., Penkhrue, W., Lumyong, S., 2022. Optimization of production of polyhydroxyalkanoates (PHAs) from newly isolated *Ensifer* sp. strain HD34 by response surface methodology. *Processes* 10 (8), 1632.
<https://doi.org/10.3390/pr10081632>
- Koller, M., 2018. A review on established and emerging fermentation schemes for microbial production of polyhydroxyalkanoate (PHA) biopolyesters. *Fermentation* 4 (2), 30. <https://doi.org/10.3390/fermentation4020030>
- Koller, M., Mukherjee, A., 2020. Polyhydroxyalkanoates—linking properties, applications, and end-of-life options. *Chem. Biochem. Eng. Q.* 34 (3), 115–129. <https://doi.org/10.15255/CABEQ.2020.1819>
- Kouzi, A.I., Puranen, M., Kontro, M.H., 2020. Evaluation of the factors limiting biogas production in full-scale processes and increasing the biogas production efficiency. *Environ. Sci. Pollut. Res.* 27 (22), 28155–28168.
<https://doi.org/10.1007/s11356-020-09035-1>
- Kovalcik, A., 2021. Recent advances in 3D printing of polyhydroxyalkanoates: a review. *Eurobiotech J.* 5 (1), 48–55. <https://doi.org/10.2478/ebtj-2021-0008>
- Kruglova, A., Kesulahti, J., Minh Le, K., Gonzalez-Martinez, A., Mikola, A., Vahala, R., 2020. Low-temperature adapted nitrifying microbial communities of Finnish wastewater treatment systems. *Water* 12 (9), 2450.
<https://doi.org/10.3390/w12092450>
- Kumar, S., Singh, E., Mishra, R., Kumar, A., Caucci, S., 2021. Utilization of Plastic Wastes for Sustainable Environmental Management: A Review. *ChemSusChem* 14 (19), 3985–4006.
<https://doi.org/10.1002/cssc.202101631>
- Kumar, V., Chopra, A.K., Kumar, A., 2017. A review on sewage sludge (Biosolids) a resource for sustainable agriculture. *Arch. Agric. Environ. Sci.* 2 (4), 340–347. <https://doi.org/10.26832/24566632.2017.020417>
- Laist, D.W., 1987. Overview of the biological effects of lost and discarded plastic debris in the marine environment. *Mar. Pollut. Bull.* 18 (6), 319–326.
[https://doi.org/10.1016/S0025-326X\(87\)80019-X](https://doi.org/10.1016/S0025-326X(87)80019-X)

- Lemoigne, M., 1926. Produit de déshydratation et de polymérisation de l'acide β -oxybutyrique. *Bull. Soc. Chim. Biol.* 8, 770–782.
- Li, H., Si, D., Liu, C., Feng, K., Liu, C., 2018. Performance of direct anaerobic digestion of dewatered sludge in long-term operation. *Bioresour. Technol.* 250, 355–364. <https://doi.org/10.1016/j.biortech.2017.11.075>
- Lim, J.W., Chiam, J.A., Wang, J.Y., 2014. Microbial community structure reveals how microaeration improves fermentation during anaerobic co-digestion of brown water and food waste. *Bioresour. Technol.* 171, 132–138. <https://doi.org/10.1016/j.biortech.2014.08.050>
- Lim, J.X., Zhou, Y., Vadivelu, V.M., 2020. Enhanced volatile fatty acid production and microbial population analysis in anaerobic treatment of high strength wastewater. *J. Water Process Eng.* 33, 101058. <https://doi.org/10.1016/j.jwpe.2019.101058>
- Liu, H., Han, P., Liu, H., Zhou, G., Fu, B., Zheng, Z., 2018. Full-scale production of VFAs from sewage sludge by anaerobic alkaline fermentation to improve biological nutrients removal in domestic wastewater. *Bioresour. Technol.* 260, 105–114. <https://doi.org/10.1016/j.biortech.2018.03.105>
- Lin, L., Xu, F., Ge, X., Li, Y., 2019. Biological treatment of organic materials for energy and nutrients production—Anaerobic digestion and composting. In: *Advances in Bioenergy Vol. 4*. Elsevier, pp. 121–181.
- Liu, X., Liu, H., Chen, Y., Du, G., Chen, J., 2008. Effects of organic matter and initial carbon–nitrogen ratio on the bioconversion of volatile fatty acids from sewage sludge. *J. Chem. Technol. Biotechnol.* 83 (7), 1049–1055. <https://doi.org/10.1002/jctb.1913>
- Liu, Y.L., Li, X., Kang, X.R., Yuan, Y.X., Du, M.A., 2014. Short chain fatty acids accumulation and microbial community succession during ultrasonic-pretreated sludge anaerobic fermentation process: Effect of alkaline adjustment. *Int. Biodeterior. Biodegrad.* 94, 128–133. <https://doi.org/10.1016/j.ibiod.2014.07.004>
- Lorang, S., Yang, Z., Zhang, H., Lü, F., He, P., 2022. Achievements and policy trends of extended producer responsibility for plastic packaging waste in Europe. *Waste Disposal Sustainable Energy* 4 (2), 91–103. <https://doi.org/10.1007/s42768-022-00098-z>
- Luo, K., Pang, Y., Yang, Q., Wang, D.B., Li, X., Lei, M., Huang, Q., 2019. A critical review of volatile fatty acids produced from waste activated sludge: enhanced strategies and its applications. *Environ. Sci. Pollut. Res.* 26 (14), 13984–13998. <https://doi.org/10.1007/s11356-019-04798-8>
- Ma, H.J., Chen, X.C., Liu, H., Liu, H.B., Fu, B., 2016. Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: alkaline or neutral pH? *Waste Manage.* 48, 397–403. <https://doi.org/10.1016/j.wasman.2015.11.029>
- Ma, J.W., Xie, S.B., Yu, L., Zhen, Y.B., Zhao, Q.B., Frear, C., Chen, S.L., Wang, Z.W., Shi, Z., 2019. pH shaped kinetic characteristics and microbial community of food waste hydrolysis and acidification. *Biochem. Eng. J.* 146, 52–59. <https://doi.org/10.1016/j.bej.2019.03.004>
- McLoughlin, S., Spillane, C., Claffey, N., Smith, P.E., O'Rourke, T., Diskin, M.G., Waters, S.M., 2020. Rumen microbiome composition is altered in sheep divergent in feed efficiency. *Front. Microbiol.* 11, 1981. <https://doi.org/10.3389/fmicb.2020.01981>

- Meereboer, K.W., Misra, M., Mohanty, A.K., 2020. Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites. *Green Chem.* 22 (17), 5519–5558. <https://doi.org/10.1039/DoGC01647K>
- Menzel, T., Neubauer, P., Junne, S., 2020. Role of microbial hydrolysis in anaerobic digestion. *Energies* 13 (21), 5555. <https://doi.org/10.3390/en13215555>
- Millican, J.M., Agarwal, S., 2021. Plastic pollution: a material problem? *Macromol.* 54 (10), 4455–4469. <https://doi.org/10.1021/acs.macromol.0c02814>
- Montiel-Jarillo, G., Carrera, J., Suárez-Ojeda, M.E., 2017. Enrichment of a mixed microbial culture for polyhydroxyalkanoates production: Effect of pH and N and P concentrations. *Sci. Total Environ.* 583, 300–307. <https://doi.org/10.1016/j.scitotenv.2017.01.069>
- Moretto, G., Valentino, F., Pavan, P., Majone, M., Bolzonella, D., 2019. Optimization of urban waste fermentation for volatile fatty acids production. *Waste Manage.* 92, 21–29. <https://doi.org/10.1016/j.wasman.2019.05.010>
- Morgan-Sagastume, F., Hjort, M., Cirne, D., Gérardin, F., Lacroix, S., Gaval, G., Karabegovic, L., Alexandersson, T., Johansson, P., Karlsson, A., Bengtsson, S., Arcos-Hernández, M.V., Magnusson, P., Werker, A., 2015. Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. *Bioresour. Technol.* 181, 78–89. <http://dx.doi.org/10.1016/j.biortech.2015.01.046>
- Morgan-Sagastume, F., Karlsson, A., Johansson, P., Pratt, S., Boon, N., Lant, P., Werker, A., 2010. Production of polyhydroxyalkanoates in open, mixed cultures from a waste sludge stream containing high levels of soluble organics, nitrogen and phosphorus. *Water Res.* 44 (18), 5196–5211. <https://doi.org/10.1016/j.watres.2010.06.043>
- Mottet, A., François, E., Latrille, E., Steyer, J.P., Déléris, S., Vedrenne, F., Carrère, H., 2010. Estimating anaerobic biodegradability indicators for waste activated sludge. *Chem. Eng. J.* 160 (2), 488–496. <https://doi.org/10.1016/j.cej.2010.03.059>
- Muthuraj, R., Valerio, O., Mekonnen, T.H., 2021. Recent developments in short- and medium-chain- length Polyhydroxyalkanoates: Production, properties, and applications. *Int. J. Biol. Macromol.* 187, 422–440. <https://doi.org/10.1016/j.ijbiomac.2021.07.143>
- Nguyen, P.Y., Marques, R., Wang, H.M., Reis, M.A.M., Carvalho, G., Oehmen, A., 2023. The impact of pH on the anaerobic and aerobic metabolism of Tetrasphaera-enriched polyphosphate accumulating organisms. *Water Res.* X 19, 100177. <https://doi.org/10.1016/j.wroa.2023.100177>
- Ochoa, C., Hernández, M.A., Bayona, O.L., Cabeza, I.O., Candela, A.M., 2021. Value-added by-products during dark fermentation of agro-industrial residual biomass: metabolic pathway analysis. *Waste Biomass Valorization* 12 (11), 5937–5948. <https://doi.org/10.1007/s12649-021-01421-1>
- Oehmen, A., Keller-Lehmann, B., Zeng, R.J., Yuan, Z., Keller, J., 2005. Optimisation of poly- β -hydroxyalkanoate analysis using gas chromatography for enhanced biological phosphorus removal systems. *J. Chromatogr. A* 1070 (1-2), 131–136. <https://doi.org/10.1016/j.chroma.2005.02.020>

- Oliveira, C.S.S., Silva, C.E., Carvalho, G., Reis, M.A., 2017. Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: Feast and famine regime and uncoupled carbon and nitrogen availabilities. *New Biotechnol.* 37, 69–79. <https://doi.org/10.1016/j.nbt.2016.10.008>
- Otitoju, M. A., Olawoye, T., Abiola, S. S., Ahmed, S., Okoma, O., 2023. Plastic waste management and recycling: a review. *Journal of Global Social Sciences* 4 (16), 60–71. <https://doi.org/10.58934/jgss.v4i16.219>
- Pang, D.G., Yang, H.J., Cao, B.B., Wu, T.T., Wang, J.Q., 2014. The beneficial effect of *Enterococcus faecium* on the in vitro ruminal fermentation rate and extent of three typical total mixed rations in northern China. *Livest. Sci.* 167, 154–160. <https://doi.org/10.1016/j.livsci.2014.06.008>
- Plácido, J., Zhang, Y., 2018. Evaluation of esterification and membrane based solvent extraction as methods for the recovery of short chain volatile fatty acids from slaughterhouse blood anaerobic mixed fermentation. *Waste Biomass Valorization* 9 (10), 1767–1777. <https://doi.org/10.1007/s12649-017-9952-7>
- Pokhrel, S.P., Milke, M.W., Bello-Mendoza, R., Buitrón, G., Thiele, J.J.W.M., 2018. Use of solid phosphorus fractionation data to evaluate phosphorus release from waste activated sludge. *Waste Manag.* 76, 90–97. <https://doi.org/10.1016/j.wasman.2018.03.008>
- Prata, J.C., da Costa, J.P., Lopes, I., Andrady, A.L., Duarte, A.C., Rocha-Santos, T., 2021. A One Health perspective of the impacts of microplastics on animal, human and environmental health. *Sci. Total Environ.* 777, 146094. <https://doi.org/10.1016/j.scitotenv.2021.146094>
- Puyol, D., Batstone, D.J., Hülsen, T., Astals, S., Peces, M., Krömer, J.O., 2017. Resource recovery from wastewater by biological technologies: opportunities, challenges, and prospects. *Front. Microbiol.* 7, 2106. <https://doi.org/10.3389/fmicb.2016.02106>
- Qian, L., Wang, S., Xu, D., Guo, Y., Tang, X., Wang, L., 2016. Treatment of municipal sewage sludge in supercritical water: a review. *Water Res.* 89, 118–131. <https://doi.org/10.1016/j.watres.2015.11.047>
- Qiu, S., Zhang, X.C., Xia, W.H., Li, Z.M., Wang, L.F., Chen, Z.P., Ge, S.J., 2023. Effect of extreme pH conditions on methanogenesis: Methanogen metabolism and community structure. *Sci. Total Environ.* 877, 162702. <https://doi.org/10.1016/j.scitotenv.2023.162702>
- Quist-Jensen, C.A., Wybrandt, L., Løkkegaard, H., Antonsen, S.B., Jensen, H.C., Nielsen, A.H., Christensen, M.L., 2018. Acidification and recovery of phosphorus from digested and non-digested sludge. *Water Res.* 146, 307–317. <https://doi.org/10.1016/j.watres.2018.09.035>
- Rabelo, C.H.S., Basso, F.C., Lara, E.C., Jorge, L.G.O., Härter, C.J., Mari, L.J., Reis, M.R.A., 2017. Effects of *Lactobacillus buchneri* as a silage inoculant or probiotic on in vitro organic matter digestibility, gas production and volatile fatty acids of low dry-matter whole-crop maize silage. *Grass Forage Sci.* 72 (3), 534–544. <https://doi.org/10.1111/gfs.12273>
- Rajmohan, K.V.S., Ramya, C., Viswanathan, M.R., Varjani, S., 2019. Plastic pollutants: effective waste management for pollution control and abatement. *Curr. Opin. Environ. Sci. Health.* 12, 72–84. <https://doi.org/10.1016/j.coesh.2019.08.006>

- Ramos-Suarez, M., Zhang, Y., Outram, V., 2021. Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste. *Rev. Environ. Sci. Bio/Technol.* 20 (2), 439–478. <https://doi.org/10.1007/s11157-021-09566-0>
- RedCorn, R.M., Hillman, E.T., Solomon, K.V., Engelberth, A.S., 2019. Xanthobacter-dominated biofilm as a novel source for high-value rhamnose. *Appl. Microbiol. Biotechnol.* 103 (11), 4525–4538. <https://doi.org/10.1007/s00253-019-09765-4>
- Regueira, A., Bevilacqua, R., Lema, J.M., Carballa, M., Mauricio-Iglesias, M., 2020. A metabolic model for targeted volatile fatty acids production by cofermentation of carbohydrates and proteins. *Bioresour. Technol.* 298, 122535. <https://doi.org/10.1016/j.biortech.2019.122535>
- Reis, M.A.M., Serafim, L.S., Lemos, P.C., Ramos, A.M., Aguiar, F.R., Van Loosdrecht, M.C.M., 2003. Production of polyhydroxyalkanoates by mixed microbial cultures. *Bioprocess Biosyst. Eng.* 25 (6), 377–385. <https://doi.org/10.1007/s00449-003-0322-4>
- Rhodes, C.J., 2019. Solving the plastic problem: From cradle to grave, to reincarnation. *Sci. Prog.* 102 (3), 218–248. <https://doi.org/10.1177/0036850419867204>
- Rorat, A., Courtois, P., Vandenbulcke, F., Lemiére, S., 2019. Sanitary and environmental aspects of sewage sludge management. In: *Industrial and Municipal Sludge*. Butterworth-Heinemann, pp. 155–180. <https://doi.org/10.1016/B978-0-12-815907-1.00008-8>
- Sakai, K., Miyake, S., Iwama, K., Inoue, D., Soda, S., Ike, M., 2015. Polyhydroxyalkanoate (PHA) accumulation potential and PHA-accumulating microbial communities in various activated sludge processes of municipal wastewater treatment plants. *J. Appl. Microbiol.* 118 (1), 255–266. <https://doi.org/10.1111/jam.12683>
- Samadhiya, K., Sangtani, R., Nogueira, R., Bala, K., 2022. Insightful advancement and opportunities for microbial bioplastic production. *Front. Microbiol.* 12, 674864. <https://doi.org/10.3389/fmicb.2021.674864>
- Sawatdeenarunat, C., Sung, S., Khanal, S.K., 2017. Enhanced volatile fatty acids production during anaerobic digestion of lignocellulosic biomass via micro-oxygenation. *Bioresour. Technol.* 237, 139–145. <https://doi.org/10.1016/j.biortech.2017.02.029>
- Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol. Mol. Biol. Rev.* 61 (2), 262–280. <https://doi.org/10.1128/mmbr.61.2.262-280.1997>
- Schoenherr, S., Ebrahimi, M., Czermak, P., 2018. Lignin degradation processes and the purification of valuable products. In: Poletto M, ed. *Lignin -Trends and Applications*. IntechOpen. <https://dx.doi.org/10.5772/intechopen.71210>
- Seiple, T.E., Coleman, A.M., Skaggs, R.L., 2017. Municipal wastewater sludge as a sustainable bioresource in the United States. *J. Environ. Manage.* 197, 673–680. <https://doi.org/10.1016/j.jenvman.2017.04.032>
- Serafim, L.S., Lemos, P.C., Oliveira, R., Reis, M.A.M., 2004. Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. *Biotechnol. Bioeng.* 87 (2), 145–160. <https://doi.org/10.1002/bit.20085>

- Sharma, R., Sharma, M., Sharma, R., Sharma, V., 2013. The impact of incinerators on human health and environment. *Rev. Environ. Health* 28 (1), 67–72. <https://doi.org/10.1515/reveh-2012-0035>
- Shishatskaya, E.I., 2008. Biomedical investigations of biodegradable PHAs. *Macromol. Symp.* 269 (1), 65–81. <https://doi.org/10.1002/masy.200850909>
- Shrestha, B., Le Brech, Y., Ghislain, T., Leclerc, S., Carré, V., Aubriet, F., Hoppe, S., Marchal, P., Pontvianne, S., Brosse, N., Dufour, A., 2017. A multitechnique characterization of lignin softening and pyrolysis. *ACS Sustain. Chem. Eng.* 5 (8), 6940–6949. <https://doi.org/10.1021/acssuschemeng.7b01130>
- Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage sludge. *Waste Manage.* 28 (2), 347–358. <https://doi.org/10.1016/j.wasman.2006.12.010>
- Sjöström, E., 1993. *Wood Chemistry: Fundamentals and Applications*. Academic Press. San Diego, CA, USA. 293 p. ISBN 978-0-08-092589-9. <https://doi.org/10.1016/C2009-0-03289-9>
- Strazzera, G., Battista, F., Tonanzi, B., Rossetti, S., Bolzonella, D., 2021. Optimization of short chain volatile fatty acids production from household food waste for biorefinery applications. *Environ. Technol. Innovation* 23, 101562. <https://doi.org/10.1016/j.eti.2021.101562>
- Sun, M., Liu, B., Yanagawa, K., Ha, N.T., Goel, R., Terashima, M., Yasui, H., 2020. Effects of low pH conditions on decay of methanogenic biomass. *Water Res.* 179, 115883. <https://doi.org/10.1016/j.watres.2020.115883>
- Suzuki, M., Tachibana, Y., Kasuya, K.I., 2021. Biodegradability of poly (3-hydroxyalkanoate) and poly (ϵ -caprolactone) via biological carbon cycles in marine environments. *Polym. J.* 53 (1), 47–66. <https://doi.org/10.1038/s41428-020-00396-5>
- Szacherska, K., Oleskowicz-Popiel, P., Ciesielski, S., Mozejko-Ciesielska, J., 2021. Volatile Fatty Acids as Carbon Sources for Polyhydroxyalkanoates Production. *Polymers* 13 (3), 321. <https://doi.org/10.3390/polym13030321>
- Toivakainen, S., Laukkanen, T., Dahl, O., 2013. Simultaneous precipitation of phosphorus in a kraft pulp mill wastewater treatment plant. *Water Sci. Technol.* 67 (2), 299–305. <https://doi.org/10.2166/wst.2012.522>
- Tyagi, V.K., Lo, S.L., 2013. Sludge: a waste or renewable source for energy and resources recovery?. *Renewable Sustainable Energy Rev.* 25, 708–728. <https://doi.org/10.1016/j.rser.2013.05.029>
- Uçaroğlu, S., Alkan, U., 2016. Composting of wastewater treatment sludge with different bulking agents. *J. Air Waste Manage. Assoc.* 66 (3), 288–295. <https://doi.org/10.1080/10962247.2015.1131205>
- Varghese, V.K., Poddar, B.J., Shah, M.P., Purohit, H.J., Khardenavis, A.A., 2022. A comprehensive review on current status and future perspectives of microbial volatile fatty acids production as platform chemicals. *Sci. Total Environ.* 815, 152500. <https://doi.org/10.1016/j.scitotenv.2021.152500>
- Verma, K., Sarkar, C., Saha, S., 2023. Biodegradable polymers for agriculture. In: *Biodegradable polymers and their emerging applications*. Springer Nature Singapore, Singapore, pp. 191–212. https://doi.org/10.1007/978-981-99-3307-5_9
- Wang, K., Yin, J., Shen, D.S., Li, N., 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect

- of pH. *Bioresour. Technol.* 161, 395–401.
<https://doi.org/10.1016/j.biortech.2014.03.088>
- Wang, Y.F., Sun, P.Z., Guo, H.X., Wang, D.B., Zhu, T.T., Liu, Y.W., 2022. Enhancing methane production from anaerobic digestion of waste activated sludge through a novel sodium percarbonate (SPC) pretreatment: reaction kinetics and mechanisms. *ACS ES&T Eng.* 2 (7), 1326–1340.
<https://doi.org/10.1021/acsestengg.1c00468>
- Yang, C., Zhang, W., Liu, R.H., Zhang, C., Gong, T., Li, Q., Wang, S.F., Song, C.J., 2013. Analysis of polyhydroxyalkanoate (PHA) synthase gene and PHA-producing bacteria in activated sludge that produces PHA containing 3-hydroxydodecanoate. *FEMS Microbiol. Lett.* 346 (1), 56–64.
<https://doi.org/10.1111/1574-6968.12201>
- Yang, X., Wen, L., Liu, X., Chen, S., Wang, Y.Y., Wan, C.L., 2015. Bio-augmentative volatile fatty acid production from waste activated sludge hydrolyzed at pH 12. *RSC Adv.* 5 (62), 50033–50039.
<https://doi.org/10.1039/C5RA04651C>
- Yen, H.W., Brune, D.E., 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresour. Technol.* 98 (1), 130–134.
<https://doi.org/10.1016/j.biortech.2005.11.010>
- Yin, J., Yu, X., Wang, K., Shen, D., 2016. Acidogenic fermentation of the main substrates of food waste to produce volatile fatty acids. *Int. J. Hydrogen Energy* 41 (46), 21713–21720.
<https://doi.org/10.1016/j.ijhydene.2016.07.094>
- Yu, H., Liu, Y.Y., Guo, N.N., Piao, W.L., Pan, Z.L., Zhu, B., Zhu, Y.M., Wu, L.B., Wan, J.L., Wei, H.Z., 2024. Recent Advances in Hydrothermal Oxidation Technology for Sludge Treatment. *Appl. Sci.* 14 (24), 11827.
<https://doi.org/10.3390/app142411827>
- Yuan, H.Y., Chen, Y.G., Zhang, H.X., Jiang, S., Zhou, Q., Gu, G.W., 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. *Environ. Sci. Technol.* 40 (6), 2025–2029.
<https://doi.org/10.1021/es052252b>
- Zacharof, M.-P., Lovitt, R.W., 2014. Recovery of volatile fatty acids (VFA) from complex waste effluents using membranes. *Water Sci. Technol.* 69 (3), 495–503. <https://doi.org/10.2166/wst.2013.717>
- Zehnder, A.J.B., 1988. *Biology of anaerobic microorganisms*. John Wiley and Sons Inc., New York.
- Zhang, B., Zhang, L.L., Zhang, S.C., Shi, H.Z., Cai, W.M., 2005. The influence of pH on hydrolysis and acidogenesis of kitchen wastes in two-phase anaerobic digestion. *Environ. Technol.* 26 (3), 329–340.
<https://doi.org/10.1080/09593332608618563>
- Zhang, M., Wu, H., Chen, H., 2014. Coupling of polyhydroxyalkanoate production with volatile fatty acid from food wastes and excess sludge. *Process Saf. Environ. Prot.* 92 (2), 171–178. <https://doi.org/10.1016/j.psep.2012.12.002>
- Zhang, Y.F., Zhang, S.Y., Li, H., Wang, C.W., Jiang, F.H., Lyu, J.F., 2020. Treatment of municipal sludge by hydrothermal oxidation process with H₂O₂. *Chemosphere* 257, 127140.
<https://doi.org/10.1016/j.chemosphere.2020.127140>
- Zhao, J.W., Yang, Q., Li, X.M., Wang, D.B., An, H.X., Xie, T., Xu, Q.X., Deng, Y.C., Zeng, G.M., 2015. Effect of initial pH on short chain fatty acid production during the anaerobic fermentation of membrane bioreactor sludge enhanced

by alkyl polyglcoside. Int. Biodeter. Biodegrad. 104, 283–289.
<http://dx.doi.org/10.1016/j.ibiod.2015.06.012>

