Tampereen yliopisto

Van Le

PRODUCTION OF BIOCHEMICALS FROM PULP AND PAPER INDUSTRY WASTE STREAMS

Master's thesis
Faculty of Engineering and Natural Sciences
Associate Professor Marika Kokko &
Associate Professor Ville Santala
May 2024

ABSTRACT

Van Le: Production of biochemicals from pulp and paper industry waste streams
Master's thesis
Tampere University
Master of Programme in Environmental engineering
May 2024

Every year there is about 16,300,000 m^3 spruce and 12,900,000 m^3 pine log removed for the production in Finnish paper and pulp industry. As a result, the industry has discharged approximately 28 billion tons of waste and sides streams, which could then be utilized as sustainable feedstocks for biochemicals production such as volatile fatty acids (VFAs). VFAs have from two to six carbon atoms as well as linear short-chain aliphatic mono-carboxylate compounds. Because of their functional groups, they have been utilized largely as important building block chemicals for the synthesis of different products such as polyhydroxyalkanoates (PHAs). The VFAs are produced from acid fermentation by which sugars in woody biomass is converted into metabolites for fermentative microorganisms. However, the woody biomass contains high amount of lignin and crystallised structure which make hydrolysis become a rate limiting step in the fermentation process, thereby reducing VFAs yield. Thus, pre-treatment is needed to cleavage the recalcitrant components of the biomass and increase soluble fractions for microorganisms to access.

The aim of this thesis was to investigate the feasibility of combining a hydrolysis method with an acid fermentation to produce VFAs and valorize VFAs rich stream for PHAs production from side and waste streams of Finnish pulp and paper industry. Bark and biosludge are the two feedstocks studied in the thesis. The study examined thermal pretreatment approaches for each feedstock and evaluated the obtained VFAs yields and PHAs yields from valorized VFAs rich stream.

The thesis found that hot water extraction at 75°C in 60 minutes could be the feasible pretreatment approach for barks and biosludge could be thermally pretreated at 165°C in 45 minutes. The acid fermentation of extracted bark could produce 0.179gAcetate/gVS_{added} which could be valorized to produce 0.118gP3HB/gVS_{added} and 0.110gPHBV/gVS_{added}. Meanwhile, the hydrolyzed biosludge could produce 0.435gAcetate/gVS_{added} and the VFAs can be used as substrates to produce 0.287 gP3HB/gVS_{added} and 0.266 gPHBV/gVS_{added}. It is not straightforward to compare the VFAs yields obtained from this thesis with other studies because of different feedstocks properties, inconsistent units and operational pretreatment conditions. Comparing the VFAs yields results with theoretical VFAs values for PHAs production, both barks and sludges show as promising feedstocks. However, compared to current industrial capacities of PHAs production, bark seems to be a more promising feedstock for large scale PHAs production. Because it is estimated that 321,970-ton discharged barks could produce 23,011 ton P3HB and 21,329 ton PHBV while 58,375 ton of discharged sludge could produce 2,409 ton P3HB and 2,233 ton PHBV annually.

The thesis existed some limitations mainly from secondary data and assumptions made for VFAs and PHAs yields calculation. The thesis suggested to have experiments in a near future utilizing real barks and biosludge which will be pretreated at the conditions examined in this study combined with acid fermentation to examine actual VFAs yields. Especially, the research for the hot water extraction of barks at higher temperature than 72°C should be done because a rough estimation from assumed methane yield showed that a higher VFAs yield (0.363 gAcetate/gVS_{added}) could be obtained from barks. Moreover, spruce barks and pine barks should be mixed rather than using spruce barks alone because spruce barks give much lower VFAs yields in the same hydrolysis condition. It was also predicted that a possible side stream in a form of liquid and solid mixture could be discharged from the acid fermentation process in VFAs production and aerobic process in PHAs production as these are the biological process. Thus, it is essential to investigate the composition and content of the side stream so that no potential resource is wasted.

Keywords: VFAs, PHAs, pulp and paper biosludge, bark, hot water extraction, thermal hydrolysis, acid fermentation.

ACKNOWLEDGEMENTS

This Master’s Thesis was done as part of isoSUS project. I feel honored to have the opportunity to work on this project plan and become knowledgeable on this novel topic.

I would like to show my sincerest thanks to my supervisors Associate Professor Marika Kokko and Associate Professor Ville Santala. I thank you for giving me the opportunity to contribute my humble knowledge on your project plan. Also, I thank you for your great guidance and valuable comments on my thesis report as well as other massive supports for my study.

I would like to thank my family and friends for sharing me joys in my ups and downs moments during my studies in Finland. Lastly, I want to thank myself for my perseverance, resilience, and determination to overcome the obstacles when I decided to school again and fill knowledge gaps from the years not in academia.

Tampere, May 2024

Le Thi Tuong Van

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

ABE	Acetone-butanol-ethanol
AD	Anaerobic Digestion
AET	Acetate-ethanol Type Fermentation
BTF	Butyrate-type Fermentation
COD	Chemical Oxigen Demand
DS	Dry Solids
HMF	Hydroxymethylfurfural
HRT	Hydraulic Retention Time
HWE	Hot Water Extraction
LTF	Lactate-type Fermentation
MAF	Mixed-acids Fermentation
MWs	Molecular Weights
OLRs	Organic Loading Rates
PHAs	Polyhydroxyalkanoates
PPI	Pulp and Paper Industry
PTF	Propionate-type Fermentation
sCOD	Soluble COD
TVS	Total Volatile Solids
VFAs	Volatile Fatty Acids
VS	Volatile Solids

.

1. INTRODUCTION

Bioeconomy is one of the key sectors in Finland, which contributed 13% of the value added generated for the country economy in 2019. According to Finnish Bioeconomy Strategy 2022 – 2035, it is targeted that the value added of bioeconomy will be doubled with the use of new bio-based products in the textile industry, pharmaceutical industry and even as battery materials. Furthermore, these products are produced by recycling materials and utilizing side streams as renewable raw materials. (Finnish government, 2022)

Volatile fatty acids (VFAs) are used as a precursor for the production of biochemicals production such as hydrogen, methane, ethanol, alkanes and polyhydroxyalkanoates (PHAs) (Singhania et al., 2013; Blasco et al., 2020). Thus, the production of VFAs has become a pressing topic due to increasing market demand and its wide range application in various industries (Blasco et al., 2020; Doddapaneni et al., 2022).

Traditionally, VFAs are produced by chemical routes, extracting a large amount of non-renewable fossil fuels as the raw material (Blasco et al., 2020; Castilla-Archilla et al.,2021). However, VFAs could be obtained from acid fermentation by which sugars in biomass is converted into metabolites for fermentative microorganisms (Llamas et al., 2020; Castilla-Archilla et al.,2021).

The consistent composition and availability of the feedstock are the two important factors contributing the feasibility of a bioprocess in terms of the yield, stability and the cost-effectiveness (Castilla-Archilla et al.,2021). Among many biomasses, wood-based biomass has been recently considered as the major contributor in many biorefinery concepts and as the key factor of the transition to the circular economy (Wenger et al., 2022; Sopelana et al., 2023) because of its low cost and abundance (Llamas et al., 2020). It is believed that the biomass can support the reduction of production costs (Wainaina et al., 2019). The side and waste streams of pulp and paper industry (PPI) in Finland is one potential source for VFAs production as the industry has discharged approximately 28 billion tons of waste and sides streams, which could then be utilized as sustainable materials in the biorefinery (Hassan et al., 2019).

However, woody biomass contains high amount of lignin and crystallised structure which hinders the efficiency of feedstock degradation. Thus, biomass pre-treatment is required to cleavage the main components of the lignocellulose and facilitate depolymerization to soluble fractions, making the biomass become more accessible for microorganisms in biological process, hereby leading to the improvement of the product yields (Zhao et al., 2022; Negi and Kesari, 2023).

Literature review shows that little attention has been devoted to the VFAs production from woody biomass. Thus, the alternative of producing VFAs via acid fermentation from Finnish PPI's waste and side streams is of special interest to this study. To enable the utilization of woody biomass from Finnish pulp and paper industry for VFAs production, it is essential to evaluate different pre-treatment methods on waste stream and side streams of the industry and the effects of the pretreated biomass on fermentation performance, VFAs and other possible biochemicals' production.

This thesis aims to examine unit processes for VFAs production and VFAs rich stream valorization by combining a hydrolysis method and acid fermentation for selected streams from the Finnish PPI and evaluate the feasibility of these combinations. To obtain these aims, this thesis deals with these main research questions:

1. Which organic waste and side streams are most feasible from the PPI in Finland for VFAs production and valorization?
2. Which thermal pretreatment method including its optimal conditions, treatment efficiency can be used to hydrolyze the identified side streams?
3. How hydrolyzed streams affect VFAs and PHAs yields?

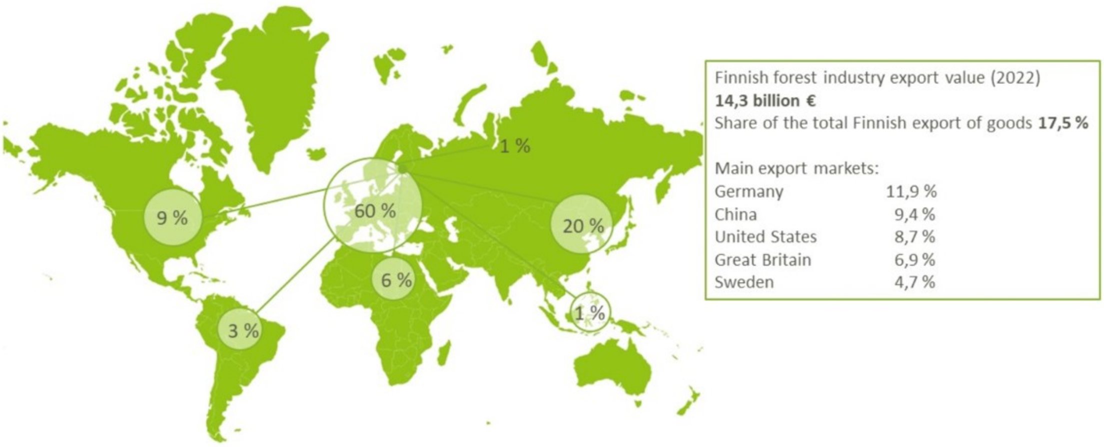
The thesis consists of seven chapters, starting with the potentials of VFAs platform application along with the potential of using waste and side streams from the pulp and paper industry in Finland, thereby leading to the rationale of the study. Then, chapter 2 gives an overview on the Finnish industry status, the waste and side streams of PPI, and its characteristics. Next, chapter 3 focuses on the hydrolysis of organic side streams from PPI by bringing up thermal pre-treatment methods. Chapter 4 presents the biological process for VFAs production and the valorization of VFAs rich stream for PHAs production. After the theoretical parts, the methodology chapter is dedicated to describe the initial data and the calculation approach for estimating of VFAs yields and PHAs yields. Chapter 6 is followed to present and discuss the obtained results. Finally, some recommendations for future research are concluded.

2. FINNISH PULP AND PAPER INDUSTRY AND ITS SIDE STREAMS CHARACTERISTICS

This chapter is divided into three sections. A brief overview of the Finnish pulp and paper industry status is described in the first section. The second section is dedicated to give details the types of waste and side streams produced from the processing of raw woody materials. The final section discusses the characteristics of the streams.

2.1 The development of pulp and paper industry in Finland

Europe is the second-largest producer of pulp and paper in the world, contributing approximately 25% of the production (Sopelana et al., 2023) and Finland is one of the largest pulp producers in Europe (Lipiäinen et al., 2022). Finnish land area is covered with 74% forests which leads the country with significant forest industry, contributing the economic development of the country. Together with Sweden, Finland forests have contributed about 50% of the virgin pulp production in Europe. The industry is export oriented with 75% of forest products exported in Finland. (Lipiäinen et al., 2022)



Finnish forest industry export value (2022)

14,3 billion €

Share of the total Finnish export of goods **17,5 %**

Main export markets:

Germany	11,9 %
China	9,4 %
United States	8,7 %
Great Britain	6,9 %
Sweden	4,7 %

Figure 1. *Finnish forest industry products markets in 2022 (Metsäteollisuus, 2023)*

It is known that the growing stock has risen over the last five decades in Finnish forests, making up the forest stock to 2.6 billion m^3 (Metsäteollisuus, 2023), in which the annual growth and harvesting from Finnish forests are 104 million m^3 and 79 million m^3, respectively (Hassan et al., 2019; Metsäteollisuus, 2022)(shown in Figure 2). Consequently, the forest growth exceeds the fellings.

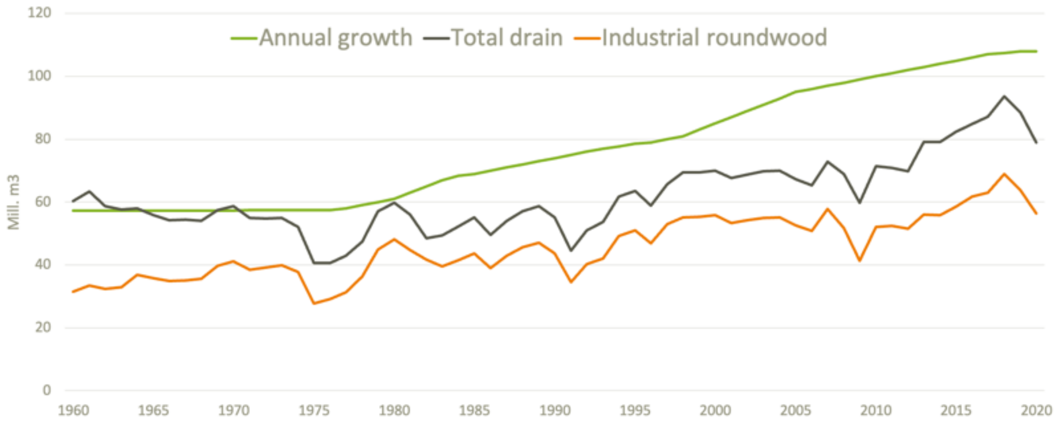


Figure 2. *Finnish forests growth over time (Metsäteollisuus, 2022)*

Spruce, pine, downy birch and silver birch are the most popular wood species in Finnish forests, originating from Finland (Metsäteollisuus, 2023). Hassan et al. (2019) indicated that 45.4% pine, 38.1% spruce, and 16.5% hardwood was processed at Finnish mills in 2013.

Virgin fibers are mainly produced from chemical and mechanical pulping processes in the Finnish PPI with high volumes as compared to recycled fibers with 5%–10%. According to the statistic in 2018, the pulp and paper industry in Finland produced 11.7 Mt of pulp. Finland is also a large manufacturer of printing and writing papers, producing the total volumes of paper was 10.5Mt in 2018. (Lipiäinen et al., 2022) So it was estimated that 16,300,000 m^3 spruce and 12,900,000 m^3 pine log are removed annually for pulp and paper processing (Luke,2022).

2.2 The waste and side streams of pulp and paper industry

Sawmills, plywood mills, paper and board mills, and pulping mills are the operating mills in Finnish PPI. Each operation generates a huge amount of side streams and waste streams at stages of the manufacturing process with various types of the streams including bark, bark chips, sawdust, wood chips, sanding dust, fiber sludge and bio-sludge. (Hassan et al., 2019)

In details, the process at sawmills and plywood mills are wood processing. Therefore, these mills generate a significant number of woodchips, sawdust and bark, namely solid wood-based side streams. These streams are mostly deployed as inputs for the production of pulp, and the generation of heat and electricity. Meanwhile, fiber sludge and primary sludge are the main side streams of paper and board mills. To differentiate between fiber sludge and primary sludge, woods is supplied to pulping process and the pulping process produces fiber sludge, whereas primary sludge comes from wastewater treatment process. (Hassan et al., 2019)

The production of pulp from chemical pulping process generates organic and inorganic components. The organic components are primary sludge from wastewater treatment, secondary sludge or biosludge from secondary wastewater treatment stage, while inorganic components are tall oil and turpentine, black liquor, green liquor, lime mud and ash from kraft pulping process, cooking of wood materials, causticizing and reburning processes, and fluidized bed boilers. It is indicated that tall oil, turpentine, and black liquor are not true waste stream products. This is because that black liquor is combusted for the own electricity use in the mill's production. After the black liquor is combusted in a recovery boiler, the green liquor is obtained as concentrated lignin which is used for causticizing and cooking chemicals. Generally, sludges contribute a significant volume of the side stream generated from pulp and paper mills, which could be served as a feedstock in biorefinery process. (Hassan et al., 2019)

Hassan et al. (2019) collected the data of wood log input and side streams discharged which are expressed in either mass or volume depending on surveyed mills. In addition, the survey also estimated in proportion of side streams out of wood log input as well as finished products. However, only proportion of each type of side stream in mills expressed as the percentage of wood log input are collected in Table 1.

Table 1. *The proportion of each type of side stream from different mills (collected from Hassan et al., 2019).*

Type of side stream	**Proportion (% wood log input)**
Sawmills	
Wood chips	38.3
Sawdust	
Bark	
Plywood mills	
Wood chips	45.2
Sawdust and bark	10.5
Paper and board mills	
Sludge	13.8

Bark	4.6
Ash	4.2
Kraft chemical pulping mills	
Bark	5.6
Biosludge	2
Black liquor	43
Green liquor dregs	1.4

2.3 Characteristics of waste streams of pulp and paper industry

In general, the main features of most of the side streams are related to woody characteristics (Haile et al., 2021). Woody materials are classified as lignocellulosic biomass (Durak, 2023). Hardwood and softwood are the main species in wood. Hardwood species are eucalyptus, poplar and birch while softwood species include pine, spruce and fir. Each type of wood has its own unique characteristics. (Li et al., 2023) In general, lignocelluloses contain organic polysaccharides including cellulose and hemicelluloses, lignin, and other elements such pectin and ash (Li et al., 2023; Alves et al., 2023; Negi and Kesari, 2023) The proportions of polysaccharides and other elements in lignocellulosic biomass are presented in Figure 3 (Negi and Kesari, 2023).

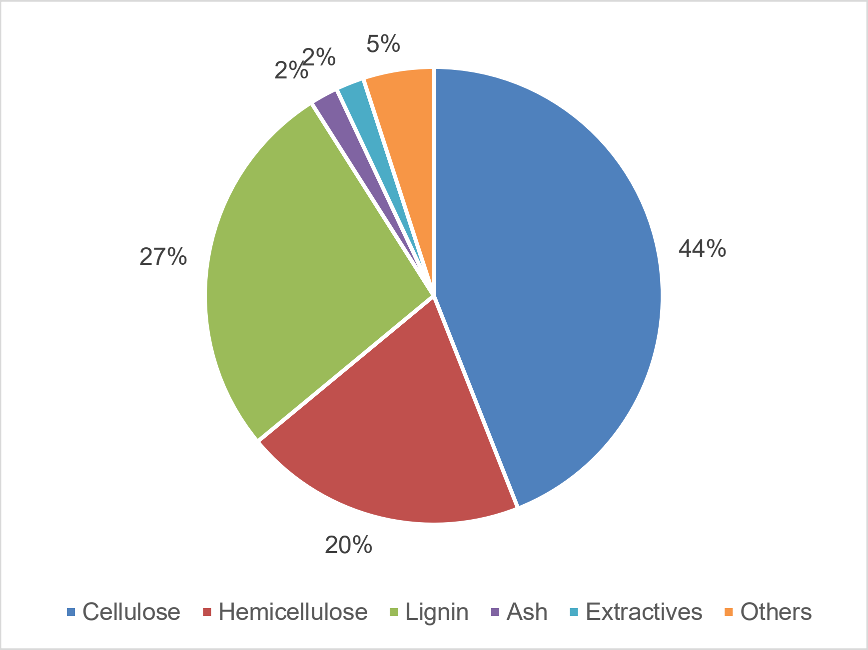


Figure 3. *The proportion of polysaccharides and other elements in lignocellulosic biomass (modified from Negi and Kesari, 2023)*

Sawdust and bark are composed of cellulose and hemicelluloses (Tripathi et al., 2020; Haile et al., 2021). In fact, cellulose and hemicellulose are the most abundant substances of lignocellulosic biomass, in which, cellulose is the most abundant polysaccharide element, comprising two D-glucose molecules linked by β-1,4-glycosidic bonds. In addition, the structure of cellulose can be amorphous and crystalline. (Negi and Kesari, 2023; Li et al., 2023) The crystal structure impedes the penetration of mild chemical acid in biomass treatment (Negi and Kesari, 2023). This crystal structure is caused by the formation of intra and inter molecular hydrogen bonding between hydroxyl groups (Millati et al.,2020; Negi and Kesari, 2023). Consequently, the bonding creates the insolubility and lesser miscibility characteristics of cellulose with organic solvents and water (Negi and Kesari, 2023).

Hemicellulose, on the other hand, is the second most common polysaccharide in lignocellulosic biomass. The hemicellulose consists of arabinose, mannose, xylose (Li et al., 2023, Alves et al., 2023), glucose, and galactose (Li et al., 2023) which are monosaccharides linked together by covalent bonds, hydrogen bonds, and lipid bonds. Furthermore, hemicellulose is linked with cellulose and lignin by covalent and non-covalent bonds, making inner matrix of the lignocellulosic feedstocks (Millati et al.,2020). The hemicelluloses of softwood and hardwood are distinct from each other (Yedro et al.,2015; Andérez Fernández et al.,2018; Tarabanko et al.,2020). Xylose is the main compound in hardwood's hemicellulose, accounting for 20% to 37% while mannose and glucomannan constitutes 16–27% of hemicellulose in softwood (Li et al., 2023).

Beside monosaccharides, hemicelluloses also contain uronic acids, acetyl group, oligosaccharides, and polysaccharides (Wan Azelee et al.,2023), in which acetyl group is found with higher amount in hardwood than softwood (Yerdo et al.2015). In the hydrolysis of hemicellulose, acetic acid is produced from the acetyl group which play as a catalyst for speeding up the fractionation of carbohydrates (Yerdo et al.,2015).

Table 2. *The distribution of cellulose, hemicellulose and lignin in hardwoods and softwoods (modified from Negi and Kesari, 2023)*

Lignocellulosic biomass	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**
Hardwoods stem	40 – 55	24 – 40	18 – 25
Softwood stem	45 – 50	25 – 35	25 – 35

After cellulose and hemicellulose, lignin is the most abundant non-polysaccharide in lignocellulosic feedstocks (Kumar et al.,2020). Lignin is an aromatic and very rigid polymer, which has cross-branched, three-dimensional polyphenylene propane complex. A matrix is formed when lignin combines with hemicellulose, making it hard to degradation. (Li et al., 2023; Alves et al., 2023) For this reason, lignin is the most recalcitrant compound in lignocellulosic biomass (Millati et al.,2020). Thus, very high temperature and acidic solvents are required to dissolve the lignin (Millati et al.,2020). Especially, for softwood, because softwood has the highest content of lignin among lignocellulose (Yedro et al.,2015; Andérez Fernández et al.,2018), more servere conditions must be applied for fractionating this type of wood.

Pectin and ash are other elements in lignocellulosic biomass. Pectin is polygalaturonic acid polysaccharide connected by α-1,4 bonds and mainly distributed in the first and middle layers of plant cell walls. The chemical resists well with heat and dissolves in water but less likely solubilizes in organic agents. Nevertheless, ash is the inorganic matter remaining after combustion and chemical treatment. (Li et al., 2023) Bark has higher amount of extractives, lower amount of polyphenols, suberin and polysaccharides (Tripathi et al., 2020) and some other compounds such as tannins, stilbene glucosides (Rasi et al., 2019).

A high amount of hemicellulose in biomass is advantageous for biorefinery process (Alves et al., 2023), containing valuable precursors such as C5 and C6 compounds which can be used to develop sustainable chemical processes (Alves et al., 2023; Negi and Kesari, 2023). However, because the structure of lignocellulose has high level of crystallinity, bonds networking, and mild solubility in organic and water, it is essential to depolymerize cellulose and hemicellulose before further processes. (Negi and Kesari, 2023)

Volatile matter is another element worth noticing in the biomass characteristics. It is found that the volatile matters value of lignocellulose biomass ranges from 74% to 94%. First, the factor of volatile matters provides information to assess the combustion ability of biomass in thermochemical process. It is more likely that biomass with higher volatile matter ignites more easily and then burn more rapidly. (Alves et al., 2023) Secondly volatile matters represent organic materials presenting in biomass (Alves et al., 2023), which significantly contributes the success of a biological process. This is because microorganisms can only be able to consume organic pollutants and convert it into specific products such as methane, VFAs (Iglesias-Iglesias et al.,2019; Goycoechea et al., 2023).

Besides volatile matter, it is needed to understand the moisture content in biomass. The moisture content will divide the biomass into wet and dry biomass. (Alves et al., 2023) For example, some lignocellulose such as sludge from pulping process has high water content known as wet biomass (Doddapaneni et al., 2022; Alves et al., 2023). The presence of high amount of water affects the calorific value of the biomass, thereby influencing the energetic use. Hence, some thermal treatment methods such as combustion, pyrolysis and gasification are not preferred. (Englatina et al., 2021; Doddapaneni et al., 2022; Alves et al., 2023) Some values regarding pH, dry solids (DS) content, volatile solids (VS), cellulose, hemicellulose and lignin in pulp and paper sludge are collected in Table 3 (Kaur et al., 2020).

Table 3. *Characteristics of pulp and paper sludge (modified from Kaur et al., 2020)*

	Primary sludge	**Secondary sludge**
pH	5.5 – 8.2	6.5 – 7.6
Dry solid content (%w/w)	39.7 – 48	1.0 – 32
VS (%w/w DS)	33 – 40	65 – 97
Cellulose (%w/w)	23.6 – 60.8	2.0 – 28
Hemicellulose (%w/w)	4.9 – 14.2	12.0 – 15.0
Lignin (%w/w)	2.2 – 20.92	16.85 – 58.0

DS = dry solids

The statistics from Hassan et al.(2019) regarding the amount of waste streams in Finnish pulp and paper industry indicated approximately 3,897,000 ton all types of sludges generated from the wastewater treatment of the pulping process. There are three types of sludge generated in a pulp mill including primary, secondary (Trumic et al., 2018; Kaur et al., 2020; Doddapaneni et al., 2022) and tertiary sludge (Saari et al., 2022). Primary sludge is produced after primary settling, whereas secondary settling produces secondary sludge (Trumic et al., 2018; Kaur et al., 2020). The tertiary sludge which is discharged from physicochemical coagulation-flocculation process, requires high treatment and disposal costs. In addition, the tertiary treatment consumes a lot of energy and chemicals. Because of these reasons, the tertiary process is not commonly applied in many pulp and paper mills. (Saari et al., 2022) Thus, in general, primary and secondary are the sludges commonly mentioned in pulp and paper making process. These two types of sludges have different proportions of components. As shown in Table 3, overall, the sludges contain high content of organic matters in forms of cellulose, hemicellulose and lignin, in which primary sludge has higher content of cellulose than secondary sludge (Trumic et al., 2018; Kaur et al., 2020). Cellulose in sludges is the product of degradation process of polysaccharides in woods. But parts of cellulose is then removed at secondary wastewater treatment, resulting in its low amount in secondary sludge. Meanwhile, the sludges consist of high level of lignin due to its rigid structure, so it is not removed after the biological treatment. (Kaur et al.,2020) In addition, secondary sludge is structured by intra-cellular water and extra-cellular polymeric substances, retaining certain amount of water even after dewatering process (Saari et al., 2022). pH of secondary sludge remains in a neutral range while that of primary sludge varies from acidic to alkaline values because chemical treatment is applied to the primary treatment stage (Kaur et al.,2020). Besides, wood chips, carbonate, ink, calcium, clay, sand, metals, and adhesives components are other elements existing in both sludge types (Haile et al., 2021; Saari et al., 2022; Doddapaneni et al., 2022).

3. THERMAL PRE-TREATMENT METHODS FOR WASTE STREAMS HYDROLYSIS

The recalcitrant structure of the streams from pulp and paper industry requires pretreatment which is the essential step to facilitate the accessibility for the microorganisms to the feedstocks in biological processes. There are some widely studied pretreatment methods for sawdust, bark and biosludge in the industry, which this chapter focuses in detail in terms of its principles, advantages, and disadvantages. The main goals of these pretreatment approaches are to reduce the lignin and hemicellulosic portion from the biomass and to improve the availability of cellulose (Kumar et al., 2020; Millati et al.,2020; Zhao et al., 2022), thereby enhancing sugar yield and minimizing the formation of inhibitory products (Modenbach et al.,2012; Millati et al.,2020; Wan Azelee et al.,2023).

3.1 Thermal hydrolysis

3.1.1 The principle of the method

Thermal hydrolysis pretreatment belongs to physical pretreatment method (Zhao et al.,2022). As mentioned above, among the waste and side streams in pulp and paper industry, this method has been used mostly for sludge. Also, it is proved that the method is commonly used and shows its effectiveness in improving the disintegration and biodegradability of sludge and biogas production in sewage sludge treatment (Choi et al.,2018, Goycoechea et al.,2023). Hence, this section focuses on the principle of the method on sludge treatment.

Physical and chemical effects are the two main effects involved in the method’s working principle (Goycoechea et al.,2023), where sludge is exposed to high temperature, inducing cell lysis and then rupturing intracellular and extracellular polymeric substances from the cell (Khanh Nguyen et al., 2021; Gahlot et al.,2022). The objectives of the method are to disintegrate cell membranes (Khanh Nguyen et al., 2021) and increase the accessible surface area of cellulose (Eskicioglu et al., 2017; Veluchamy et al., 2017), from which recalcitrant organic matters are transformed into soluble forms for microorganisms digestion (Khanh Nguyen et al., 2021; Gahlot et al.,2022).

Temperature, exposure time (Veluchamy et al., 2017; Choi et al.,2018; Fang et al.,2020; Goycoechea et al.,2023), moisture content and size of the particle (Veluchamy et al., 2017) are the main important factors controlling the performance of the method. However, it is indicated that temperature is the most influencing parameter for this method

(Bougrier et al.,2007). Low-temperature and high-temperature are more frequently employed in the thermal hydrolysis, in which low-temperature pretreatment utilizes the temperatures below 100°C while high-temperature employs the temperatures above 100°C (Khanh Nguyen et al., 2021; Gahlot et al.,2022). About the waste type, wet organic waste and high content of lignocellulosic waste which are difficult to microbiologically hydrolyze into simpler forms are applicable in thermal hydrolysis (Gahlot et al.,2022).

For the thermal hydrolysis of pulp mill secondary sludge or biosludge, most studies were carried out at the temperature range from 80°C to 175°C in 10 minutes to three hours to evaluate methane yield (Veluchamy et al.,2017; Fioreze et al.,2022). The results show there is enhancement of methane production from pretreated biosludge. Kinnunen et al. (2015) stated that the temperature above 150°C is required to improve the hydrolysis of hemicellulose for pulp and paper industry biosludge which corresponds to the optimal temperature of 165°C implemented in commercial technologies for sewage biosluge (Goycoechea et al.,2023). But according to sewage sludge pretreatment, Bougrier et al. (2007) argued that a severe temperature condition from 170°C to 200°C in thermal hydrolysis can solubilize more organic matters in sewage sludge. Consequently, biogas production was observed to increase by about 40% to 100% when the pretreatment was conducted from 160°C to 180°C (Bougrier et al.,2008). But it is also said that the extreme condition will not necessarily increase methane production from pretreated stream (Bougrier et al.,2008; Goycoechea et al.,2023), especially above 190°C (Bougrier et al.,2008) because of the formation of refractory compounds from the Maillard reaction between carbohydrates and amino acids (Bougrier et al.,2007; Sayara et al.,2019).

3.1.2 Advantages and disadvantages of thermal hydrolysis

The utilization of thermal hydrolysis for sludge pretreatment has increased solubilization of the sludge and thereby enhanced the products yield in anaerobic process. Furthermore, there are other numerous benefits which can be obtained from the method. First, sludge dewaterability is enhanced and the odor is removed. Second, the approach improves substrate biodegradability, so increasing organic loading rates (OLRs) (Gahlot et al.,2022) and reducing significantly the volume of an anaerobic reactor due to the need of shorter digestion time (Ngo et al.,2021; Gahlot et al.,2022). In addition, the method is also non-toxic and environment friendly (Zhao et al., 2022).

Besides some advantages, the method has some major drawbacks such as high capital costs, energy intensiveness, and specialized reactor requirement (Gahlot et al.,2022). Another drawback is the formation of toxic compounds at higher temperatures (Zhao et al.,2022). In details, lignin starts to be solubilized at 160°C (Kinnunen et al.,2015) or

180°C (Sayara et al.,2019; Millati et al.,2020), causing the formation of phenolic compounds (Teghammar et al., 2010; Kinnunen et al.,2015). Furthermore, other inhibitors such as furfural and hydroxymethylfurfural (HMF) are also produced during the degradation of hemicellulose and lignin when the temperature exceeds 160°C (Teghammar et al., 2010; Sayara et al.,2019; Zhao et al.,2022) because the high temperature can degrade some sugars and produce these inhibitors (Andérez Fernández et al.,2018). These inhibitory compounds will inhibit the growth of anaerobic microorganisms (Sayara et al.,2019).

3.2 Steam explosion

3.2.1 The principle of the method

Steam explosion is one of the hydrothermal pretreatment approaches in which water in vapor phase is used to pretreat biomass (Teghammar et al., 2010). The steam explosion is the efficient pretreatment for pulp and paper industry waste streams (Vivekanand et al., 2013). In this process, biomass is placed under the temperature range from 150°C to 260°C with the pressure of 0.5 to 5 MPa (Modenbach et al.,2012). The process can take place in a wide range of time from several seconds to a few minutes (Modenbach et al.,2012; Cantero et al.,2019). The method does not utilize chemicals (Jonsson et al., 2016) but steam as reagent in its process (Modenbach et al.,2012).

There are two steps involved in this process consisting of auto-hydrolysis step and explosion step (Baig et al., 2019; Zhao et al.,2022). In the auto-hydrolysis step, cellulose from woody biomass is separated via breaking the β-O-4 bond of lignin (Guigou et al.,2019; Zhao et al.,2022) as well as hemicellulose is partially hydrolyzed (Baig et al., 2019). Acetic acid is also formed from the hemicellulose's O-acetyl groups (Guigou et al.,2019; Cantero et al.,2019). The acetic acid plays as catalyst to further fractionation of the soluble hemicellulose oligomers (Baig et al., 2019; Cantero et al.,2019). This step can depolymerize lignin partially to lower molecular weight units (Guigou et al.,2019; Baig et al., 2019) or transform lignin structure (Cantero et al.,2019). In the explosion step, when the steam is released under the rapid pressure drop to atmosphere level, the biomass particles are destroyed into smaller pieces, resulting in its morphological and chemical changes (Vivekanand et al., 2013; Baig et al., 2019; Millati et al.,2020).

Temperature and pressure are the two key parameters in steam explosion process (Modenbach et al.,2012). However, the structural and chemical properties of the biomass significantly affect the performance of pretreatment. Specifically, as discussed in chapter 2, hardwood and softwood have different compounds in its hemicellulose as well as the levels of lignin content. Because of this reason, the lower temperature is applied for hardwood (Yedro et al.,2015).

3.2.2 Advantages and disadvantages of steam explosion

Steam explosion pretreatment is advantageous due to the fact that it leads to the reduction in biomass size but increase its porous size, allowing for better accessibility of the carbohydrates for microorganisms in bioprocess. Moreover, the process also aids to separate biomass into fibers, minimizing the biomass loss. (Modenbach et al.,2012; Baig et al., 2019) Some studies deployed steam explosion to investigate ethanol production. For example, Eucalyptus grandis sawdust was pretreated in steam explosion 200°C for 10 min, producing 75.6g ethanol/L by simultaneous saccharification and fermentation (Rochón et al.,2020). In other study, Asada et al. (2019) implemented steam explosion for mixed softwood sawdust pretreatment at the pressure of 45 atm in 5 minutes and 66.7 g ethanol/L was obtained by the simultaneous saccharification and fermentation of the pretreated stream.

Cambi is the commercial technology which has been successfully installed worldwide over 20 years in sludge pretreatment using steam explosion. The technology has applied the temperature from 160°C to 180°c under the pressure of 6 bars with the reaction time up to 30 minutes to hydrolyze organic matters in sludge. The hydrolyzed sludge then undergoes sudden depressurization to atmospheric pressure to rupture cell walls of the biomass. The practice proves that the technology has enhanced biogas production by 150% through solubilizing more organic matters and converting up to 70% of these organic matters into biogas. (Gahlot et al.,2022)

The steam explosion pretreatment utilized steam without catalyst addition, thus there is no chemical cost required as well as no environmental pollution. So the method is also environmental-friendly (Guigou et al.,2019; Zhao et al., 2022). In addition, because the chemical is eliminated, reactors do not need to use corrosion resistant materials (Eskicioglu et al., 2017; Andérez Fernández et al.,2018). However, this pretreatment approach applies high temperature and pressure to fractionate woody biomass, so the energy consumption is intensive (Modenbach et al.,2012; Sayara et al.,2019; Zhao et al.,2022). Therefore, there is some arguments about the necessity of explosion step because the results from some experiments with and without explosion step showed that there is no significant effect in pretreatment efficiency between these steps (Baig et al., 2019). Furthermore, it is suggested that high solids waste is more suitable for this pretreatment method because less volume of water is needed for the process (Modenbach et al.,2012).

Another point worth mentioning is that the steam explosion pretreatment is more effective for hardwood than softwood (Vivekanand et al., 2013; Zhou et al., 2022). This is because of the properties of softwood which contains less amount of acetyl groups in its hemicellulose structure (Jonsson et al., 2016; Zhou et al., 2022). As a result, this method is more cost-effective for hardwood stream (Zhou et al., 2022).

Regarding potential inhibitors, because this process conducts under high temperature, so similar to the thermal hydrolysis under severe pretreatment conditions, the formation of furfural, HMF or phenol derivatives can occur (Teghammar et al., 2010; Yedro et al., 2015; Sayara et al.,2019; Millati et al.,2020) from the dehydration of D-glucose (Ahmed et al.,2019) with high toxicity (Wan Azelee et al., 2023). The steam explosion can solubilize the lignin of biomass in the hydrolysis step but soluble lignin can be recondensed and precipitated on pretreated surfaces of the biomass, hindering the biomass' degradability and reducing methane yield (Hendriks et al.,2009; Baig et al., 2019).

3.3 Hot water extraction

3.3.1 The principles of hot water extraction

Hot water extraction (HWE) is another hydrothermal pretreatment method (Ghimire et al., 2021; Leszczyński et al., 2023). Generally, the principle of the hydrothermal pretreatment can be applied to the hot water extraction method. However, unlike steam explosion, HWE is carried out in aqueous environment at mild temperatures varying from 140°C to 240°C, and the sufficient high pressure is used at which water is kept in liquid state (Gallina et al., 2018; Cantero et al.,2019; Ghimire et al., 2021). This condition can make the water molecules become good solvent to catalyze the removal of hemicellulose from lignocellulose and making cellulose more accessible for microorganisms (Gallina et al., 2018). Hemicellulose and low molecular weight lignin are the two main products obtained after the process (Wan Azelee et al., 2023) but only up to 12% of the amount of lignin in feedstocks is extracted (Eskicioglu et al., 2017).

Temperature and time are the extraction conditions to control the further autohydrolysis of hemicellulose into oligomers and monomers (Gallina et al., 2018; Wolf et al.,2022). Even though the temperature of hot water extraction is theoretically defined as mentioned above, some studies conducted this approach under the temperature 95°C to 155°C (Ruiz et al.,2020; Wolf et al.,2022) or 75°C (Rasi et al.,2019). The extraction time is mostly studied in 20 minutes to 120 minutes (Wolf et al.,2022; Cantero et al.,2019). Rasi et al.(2019) used the hot water extraction method to extract spruce bark and pine bark at 75°C in 60 minutes and the results showed that the biomethane potentials from extracted barks were increased to 99 $mLCH_4/gVS_{added}$ for pine bark and to 55

$mLCH_4/gVS_{added}$ for spruce bark while the methane yields from unpretreated pine bark and spruce bark are 53 $mLCH_4/gVS_{added}$ and 46 $mLCH_4/gVS_{added}$, respectively.

3.3.2 The advantages and disadvantages of hot water extraction

As hot water extraction method is a hydrothermal pretreatment, chemicals are not added in the process. So the method is considered as an environmental-friendly (Ghimire et al., 2021; Wolf et al.,2022; Wan Azelee et al., 2023). Furthermore, the hot water extraction can reduce operation cost (Andérez Fernández et al., 2018; Wan Azelee et al., 2023) when water as the extraction solvent can be recovered for further use (Wan Azelee et al., 2023) and no biomass drying is needed (Andérez Fernández et al., 2018).

Hot water extraction improves the biodegradability of lignocellulosic biomass (Rasi et al.,2019). Besides, the approach also promotes the separation of high-grade hemicelluloses which could be further extracted and converted into high valued bioproducts such as such as antioxidant phenols, stilbenes, flavonoids and terpenes, bringing another economic profitability through the application of these products in in pharmaceutical, nutraceutical and cosmetic industries (Ruiz et al.,2020; Wolf et al.,2022). It is also stated that inhibitory products are generated with relatively low content during the process (Sayara et al.,2019; Leszczyński et al., 2023; Mohanakrishna et al.,2023) because of high water input (Sayara et al.,2019). However, like the steam explosion method, hot water extraction has a drawback in terms of high energy consumption because energy is required for high temperatures and pressures (Wan Azelee et al., 2023).

4. BIOLOGICAL PROCESS FOR VFAS PRODUCTION AND VALORIZATION

Acid fermentation is a stage of anaerobic digestion process. Thus chapter 4 firstly delivers a brief overview of the process of anaerobic digestion from which how VFAs is produced from the acid fermentation stage is discussed. In detail, VFAs compositions and yields are affected by metabolic pathways and operational parameters. Thus the metabolic pathways and these parameters are also clarified. Some studies related to VFAs production are taken as examples to see the potential of VFAs production from different feedstocks. In addition, because this thesis also deals with the evaluation of PHAs yields valorized from VFAs rich stream, a short discussion regarding VFAs valorization process into PHAs is given as well.

4.1 Overview of an acid fermentation-related process and metabolic pathways of acid fermentation

4.1.1 Anaerobic digestion

Anaerobic digestion (AD) is a well-established method to valorize different types of waste materials to recover resources (Llamas et al., 2020; Castilla-Archilla et al., 2021). In the AD process, the organic compound is degraded by mixed culture of bacteria and archaea under the absence of oxygen (Wainaina et al., 2019). The aim of the AD process is to convert carbohydrates, proteins and lipids in feedstock into value-added products such as biogas, VFAs or hydrogen (Wainaina et al., 2019; Llamas et al., 2020) while producing a small amount of biomass (Singhania et al., 2013). In general, methane and carbon dioxide are the two main products of the AD process (Singhania et al., 2013; Wainaina et al., 2019).

There are four steps including hydrolysis, acidogenesis, acetogenesis and methanogenesis involved in the process (Zhou et al., 2018; Castilla-Archilla et al., 2021). A variety of microbes works at different stages of the process including hydrolytic, acidogenic, hydrogen-producing, acetate-forming microbes as well as methanogens and these microbes act in a syntrophic manner to produce mentioned-above products (Wainaina et al., 2019).

The process firstly starts with hydrolysis which breaks down large polymers into monomers by enzymes from the hydrolytic microorganisms, producing fermented sugars, amino acids and lipids (Wainaina et al., 2019; Mohanakrishna et al.,2023). Next, acidogenic fermentations produce VFAs (Singhania et al., 2013; Wainaina et al., 2019), hydrogen (Wainaina et al., 2019) and CO_2 (Zhou et al., 2018) by acidogens by converting the fermented sugars and amino acids into pyruvic acid through different metabolic pathways such as the Embden-Meyerhof pathway, and Pentose Phosphate pathway, Bifidus pathway (Wainaina et al., 2019). Volatile acids are broken into acetic acid and hydrogen in the acetogenesis step (Singhania et al., 2013; Wainaina et al., 2019). Finally, methanogens involved in the methanogenesis step of the AD process will convert acetate, formaldehyde, hydrogen and carbon dioxide into methane (Singhania et al., 2013; Wainaina et al., 2019) and carbon dioxide (Wainaina et al., 2019). Many authors indicated that more than 65% and up to 90% of methane is produced from acetate (Mountfort and Asher, 1978; Weber et al.,1984; Li et al.,2020). The four steps of the AD process are presented in Figure 4.

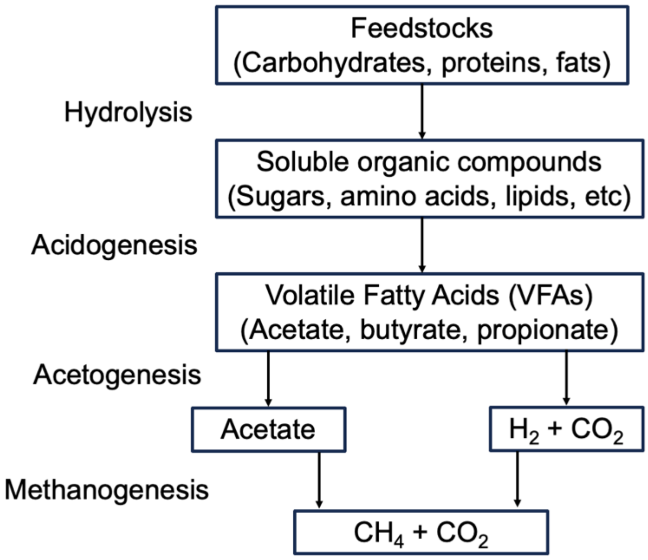


Figure 4. *Stages in the AD process (modified from Mohanakrishna et al.,2023)*

The AD is a low-cost treatment process (Singhania et al., 2013). In addition, besides biogas produced from the AD process can be recovered to generate heat and electricity as renewable energy or used as transport fuel, digestate is also produced and utilized as biofertilizer (Wainaina et al., 2019).

4.1.2 Acid fermentation and volatile fatty acids production

The AD process could also be used to produce VFAs (Greses et al., 2020). VFAs including acetic, propionic, iso-butyric, butyric, iso-valeric, valeric and caproic acids (Llamas et al., 2020; Luo et al., 2022) are the intermediates produced in acidogenesis and acetogenesis phases of the AD process (Zhou et al., 2018). In other words, VFAs production is obtained from acidogenic fermentation (Luo et al., 2022). There are several metabolic pathways during acidogenic fermentation which play an important role in the concentrations and distribution of VFAs such as acetate-ethanol type fermentation (AET), acetone-butanol-ethanol (ABE), propionate-type fermentation (PTF), butyrate-type fermentation (BTF), mixed-acids fermentation (MAF), lactate-type fermentation (LTF) as shown in the Figure 5. Each pathway is defined by major products produced from the pathway. For example, the main products of AET pathway are acetate and ethanol while ABE pathway is termed for its acetone, butanol and ethanol products. PTF and BTF pathways are dominated by propionate and butyrate, respectively. Mixture of acetate, propionate, butyrate and valerate are produced with the same amount in MAF and lactic acid is converted from glucose in LTF pathway. However, regardless of the pathways, pyruvate is the important control point which can be converted into different products depending on the distributions of major soluble compounds, environmental conditions, and inoculum. (Zhou et al., 2018)

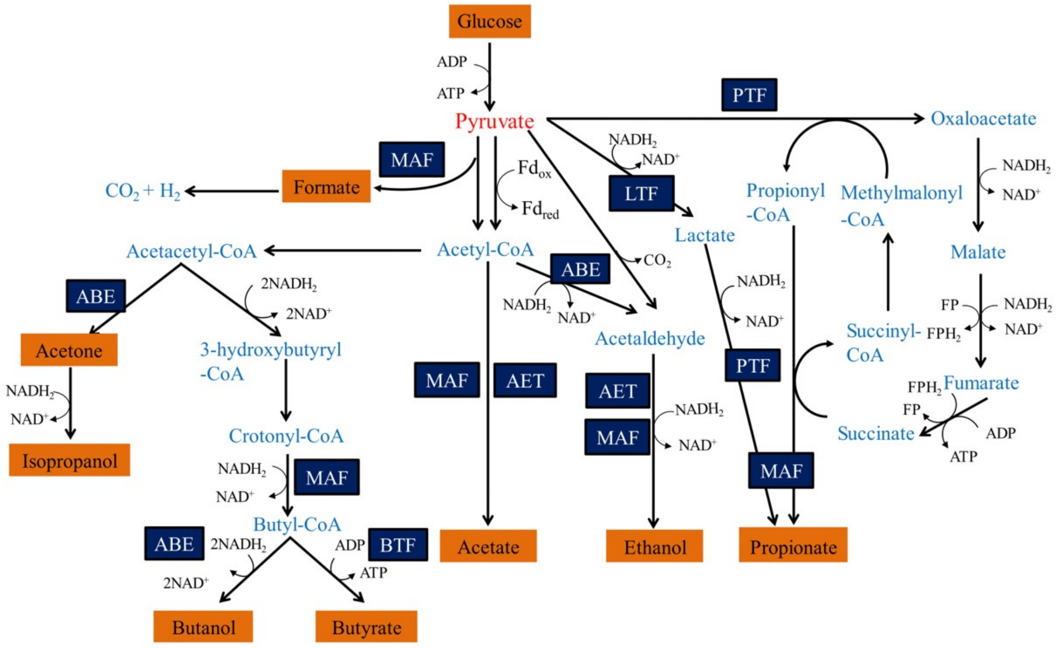


Figure 5. *Metabolic pathways in acidogenic fermentation process (Zhou et al., 2018)*

The metabolic pathway of AET yields three mol acetate from one mol glucose while ABE and LTF pathways produce no acetate. Meanwhile, two third mol acetate and one half mol acetate are obtained from one mol glucose in the PTF and BTF pathways, respectively. (Zhou et al., 2018) Tampio et al. (2019) conducted the experiment of VFAs production from food waste and cow slurry, and they pointed out that acetate is the main organic acid of total VFAs, accounting for 66% to 80% of the total. Thus, this thesis only focuses on the detailed description of AET metabolic pathway.

Regarding the AET pathway, acetate can be generated from pyruvate through acetyl-CoA pathway. On the other hands, ethanol or longer chain fatty acids such as propionate and butyrate can be oxidized simultaneously to produce acetate. A series of biochemical reactions occurs in the AET metabolic pathway are presented as following, in which Eq.1 to Eq.5 express the syntrophic oxidation of ethanol and longer chain fatty acids into acetate and Eq.5 illustrates the conversion of glucose into ethanol as another possible product in the pathway. (Zhou et al., 2018)

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ (Eq.1)

$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$ (Eq.2)

$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$ (Eq.3)

$CH_3CH_2OH + 2H_2O \rightarrow CH_3COOH + 2H_2$ (Eq.4)

$C_6H_{12}O_6 + H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2H_2 + 2CO_2$ (Eq.5)

A variety of fermentative microorganisms have participated in acid fermentation. But the formation of specific acid is optimized by a certain type of microbes. Take acetate production as an example, acetate production is commercially done with *Acetobacteriaceae, Gluconacetobacter* and *Gluconobacter*. However, butyrate is produced by *Clostridium butyricum* and *Propionibacterium sp.* is the propionate producer. (Lv et al., 2022)

The accumulation of higher yields of VFAs in fermentation is improved if the rate of hydrolysis step is improved. This is because the hydrolysis is the rate-determining step in the fermentation process. (Wang et al., 2014; Xiang et al., 2023; Zhu et al., 2023) Thus, it is believed that the increased hydrolysis rate will enhance the generation of soluble substrates for acidogenesis step because acidogenic fermentation could enhance VFAs production up to 30% (Xiang at al., 2023). For example, food waste contains high amount of lipids which do not favor the fermentative microorganisms (Blasco et al., 2020). Meanwhile, for lignocellulose biomass, due to its lignin structure interwoven with cellulose and hemicellulose, the biomass becomes rigid to be digested by microorganisms (Wainaina et al., 2019). Hence, these types of materials need to be pretreated to increase solubilized organic matters. Some pretreatment methods can be used to promote the fermentation efficiency such as thermal treatment, ultrasonic treatment, alkaline treatment, advanced oxidation, and enzymatic pretreatment (Xiang et al., 2023; Zhu et al., 2023).

The activity of methanogens must be inhibited to shift the production of the AD process's end products to the accumulation of VFAs (Wang et al., 2014; Castilla-Archilla et al., 2021; Cai et al., 2024). To prevent the growth of methanogenesis microorganisms, the OLR and the ammonium concentration could be increased (Castilla-Archilla et al., 2021) or methanogenic inhibitors can be added to the AD system (Cai et al., 2024). Using heat shock pretreatment is another inhibition way for the methanogens growth (Jariyaboon et al., 2023; Cai et al., 2024). This is because that methanogens are the most sensitive microorganisms in the AD process (Greses et al., 2020) and very sensitive to heat (Mohanakrishna et al.,2023).

4.1.3 The effects of operational parameters on VFAs yields

The yields of VFAs are majorly influenced by different parameters such as the characteristics of waste stream, pH, (Zhou et al., 2018; Lv et al., 2022; Jariyaboon et al., 2023), temperature, hydraulic retention time (HRT) (Zhou et al., 2018; Lv et al., 2022; Jariyaboon et al., 2023), and OLR (Lv et al., 2022; Zhou et al., 2018). First, for the characteristics of waste stream, it is considered as one of the most critical elements influencing the formation and the composition of VFAs (Zhou et al., 2018; Lv et al., 2022; Jariyaboon et al., 2023). As already mentioned in the previous section about the VFAs formation, VFAs yield is relatively low if there is no pretreatment implemented for lignocellulose materials. About the composition of VFAs distribution, there is existence of different metabolic pathways in the fermentation process. So the type of substrate will determine the dominating pathway for a specific proportion of organic acids in VFAs. (Atasoy et al.,2018) Lv et al. (2022) stated that the feedstock with high amount of carbon will promote the production of ethanol and butyrate. But the protein-rich streams will have tendency to generate propionate (Lv et al.,2022), iso-valeric acids (Atasoy et al.,2018) and valerate (Atasoy et al.,2018; Lv et al.,2022).

Some research state that fermentative microorganisms show higher specific growth rate when cultivated at pH of above 8.0 or below 6.0 (Jariyaboon et al., 2023). For instance, Atasoy et al.(2018) reviewed from some studies that pH from 9 to 11 could promote the activity of acid-producing microorganisms as well as inhibit the growth of methanogens, thereby improving the yield of VFAs while Wang et al.(2014) showed pH of 5.0 to 6.5 is the optimal one for effective fermentation. The change of pH also affects VFAs composition. For example, glucose is fermented at thermophilic condition (from 55°C to 60°C) and pH of 4, it was observed that acetate, butyrate, and hydrogen are generated as the main products. In contrast, when pH is adjusted to 7, acetate, ethanol, and propionate are produced. (Jariyaboon et al., 2023) The microbial community in medium also changes with the variation of pH. For example, *Clostridia* was found abundant at pH 8 or 9. (Atasoy et al.,2018)

The growth of microorganisms, enzyme activity and hydrolysis rate are influenced by temperature (Atasoy et al.,2018). Atasoy et al. (2018) also stated that thermophilic condition favors the hydrolysis rate, resulting in the higher generation of VFAs. However, it seems that there is no distinct difference in VFAs yield with regards to thermophilic and mesophilic fermentation. Thus, mesophilic temperature is considered as the optimal condition as compared to thermophilic temperature in terms of economic perspective. (Atasoy et al.,2018)

Another parameter affecting VFAs production is HRT. It is pointed out that HRT influences the quantity and composition of produced VFAs (Atasoy et al.,2018; Zhou et al., 2018). More VFAs are generated when the retention time is longer due to the fact that the microorganisms with slower growth rate can stay longer in bioreactors to digest substrate (Zhou at al., 2018; Wang et al., 2022). However, a specific HRT depends on feedstocks and other operational conditions (Atasoy et al.,2018). For example, VFAs production from mixed waste was seen to increase when HRT was increased from 1 to 2 days (Zhou et al., 2018). About the HRT effect on VFAs composition, caproate was detected in VFAs composition after acidogenic fermentation of vegetable and salad waste in 20 to 30 days besides butyrate and acetate (Atasoy et al.,2018).

Regarding OLR, Zhou et al.(2018) and Gottardo et al.(2022) indicated that the increased amount of VFAs is produced proportionally with the rising of OLR. Because OLR means the availability of substrate. For example, in the experiment with food waste, it was observed that when OLR increased from 5 $gL^{-1}d^{-1}$ to 13 $gL^{-1}d^{-1}$, this resulted in the increased formation of VFAs (Zhou et al., 2018). In fact, the high value of OLR from 7.5 $gCOD.L^{-1}d^{-1}$ to 10 $gCOD.L^{-1}d^{-1}$ favors the fermentation, contrasting to the lower value which promotes the VFAs conversion into biogas (Gottardo et al.,2022). In addition, OLR also affect the VFAs composition. Acetate and valerate are more produced at high OLR while propionate and butyrate are related to low OLR. It is also worth noticing that when the OLR is high, the medium has become very viscous and led to unstable operation. (Zhou et al., 2018)

4.2 VFAs production from organic feedstocks

To clarify the effects of operational parameters mentioned in section 4.1.3 on VFAs production, this section discusses some studies on VFAs production using different types of feedstocks and operational conditions for the studies' experiments. There are several types of wastes observed in VFAs production studies including food waste (Yin et al.,2014; Wang et al.,2014), poultry litter (Kuruti et al.,2017), municipal solid waste (Garcia-Aguirre et al.,2017), and sludge (Jankowska et al.,2015; Chen et al.,2017; Esteban-

Gutiérrez et al.,2018; Liu et al.,2019). Depending on the characteristics of studied feedstocks or substrates, acidic environment or alkaline environment can be applied for optimal VFAs yields. In addition, various ranges of OLRs were used to evaluate the impacts of OLRs on VFAs yields. The VFAs yields and experimental details from studies are presented in the Table 4.

Each study in Table 4 has its different research objectives, thus VFA results were calculated and expressed in inconsistent units. Furthermore, there are some feedstocks which are recalcitrant to degrade by microorganisms, which needs to firstly undergo pretreatment step before acid fermentation. Thus, another possibility for VFAs yield expression is not based on initial organic matters in feedstocks but on hydrolyzed compounds after pretreatment. For example, Liu et al.(2019) thermally pretreated sewage sludge with alkaline catalyst and utilized the pretreated substrate for acid fermentation. However, VFAs yield was estimated based on the initial VS of the feedstock. Whereas, the feedstocks in the studies of Yin et al.(2014) and Kuruti et al.(2017) also underwent the same manner in their experiments but VFAs yields were expressed based on the VS of pretreated streams.

Table 4. *Summaries of VFAs yields collected from different studies.*

Feedstocks	**Optimal conditions**					**VFAs yields**	**References**
	pH	**HRT (days)**	**Temperature**	**OLRs**	**Microbial community**		
Co-substrates of food waste and biological sludge	9	6	37°C	7.7 kgVS/m^{-3} d^{-1}	n.m.	0.77 g/gVS$_{initial}$	Moretto et al.,2019
Food waste	6	15	30°C	n.m	n.m.	0.908g/gVS$_{added}$	Yin et al.,2014
Food waste	6	20	30°C	n.m	n.m.	0.918g/gVSS	Wang et al.,2014
Poultry litter	5.5	4	35°C	n.m	n.m.	0.67 kg/kgVS$_{added}$	Kuruti et al.,2017
Cattle manure	5.5	4	35°C	n.m	n.m.	0.43 kg/kgVS$_{added}$	Kuruti et al.,2017
Mixture of primary sludge and waste activated sludge	10	15	35°C	0.5gVS/100 mL	n.m.	0.62 g/gVS$_{initial}$	Jankowska et al.,2015
Sewage sludge	10 - 11	14	n.m	3.0 kg VS/ m^{-3} d^{-1}	n.m.	261.32 mg/gVSS$_{added}$	Liu et al.,2018
Sewage sludge	uncontrolled	10	37°C	1600mgCOD/L^{-1}d^{-1}	*Proteobacteria* (37%), *Bacteroidetes* (33%) and *Firmicutes* (25%)	0.405 mg/VSS$_{added}$	Iglesias-Iglesias et al.,2019
Sewage sludge	10	20	37°C	11 kgCOD/m^{-3} d^{-1}	n.m	0.38 kg/kgVS$_{initial}$	Liu et al.,2019
Sewage sludge	10	10	55°C	n.m	n.m	0.4 g/gCOD$_{in}$	Esteban-Gutiérrez et al.,2018

Feedstocks	Optimal conditions					VFAs yields	References
	pH	HRT (days)	Temperature	OLRs	Microbial community		
Mixture of primary and secondary sludge	8.9	n.m	55°C	n.m	*Clostridia* (59.16%)	423 mg/gVSS	Chen et al.,2017
Organic fraction of municipal solid waste (OFMSW)	5	3.3	55°C	20.5 ± 0.7 kgTVS/$m^{-3}d^{-1}$	n.m.	0.90 g/g sCOD	Valentino et al., 2018
Paper mill activated sludge	6	0.67	30°C	8750 mgCOD/L	n.m.	0.84 kg/kg sCOD	Bengtsson et al.,2008

n.m. = not mentioned

4.3 VFAs-rich stream valorization into PHAs

4.3.1 Overview of PHAs

VFAs can be used as a promising substrate in biodegradable polyhydroxyalkanoate (PHAs) synthesis for plastic production (Zhou et al., 2018; She et al., 2020; Xiang et al., 2023) or lipids synthesis for biodiesel production (Llamas et al., 2020; Castilla-Archilla et al.2021). This thesis deals with the evaluation of PHAs yields from VFAs rich stream, thus this part discusses how VFAs rich stream can be valorized to synthesize PHAs.

Polyhydroxyalkanoates (PHAs) are intracellular polymers consisting of different hydroxycarboxylic acids and synthesized by specific microorganisms shown in Table 5 under the excess carbon sources and the limitation of nutrients such as nitrogen and phosphorous (Ye et al., 2018; Kacanski et al., 2022; Ranganadhareddy, 2023). PHAs are structured by monomers with 3 to 5 carbon atoms monomers to form short-chain-length PHAs or monomers with 6 to 14 carbon atoms to form medium-chain-length PHAs. Furthermore, monomers can be further synthesized into homopolymers, random copolymers (Yang et al.,2018, Ye et al.,2018; Tan et al., 2021), block copolymers, or functional polymers (Tan et al., 2021). Thus, the properties of PHAs are determined by constitutive monomers (Yang et al., 2018; Kacanski et al., 2022). In general, PHAs are biocompatible, biodegradable (Tan et al., 2021; Kacanski et al., 2022), edible, nontoxic degraded and partially comparable to chemical plastics (Tan et al., 2021; Agnihotri et al., 2022) such as poly(3-hydroxybutyrate)(P3HB) (Ye et al., 2018).

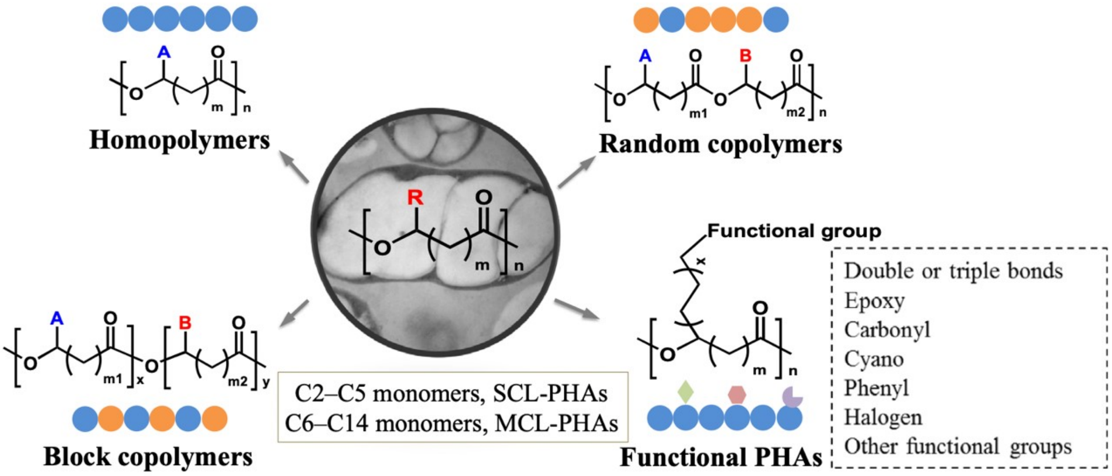


Figure 6. *Different types of PHAs structure (Tan et al., 2021)*

Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)(Tan et al., 2021), poly(4-hydroxybutyrate)(Ye et al., 2018), P3HB, poly(3-hydroxy-butyrate-co-3-hydroxyvalerate)(P3HB-co-3HV or PHBV), and poly(3-hydroxybutyrate-co-4-hydroxybutyrate)(Ye et al., 2018; Tan et al., 2021) are the PHAs produced at industrial scale by current industrial biotechnology with conventional microorganisms hosts (Tan et al., 2021). The current industrial bioprocess has used sugars as the main carbon source for PHAs synthesis, contributing to high production cost and leading to the uncompetitive price with up to six times higher compared to fossil-based plastics (Chen et al., 2018; Tan et al., 2021; Agnihotri et al., 2022). To compete with PHAs products from fossil-based plastics, VFAs (Agnihotri et al., 2022; Vu et al., 2021) or acetate (Chen et al., 2018; Vu et al., 2021) in particular are considered as the cost-effective substrates focused on many research for its feasibility in PHAs production not only economic reasons, but also to remove links to the food/feed production chains.

4.3.2 VFAs rich stream for PHAs valorization

Regarding the properties of VFAs rich stream, VFAs contains acetic, propionic, butyric and valeric acid (Bengtsson et al.,2008; Albuquerque et al., 2010; Vu et al.,2021). As mentioned earlier, acetic acid accounts for up to 80% of VFAs. The beneficial property of acetate is that it forms easily a homogeneous mixture when added in a medium, which makes the mass transfer to cell membrane become more conveniently (Gong et al., 2022). Another advantage of acetate is that metabolic pathways of acetate by microorganisms is simpler than that of glucose. While glucose metabolism needs multiple steps including the conversion to pyruvate via glycolysis before being converted into acetyl-CoA which is the precursor for PHAs production, the acetyl-CoA is converted directly from acetate. (Saika et al.,2014; Vu et al.,2021; Gong et al.,2022)

However, even though acetate can be used as a carbon source by many microbes such as *Bacillus sp.*, *Ralstonia eutropha* (Bugnicourt et al., 2014; Park et al., 2024), *Halomonas sp.* (Chen et al., 2018; Park et al., 2024), *E.coli* (Ye et al., 2018; Chen et al., 2018), *Comamonas sp.*, and *Pseudomonas sp.* (Park et al., 2024), acetic acid can be acidic and toxic at concentrations even lower than 5g/L (Gong et al.,2022), thereby inhibiting the growth of bacteria (Chen et al., 2018; Kacanski et al., 2022; Gong et al., 2022; Park et al., 2024). In addition to the toxicity, low energy content is another drawback of using acetate as a carbon source. In fact, one mol glucose can generate 38 ATPs, whereas one mol acetate produces 10 ATPs. (Gong et al.,2022)

Another issue worth mentioning is that the type of acid existing in VFAs stream affects the types of PHAs products. In details, acetic and butyric acid are classified as even-numbered VFAs while propionic and valeric acid are odd-numbered ones. It is proved that PHB is synthesized from even-numbered VFAs and PHV is correlated with odd-numbered VFAs. (Zhang et al.,2014; Ferre-Guell et al.,2018)

In terms of metabolic pathway for producing PHAs, VFAs are first obtained from acid fermentation. Then selected cultures consume the VFAs and transform them into PHAs in an aerobic process. (Pittmann et al., 2014; Agnihotri et al., 2022) The common pathway for PHB synthesis from acetate starts with the conversion of acetic acid into acetyl-CoA (Vu et al., 2021; Narayanan et al., 2021; Park et al.,2024), shown in Eq.(6). Then two molecules of acetyl-CoA are combined to form acetoacetyl-CoA by PhaA (PHA-specific ß-ketothiolase) in Eq.(7). Subsequently, acetoacetyl-CoA is reduced to hydroxybutyryl-CoA by PhaB (Acetoacetyl-CoA reductase) as in Eq.(8). The monomer hydroxybutyryl-CoA is then polymerized via an ester bond by PhaEC (PHB synthase) (Bugnicourt et al., 2014; Ranganadhareddy, 2023), expressed in Eq.(9). The pathway involved these following fundamental reactions (Vu et al., 2021):

CH_3COOH (acetic acid) + CoA-HS → CH_3COS-CoA (acetyl-CoA) + H_2O Eq. (6)

$2CH_3COS$-CoA (acetyl-CoA) – CoA-HS → $C_4H_5\,O_2S$-CoA (acetoacetyl-CoA) Eq. (7)

$C_4H_5O_2S$-CoA (acetoacetyl-CoA) + NADH → $C_4H_6O_2S$-CoA (3-hydroxybutyrul-CoA) + NAD^+ Eq. (8)

$C_4H_6O_2S$-CoA (3-hydroxybutyrul-CoA) – CoA-HS → $n(C_4H_6O_2)$ (polyhydroxybutyrate) Eq. (9)

4.3.3 Studies on PHAs production from VFAs

Although there are several studies in India and Portugal conducted on available carbon sources in feedstocks or substrates for PHAs accumulation such as cow dung (Chandani Devi et al.,2018), sludge (Narayanan et al., 2021), hydrolyzed whey (Pais et al., 2016), this section only focuses on the studies using VFAs or acetate for PHAs production. In general, there are two stages in PHAs production process. First, biomass is enriched in a balanced carbon and nutrients medium. Next, the accumulation of PHA is triggered within cells under limiting one of essential nutrients such as nitrogen or phosphorous. (Chakraborty et al.,2009)

There are bioprocess parameters affecting the conversion efficiency from VFAs to PHAs such as pH, temperature, nutrients and feeding regime of VFAs (Kedia et al.,2014), which are collected from studies in Table 5. Most studies adjusted temperature in a similar range, but pH is controlled or uncontrolled which depends on feeding regime mode. More specifically, if controlling pH is done to optimal value which is in accordance with the success of previous studies, VFAs are added continuously to a medium. In contrast, if pH is not controlled, there is higher possibility of inhibition to microbes caused by acidic VFAs. Thus, VFAs are pulsed in a medium by single time, meaning that only one VFAs feeding in 24 hours. (Kedia et al.,2014) Regarding nutrients, it is worth noted that even though PHAs is produced under excess of carbon and limited nutrients, nutrients are needed at the beginning stage for cell's growth. When the nutrients are depleted, microbes start accumulating carbon sources from VFAs for PHAs production. (Chakraborty et al.,2009; Lee et al,2014) In the biomass enrichment phase, carbon sources can be from VFAs (Bengtsson et al.,2008) or glycerol (Chakraborty et al.,2009).

In the PHAs production process, besides substrates, choosing bacterial strains for PHA production is of the utmost importance (Vu et al., 2021). In fact, the current industrial bioprocess operation is expensive because there is requirement regarding full sterilization for the conventional hosts (Tan et al., 2021) and pure culture cultivations (Agnihotri et al., 2022). To handle the challenges of sterility requirements, extremophilic bacteria has been employed because they are able to grow fast, be cultivated in unsterile environment, seawater and low-cost mixed substrates (Tan et al., 2021; Ye et al., 2018). For instance, Mittal et al.(2023) engineered thermophilic bacteria *Bacillus sonorensis* isolated from hot springs to synthesize PHB from pulp and paper wastewater. Additionally, mixed microbial cultures are also employed to reduce the production cost (Agnihotri et al., 2022). The advantages of using mixed microbial cultures are no sterility requirement in the production process as well as the flexibility of substrate quality (Kacanski et al., 2022; Agnihotri et al., 2022), contributing to 50% reduction for the production cost (Agnihotri et al., 2022). For example, Munir et al.(2022) conducted an experiment of PHAs production from synthetic VFAs using waste activated sludge as mixed microbial culture. However, when it comes to desired properties for PHAs products, pure culture has more advantages to ensure the products quality over than mixed microbial cultures by genetic engineering or reprogramming the metabolic pathway for a selected strain (Kacanski et al., 2022).

Table 5. *VFAs or acetate conversion to PHAs studies*

Feedstocks	**Culture**	**Temperature**	**HRT (hrs)**	**pH**	**Titers**	**PHAs yields**	**PHAs content (%CDW)**	**References**
Pretreated pulp and paper wastewater	*Bacillus sonorensis NAM5*	50ºC	72	7	5.28 ± 0.11 g/L	n.m.	n.m.	Mittal et al.,2023
Paper mill activated sludge	Municipal sludge	30ºC	25	7.3	n.m.	0.11kg/kgsCOD	48%	Bengtsson et al.,2008
Fermented waste paper (pretreated)	*Cupriavidus necator*	30ºC	72	n.m.	n.m.	n.m.	53.50%	Al Battashi et al., 2021
Synthetic VFAs with the ratio of 5 Acetatic : 1Propionic : 4Butyric acid	*Cupriavidus necator*	30ºC	72	n.m.	n.m.	0.31g/gVFAs	56.98%	Al Battashi et al., 2021
Synthesized VFAs (Acetic acid ≥ 99.85% and propionic acid ≥ 99.5%)	Waste activated sludge	37ºC	8	uncontrolled	n.m.	0.32g/gVSS	32%	Munir et al.,2022

Feedstocks	**Culture**	**Temperature**	**HRT (hrs)**	**pH**	**Titers**	**PHAs yields**	**PHAs content (%CDW)**	**References**
Fermented food waste	*Bacillus megaterium ATCC 14945*	37°C	48	uncontrolled	0.16 gPHAs/L	0.23g/gVFAs	8–9%	Vu et al.,2021
Fermented food waste	*Haloferax mediterranei*	37°C	144	7.0	n.m.	0.41 to 0.54 g PHBV/gAcetate	n.m.	Wang et al.,2021
Fermented sludge	*Cupriavidus necator*	30°C	29	6.9 – 7.1	4.9g/L	0.33g/gAcetate	n.m.	Kedia et al.,2014
Acetate	*Halomonas sp. YLGW01*	30°C	12	n.m.	0.72 g/L	0.075 g/gAcetate	n.m.	Park et al.,2024
Acetate	Engineered *E. coli*	37°C	48	n.m.	2.15 gP3HB4HB/L	1.99g P3HB4HB/gAcetate	n.m.	Chen et al.,2018
Acetate	*Ralstonia eutropha*	30°C	48	7.5 – 8.5	2.9 g/L	n.m.	29.3%	Chakraborty et al.,2009

n.m. = not mentioned
CDW = Cell Dry Weight

5. METHODOLOGY

Chapter 5 begins with the description of case studies. There are two case studies developed in this thesis and the reasons for selecting case studies are clarified. A pretreatment method for each feedstock is discussed, followed by the process design of hydrolyzing the feedstock to VFAs and converting the VFAs rich stream into PHAs. To calculate VFAs yields and PHAs yields, initial data and steps for the calculation are also mentioned.

5.1 Case study description

5.1.1 Feedstock selection

Bark and biosludge (referred to as sludge) are the two main streams utilized as carbon sources in case studies. There are two reasons for this choice. First, when considering the volumes of side streams, barks and sludge are the two main biomass accounting for 13.7% and 14.1% of the side and waste streams of Finnish pulp and paper industry (Hassan et al., 2019). More importantly, it was investigated that the current utilization of sludge was only 50% while the residual bark was reused with 80% to 100% (Hassan et al., 2019). A question was raised with the remaining portion of unused biomass, the left 50% for sludge and 20% for barks. Currently these unused streams are disposed for landscaping or incinerating (Hassan et al., 2019; Kaur et al., 2020), leading to waste potential resources in the streams. So the thesis predicted that bark and sludge are available with excess amount in near future, which can be utilized for other purpose such as the biomass valorization to VFAs. Second reason worth considering is the composition of the biomass. As indicated in chapter 2, biosludge contains 65% to 97% of organic compounds out of total dry solids and polysaccharides are available in barks, which are the huge carbon source for biomass conversion into value-added products such as VFAs and PHAs.

5.1.2 Pretreatment method selection

As indicated in chapter 4, hydrolysis is the rate-limiting step in anaerobic treatment of lignocellulosic biomass. Thus, pre-treatment is needed for the biomass to speed up the hydrolysis and improve the efficiency of bioprocess. Chapter 3 showed that hydrothermal and hot water extraction are the most often studied approaches for pretreating barks

(Eskicioglu et al.,2017; Rasi et al.,2019) while sludge from pulp and paper mills is pretreated mainly by thermal hydrolysis (Kinnunen et al.,2015; Goycoechea et al.,2023) or rarely by torrefaction (Doddapaneni et al.,2022). The publications results related to methane yields obtained from these pre-treatment methods along with its treatment conditions were gathered in Table 6 for bark and Table 7 for sludge. The values of methane yield are presented as the volume of methane produced from the amount of VS or COD obtained after pre-treatments ($mLCH_4/gVS_{added}$ or $mLCH_4/gCOD_{added}$). The choice of a pretreatment method in this thesis is made based on the methane yields, carbon loss and energy efficiency.

Table 6. *Methane yields from pre-treated bark after selected pretreatments.*

Pretreatment	**Temperature (°C)**	**Time (mins)**	**Pressure (bar)**	**Methane yield**
Hot water extraction$^{(1)}$	75	60	n.m.	99 $mLCH_4/gVS_{added}$
Hydrothermal pre-treatment with CO_2 as catalyst $^{(2)}$	171	30	50	253 ± 5 $mLCH_4/gCOD_{added}$ for liquid fraction. 98 ± 5 $mLCH_4/g VS_{added}$ for solid fraction

$^{(1)}$Rasi et al.(2019); $^{(2)}$ Eskicioglu et al.(2017)
n.m. = not mentioned

As can be seen in Table 6 that the hydrothermal pretreatment with CO_2 as catalyst has higher performance than the hot water extraction with higher methane yield of ca.253 ml $CH_4/gCOD_{added}$ for liquid fraction and ca.98 ml CH_4/gVS_{added} for solid fraction. However, the methane yields produced in the liquid fraction and the solid fraction were calculated with inconsistent units. The methane yield from the liquid fraction was measured from the amount of COD in the obtained liquid fraction. But the amount of COD can count inorganic matters presented in the fraction besides organic matters, causing the difficulty in determining the precise value of methane produced from organic matters. But when just looking at the methane yield from organic matters, hot water extraction from the study of Rasi et al.(2019) produced quite the same yield of methane as the yield from solid fraction of hydrothermal pretreatment with CO_2 as catalyst method. However, the hot water extraction at 75°C consumes less energy and does not need chemicals to catalyze the pretreatment. So hot water extraction is selected to pretreat bark in this study.

For sludge (as shown in Table 7), the feedstock torrefaction method could aid the AD process to yield up to 772 $mLCH_4/gVS_{added}$ as compared to the low yield from thermal hydrolysis method. However, the torrefaction method may not be a good choice in terms of energy intensive consumption and carbon loss. The torrefaction is usually operated at high temperature of about 275°C to 300°C (Sarvaramini et al.,2014; Doddapaneni et al.,2022) which consumes a large amount of energy. In addition to energy consumption, under this high temperature, higher fixed carbon products are produced (Cantero et al.,2019), leading to the loss of organic source for microbes in biological process and consequently reducing yields. Meanwhile, the thermal hydrolysis is considered as an environmental-friendly method and widely applied in many research not only for pulp and paper sludge (Kinnunen et al.,2015; Veluchamy et al.,2017; Fioreze et al.,2022; Goycoechea et al.,2023) but also for sewage sludge pretreatment (Liu et al.,2019). So thermal hydrolysis is selected to pre-treat the sludge.

Table 7. *Methane yields from pre-treated biosludge after selected pretreatments.*

Pretreatment	**Temperature (°C)**	**Time (mins)**	**Methane yield**
Thermal hydrolysis by autoclave (1)	80	120	49 $NmLCH_4/gVS_{added}$
	105	20	77 $NmLCH_4/gVS_{added}$
	121	20	90 $NmLCH_4/gVS_{added}$
	134	20	108 $NmLCH_4/gVS_{added}$
Thermal hydrolysis (2)(*)	140	45	187 $NmLCH_4/gVS_{added}$
	165	45	241 $NmLCH_4/gVS_{added}$
Thermal hydrolysis by hot air oven (3)	80	90	303 mL/gVS_{added}
Torrefaction (4)	275	60	481 - 772 mL/gVS_{added}
	300	60	

(1)Kinnunen et al.(2015); (2)Goycoechea et al.(2023); (3)Veluchamy et al.(2017); (4) Doddapaneni et al.(2022)
(*) not mention how the sludge was heated.

Regarding the selection of hydrolysis conditions for the selected method, as observed the results from studies in Table 7, when increasing the temperature in pretreating the same feedstock, the methane yield has increased. This corresponds to the argument discussed in chapter 3 that temperature has huge impact on the pretreatment efficiency.

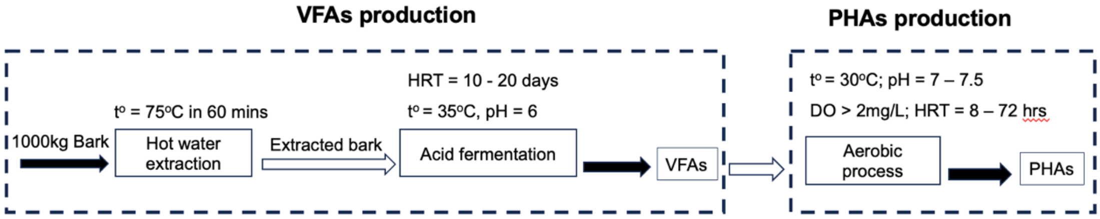
However, it is not straightforward to determine how high the temperature should be when the characteristics from studied sludge are not the same. For example, the value of organic matter from experimentally pretreated sludge under 150°C in 10 minutes was used to calculate theoretical biomethane potential, which yielded 314 – 360mLCH_4/gVS$_{added}$ (Fioreze et al.,2022). However, in reality, in another study, Goycoechea et al.(2023) showed that 241 NmLCH_4/gVS$_{added}$ could be obtained from thermally pretreated sludge at 165°C in 45 minutes. Meanwhile, the temperature of 80°C was applied in both studies of Kinnunen et al.(2015) and Veluchamy et al.(2017) but the volume of methane produced was a huge difference.

With the aim of reducing the energy consumption but not decreasing the pretreatment efficiency, the results from the study of Kinnunen et al.(2015) for sludge in Finnish PPI were inherited in this thesis. Regarding pretreatment temperature for the sludge, the temperature in this thesis was increased to 165°C which is now widely applied in commercial plant. Furthermore, the retention time is expanded to 45 minutes. The hypothesis for this thermal hydrolysis condition is that it will increase the degradability of sludge and improve the methane yield to 240 mLCH_4/gVS$_{added}$ from pretreated stream.

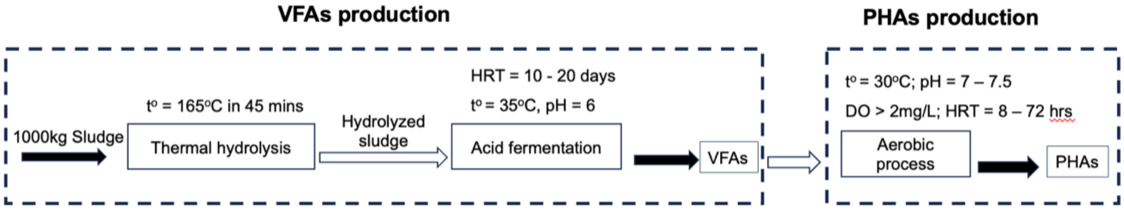
For barks, as indicated in chapter 3, hot water extraction could be conducted at the temperature up to 230°C. However, there is limited experimental data in general and in Finland particularly about methane yield from extracted barks at such high temperatures. Thus this thesis utilized the experimental conditions from Rasi et al.(2019) for the hot water extraction of pine bark, occurring at 75°C in 60 minutes and expect to obtain the same methane yield of 99 mLCH_4/gVS$_{added}$ from pretreated bark.

5.1.3 The unit process selection for VFAs and PHAs production

This part will discuss the process for VFAs production and how VFAs are converted into a desired product. PHAs are chosen to be the desired product in this thesis. In details, for VFAs production, biomass is first pretreated under the selected pretreatment approach and its operational conditions. Then the pretreated stream with high OLR is transported to acid fermentation unit for VFAs production. With the purpose of reducing huge cost from the downstream processing, downstream units to collect pure VFAs such as filtration, centrifugation, extraction are not taken into account. Thus the thesis attempts to utilize the produced VFAs diluted stream directly for microorganisms to accumulate PHAs in an aerobic unit. The VFAs and PHAs production unit process for both sludge and bark is designed in a same manner. Instead of using a lab-scale amount of initial feedstocks for the estimation of VFAs production, 1000 kg of bark and 1000kg of thickened sludge are used as inputs of the production process in this thesis so that a bigger picture can be visualized.



a) Schematic of VFAs and PHAs production process for barks.



b) Schematic of VFAs and PHAs production process for sludge.

***Figure 7.** Schematic of VFAs and PHAs production process for barks and sludge.*

As the detailed description of the production process, 1000 kg of bark is prepared for the process. First, the amount of bark is depolymerized by hot water extraction at 75°C in 60 minutes, producing extracted bark. In the meantime, 1000kg of sludge is thermally pretreated at 165°C in 45 minutes, producing hydrolyzed sludge. In both cases, without purification and extracted step, the pretreated stream is then directly transferred to acid fermenter which is set up with the same operational conditions for the VFAs production. The process design for bark and biosludge is presented in Figure 7a and Figure 7b, respectively.

The operative conditions for a fermentation process and PHAs production process are chosen based on previous studies (presented in Table 4). For a fermentation process, the fermentation process is designed to be operated in mesophilic condition at 35°C. The OLR of pretreated bark and sludge could be chosen from 5 $gL^{-1}d^{-1}$ to 13 $gL^{-1}d^{-1}$ to inhibit the growth of methanogens microorganism, starting from smaller OLR to acclimate the inoculum to the pretreated stream and avoid shocking condition for microorganisms. The hydraulic retention time (HRT) is directly related to specific OLR. However, the amount of VS measured in each pretreated stream is the initial value for the OLR. Therefore, a precise HRT is determined later when a real experiment is set up. But as referred to

Table 4, HRTs for the sludge and barks in this thesis is chosen in the range from 10 to 20 days.

With regard to pH factor, as can be seen in Table 4 that the pH value in the studies for sludges were adjusted to be in a wide range from acidic to alkaline. However, chapter 2 showed that the pH of biosludge is usually in slightly neutral range from 6.5 to 7.6. Thus, this thesis tries to apply pH 6 for VFAs production from sludge with the purpose of preventing from high chemical consumption for pH adjustment. Unfortunately, there is no reference for bark experiment. But with the same aim of chemical reduction, pH 6 is used for the acid fermentation of bark.

Next, for the PHAs production process, PHAs accumulating-microorganisms in aerobic unit are fed with the obtained VFAs stream from the upstream pretreatment process of bark and sludge. A sequencing batch reactor (SBR) is used in this thesis. This is because this kind of reactor was widely utilized in most studies on PHAs production (Bengtsson et al.,2008; Valentino et al., 2018; Valentino et al., 2020; Morgan-Sagastume et al.,2010). The reactor is operated in batch mode and under feast and famine regimes. The aim of the feast and famine regime is to stimulate the growth of microorganisms and biomass enrichment in the feast phase and then promote the metabolic activity inside the microorganisms under nutrients limited in the famine phase (Morya et al.,2023). The concentration of dissolved oxygen (DO) is used to monitor the aerobic condition (Bengtsson et al.,2008) and the feast and famine status (Morgan-Sagastume et al.,2010). The level of DO must be above 2mg/L to maintain aerobic condition (Bengtsson et al.,2008; Morgan-Sagastume et al.,2010). The feast regime is controlled with the high availability of VFAs stream and associated with low DO levels because of high respiration, meaning that VFAs along with some nutrients supplemented are used as substrates in the growth phase of microbes. In the meantime, the famine phase is in line with limited amount of VFAs and higher DO levels. (Morgan-Sagastume et al.,2010)

The thesis selected the OLRs for PHAs production process from 2.0 to 4.4 gCOD.$L^{-1}d^{-1}$. This selection was based on some OLRs of VFAs from other substrates such as 2.5 gCOD.$L^{-1}d^{-1}$ (Valentino et al.,2018) for organic fraction of municipal solid waste, 3.5 gCOD.$L^{-1}d^{-1}$ (Crognale et al.,2019) for urban waste, or 2.0 to 4.4 gCOD.$L^{-1}d^{-1}$ for mixture of food waste and sewage sludge (Valentino et al.,2020).

Because the PHAs accumulation process takes place under limited nutrients, so the ratio COD:N:P should be taken into account. This thesis used the same ratio of 100:0.030:0.0015 which was done in a study of Bengtsson et al.(2008). As referred the operational parameters in Table 5, the thesis applies the same temperature maintaining at 30°C and the same pH adjusting to 7.0 – 7.5. As the same as the acid fermentation process, HRT is a dependent value. However, the PHAs accumulation process is seen in Table 5 to take place in the range from 8 hours to 72 hours. Thus, the thesis also chose HRT of 8 hours to 72 hours. Mixed microbial culture is used in this thesis. The culture could be obtained from activated sludge of a municipal wastewater treatment plant.

5.2 Inital data

The data in this study were collected from various sources consisting of articles and Google Search. The calculation in this study was done in reverse way using the yield of methane from articles discussed in section 5.1.2. In addition, for evaluating the efficiency of pretreatment process, the methane yields from untreated sludge and barks from the same experiments are also collected as control values. All data are presented in Table 8.

The percentage of VS in each feedstock was retrieved from Eskicioglu et al.(2017) for bark and Kinnunen et al.(2015) for sludge, thereby evaluating the available organic matters in each stream. This percentage is understood as the mass of VS in total dry solids. Regarding dry matter content in sludge, the sludge stream is assumed to have the same dry content of 20% as that analyzed in Doddapaneni et al. (2022). The dry content of barks is based on moisture content. The moisture content varies depending on fresh barks and stored barks. It is reported that the average moisture content of fresh barks is about 47.5% and this content decreased to 31.4% after 8 weeks storage. (Routa et al.,2021) This thesis took the average of the moisture content of fresh barks and stored barks for the calculation, which is 39.5%. Therefore, the dry content of barks is 60.5%.

Table 8. *Methane yields for untreated and pretreated biomass and VS percentage in feedstocks.*

Feedstocks	**Methane yields from pretreated biomass**	**%Dry content**	**VS (% w/w DS)**	**Methane yields from untreated biomass (control values)**
Sludge	240 mL/gVS$_{added}$ (1)	20 (5)	72 (2)	45 mL/g VS$_{added}$ (2)
Bark	99 mL/gVS$_{added}$ (3)	60.5	99.9 (4)	53 mL/g VS$_{added}$ (3)

(1)Goycoechea et al.(2023); (2)Kinnunen et al.(2015); (3)Rasi et al.(2019); (4)Eskicioglu et al.(2017); (5)Doddapaneni et al. (2022)

To compare with current PHAs commercial production capacity, the expected amount of P3HB and PHBV from each feedstock produced annually are determined from the available mass of residual barks and sludge. Thus the amounts of waste and side streams from wood processings, pulp and paper production are collected in Table 9. Because of the difference in the collected data for bark stream, so the density of bark was obtained from Google search to convert the volumetric value of bark into mass value. Bark has a density range from 333 to 551 kg/m^3. So it is assumed that the used density value for bark is 333 kg/m^3.

Table 9. *The amounts of waste and side streams from wood processings, pulp and paper production (Hassan et al.,2019).*

Biomass	**Unit**	**Amount**
Sludge		
Biosludge from paper and board mills	ton	8,775
Biosludge from semi-mechanical pulp mills	ton	7,000
Biosludge from chemical pulp mills	ton	42,600
Total amount of sludge	**ton**	**58,375**
Bark		
Bark from sawmills	m^3	49,091
Bark from plymills	m^3	141,892
Bark from paper and board mills	ton	37,735
Bark from chemical pulp mills	ton	120,638
Bark from semi-mechanical pulp mills	ton	100,000
Total amount of bark	**ton**	**321,970**

Regarding the initial data for PHAs calculation, P3HB [$(C_4H_6O_2)_n$] and PHBV [$(C_9H_{14}O_4)_n$] are the two targeted PHAs in this calculation. To proceed the calculation, it is needed to know how many monomers are polymerized in these PHAs, meaning that the number of "n" must be identified. Therefore, the molecular weights (MWs) of P3HB and PHBV are required for the calculation of "n". Pais et al.(2016) showed that PHBV accumulated by *Haloferax mediterranei* in cheese whey medium has the polymer presented a molecular mass of 4.4×10^5 g/mol. In the meantime, the absolute MW of P3HB by recombinant *E.coli* in ethanol medium was 5.8×10^6 g/mol (Tsuge, 2016). It is assumed that microorganisms can utilize carbon source from obtained VFAs from this thesis study to produce the same MWs for P3HB and PHBV.

5.3 Estimations on the biochemical production from each waste stream

5.3.1 Estimation of VFAs yields

VFAs yield from the acid fermentation of sludge and bark is estimated in this calculation. Tampio et al. (2019) and Atasoy et al.(2018) stated that acetate accounts for up to 80% of the total VFAs. Thus, the yield of acetate is presentative of the VFAs yield in this thesis' calculation. The VFAs yield can be calculated as the mass of acetate per gram of VS from pretreated stream (gAcetate/gVS$_{added}$) or the mass of acetate per gram of VS from initial biomass (gAcetate/gVS$_{initial}$). However, the methane yields in Table 8 were presented as the volume of methane produced from the amount of VS added after pretreatment. Hence, this thesis deals with the VFAs yield against the amount of VS from pretreated stream (gAcetate/gVS$_{added}$) from which the research question regarding the pretreatment efficiency of hot water extraction for bark and thermal hydrolysis for sludge will be answered. Besides, the thesis also calculates the acetate yield against the initial input of feedstocks to visualize the potential of VFAs production from large amount of input feedstocks (gAcetate/g$_{sludge/bark}$ or kgAcetate/kg$_{sludge/bark}$ equivalent).

As described in chapter 4, 60% to 90% of acetate is converted into methane. Thus, it is assumed in this thesis that 70% of acetate is converted into methane in AD to simplify the calculation as expressed in Eq.10.

$CH_3COOH \rightarrow CH_4 + CO_2$ (Eq.10)

The calculation starts with the proposed yield of methane from pretreated sludge and barks in Table 8, which are at standard temperature and pressure (STP) conditions (273.15°K, 1.01 bar). According to the ideal gas law, the molecular volume of gas at STP is 22.4 liter per mol. Thus, by applying the ideal gas law formula at STP conditions, the molar yield of methane per one gram of VS added is obtained.

$$\text{Molar yield of methane } (\text{molCH}_4/\text{gVS}_{\text{added}}) = \frac{70\% * \text{Methane yield}(\text{L}/\text{gVS}_{\text{added}})}{22.4(\text{L}/\text{mol})} \quad \text{(Eq.11)}$$

Following the stoichiometry balance of the Eq.10, one can get the molar yield of acetate from the molar yield of methane estimated from Eq.11. Consequently, the mass yield of acetate is estimated by multiplying the molar yield with the MW of the acetate (Eq.12).

$$\text{Acetate yield } \left(\frac{\text{gAcetate}}{\text{gVS}_{\text{added}}}\right) = \text{Molar yield of acetate}\left(\frac{\text{molAcetate}}{\text{gVS}_{\text{added}}}\right) * 58\left(\frac{\text{gAcetate}}{\text{molAcetate}}\right) \quad \text{(Eq.12)}$$

To estimate the acetate yield against initial mass of feedstock, it is needed to know the total mass of acetate produced from the initial mass of sludge and bark (1000kg for each). To obtain this value, it is essential to firstly obtain the mass of VS in the initial mass of feedstocks after pretreatment. Unfortunately, there are not so many studies investigating the changes of VS so that a clear conclusion about the percentage loss of VS after pretreatment could be made. However, as observed on the results in the study of Yin et al.(2014), they showed that about 6.8% of VS in food waste is lost in hydrothermal pretreatment at the temperature of 180°C and the loss is much lower when the temperature is less than 180°C. Because the proposed pretreatment temperatures for bark and sludge in this thesis are 75°C and 165°C, respectively, hence it is assumed that the percentage of VS in the feedstocks after pretreatment process does not change a lot and remains the same as that before the pretreatment. So the mass of VS after pretreatment was done by multiplying the percentage of VS (72% for sludge and 99.9% for bark) with the amount of dry solids, in which the amount of dry solids is obtained from the dry content of each feedstock indicated in Table 8 in 1000kg initial input. After the mass of VS for each feedstock after the pretreatment is obtained, one can get the total amount of acetate produced by organic matters in 1000kg of each feedstock, in turn which is converted to the amount of VFAs from one kg of feedstock (shown in Eq.13).

$$\text{Acetate yield against initial mass of feedstock}\left(\frac{\text{gAcetate}}{\text{g}_{\text{bark/sludge}}}\text{ or }\frac{\text{kgAcetate}}{\text{kg}_{\text{bark/sludge}}}\right) = \frac{\text{Mass of VS after pretreatment }(\text{kgVS}_{\text{added}})*\text{Acetate yield}(\text{g/gVS}_{\text{added}})}{1000\text{kg}} \quad \text{(Eq.13)}$$

5.3.2 Estimation of PHAs yields

The yields of P3HB and PHBV are the mass of the biopolymers produced from one gram of VS added ($\text{gP3HB/gVS}_{\text{added}}$ or $\text{gPHBV/gVS}_{\text{added}}$), which are obtained from the yields of acetate through stoichiometric equations of the reactions from substrate to product. The number of monomers of P3HB and PHBV, which are figured out from the MW, are 67442 and 2366 (degree of polymerization or DP), respectively. Thus, the molecular formula for P3HB is $(C_4H_6O_2)_{67442}$ and that for PHBV is $(C_9H_{14}O_4)_{2366}$.

The reactions of PHAs production can be divided into two parts. First, acetate is oxidized to produce energy (ATP) for the growth of microorganism (Eq.14). Then, PHAs is produced through Kerbs cycle (Eq.15 and Eq.16).

$$C_2H_4O_2 + O_2 \rightarrow CO_2 + H_2O + ATP \qquad \text{(Eq.14)}$$

$$C_2H_4O_2 + ATP \rightarrow (C_4H_6O_2)_{67442} + CO_2 + H_2O \qquad \text{(Eq.15)}$$

$$C_2H_4O_2 + ATP \rightarrow (C_9H_{14}O_4)_{2366} + CO_2 + H_2O \qquad \text{(Eq.16)}$$

The estimation of maximal PHAs production is based on balancing stoichiometric equations from acetate to P3HB (Eq.15) and acetate to PHBV (Eq.16), but not using the restrictions of natural production pathways.

$aC_2H_4O_2 + bCO_2 + cH_2O \rightarrow (C_4H_6O_2)_{67442}$ (Acetate to P3HB) (Eq.17)

$aC_2H_4O_2 + bCO_2 + cH_2O \rightarrow (C_9H_{14}O_4)_{2366}$ (Acetate to PHBV) (Eq.18)

These equations have unknown coefficients a, b and c which need to be solved. After solving the mathematical equation systems, 151744.5, -33721 and -101163 are the values of a, b and c, respectively for Eq.17 while 12421.5, -3549 and -8281 are the respective coefficients of Eq.18. The balanced stoichiometric equations are given below, in which Eq.19 and Eq.20 present for P3HB production and PHBV production, respectively.

$151744.5C_2H_4O_2 \rightarrow (C_4H_6O_2)_{67442} + 33721CO_2 + 101163H_2O$ (Eq.19)

$12421.5C_2H_4O_2 \rightarrow (C_9H_{14}O_4)_{2366} + 3549CO_2 + 8281H_2O$ (Eq.20)

The yields of P3HB and PHBV can be obtained from the acetate yield and the coefficients in Eq.19 and Eq.20, respectively. The way of calculating acetate yield was already presented in section 5.3.1. The formulas for the P3HB and PHBV yields calculation are expressed in Eq.21 and Eq.22.

$$\text{P3HB yield }(\frac{\text{gP3HB}}{\text{gVS}_{\text{added}}}) = \text{Molar yield of Acetate}(\frac{\text{molAcetate}}{\text{gVS}_{\text{added}}}) * \frac{1*\text{MW of P3HB}}{151744.5} \text{(Eq.21)}$$

$$\text{PHBV yield }(\frac{\text{gPHBV}}{\text{gVS}_{\text{added}}}) = \text{Molar yield of Acetate}(\frac{\text{molAcetate}}{\text{gVS}_{\text{added}}}) * \frac{1*\text{MW of PHBV}}{12421.5} \text{(Eq.22)}$$

To estimate P3HB and PHBV yields against initial mass of feedstock (kg P3HB/$kg_{sludge/bark}$ or kg PHBV/$kg_{sludge/bark}$), the same calculation approach as for acetate yield against initial mass of feedstock in the section 5.3.1 was used. Besides the yields calculation, to compare the potential capacity of P3HB and PHBV production in this thesis with the current capacity of PHAs production in commercial plants, after the PHAs yields against initial mass of feedstock are obtained, with the data of potentially available amount of sludge and barks in Table 9, one can get the amount of PHAs produced from the waste streams annually in Finland.

6. RESULTS AND DISCUSSION

This chapter presents the results of VFAs yields calculated for bark and sludge. From these results, the efficiency of pretreatment approaches for each feedstock is discussed. Then the estimated amounts of PHAs yields are also given to evaluate the potential of VFAs valorization and compare with the capacities of current operating PHAs production plants.

6.1 VFAs yields from the acid fermentation of selected waste streams

6.1.1 VFAs yield from the acid fermentation of bark

The VFAs yields from bark with and without pretreatment were calculated. The VFAs yield from extracted bark is 0.179 $gAcetate/gVS_{added}$, nearly two times that of the bark without pretreatment (0.096 $gAcetate/gVS_{added}$). The results pointed out that hot water extraction at 75°C in 60 minutes is effective for generating higher yield of VFAs. In fact, it is seen from the methane yields from experimental data that the anaerobic digestion of untreated bark produced 53 $mLCH_4/gVS_{added}$ while pretreated bark contains more organic matters for the digestion with higher methane yield of 99 $mLCH_4/gVS_{added}$ (Rasi et al.,2019).

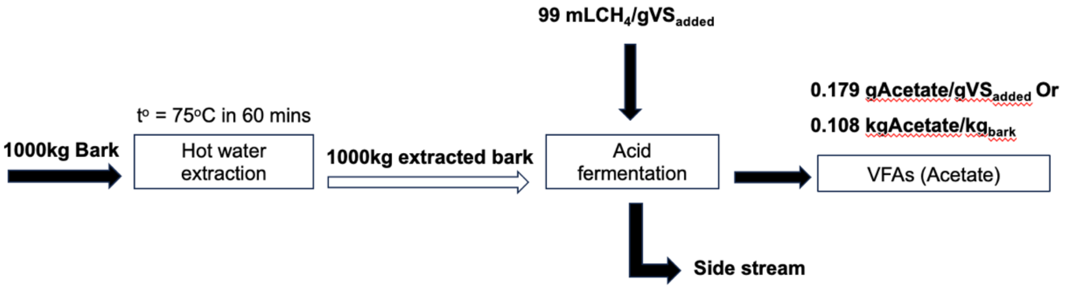


Figure 8. *VFAs yields from bark.*

Besides the VFAs yield from the VS of pretreated bark, the yield of VFAs from mass of input bark was estimated. The result shows that 1000kg bark fed could produce 108kgAcetate or 0.108$kgAcetate/kg_{bark}$. In one study of Yin et al.(2014), they demonstrated that one gram of food waste fed can produce 0.587g VFAs or 0.587$kgVFAs/kg_{food-waste}$ approximately. This result from the study of food waste is higher as compared to that from this study. A first reason could be that even though the food waste in Yin et al.(2014) study is characterized with the higher percentage of VS, about 93%. Therefore, higher amount of organic compounds is readily available for a bioprocess. A second reason could be that a hydrothermal pretreatment step for the food waste was conducted at higher temperature, 160°C. As a result, higher VFA yield of 0.908 g/g VS_{added} was obtained, consequently leading to higher yield of VFAs against the fed feedstock. In fact, this is in line with the theoretical part about the effect of higher temperature on degrading more recalcitrant compounds into soluble ones. Furthermore, as indicated in chapter 3, softwood requires higher temperature than hardwood for pretreatment. Hence, if softwood bark in this thesis is extracted at the temperature above 75°C, VFAs yield could be higher than the estimated yield. Given that 200 $mLCH_4/gVS_{added}$ is obtained after a higher temperature is applied for bark pretreatment, it is expected that the yield of VFAs is doubled (0.363 $gAcetate/gVS_{added}$). However, when increasing the temperature particularly higher than 120°C (Yin et al.,2014), the loss of VS should be taken into account carefully.

However, as the theory part showed, besides temperature, another factors affecting VFAs yields worth mentioning are pH and OLRs. There is no real waste stream tested yet in this thesis. So more attention should be paid for these factors when setting up a real experiment. In fact, the methane yield taken from Rasi et al.(2019) was observed at pH 7 to 8 because this study aimed to examine methane production. However, as shown in Figure 7a, this thesis proposed that the VFAs production is conducted at pH 6. Thus, the yield of 0.179 $gAcetate/gVS_{added}$ is assumedly obtained at pH 6. A question is raised when comparing to other VFAs experiments conditions. For example, Moretto et al.(2019) conducted established a VFAs experiment with urban organic waste (mixture of food waste and biological sludge) at pH 9. The waste also has high amount of VS content (80%), which was also thermally pretreated at low temperature of 72°C but 0.77 gVFAs was produced from one gram initial VS of the urban organic waste in mesophilic condition of acid fermentation (Moretto et al.,2019). Therefore, it is suggested that in a real experiment in a near future, an acidic or alkaline pH could be examined for the acid fermentation of barks.

Regarding OLR, the study of Moretto et al.(2019) resulted a high yield of $0.77gVFAs/gVS_{initial}$ obtained at higher OLR of $7.7kgVS/m^{-3}d^{-1}$ and HRT of 6 days. Furthermore, the experiment pointed out that higher OLRs ($9.3kgVS/m^{-3}d^{-1}$ and $11.3kgVS/m^{-3}d^{-1}$) than the optimal OLR of $7.7kgVS/m^{-3}d^{-1}$ showed decreasing VFAs yields. Hence, a real experiment for the barks acid fermentation could start from lower OLR limit in the theoretical range (in Chapter 4) such as 5 g $VS/d^{-1}L^{-1}$ and increase gradually to higher OLRs in the range.

It is worth noticing that the structural and chemical properties of a specific plant affect significantly the pretreatment performance (Yerdo et al.,2015). In fact, the methane yield from pretreated bark used for VFAs calculation in this thesis is the yield from pine bark, whereas the methane yield from extracted spruce bark showed much lower value. However, chapter 2 gives that pine and spruce woods account for 45.4% and 38.1%, respectively. The remaining portion is hardwood. Thus, a relatively big share of spruce bark is observed. However, much lower VFAs yield is expected if spruce bark is used, about 0.100 gAcetate/g VS_{added} (55 $mLCH_4/gVS_{added}$). So in a real experiment, VFAs yield can be lower than the expected yield in this study if spruce bark is deployed. It is important to select an optimal condition to pretreat the spruce bark for high yield of VFAs. A suggestion is that the barks of these wood types should be mixed and pretreated at higher temperature than 75°C for a better result.

Besides VFAs are the main products of acid fermentation process, as a bioprocess, the acid fermentation can discharge a side stream in a form of liquid and solid mixture which could be valorized for other purposes. Thus, it is essential to investigate the composition and content of the side stream so that no potential resource is wasted.

6.1.2 VFAs yield from the acid fermentation of sludge

The results from calculations show that VFAs yield from thermally pretreated sludge is $0.435kgAcetate/kgVS_{added}$. Meanwhile, if sludge is not pretreated, the amount of VFAs per one gram VS of fed sludge is 0.082 gram. With referencing to methane yields in Table 7, untreated sludge produced only 45 mL CH_4 per gram VS, whereas when the temperature was increasing gradually to 134°C in the study of Kinnunen et al.(2015), the methane yields had an increasing tendency. This indicates that the thermal hydrolysis could break extracellular polymeric structures of sludge. The results expected that thermal hydrolysis at 165°C in 45 minutes could improve bioconversion efficiency and enhance VFAs yield (Goycoechea et al.,2023).

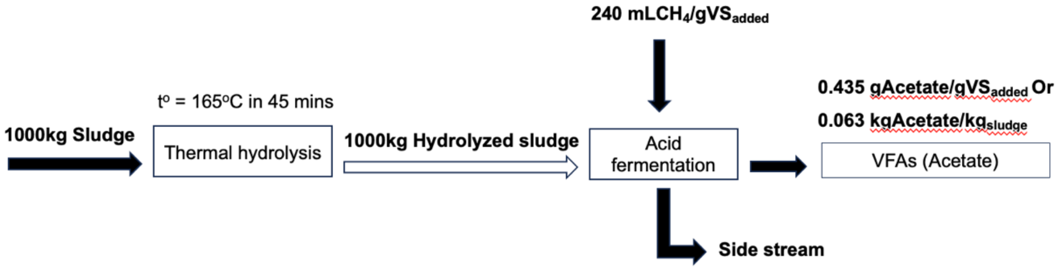


Figure 9. *VFAs yields from sludge.*

VFAs yield against fed sludge was also computed. The result shows that one kg sludge fed produces 0.063 kgAcetate. While the VFAs yields against the amount of VS from pretreated sludge is 2.5-times higher than that of bark, the yield from initial input of barks is nearly two times higher than that from fed sludge. The main reason for this low yield comes from only 20% of dry sludge can be taken from the original feedstock, from which the low amount of VS can be obtained.

In comparison with the VFAs yield from other feedstocks, it is not straightforward to compare such a result among sludges because there is no consistency in calculations among studies. The VFAs yield in this thesis is calculated based on the mass of acetate produced against VS added after pretreatment, not all VFAs produced in the acid fermentation. Meanwhile, Liu et al.(2018) as an example aimed to evaluate the potential of VFAs production from sewage sludge based on the mass of VFAs per volatile suspended solids (VSS). Another reason is different thermal pretreatment conditions which lead to incomparable VFAs yields. Specifically, the percentage of VS in the sludge of this thesis used is about 72%. In the study of Moretto et al.(2019) already mentioned above, the VS percentage of waste in the study has similar range with this thesis' sludge. However, Moretto et al.(2019) did a pretreatment experiment at 72°C but the yields were already much higher than that of this study while this study aims to achieve the calculated yield at 165°C. The difference in feedstock properties could be another reason contributing the challenges of VFAs yields comparison. For example, Liu et al.(2019) proved that sewage sludge with 45% VS was pretreated at 105°C and produced 0.38 kg VFAs/kg$VS_{initial}$ in anaerobic dynamic membrane reactor. It can be seen that the sewage sludge is characterized with lower percentage of VS than this study's sludge but the VFAs from the feedstock is not far different from this study's result. Without looking at the sewage sludge characteristic and thermal temperatures pretreatment, it can be said that the VFAs yield in this thesis (0.435gAcetate/gVS_{added} or 0.435kgAcetate/kgVS_{added} equivalent) is higher than that yield from the sewage sludge study of Liu et al.(2019). But it cannot be concluded which study performs more efficiently than the others in this context.

It is worth noticing that the thermal operating condition assumed in this thesis was taken from the study of Goycoechea et al.(2023) with the hypothesis that the Finnish PPI could produce the same methane yield in this condition. However, Goycoechea et al.(2023) utilized sludge from a Kraft cellulose production plant for their experiment in Uruguay. In fact, the difference in geography can already cause the inconsistency in waste characteristics due to the genre of planted woods even if the waste comes from the same process in PPI. In this case, Uruguay and Finland might have distinct practice in pulp and paper processing as well as the geographical difference. Thus there might be smaller or larger amount of VFAs in a real experiment. However, 165°C in 45 minutes is a promising thermal hydrolysis condition for PPI biosludge. This is because Kinnunen et al.(2015) concluded in their experiment for a sample from Finnish PPI that there is an increasing methane yield for pretreated biosludge at rising temperatures of 80°C, 105°C, 121°C and 134°C. It is expected that this higher temperature can enhance the solubility of the organic matters and produce a similar yield as calculated.

6.2 PHAs yield from barks and sludge

Regarding the PHAs yield from barks, as shown in Figure 10 and Figure 11, the P3HB and PHBV yields were calculated based on the mass of these PHAs produced from VFAs rich stream obtained from the acid fermentation of pretreated bark. The maximal theoretical yields of these PHAs were found about 0.118 gP3HB/gVS$_{added}$ and 0.110 gPHBV/gVS$_{added}$ from 0.179 gVFAs/gVS$_{added}$. It can be seen that the yields between P3HB and PHBV are not far different from each other. This could be affected by the assumed MWs for these polymers. When comparing these PHAs yields in this thesis to other studies, the yields are low. In fact, it is not straightforward to indicate the reason of this low yield. This is because the PHAs yields were theoretically obtained from VFAs loading calculated against the amount of VS (0.179 gVFAs/gVS$_{added}$). In the meantime, Vu et al.(2021) experimented with VFAs from fermented food waste and got higher yield with 0.23 gPHAs/gVFAs. However, the experiment was done with VFAs concentration of 5gVFAs/L. In the study of Al et al.(2021), they also obtained 0.31 gPHAs from one gram synthetic VFAs in 10 gVFAs/L medium.

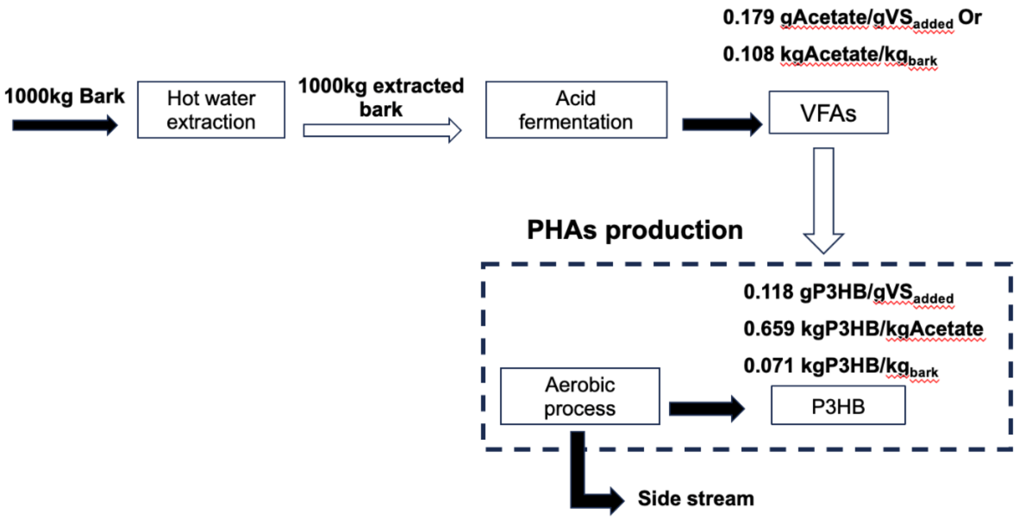


Figure 10. *A P3HB yield from bark-derived VFAs.*

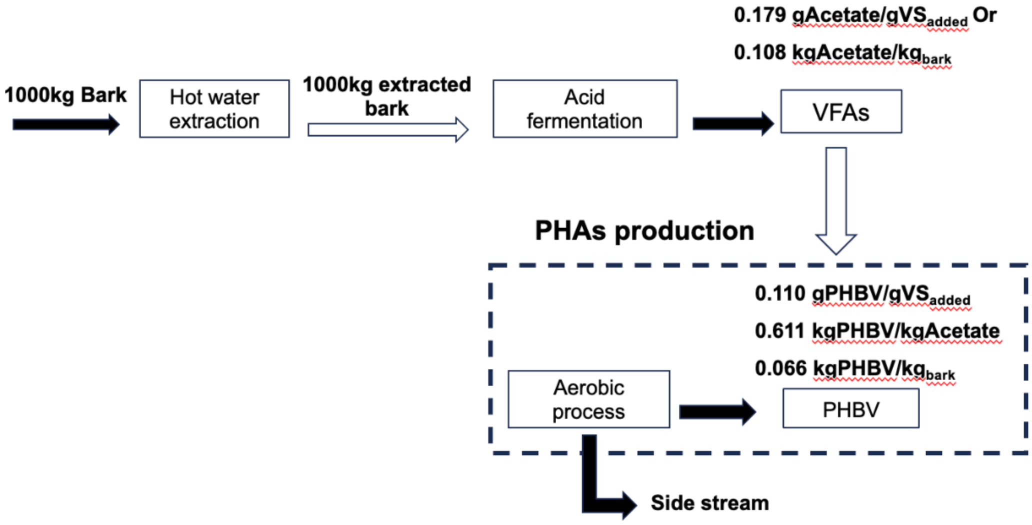


Figure 11. *A PHBV yield from bark-derived VFAs.*

Arslan et al.(2016) in Wang et al.(2021) stated that a range of VFAs for PHAs production is from 0.1 – 0.6g per one gram VS. This indicates that the VFAs yields from fermented barks in this study is reasonable. However, to obtain the theoretical maximum yields, substrate loadings should be carefully selected in a real experiment in future. This is because a too high substrate loading can inhibit the growth of microbes, thereby killing biomass and consequently decreasing the concentration of PHAs (Wang et al.,2021). In addition to the substrate loading, microbial culture plays an important role in storing PHAs content. This thesis assumed to utilized mixed microbial culture for production cost reduction. However, it is worthy noted that each microbe's species can accumulate different amount of PHAs (Ben et al.,2016). Thus, it is suggested to check the mixed microbial culture with a high storage capacity.

When it comes to possible capacities of P3HB and PHBV estimated per year from barks derived VFAs, with approximately 321,970-ton discharged barks every year, 23,011 ton P3HB and 21,329 ton PHBV could be produced annually. According to Wang et al.(2022), PHAs is currently produced at industrial scale with the range from 10,000 metric ton/year to 50,000 metric ton/year. So the capacities calculated in this thesis show a promising future for PHAs production from barks at a large scale. Similar to VFAs production, it is predicted that there can be a side stream from the aerobic process in PHAs production as a form of liquid and solid mixture which needs to be considered.

About the PHAs yields from sludge, the amount of P3HB produced against VS added from fermented sludge derived-VFAs is 0.287g/gVS$_{added}$ while 0.266g/gVS$_{added}$ is the yield for PHBV. It can be seen that the yields of P3HB and PHBV obtained from sludge are more than two times higher than those from barks. The results are quite understandable because there is more than doubled amount of VFAs (0.435gVFAs/gVS$_{added}$) produced from the acid fermentation of sludge as compared to that from barks. The thesis tried to estimate the amounts of these two PHAs from obtained VFAs in the acid fermentation process for sludge. However, because the estimations are simply done based on the mass of acetate, stoichiometry coefficients and MWs, the mass of acetate appears in a numerator and denominator of one calculation which is then cancelled each other. As a result, the P3HB yields against VFAs are the same for the barks case and the sludge case (0.659kgP3HB/kgAcetate). The same situation is repeated for PHBV yields against VFAs (0.611kgPHBV/kgAcetate).

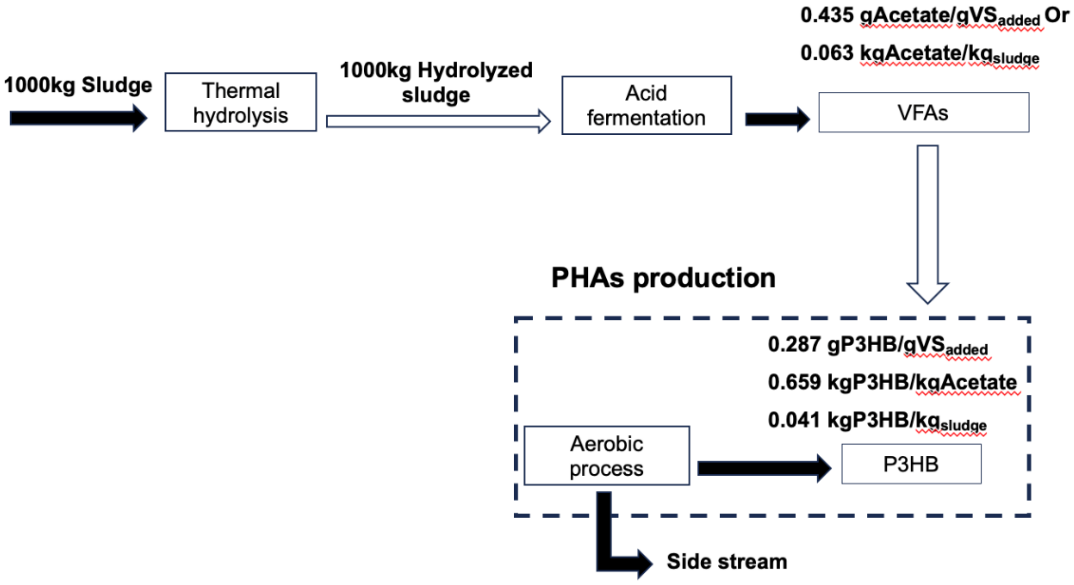


Figure 12. *A P3HB yield from sludge-derived VFAs.*

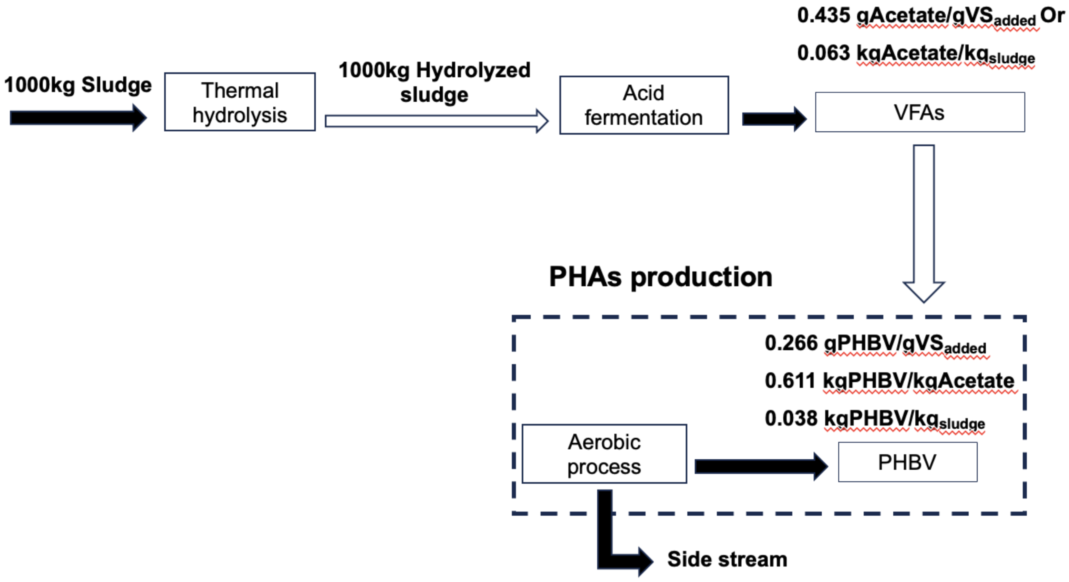


Figure 13. *A PHBV yield from sludge-derived VFAs.*

Regarding PHAs yields against the initial mass of sludge, even though sludge contains high content of VS in total solids (72%), only 0.041 kgP3HB/kg$_{sludge}$ and 0.038kgPHBV/kg$_{sludge}$ could be produced. The reason was already explained in the VFAs yield from fed sludge. All results are presented in the Figure 12 and Figure 13.

Besides the issue of substrate loadings mentioned in the previous section, it is essential to evaluate potential inhibitors in pretreated sludge. As the proposed temperature is 165°C, but chapter 3 showed that some inhibitors such as furfural and hydroxymethylfurfural (HMF) can be formed when the temperature exceeds 160°C. It is not clear yet the impacts of these inhibitors on PHAs yields. However, it is observed that phenols in cashew wastewater affect cellular function to death, decreasing biomass formation and then PHAs accumulation in cells. (Katagi et al.,2023)

This thesis assumed acetate is the main product of an acid fermentation. However, chapter 4 already indicated that there are several metabolic routes in an acid fermentation as well as the impacts of the fermentation conditions, resulting in various VFAs compositions. Therefore, VFAs compositions may be distributed differently in a real experiment. Consequently, PHAs products may change because of the correlation of VFAs.

Regarding estimated annual capacities of P3HB and PHBV production from sludge, it is seen that 58,375 ton of discharged sludge could produce 2,409 ton P3HB and 2,233 ton PHBV yearly. Compared to the capacities from bark, these values are much lower because of the lower PHAs yield from initial amount of sludge. In addition, the discharged amount of bark is 6-fold higher than sludge. Therefore, sludge seems not to be considered as a promising feedstock for PHAs production at commercial scales.

6.3 Limitations of the study

There are some limitations in this study. First, data for the estimations are secondary, which were collected from the experimental and survey results of articles and Google search. Thus, there are some bias for the data which were primarily produced by the authors for their purpose and consequently the data do not meet the current study' needs. For example, pretreatment conditions from other studies were used for this thesis' hypothesis. However, these conditions were assigned for other feedstocks which do not share the same properties with the feedstocks in this thesis. In addition, the conditions were applied for the purpose of investigating potential methane production, not for VFAs production. So for a real sample in a near future experiment, there might be distinct differences compared to the results obtained in this thesis. Secondly, the secondary data are not timely. For example, the data of waste streams volumes were collected in 2016 – 2017, causing the estimation for PHAs capacities may not reflect precisely current real situation.

Another limitation of the thesis is that the calculations for the yields of P3HB and PHBV were done by using the stoichiometric way which does not use the restriction of the existing natural production pathways. Many assumptions were also made to simplify the calculations, which may have influenced the correctness of the estimations. For instance, the data of methane yields from AD process were obtained from other studies for this thesis' VFAs calculation. Some other data such as the percentage of VS, dry content, MWs of PHB and PHBV, as well as the percentage of acetate conversion to methane were also assumed from theoretical values. In addition, the loss of VS was also negligible due to the lack of data. Therefore, future research on a real experiment for the topic is required to provide more accurate answers on the proposed research questions.

7. CONCLUSIONS

This study found that bark and sludge are the most feasible streams from Finnish PPI, which could be accessible and sustainable low-cost feedstocks for VFAs production. This study also demonstrated the efficient performance of pretreatment approaches in overcoming hydrolysis step in acid fermentation and promoting bioavailable substrates for microorganisms, contributing to the enhancement of VFAs yields. A hot water extraction at 72°C in 60 minutes could be applied for breaking recalcitrant compounds in barks, whereas thermal hydrolysis at 165°C in 45 minutes could hydrolyze pulp and paper sludge into soluble organic matters.

It is estimated that the acid fermentation of extracted barks could produce 0.179gAcetate/gVS$_{added}$ or 0.108kgAcetate/kg$_{bark}$. This VFAs could be valorized to produce 0.118gP3HB/gVS$_{added}$ (0.071kgP3HB/kg$_{bark}$) and 0.110gPHBV/gVS$_{added}$ (0.066kgPHBV/kg$_{bark}$). However, the study also estimated a higher VFAs yield (0.363 gAcetate/gVS$_{added}$) from barks could be obtained based on the assumed methane yield at higher temperature than 72°C. Moreover, spruce barks and pine barks should be mixed rather than using spruce barks alone because spruce barks give much lower VFAs yields in the same hydrolysis condition.

Meanwhile, for sludge, the hydrolyzed sludge could yield 0.435gAcetate/gVS$_{added}$ or 0.063 kgAcetate/kg$_{sludge}$ which can be the substrates for microbes to accumulate and convert into 0.287gP3HB/gVS$_{added}$ (0.041 kgP3HB/kg$_{sludge}$) or 0.266 gPHBV/gVS$_{added}$ (0.038 kgPHBV/kg$_{sludge}$). There are some challenges in comparing the VFAs yields obtained in this thesis with other studies because of the differences in feedstocks properties, research aims and pretreatment conditions. But according to the VFAs yields against VS results, both barks and sludges show as promising feedstocks for PHAs production. However, based on the capacity's results, this thesis also pointed out that bark is a more promising feedstock for PHAs production at large scales.

Further research on real barks and sludge should be conducted to overcome given study limitations. After influent parameters of the real feedstocks are tested, some operational conditions for acid fermentation and PHAs production such as OLRs, pH, temperature, HRTs, C/N can be determined more specifically. Furthermore, more attention should be paid on potential side streams from the acid fermentation process of VFAs production and the aerobic process of PHAs production.

Overall, this study opened up a possibility of combining a pretreatment unit with acid fermentation for side and waste streams from Finnish PPI to produce VFAs and valorize VFAs rich stream. The VFAs produced from the waste and side streams can be served as sustainable platform chemicals for PHAs production. In addition, the VFAs production without further downstream processing can reduce PHAs production cost, aiding to lower bio-PHAs price and compete with sugar-based PHAs.

REFERENCES

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

Ahmed, B., Aboudi, K., Tyagi, V. K., Álvarez-Gallego, C. J., Fernández-Güelfo, L. A., Romero-García, L. I., & Kazmi, A. A. (2019). Improvement of Anaerobic Digestion of Lignocellulosic Biomass by Hydrothermal Pretreatment. *Applied Sciences*, *9*(18), 3853-. https://doi.org/10.3390/app9183853

Al Battashi, H., Al-Kindi, S., Gupta, V. K., & Sivakumar, N. (2021). Polyhydroxyalkanoate (PHA) Production Using Volatile Fatty Acids Derived from the Anaerobic Digestion of Waste Paper. *Journal of Polymers and the Environment*, *29*(1), 250–259. https://doi.org/10.1007/s10924-020-01870-0

Albuquerque, M. G. E., Torres, C. A. V., & Reis, M. A. M. (2010). Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: Effect of the influent substrate concentration on culture selection. *Water Research (Oxford)*, *44*(11), 3419–3433. https://doi.org/10.1016/j.watres.2010.03.021

Alves, E. P. R., Salcedo-Puerto, O., Nuncira, J., Emebu, S., & Mendoza-Martinez, C. (2023). Renewable Energy Potential and CO2 Performance of Main Biomasses Used in Brazil. *Energies (Basel)*, *16*(9), 3959-. https://doi.org/10.3390/en16093959

Andérez Fernández, M., Rissanen, J., Pérez Nebreda, A., Xu, C., Willför, S., García Serna, J., Salmi, T., & Grénman, H. (2018). Hemicelluloses from stone pine, holm oak, and Norway spruce with subcritical water extraction − comparative study with characterization and kinetics. *The Journal of Supercritical Fluids*, *133*, 647–657. https://doi.org/10.1016/j.supflu.2017.07.001

Arslan, D., Steinbusch, K. J. J., Diels, L., Hamelers, H. V. M., Strik, D. P. B. T. B., Buisman, C. J. N., & De Wever, H. (2016). Selective short-chain carboxylates production: A review of control mechanisms to direct mixed culture fermentations. *Critical Reviews in Environmental Science and Technology*, *46*(6), 592–634. https://doi.org/10.1080/10643389.2016.1145959

Asada, C., Sasaki, C., & Nakamura, Y. (2019). High Concentration Ethanol Production from Mixed Softwood Sawdust Waste. *Waste and Biomass Valorization*, *10*(2), 433–439. https://doi.org/10.1007/s12649-017-0073-0

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. *Bioresource Technology*, *268*, 773–786. https://doi.org/10.1016/j.biortech.2018.07.042

Baig, K. S., Wu, J., & Turcotte, G. (2019). Future prospects of delignification pretreatments for the lignocellulosic materials to produce second generation bioethanol. *International Journal of Energy Research*, *43*(4), 1411–1427. https://doi.org/10.1002/er.4292

Ben, M., Kennes, C., & Veiga, M. C. (2016). Optimization of polyhydroxyalkanoate storage using mixed cultures and brewery wastewater: Optimization of polyhydroxyalkanoate storage using mixed. *Journal of Chemical Technology and Biotechnology (1986)*, *91*(11), 2817–2826. https://doi.org/10.1002/jctb.4891

Bengtsson, S., Werker, A., Christensson, M., & Welander, T. (2008). Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. *Bioresource Technology*, *99*(3), 509–516. https://doi.org/10.1016/j.biortech.2007.01.020

Blasco, L., Kahala, M., Tampio, E., Vainio, M., Ervasti, S., & Rasi, S. (2020). Effect of Inoculum Pretreatment on the Composition of Microbial Communities in Anaerobic Digesters Producing Volatile Fatty Acids. *Microorganisms (Basel)*, *8*(4), 581-. https://doi.org/10.3390/microorganisms8040581

Bougrier, C., Delgenès, J. P., & Carrère, H. (2007). Impacts of thermal pre-treatments on the semi-continuous anaerobic digestion of waste activated sludge. *Biochemical Engineering Journal*, *34*(1), 20–27. https://doi.org/10.1016/j.bej.2006.11.013

Bugnicourt, E., Cinelli, P., Lazzeri, A., & Alvarez, V. (2014). Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging. *Express Polymer Letters*, *8*(11), 791–808. https://doi.org/10.3144/expresspolymlett.2014.82

Cai, F., Lin, M., Wang, L., Song, C., Jin, Y., Liu, G., & Chen, C. (2024). Enhancing acidification efficiency of vegetable wastes through heat shock pretreatment and initial pH regulation. *Environmental Science and Pollution Research International*, *31*(1), 1079–1093. https://doi.org/10.1007/s11356-023-31025-2

Cantero, D., Jara, R., Navarrete, A., Pelaz, L., Queiroz, J., Rodríguez-Rojo, S., & Cocero, M. J. (2019). Pretreatment Processes of Biomass for Biorefineries: Current Status and

Prospects. *Annual Review of Chemical and Biomolecular Engineering*, *10*(1), 289–310. https://doi.org/10.1146/annurev-chembioeng-060718-030354

Castilla-Archilla, J., Papirio, S., & Lens, P. N. L. (2021). Two step process for volatile fatty acid production from brewery spent grain: Hydrolysis and direct acidogenic fermentation using anaerobic granular sludge. *Process Biochemistry (1991)*, *100*, 272–283. https://doi.org/10.1016/j.procbio.2020.10.011

Chakraborty, P., Gibbons, W., & Muthukumarappan, K. (2009). Conversion of volatile fatty acids into polyhydroxyalkanoate by Ralstonia eutropha. *Journal of Applied Microbiology*, *106*(6), 1996–2005. https://doi.org/10.1111/j.1365-2672.2009.04158.x

Chandani Devi, N., Mazumder, P. B., & Bhattacharjee, A. (2018). Statistical Optimization of Polyhydroxybutyrate Production by Bacillus Pumilus H9 Using Cow Dung as a Cheap Carbon Source by Response Surface Methodology. *Journal of Polymers and the Environment*, *26*(8), 3159–3167. https://doi.org/10.1007/s10924-018-1194-7

Chen, J., Li, W., Zhang, Z.-Z., Tan, T.-W., & Li, Z.-J. (2018). Metabolic engineering of Escherichia coli for the synthesis of polyhydroxyalkanoates using acetate as a main carbon source. *Microbial Cell Factories, 17*(1), 102–102. https://doi.org/10.1186/s12934-018-0949-0

Chen, Y., Jiang, X., Xiao, K., Shen, N., Zeng, R. J., & Zhou, Y. (2017). Enhanced volatile fatty acids (VFAs) production in a thermophilic fermenter with stepwise pH increase – Investigation on dissolved organic matter transformation and microbial community shift. *Water Research (Oxford), 112,* 261–268. https://doi.org/10.1016/j.watres.2017.01.067

Choi, J.-M., Han, S.-K., & Lee, C.-Y. (2018). Enhancement of methane production in anaerobic digestion of sewage sludge by thermal hydrolysis pretreatment. *Bioresource Technology, 259,* 207–213. https://doi.org/10.1016/j.biortech.2018.02.123

Crognale, S., Tonanzi, B., Valentino, F., Majone, M., & Rossetti, S. (2019). Microbiome dynamics and phaC synthase genes selected in a pilot plant producing polyhydroxyalkanoate from the organic fraction of urban waste. *The Science of the Total Environment*, *689*, 765–773. https://doi.org/10.1016/j.scitotenv.2019.06.491

Doddapaneni, T. R. K. C., Cahyanti, M. N., Orupõld, K., & Kikas, T. (2022). Integrating Torrefaction of Pulp Industry Sludge with Anaerobic Digestion to Produce Biomethane and Volatile Fatty Acids: An Example of Industrial Symbiosis for Circular Bioeconomy. *Fermentation (Basel), 8*(9), 453-. https://doi.org/10.3390/fermentation8090453

Durak, H. (2023). Comprehensive Assessment of Thermochemical Processes for Sustainable Waste Management and Resource Recovery. *Processes, 11*(7), 2092-. https://doi.org/10.3390/pr11072092

Englatina I.N.C. Assis, & Evans M.N. Chirwa. (2021). Physicochemical Characteristics of Different Pulp and Paper Mill Waste Streams for Hydrothermal Conversion. *Chemical Engineering Transactions*, *86*. https://doi.org/10.3303/CET2186102

Eskicioglu, C., Monlau, F., Barakat, A., Ferrer, I., Kaparaju, P., Trably, E., & Carrère, H. (2017). Assessment of hydrothermal pretreatment of various lignocellulosic biomass with CO2 catalyst for enhanced methane and hydrogen production. *Water Research (Oxford), 120*, 32–42. https://doi.org/10.1016/j.watres.2017.04.068

Esteban-Gutiérrez, M., Garcia-Aguirre, J., Irizar, I., & Aymerich, E. (2018). From sewage sludge and agri-food waste to VFA: Individual acid production potential and up-scaling. *Waste Management (Elmsford), 77*, 203–212. https://doi.org/10.1016/j.wasman.2018.05.027

Fang, W., Zhang, X., Zhang, P., Wan, J., Guo, H., Ghasimi, D. S. M., 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. *Journal of Environmental Sciences (China), 87,* 93–111. https://doi.org/10.1016/j.jes.2019.05.027

Ferre-Guell, A., & Winterburn, J. (2018). Biosynthesis and Characterization of Polyhydroxyalkanoates with Controlled Composition and Microstructure. *Biomacromolecules*, *19*(3), 996–1005. https://doi.org/10.1021/acs.biomac.7b01788

Finnish Government (2022). Bioeconomy Strategy 2022 – 2035 Sustainably towards higher value added. Available: https://valtioneuvosto.fi/en/-//1410877/bioeconomy-strategy-2022-2035-sustainably-towards-higher-value-added. Accessed: 15 January 2024.

Fioreze, M., Labotić, L., Torres, C. M. M. E., & Silva, C. M. (2022). Effects of pretreatments on the solubilization and theoretical methane production of waste activated sludge from a Brazilian eucalyptus kraft pulp mill. *Bioresources, 17*(3), 5300–5318. https://doi.org/10.15376/biores.17.3.5300-5318

Gahlot, P., Balasundaram, G., Tyagi, V. K., Atabani, A. E., Suthar, S., Kazmi, A. A., Štěpanec, L., Juchelková, D., & Kumar, A. (2022). Principles and potential of thermal hydrolysis of sewage sludge to enhance anaerobic digestion. *Environmental Research, 214*, 113856–113856. https://doi.org/10.1016/j.envres.2022.113856

Gallina, G., Cabeza, Á., Grénman, H., Biasi, P., García-Serna, J., & Salmi, T. (2018). Hemicellulose extraction by hot pressurized water pretreatment at 160°C for 10 different woods: Yield and molecular weight. *The Journal of Supercritical Fluids, 133*, 716–725. https://doi.org/10.1016/j.supflu.2017.10.001

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. *Bioresource Technology*, *244*(Pt 1), 1081–1088. https://doi.org/10.1016/j.biortech.2017.07.187

Ghimire, N., Bakke, R., & Bergland, W. H. (2021). Mesophilic Anaerobic Digestion of Hydrothermally Pretreated Lignocellulosic Biomass (Norway Spruce (Picea abies)). *Processes, 9*(2), 190-. https://doi.org/10.3390/pr9020190

Gong, G., Wu, B., Liu, L., Li, J., Zhu, Q., He, M., & Hu, G. (2022). Metabolic engineering using acetate as a promising building block for the production of bio-based chemicals. *Engineering Microbiology*, *2*(4), 100036-. https://doi.org/10.1016/j.engmic.2022.100036

Gottardo, M., Bolzonella, D., Adele Tuci, G., Valentino, F., Majone, M., Pavan, P., & Battista, F. (2022). Producing volatile fatty acids and polyhydroxyalkanoates from foods by-products and waste: A review. *Bioresource Technology, 361*, 127716–127716. https://doi.org/10.1016/j.biortech.2022.127716

Goycoechea, N., Borges, I., Castello, E., & Borzacconi, L. (2023). Improvements in the anaerobic digestion of biological sludge from pulp and paper mills using thermal pretreatment. *Waste Management & Research, 41*(8), 1331–1341. https://doi.org/10.1177/0734242X231154198

Greses, S., Tomás-Pejó, E., & Gónzalez-Fernández, C. (2020). Agroindustrial waste as a resource for volatile fatty acids production via anaerobic fermentation. *Bioresource Technology*, *297*, 122486–122486. https://doi.org/10.1016/j.biortech.2019.122486

Guigou, M., Cabrera, M. N., Vique, M., Bariani, M., Guarino, J., Ferrari, M. D., & Lareo, C. (2019). Combined pretreatments of eucalyptus sawdust for ethanol production within a biorefinery approach. *Biomass Conversion and Biorefinery, 9*(2), 293–304. https://doi.org/10.1007/s13399-018-0353-3

Haile, A., Gelebo, G. G., Tesfaye, T., Mengie, W., Mebrate, M. A., Abuhay, A., & Limeneh, D. Y. (2021). Pulp and paper mill wastes: utilizations and prospects for high value-added biomaterials. *Bioresources and Bioprocessing, 8*(1), 1–22. https://doi.org/10.1186/s40643-021-00385-3

Hassan, Md. K., Villa, A., Kuittinen, S., Jänis, J., & Pappinen, A. (2019). An assessment of side-stream generation from Finnish forest industry. *Journal of Material Cycles and Waste Management, 21*(2), 265–280. https://doi.org/10.1007/s10163-018-0787-5

Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology, 100*(1), 10–18. https://doi.org/10.1016/j.biortech.2008.05.027

Iglesias-Iglesias, R., Campanaro, S., Treu, L., Kennes, C., & Veiga, M. C. (2019). Valorization of sewage sludge for volatile fatty acids production and role of microbiome on acidogenic fermentation. *Bioresource Technology, 291*, 121817–121817. https://doi.org/10.1016/j.biortech.2019.121817

Jankowska, E., Chwiałkowska, J., Stodolny, M., & Oleskowicz-Popiel, P. (2015). Effect of pH and retention time on volatile fatty acids production during mixed culture fermentation. *Bioresource Technology*, *190*, 274–280. https://doi.org/10.1016/j.biortech.2015.04.096

Jariyaboon R, Hayeeyunu S, Usmanbaha N, Ismail SB, O-Thong S, Mamimin C, Kongjan P. Thermophilic Dark Fermentation for Simultaneous Mixed Volatile Fatty Acids and Biohydrogen Production from Food Waste. *Fermentation*. 2023; 9(7):636. https://doi.org/10.3390/fermentation9070636

Jonsson, L. J., & Martin, C. (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology, 199*, 103–112. https://doi.org/10.1016/j.biortech.2015.10.009

Kacanski, M., Pucher, L., Peral, C., Dietrich, T., & Neureiter, M. (2022). Cell Retention as a Viable Strategy for PHA Production from Diluted VFAs with Bacillus megaterium. *Bioengineering (Basel), 9*(3), 122-. https://doi.org/10.3390/bioengineering9030122

Katagi, V. N., Manasa S, Raghavendra P, Bhat, S. G., & M S, D. (2023). Valorization of cashew industry wastewater as a carbon and nutrient source for the microbial growth and production of the polyhydroxyalkanoates: A potential biopolymer by Bacillus species. *Cogent Engineering, 10*(2). https://doi.org/10.1080/23311916.2023.2269652

Kaur, R., Tyagi, R. D., & Zhang, X. (2020). Review on pulp and paper activated sludge pretreatment, inhibitory effects and detoxification strategies for biovalorization. *Environmental Research, 182*, 109094-. https://doi.org/10.1016/j.envres.2019.109094

Kedia, G., Passanha, P., Dinsdale, R. M., Guwy, A. J., & Esteves, S. R. (2014). Evaluation of feeding regimes to enhance PHA production using acetic and butyric acids by a pure culture of Cupriavidus necator. *Biotechnology and Bioprocess Engineering, 19*(6), 989–995. https://doi.org/10.1007/s12257-014-0144-z

Khanh Nguyen, V., Kumar Chaudhary, D., Hari Dahal, R., Hoang Trinh, N., Kim, J., Chang, S. W., Hong, Y., Duc La, D., Nguyen, X. C., Hao Ngo, H., Chung, W. J., & Nguyen, D. D. (2021). Review on pretreatment techniques to improve anaerobic digestion of sewage sludge. *Fuel (Guildford), 285,* 119105-. https://doi.org/10.1016/j.fuel.2020.119105

Kinnunen, V., Ylä-Outinen, A., & Rintala, J. (2015). Mesophilic anaerobic digestion of pulp and paper industry biosludge–long-term reactor performance and effects of thermal pretreatment. *Water Research (Oxford), 87*, 105–111. https://doi.org/10.1016/j.watres.2015.08.053

Kumar, V., Yadav, S. K., Kumar, J., & Ahluwalia, V. (2020). A critical review on current strategies and trends employed for removal of inhibitors and toxic materials generated during biomass pretreatment. *Bioresource Technology, 299*, 122633–122633. https://doi.org/10.1016/j.biortech.2019.122633

Kuruti, K., Nakkasunchi, S., Begum, S., Juntupally, S., Arelli, V., & Anupoju, G. R. (2017). Rapid generation of volatile fatty acids (VFA) through anaerobic acidification of livestock organic waste at low hydraulic residence time (HRT). *Bioresource Technology*, *238*, 188–193. https://doi.org/10.1016/j.biortech.2017.04.005

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2014). A review of the production and applications of waste-derived volatile fatty acids. *Chemical Engineering Journal (Lausanne, Switzerland : 1996), 235*, 83–99. https://doi.org/10.1016/j.cej.2013.09.002

Leszczyński, M., & Roman, K. (2023). Hot-Water Extraction (HWE) Method as Applied to Lignocellulosic Materials from Hemp Stalk. *Energies (Basel), 16*(12), 4750-. https://doi.org/10.3390/en16124750

Li, P., Xu, Y., Yin, L., Liang, X., Wang, R., & Liu, K. (2023). Development of Raw Materials and Technology for Pulping—A Brief Review. *Polymers, 15*(22), 4465-. https://doi.org/10.3390/polym15224465

Li, Q., Liu, Y., Yang, X., Zhang, J., Lu, B., & Chen, R. (2020). Kinetic and thermodynamic effects of temperature on methanogenic degradation of acetate, propionate, butyrate and valerate. *Chemical Engineering Journal (Lausanne, Switzerland : 1996), 396*, 125366-. https://doi.org/10.1016/j.cej.2020.125366

Lipiäinen, S., Kuparinen, K., Sermyagina, E., & Vakkilainen, E. (2022). Pulp and paper industry in energy transition: Towards energy-efficient and low carbon operation in Finland and Sweden. *Sustainable Production and Consumption*, *29*, 421–431. https://doi.org/10.1016/j.spc.2021.10.029

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. *Bioresource Technology*, *260*, 105–114. https://doi.org/10.1016/j.biortech.2018.03.105

Liu, H., Wang, L., Zhang, X., Fu, B., Liu, H., Li, Y., & Lu, X. (2019). A viable approach for commercial VFAs production from sludge: Liquid fermentation in anaerobic dynamic membrane reactor. *Journal of Hazardous Materials*, *365*, 912–920. https://doi.org/10.1016/j.jhazmat.2018.11.082

Llamas, M., Dourou, M., González-Fernández, C., Aggelis, G., & Tomás-Pejó, E. (2020). Screening of oleaginous yeasts for lipid production using volatile fatty acids as substrate. *Biomass & Bioenergy*, *138*, 105553-. https://doi.org/10.1016/j.biombioe.2020.105553

Llamas, M., Magdalena, J. A., González-Fernández, C., & Tomás-Pejó, E. (2020). Volatile fatty acids as novel building blocks for oil-based chemistry via oleaginous yeast fermentation. *Biotechnology and Bioengineering*, *117*(1), 238–250. https://doi.org/10.1002/bit.27180

Llamas, M., Tomás-Pejó, E., & González-Fernández, C. (2020). Volatile fatty acids from organic wastes as novel low-cost carbon source for Yarrowia lipolytica. *New Biotechnology*, *56*, 123–129. https://doi.org/10.1016/j.nbt.2020.01.002

Luke (2022). Log removals reached a new record in 2021. Available: https://www.luke.fi/en/news/log-removals-reached-a-new-record-in-2021#:~:text=According%20to%20an%20estimate%20prepared,year%20period%20of%202016–2025.. Accessed: 9th August 2023.

Luo, J., Li, Y., Li, H., Li, Y., Lin, L., Li, Y., Huang, W., Cao, J., & Wu, Y. (2022). Deciphering the key operational factors and microbial features associated with volatile fatty acids production during paper wastes and sewage sludge co-fermentation. *Bioresource Technology*, *344*(Pt B), 126318–126318. https://doi.org/10.1016/j.biortech.2021.126318

Lv, N., Cai, G., Pan, X., Li, Y., Wang, R., Li, J., Li, C., & Zhu, G. (2022). pH and hydraulic retention time regulation for anaerobic fermentation: Focus on volatile fatty acids production/distribution, microbial community succession and interactive correlation. *Bioresource Technology*, *347*, 126310–126310. https://doi.org/10.1016/j.biortech.2021.126310

Metsäteollisuus (2022) Forest resources in Finland. Available: https://www.metsateollisuus.fi/newsroom/forest-resources-in-finland. Accessed: 20 January 2024.

Metsäteollisuus (2023) Facts about Finnish forests. Available: https://www.metsateollisuus.fi/newsroom/facts-about-finnish-forests. Accessed: 20 January 2024.

Metsäteollisuus (2023) Forest industry export market. Available: https://www.metsateollisuus.fi/newsroom/forest-industry-export-market. Accessed: 20 January 2024.

Millati, R., Wikandari, R., Ariyanto, T., Putri, R. U., & Taherzadeh, M. J. (2020). Pretreatment technologies for anaerobic digestion of lignocelluloses and toxic feedstocks. *Bioresource Technology, 304*, 122998–122998. https://doi.org/10.1016/j.biortech.2020.122998

Mittal, M., Bhuwal, A., Sharma, P., & Aggarwal, N. K. (2023). Utilization of pulp and paper industrial wastewater for production of polyhydroxybutyrate by Bacillus sonorensis NAM5. *Systems Microbiology and Biomanufacturing, 3*(4), 805–818. https://doi.org/10.1007/s43393-023-00164-5

Modenbach, A. A., & Nokes, S. E. (2012). The use of high-solids loadings in biomass pretreatment-a review. *Biotechnology and Bioengineering, 109*(6), 1430–1442. https://doi.org/10.1002/bit.24464

Mohanakrishna, G., Sneha, N. P., Rafi, S. M., & Sarkar, O. (2023). Dark fermentative hydrogen production: Potential of food waste as future energy needs. *The Science of the Total Environment, 888*, 163801–163801. https://doi.org/10.1016/j.scitotenv.2023.163801

Moretto, G., Valentino, F., Pavan, P., Majone, M., & Bolzonella, D. (2019). Optimization of urban waste fermentation for volatile fatty acids production. *Waste Management (Elmsford), 92*, 21–29. https://doi.org/10.1016/j.wasman.2019.05.010

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 Research (Oxford), 44*(18), 5196–5211. https://doi.org/10.1016/j.watres.2010.06.043

Morya, R., Andrianantenaina, F. H., Pandey, A. K., Yoon, Y. H., & Kim, S.-H. (2023). Polyhydroxyalkanoate production from rice straw hydrolysate: Insights into feast-famine dynamics and microbial community shifts. *Chemosphere (Oxford), 341*, 139967–139967. https://doi.org/10.1016/j.chemosphere.2023.139967

Mountfort, D. O., & Asher, R. A. (1978). Changes in proportions of acetate and carbon dioxide used as methane precursors during the anaerobic digestion of bovine waste. *Applied and Environmental Microbiology, 35*(4), 648–654. https://doi.org/10.1128/aem.35.4.648-654.1978

Munir, S., & Jamil, N. (2020). Polyhydroxyalkanoate (PHA) production in open mixed cultures using waste activated sludge as biomass. *Archives of Microbiology, 202*(7), 1907–1913. https://doi.org/10.1007/s00203-020-01912-0

Narayanan, M., Kandasamy, G., Murali P, Kandasamy, S., Ashokkumar, V., Nasif, O., & Pugazhendhi, A. (2021). Optimization and production of polyhydroxybutyrate from sludge by Bacillus cereus categorized through FT-IR and NMR analyses. *Journal of Environmental Chemical Engineering, 9*(1), 104908-. https://doi.org/10.1016/j.jece.2020.104908

Negi, A., & Kesari, K. K. (2023). Light-Driven Depolymerization of Cellulosic Biomass into Hydrocarbons. *Polymers, 15*(18), 3671-. https://doi.org/10.3390/polym15183671

Ngo, P. L., Udugama, I. A., Gernaey, K. V., Young, B. R., & Baroutian, S. (2021). Mechanisms, status, and challenges of thermal hydrolysis and advanced thermal hydrolysis processes in sewage sludge treatment. *Chemosphere (Oxford), 281*, 130890–130890. https://doi.org/10.1016/j.chemosphere.2021.130890

Pais, J., Serafim, L. S., Freitas, F., & Reis, M. A. M. (2016). Conversion of cheese whey into poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by Haloferax mediterranei. *New Biotechnology, 33*(1), 224–230. https://doi.org/10.1016/j.nbt.2015.06.001

Park, J.-K., Jeon, J.-M., Yang, Y.-H., Kim, S.-H., & Yoon, J.-J. (2024). Efficient polyhydroxybutyrate production using acetate by engineered Halomonas sp. JJY01 harboring acetyl-CoA acetyltransferase. *International Journal of Biological Macromolecules, 254*(Pt 1), 127475–127475. https://doi.org/10.1016/j.ijbiomac.2023.127475

Pittmann, T., & Steinmetz, H. (2014). Polyhydroxyalkanoate production as a side stream process on a municipal waste water treatment plant. *Bioresource Technology, 167*, 297–302. https://doi.org/10.1016/j.biortech.2014.06.037

Ranganadhareddy, A. (2023). A Review on Biotechnological Approaches for the Production of Polyhydroxyalkanoates. *Journal of Biochemical Technology, 14*(2), 12–17. https://doi.org/10.51847/Hxh14VrhOr

Rasi, S., Kilpeläinen, P., Rasa, K., Korpinen, R., Raitanen, J.-E., Vainio, M., Kitunen, V., Pulkkinen, H., & Jyske, T. (2019). Cascade processing of softwood bark with hot water extraction, pyrolysis and anaerobic digestion. Bioresource Technology, 292, 121893–121893. https://doi.org/10.1016/j.biortech.2019.121893

Rochón, E., Cabrera, M. N., Scutari, V., Cagno, M., Guibaud, A., Martínez, S., Böthig, S., Guchin, N., Ferrari, M. D., & Lareo, C. (2022). Co-production of bioethanol and xylosaccharides from steam-exploded eucalyptus sawdust using high solid loads in enzymatic hydrolysis: Effect of alkaline impregnation. *Industrial Crops and Products, 175*, 114253–. https://doi.org/10.1016/j.indcrop.2021.114253

Routa, J., Brännström, H., Hellström, J., & Laitila, J. (2021). Influence of storage on the physical and chemical properties of Scots pine bark. *Bioenergy Research*, *14*(2), 575–587. https://doi.org/10.1007/s12155-020-10206-8

Ruiz, H. A., Conrad, M., Sun, S.-N., Sanchez, A., Rocha, G. J. M., Romaní, A., Castro, E., Torres, A., Rodríguez-Jasso, R. M., Andrade, L. P., Smirnova, I., Sun, R.-C., & Meyer, A. S. (2020). Engineering aspects of hydrothermal pretreatment: From batch to continuous operation, scale-up and pilot reactor under biorefinery concept. *Bioresource Technology, 299*, 122685–122685. https://doi.org/10.1016/j.biortech.2019.122685

Saari, J., Sermyagina, E., Kuparinen, K., Lipiäinen, S., Kaikko, J., Hamaguchi, M., & Mendoza-Martinez, C. (2022). Improving Kraft Pulp Mill Energy Efficiency through Low-Temperature Hydrothermal Carbonization of Biological Sludge. *Energies (Basel), 15*(17), 6188-. https://doi.org/10.3390/en15176188

Saika, A., Watanabe, Y., Sudesh, K., & Tsuge, T. (2014). Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxy-4-methylvalerate) by recombinant Escherichia coli expressing leucine metabolism-related enzymes derived from Clostridium difficile. *Journal of Bioscience and Bioengineering, 117*(6), 670–675. https://doi.org/10.1016/j.jbiosc.2013.12.006

Sarvaramini, A., & Larachi, F. (2014). Integrated biomass torrefaction – Chemical looping combustion as a method to recover torrefaction volatiles energy. *Fuel (Guildford)*, *116*, 158–167. https://doi.org/10.1016/j.fuel.2013.07.119

Sayara, T., & Sánchez, A. (2019). A Review on Anaerobic Digestion of Lignocellulosic Wastes: Pretreatments and Operational Conditions. *Applied Sciences, 9*(21), 4655-. https://doi.org/10.3390/app9214655

She, Y., Hong, J., Zhang, Q., Chen, B.-Y., Wei, W., & Xin, X. (2020). Revealing microbial mechanism associated with volatile fatty acids production in anaerobic acidogenesis of waste activated sludge enhanced by freezing/thawing pretreatment. *Bioresource Technology, 302*, 122869–122869. https://doi.org/10.1016/j.biortech.2020.122869

Sikarwar, V. S., Pohořelý, M., Meers, E., Skoblia, S., Moško, J., & Jeremiáš, M. (2021). Potential of coupling anaerobic digestion with thermochemical technologies for waste valorization. *Fuel (Guildford), 294*, 120533-. https://doi.org/10.1016/j.fuel.2021.120533

Singhania, R. R., Patel, A. K., Christophe, G., Fontanille, P., & Larroche, C. (2013). Biological upgrading of volatile fatty acids, key intermediates for the valorization of biowaste through dark anaerobic fermentation. *Bioresource Technology, 145*, 166–174. https://doi.org/10.1016/j.biortech.2012.12.137

Sopelana, A., Oleaga, A., Cepriá, J. J., Bizjak, K. F., Paiva, H., Rios-Davila, F.-J., Martinez, A. H., & Cañas, A. (2023). Enhancing Circular Business Model Implementation in Pulp and Paper Industry (PPI): A Phase-Based Implementation Guide to Waste Valorisation Strategies. *Sustainability (Basel, Switzerland), 15*(24), 16584-. https://doi.org/10.3390/su152416584

Tampio, E. A., Blasco, L., Vainio, M. M., Kahala, M. M., & Rasi, S. E. (2019). Volatile fatty acids (VFAs) and methane from food waste and cow slurry: Comparison of biogas and VFA fermentation processes. Global Change Biology. *Bioenergy, 11*(1), 72–84. https://doi.org/10.1111/gcbb.12556

Tan, D., Wang, Y., Tong, Y., & Chen, G.-Q. (2021). Grand Challenges for Industrializing Polyhydroxyalkanoates (PHAs). *Trends in Biotechnology (Regular Ed.), 39*(9), 953–963. https://doi.org/10.1016/j.tibtech.2020.11.010

Teghammar, A., Yngvesson, J., Lundin, M., Taherzadeh, M. J., & Horváth, I. S. (2010). Pretreatment of paper tube residuals for improved biogas production. *Bioresource Technology, 101*(4), 1206–1212. https://doi.org/10.1016/j.biortech.2009.09.029

Tripathi, S. K., Alam, I., & Bhardwaj, N. K. (2020). Effect of bark content in mixed hardwood chips on pulp and papermaking properties. *Nordic Pulp & Paper Research, 35*(3), 325–331. https://doi.org/10.1515/npprj-2020-0017

Trumic, M. S., Trumic, M. Z., Bogdanovic, G., & Andric, L. (2018). Pulp and paper mill sludge utilization possibilities in terms of environmental protection. *Quaestus (Timiȯara)*, *12*, 240–243.

Tsuge, T. (2016). Fundamental factors determining the molecular weight of polyhydroxyalkanoate during biosynthesis. *Polymer Journal, 48*(11), 1051–1057. https://doi.org/10.1038/pj.2016.78

Valentino, F., Gottardo, M., Micolucci, F., Pavan, P., Bolzonella, D., Rossetti, S., & Majone, M. (2018). Organic Fraction of Municipal Solid Waste Recovery by Conversion into Added-Value Polyhydroxyalkanoates and Biogas. *ACS Sustainable Chemistry & Engineering, 6*(12), 16375–16385. https://doi.org/10.1021/acssuschemeng.8b03454

Valentino, F., Lorini, L., Gottardo, M., Pavan, P., & Majone, M. (2020). Effect of the temperature in a mixed culture pilot scale aerobic process for food waste and sewage sludge conversion into polyhydroxyalkanoates. *Journal of Biotechnology, 323*, 54–61. https://doi.org/10.1016/j.jbiotec.2020.07.022

Veluchamy, C., & Kalamdhad, A. S. (2017). Enhanced methane production and its kinetics model of thermally pretreated lignocellulose waste material. *Bioresource Technology, 241*, 1–9. https://doi.org/10.1016/j.biortech.2017.05.068

Veluchamy, C., & Kalamdhad, A. S. (2017). Influence of pretreatment techniques on anaerobic digestion of pulp and paper mill sludge: A review. *Bioresource Technology, 245*(Pt A), 1206–1219. https://doi.org/10.1016/j.biortech.2017.08.179

Vivekanand, V., Olsen, E. F., Eijsink, V. G. H., & Horn, S. J. (2013). Effect of different steam explosion conditions on methane potential and enzymatic saccharification of birch. *Bioresource Technology, 127*, 343–349. https://doi.org/10.1016/j.biortech.2012.09.118

Vu, D. H., Wainaina, S., Taherzadeh, M. J., Åkesson, D., & Ferreira, J. A. (2021). Production of polyhydroxyalkanoates (PHAs) by Bacillus megaterium using food waste acidogenic fermentation-derived volatile fatty acids. *Bioengineered, 12*(1), 2480–2498. https://doi.org/10.1080/21655979.2021.1935524

Wainaina, S., Lukitawesa, Kumar Awasthi, M., & Taherzadeh, M. J. (2019). Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review. *Bioengineered, 10*(1), 437–458. https://doi.org/10.1080/21655979.2019.1673937

Wan Azelee, N. I., Mahdi, H. I., Cheng, Y.-S., Nordin, N., Illias, R. M., Rahman, R. A., Shaarani, S. M., Bhatt, P., Yadav, S., Chang, S. W., Ravindran, B., & Ashokkumar, V.

(2023). Biomass degradation: Challenges and strategies in extraction and fractionation of hemicellulose. *Fuel (Guildford), 339*, 126982-. https://doi.org/10.1016/j.fuel.2022.126982

Wang, K., & Zhang, R. (2021). Production of Polyhydroxyalkanoates (PHA) by Haloferax mediterranei from Food Waste Derived Nutrients for Biodegradable Plastic Applications. *Journal of Microbiology and Biotechnology, 31*(2), 338–347. https://doi.org/10.4014/JMB.2008.08057

Wang, K., Hobby, A. M., Chen, Y., Chio, A., Jenkins, B. M., & Zhang, R. (2022). Techno-Economic Analysis on an Industrial-Scale Production System of Polyhydroxyalkanoates (PHA) from Cheese By-Products by Halophiles. *Processes*, *10*(1), 17-. https://doi.org/10.3390/pr10010017

Wang, K., Yin, J., Shen, D., & Li, N. (2014). Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: Effect of pH. *Bioresource Technology, 161*, 395–401. https://doi.org/10.1016/j.biortech.2014.03.088

Wang, Z., Wang, W., Li, P., Leng, Y., & Wu, J. (2022). Continuous Production of Volatile Fatty Acids (VFAs) from Swine Manure: Determination of Process Conditions, VFAs Composition Distribution and Fermentation Broth Availability Analysis. *Water (Basel), 14*(12), 1935-. https://doi.org/10.3390/w14121935

Weber, H., Kulbe, K. D., Chmiel, H., & Trösch, W. (1984). Microbial acetate conversion to methane: kinetics, yields and pathways in a two-step digestion process. *Applied Microbiology and Biotechnology, 19*(4), 224–228. https://doi.org/10.1007/BF00251840

Wenger, J., Pichler, S., Näyhä, A., & Stern, T. (2022). Practitioners' Perceptions of Co-Product Allocation Methods in Biorefinery Development—A Case Study of the Austrian Pulp and Paper Industry. *Sustainability (Basel, Switzerland), 14*(5), 2619-. https://doi.org/10.3390/su14052619

Wolf, M., Berger, F., Hanstein, S., Weidenkaff, A., Endreß, H.-U., Oestreich, A. M., Ebrahimi, M., & Czermak, P. (2022). Hot-Water Hemicellulose Extraction from Fruit Processing Residues. *ACS Omega, 7*(16), 13436–13447. https://doi.org/10.1021/acsomega.1c06055

Xiang, Z., Huang, X., Chen, H., Liu, B., Liu, Z., Dong, W., & Wang, H. (2023). Insights into thermal hydrolysis pretreatment temperature for enhancing volatile fatty acids production from sludge fermentation: Performance and mechanism. *Bioresource Technology, 379*, 129032–129032. https://doi.org/10.1016/j.biortech.2023.129032

Yang, J. E., Park, S. J., Kim, W. J., Kim, H. J., Kim, B. J., Lee, H., Shin, J., & Lee, S. Y. (2018). One-step fermentative production of aromatic polyesters from glucose by metabolically engineered Escherichia coli strains. *Nature Communications, 9*(1), 79–79. https://doi.org/10.1038/s41467-017-02498-w

Ye, J., Huang, W., Wang, D., Chen, F., Yin, J., Li, T., Zhang, H., & Chen, G. (2018). Pilot Scale-up of Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) Production by Halomonas bluephagenesis via Cell Growth Adapted Optimization Process. *Biotechnology Journal, 13*(5), e1800074-n/a. https://doi.org/10.1002/biot.201800074

Yedro, F. M., Cantero, D. A., Pascual, M., García-Serna, J., & Cocero, M. J. (2015). Hydrothermal fractionation of woody biomass: Lignin effect on sugars recovery. *Bioresource Technology, 191*, 124–132. https://doi.org/10.1016/j.biortech.2015.05.004

Yin, J., Wang, K., Yang, Y., Shen, D., Wang, M., & Mo, H. (2014). Improving production of volatile fatty acids from food waste fermentation by hydrothermal pretreatment. *Bioresource Technology*, *171*, 323–329. https://doi.org/10.1016/j.biortech.2014.08.062

Zhang, M., Wu, H., & Chen, H. (2014). Coupling of polyhydroxyalkanoate production with volatile fatty acid from food wastes and excess sludge. *Process Safety and Environmental Protection*, *92*(2), 171–178. https://doi.org/10.1016/j.psep.2012.12.002

Zhao, L., Sun, Z.-F., Zhang, C.-C., Nan, J., Ren, N.-Q., Lee, D.-J., & Chen, C. (2022). Advances in pretreatment of lignocellulosic biomass for bioenergy production: Challenges and perspectives. *Bioresource Technology, 343*, 126123–126123. https://doi.org/10.1016/j.biortech.2021.126123

Zhou, M., & Tian, X. (2022). Development of different pretreatments and related technologies for efficient biomass conversion of lignocellulose. *International Journal of Biological Macromolecules, 202*, 256–268. https://doi.org/10.1016/j.ijbiomac.2022.01.036

Zhou, M., Yan, B., Wong, J. W. C., & Zhang, Y. (2018). Enhanced volatile fatty acids production from anaerobic fermentation of food waste: A mini-review focusing on acidogenic metabolic pathways. *Bioresource Technology*, *248*(Pt A), 68–78. https://doi.org/10.1016/j.biortech.2017.06.121

Zhu, R., Zhao, S., Ju, C., Yang, Q., Cui, C., Wu, L., Wang, M., Feng, L., & Wu, Y. (2023). Ultrasonic-assisted hypochlorite activation accelerated volatile fatty acids production during sewage sludge fermentation: Critical insights on solubilization/hydrolysis stages and microbial traits. *Bioresource Technology, 383*, 129233–129233. https://doi.org/10.1016/j.biortech.2023.129233