Dissertationes Forestales 324

Potential of neglected biomass and industrial side-streams utilization in biofuels production

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Doctoral dissertation

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ABSTRACT

The utilization of forest side-streams is associated with the applied bioenergy technology that must be impellent to support the increasing demand for biofuels and resources while lowering greenhouse gas (GHG) emission from the transport sector. This study aimed to estimate potential biofuel production from eutrophic (EL) and mesotrophic (ML) lake bottom biomass and the manufacturing side-streams from the pulp and paper mill (PI – PVIII). Theoretical biogas and bioethanol productions were modeled by Aspen Plus® simulation through 1) saccharification and fermentation, 2) gasification and mixed alcohol synthesis, 3) gasification-syngas fermentation, and (4) anaerobic digestion processes. In addition, the different process stages of the pulp and paper side-streams was studied by ABE fermentation using *Clostridium acetobutylicum* DSM 1731.

The bioethanol produced from EL and ML biomass from indirect gasification and mixed alcohol synthesis were 244.5 L/t and 57.1 L/t, whereas the yields from saccharification and fermentation were 137 L/t and 40 L/t, respectively. The EL biomass produced the most profitable bioethanol production from the latter process. The ML and EL biomass produced biogas of 38.9 mL/g volatile solid and 136.6 mL/g volatile solid, respectively. The ash from the EL and ML biomass and the dried samples of PI and PIII could be used as fertilizer because the harmful elements for Finnish fertilizer products were below the detectable limit. The primary sludge (PII) sample had found high N and P concentrations and cadmium (Cd) concentration (3 mg/kg), which exceeded the Cd limit for Finnish fertilizer products (1.5 mg/kg). However, wet primary sludge (PII) forming 300,000 tonnes/year (72600 dry tonnes) produced anhydrous ethanol about 3011 kg/h (24,090 tonnes/year) when PII was used for the gasification-syngas fermentation process in the bioethanol plant model.

Three pulp and paper side-streams (PI, PII and PIII) with unwashing and water washing were pretreated with dilute acid (0.2% H_2SO_4 at 180 °C for 10 min), followed by saccharification and ABE fermentation. The results suggested that water washing did not affect the PII and PIII prehydrolysate sugar recovery, as well as enzyme hydrolysis of the rejects from kraft pulping (PI) did not require prewashing before dilute acid pretreatment. In addition, the unwashed PI side-stream yielded the highest ABE concentration of 12.8 g/L, compared to the unwashed PII and PIII side-streams, 5.2 g/L, and 6.3 g/L, respectively. The side-streams from different process stages in pulp and paper mill were concluded to be high potential feedstocks for biofuels production due to their chemical compositions. The unwashed PI was suitable feedstock for butanol production, while PII could be fully utilized in the integrated gasification-syngas fermentation process. Primary sludge (PII) was found to be a promising feedstock for bioethanol and an internal rate of return (IRR) of 15 % can be obtained by two implementations. One was a cost-competitive ethanol selling price (ESP) of €0.61–0.71/L with an ethanol subsidy of €150/t at different tax rates, and the other was an ESP of €0.60–0.70/L with the imposition of a €20/t gate fee. In the future, the addition of an integrated biofuel production operations unit, installed close to a pulp and paper mill, could utilize the different pulp side-streams and create further revenues to the mill owners.

Keywords: Acetone-butanol-ethanol, Aspen Plus, Gasification-syngas fermentation, Finnish lakes, Pulp and paper, Primary sludge, Techno-economic analysis

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I believe that people who start a PhD intending to finish it on time. However, walking towards the PhD journey is not easy, and the doctoral process makes me feel isolating that the dissertation miles are longer than expected.

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LIST OF ORIGINAL ARTICLE

The thesis is a summary of the following articles, which are referred to in the text by their Roman numerals I-III. Articles I and II have been reprinted with the permission of the publishers. Article III is the author's version of a submitted manuscript.

I	Sandar S., Tibebu T.D., Shromova A.O., Kuittinen S., Turunen O., Pappinen A. (2019). Lake bottom biomass as a potential source for the biorefining industry. Bioresource Technology Reports (7) 100282: https://doi.org/10.1016/j.biteb.2019.100282
II	Sandar S., Yang M., Turunen O., Vepsäläinen J., Pappinen A., Kuittinen S. (2020). Acetone-butanol-ethanol fermentation from different pulp and paper manufacturing process side-streams. Bioresources 15(4): 9265–9290. https://doi.org/10.15376/biores.15.4.9265-9290
III	Sandar S., Shromova A.O., Kuittinen S., Turunen O., Pappinen A. (2021). Pulp and paper process side-streams into bioethanol: an economic feasibility study of the gasification-syngas fermentation process. Manuscript submitted to Waste and Biomass Valorization Journal.

The author's contribution

Sandra Sandar was the primary author of all the Articles and was responsible for all laboratory analyses and investigation. Dessie Tibebu participated in biogas experiment for Article I. Ming Yang participated in executing the enzymatic hydrolysis experiment for Article II. Jouko Vepsäläinen mainly contributed by analysing the NMR performance of the sugar samples and commented on the manuscript of Article II. Olga Shromova contributed for simulation and modeling of Aspen Plus in Articles I and III, and partially writing in Article III. Ossi Turunen contributed by commenting on all manuscripts. Ari Pappinen and Suvi Kuittinen developed research ideas for Articles I-III and improved the articles by commenting on and editing the manuscripts.

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LIST OF ABBREVIATIONS AND TERMS

ABE	Acetone-butanol-ethanol
AD	Anaerobic digestion
AIL	Acid soluble lignin
ASL	Acid insoluble lignin
APEA	Aspen Process Economic Analyzer
CAPEX	Capital expenditures
CBH	Cellobiohydrolases
CBP	Consolidated bioprocessing
CEL	Celluclast 1.5 L and Novozyme 188
DM	Dry matter
DME	Dimethyl ether
DNS	3,5-dinitrosalicylic acid
DP	Degree of polymerization
EL	Eutrophic lake
FPU	Filter paper unit
GHG	Greenhouse gas
HHV	Higher heating value
HMF	5-hydroxymethylfurfural
HVO	Hydrotreated vegetable oil
ICP-OES	Inductively coupled plasma optical emission spectrometer
IEA	International Energy Agency
IRR	Internal rate of return
LOI	Loss-on-ignition
MC	Moisture content
MESP	Minimum ethanol selling price
ML	Mesotrophic lake
MSW	Municipal solid waste
nkat	Nanokatal
NMR	Nuclear magnetic resonance
NREL	National Renewable Energy Laboratory
NRTL	Non-random-two-liquid
OPEX	Operating expenses
PPMS	Pulp and Paper manufacturing side-streams
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
SSCF	Simultaneous saccharification and co-fermentation
TEA	Techno-economic analysis
UN	Untreated original sample (control)
UWP	Unwashed-pretreated sample with dilute sulfuric acid
W	Washed and unpretreated sample
WP	Washed and pretreated sample
XYL	endo-1,4-b-xylanase

1. INTRODUCTION

During the last decade(s), concerns about climate change, rising energy consumption and disadvantages of petroleum-derived transportation fuels (GHG emissions, resource depletion, pollution, and unbalanced supply-demand relations) have resulted in greater demand for renewable fuels (Hamelinck et al. 2005). In particular, the advanced transport biofuels such as biodiesel, bio-oils, bioethanol, biobutanol, bio-synthetic gas, hydrotreated vegetable oil (HVO), biomethane, and biohydrogen production from the biochemical and thermochemical processes of low-cost feedstocks has been considered to replace fossil fuels. Global transport biofuel production is expected to increase 190 billion litres (L) in 2024 compared to 154 billion L in 2018 (IEA 2019). Recently, the European Union has preset a target to produce advanced biofuels and biogas at least 0.2% in 2022, 1% in 2025, and 3.5% in 2030 (European Commission 2018). By 2020, the target for renewable fuels used in the transport sector is 10% for each European Union member state and Finland targets a 30% share for the use of transport biofuels in 2030, reducing the CO_2 emission by 80% in 2050 from 1990 level (Toivanen et al. 2017).

Of all liquid biofuels, ethanol is currently the most produced transport fuel on a high scale worldwide, corresponding to about 73% of the 135.3 billion L of biofuel generated in 2016 (Branco et al. 2019). Bioethanol has several advantages, such as low boiling point, higher heat of vaporization, high octane number (108), and comparable energy content with blended gasoline with up to 85% (v/v) in vehicles (Sindhu et al. 2019). Bioethanol production is technically mature, but the relatively lower energy content and hygroscopicity of bioethanol limits its application scope compared to biodiesel and butanol. The cold flow behaviour such as cloud point and pour point, and fat and oil resources are limited for biodiesel production (Liu et al. 2011). Butanol is superior to ethanol due to its unique properties such as high energy content, low vapor pressure, high hydrophobicity, blending efficiency with gasoline at any percentage, and high flash point, non-corrosiveness, and no need for modification of the existing combustion engine) (Jin et al., 2011; Karimi et al., 2015; Tashiro et al., 2013).

The two major pathways of conversion from solid biomass into biofuels are biochemical (fermentation and digestion) and thermochemical (gasification and pyrolysis) processes. Biochemically, ethanol or butanol is produced from biomass conversion into reducing sugars through pretreatment and saccharification followed by microbial fermentation (Shen et al. 2015). However, major cost constraints in fermentative ethanol and butanol production are the processing costs which involve the cost of feedstock, using expensive pretreatment and enzymes, inability to degrade lignin, detoxification, ethanol recovery and butanol low productivity that makes the process economically unviable (Tashiro et al. 2013; Pardo-Planas et al. 2017). In thermochemical process, biomass gasified at higher temperature (800-1000 ^{o}C) with a limited amount of oxygen, producing syngas as the prime product (Basu 2010; Shen et al. 2015). However, syngas production has some disadvantages, for example, biomasses need drying, and the contaminants produced from gasification suppress product yield. These challenges from both processes can be solved by the combination of the biochemical and thermochemical platforms by utilizing all biomass components that can provide high carbon conversion efficiency for desired products and higher yields (Brown 2007; Shen et al. 2015). Aside higher process efficiencies, concepts for combination of gasification and fermentation can provide better syngas quality and purity, and lower investment costs (Heidenreich and Foscolo 2015).

Based on the source of feedstock (food, non-food, and algal biomass), biofuels are classified into three generation: first, second and third ((Navas-Anguita et al. 2019). First-generation biofuel generated by conventional biofuel technologies on a commercial scale includes ethanol by the fermentation of sugar or starch from food crops, biodiesel production by transesterification of oil-crops, and biogas derived through anaerobic digestion (IEA 2011; Sindhu et al. 2019). The production of ethanol from first-generation has raised some ethical and sustainability concerns such as "food vs. fuel" debate, cost of the feedstock, and the price of the products (Lennartsson et al. 2014). Second- generation is produced by advanced biofuel technologies on pilot phase using non-food crop lignocellulosic feedstocks. Fermentative ethanol and butanol production from second- or third-generation have better performance in terms of energy balance, GHG emissions and land-use requirements than conventional biofuels production (IEA 2011). It has been reported that cellulosic ethanol has the best option to reduce 86% GHG emissions (Wang et al. 2007). Recently, the European Union directive on the promotion of the use of energy from renewable sources mandates for advanced biofuels that produce from different bio-waste and lignocellulosic materials include biomass fraction of wastes and residues from forestry and forest-based industries (European Commission 2018).

The use of these feedstocks for the production of biofuels and the advantage of applying combined conversion technology can offer three significant economic benefits: (1) the cost of feedstock is zero, (2) utilizing of the whole biomass including lignin, and (3) elimination of expensive pretreatment processes and enzymes (Kang et al. 2010; Pardo-Planas et al. 2017). Finding new resources and putting waste back into the production stream in biorefineries are essential to the adoption of advanced biofuels as a primary energy source. Therefore, in this research, neglected biomass accumulated on the Finnish lake bottom as a new and locally abundant non-food biomass and Finnish PPMS as a turning waste into resource were investigated for the production of advanced biofuels – ethanol and butanol.

2. LITERATURE REVIEW

2.1 lignocellulosic biomass as a feedstock for biofuels

Globally, the only source of sustainable and renewable feedstock is lignocellulosic biomass. It is the most abundant carbon source and a promising alternative to fossil resources to produce transport fuels, chemicals, and materials. The definition of biomass by the European Directive 2009/23/EC is "the biodegradable fraction of products, waste and residues from biological origin from agriculture (including plant and animal substances), forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste" (European Commission 2009). Figure 1 illustrates possible lignocellulosic feedstocks that can be used in a biorefinery.

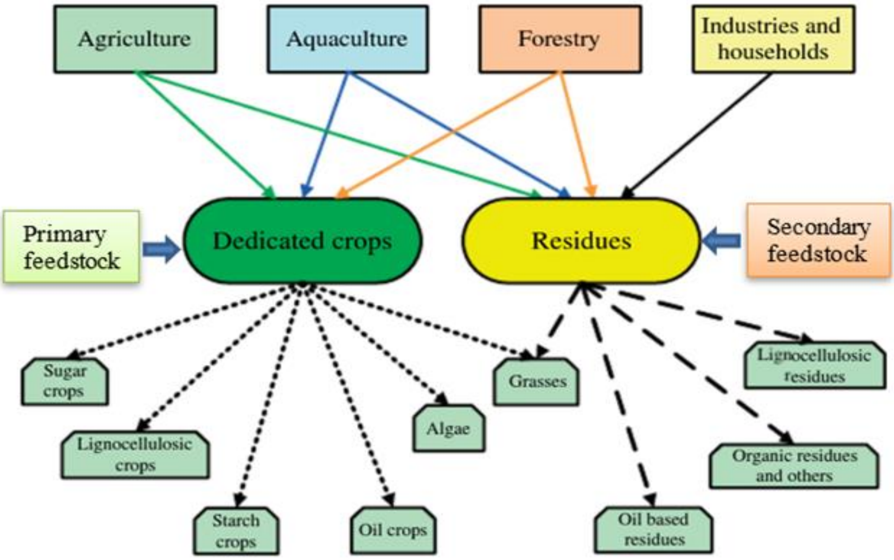


Figure 1. The classified feedstocks utilized for biorefinery (Modified from Cherubini et al., 2009).

They are classified into two groups: (i) dedicated/nonwaste/fresh materials, called primary feedstock such as green plant materials, bushes, plant shoots, algal biomass, oil-, energy-, starch-, and sugar-crops, and (ii) residual/waste/by-products, called secondary feedstock such as residues from agriculture and forestry, industrial side-streams, and organic waste (Cherubini et al. 2009; Badgujar and Bhanage 2018). Biomass feedstocks used for energy (> 80%) are sourced from wood and shrubs while the rest feedstocks are from energy crops, agricultural residues and by-products, and from various commercial and post-consumer waste (Menon and Rao 2012). In particular, promising feedstocks in agricultural residues and energy crops are corn stover and wheat straw, and switchgrass and miscanthus, respectively, while waste streams are food waste and PPMS (Alonso et al. 2012).

Two kinds of energy efficiency usage categorize biomass: traditional biomass (wood, straws and dung) is used as low-efficiency energy, for example cooking and heating while high-efficiency energy biomass conversion is found in chemical, forest products, food, and pulp and paper industry (Menon and Rao 2012). Sustainable raw materials are utilized more in second-generation biorefineries to avoid the criticism attributed to first-generation biorefineries, for example, various side-streams generated from industrial and municipal solid wastes. The pulp and paper industry is one of the major producers of solid wastes in Finland (Nurmesniemi et al. 2007). In 2018, the Finnish pulp and paper mill generated about 3.9 million tons of primary and secondary sludges, and approximately 68,000 tons of waste as landfill (FFI 2018; Hassan et al. 2019). These residues from pulp and paper industry are considered as waste although they can be utilized as resources (Nurmesniemi et al. 2007). Makkonen et al. (2002) reported that materials, which are utilized or which potentially will be utilized, cannot be defined as wastes as well as materials will not be recycled in the future, cannot be named as by-products. A possible new source of lignocellulosic feedstock could be biomass from lake bodies, in which nutrients have been accumulated from forest management, agricultural, and industrial activity areas. These biomasses contain structural carbohydrates, which can be hydrolyzed to sugars and converted to fuels and chemicals by a biochemical or thermochemical conversion. Therefore, the utilization of neglected biomass, waste and industrial side-streams, in the forest industry is a growing trend towards advanced biofuels.

2.2 Components of lignocellulosic raw materials

Botanically, lignocellulosic raw materials refer to gramineous plants (i.e. grasses), hardwoods and softwoods as monocotyledons, eudicotyledons, and gymnosperms, respectively (Kallioinen 2014). They are primarily constituted of cellulose, hemicellulose, and lignin, other extractable components such as pectin, protein, minerals (ash), and extractives. The source of lignocellulosic biomass is the plant cell wall, which determines the structural integrity of the plant, and in defence against pathogens and insects (Sticklen 2008). Figure 2 illustrates the primary cell walls that abundant with cellulose, hemicellulose and pectin, can be hydrolysed to give sugar-to-ethanol production whereas the plant secondary cell wall contains cellulose, hemicellulose and lignin (Sticklen 2008). However, the structure and composition of lignocellulosic feedstocks highly depends on its source, tissue, age, cell type, and plant taxa, and fibrillary network within each cell wall layer (Ding and Himmel 2006). In general, lignocellulosic biomass mainly contains about 30–60% cellulose, 20–40% hemicellulose, and 15–35% lignin (Lynd et al. 2002; Menon and Rao 2012; Alonso et al. 2012; Nanda et al. 2013).

Cellulose, the most abundant materials in the natural world, is a linear chain of thousands of β (1→4) linked D-glucose units, in which the repeat unit of cellobiose established with the degree of polymerization (DP) as high as 15 000 (Alén 2000). It is composed of 15–30% of the dry mass of primary and up to 40% of secondary cell walls to form cellulose to be packed into microfibrils through intra- and intermolecular hydrogen bonding which allow a rigid crystalline that embeds for mechanical strength (Fengel and Wegener 1983; Sticklen 2008) (Figure 2). Although crystalline structure (highly organized) is the major proportion of cellulose, low DP cellulose is responsive degradation in its amorphous structure (unorganized), which is scaffolded by hemicellulose and lignin. Cellulose has been used for the commercial cellulosic ethanol, chemicals and other biofuels production via catalytic process as fermentable sugars have been produced from cell wall polysaccharides through saccharification using cellulases and hemicellulases (Sticklen 2008; Alonso et al. 2012). Hemicellulose is a short-chain branched hetero-polysaccharide, made up of different monosaccharides include pentoses (D-xylose, L-arabinose), hexoses (D-mannose, D-glucose and D-galactose) and uronic acids (e.g., 4-omethylglucuronic, D-glucuronic, and D-galactouronic acids) (Alonso et al. 2012). It is amorphous with a low DP (100-200 repeating units) that as such, chemical hydrolysis of hemicellulose are easier than cellulose (Alén 2000; Kucharska et al. 2018). Hemicellulose serves as a connection between the lignin and the cellulose fibers to form a cross-linked network of heterogeneous mixture of pentoses and hexoses in the cell walls (Hendriks and Zeeman 2009) (Figure 2).

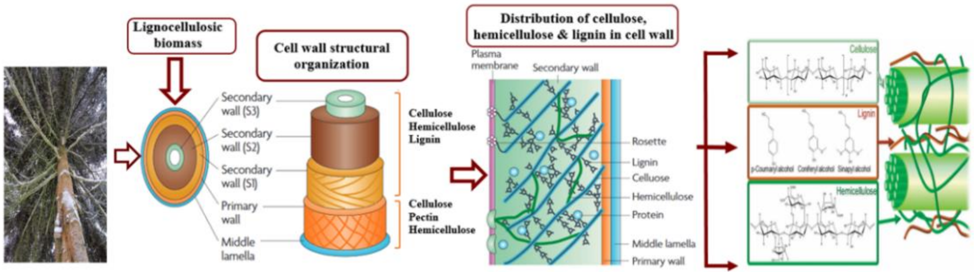


Figure 2. Representative structure of lignocellulose with cellulose, hemicellulose, lignin, and their building blocks (Modified from Alonso et al., 2012; Menon and Rao, 2012; Sticklen, 2008).

The fractions of xylan, heteroxylan, galactomannan in hemicellulose vary based on the composition and intra-structural bonding in different wood species, for example, the dominant component of hemicellulose in softwood is glucomannan while xylan is mainly found in hardwoods, grasses and straws (Fengel and Wegener 1983; Singh and Chaudhary 2016). The most studied sugar in hemicellulose is xylose, as it can be converted to the value-added products such as ethanol, furfural and xylitol. Lignin is an amorphous, complex aromatic polymer including phenylpropane units, and aromatic alcohols (*p*-coumaryl, coniferyl, and sinapyl) with the DP in the range of 450-500 units (Singh and Chaudhary 2016). The biosynthesis of lignin stems from the polymerization of aromatic units (monolognols), and named as *p* -hydroxyphenyls (H), guaicyls (G), and syringyls (S) units (Calvo-Flores and Dobado 2010). The ratio of H/G/S units in the lignin is highly dependent on plant taxonomy, for example, G and S units with trace amounts of H units are mainly in hardwood while G units in softwood, whereas grass lignins consists of all three units with different ratios (Buranov and Mazza 2008; Chundawat et al. 2011).

Lignin embeds the cellulose microfibrils of the secondary cell wall (Figure 2) that is responsible for impermeability and resistance against microbial and chemical degradation (Singh and Chaudhary 2016). It cannot be solubilized from the walls without disrupting its structure and therefore it is impractical to extract lignin in pure form (Bowsher et al. 2008). The lignin's solubility in neutral, alkaline or acid environments also relies on the precursor (Grabber 2005). However, researchers have studied using a low melting solvent mixture to extract as much as possible percentage of lignin for the production of valuable food and industrial products such as vanillin, ferulic acid, and lignans (Buranov and Mazza 2008). Lignin has about 40% of energy of the biomass due to its high carbon content and thus highly lignified material is an excellent fuel source, possessing a greater energy value than cellulose and hemicellulose when burned (Zhang et al. 2012; Alonso et al. 2012).

2.3 Biomass to biofuels conversion

Unlike food crops, lignocellulosic feedstocks cannot be directly fermented and are more difficult to process than simpler sugars and starches. According to IEA Bioenergy Task 42, the biorefinery systems can be classified as feedstocks, conversion processes, products and platforms (Cherubini et al. 2009). Biomass-to-energy transforming carriers are thermal (combustion), physical/mechanical (pressing, extraction), chemical (synthesis, transesterification), thermochemical (carbonization, liquefaction, gasification, pyrolysis), and biochemical (fermentation or aerobic and anaerobic decomposition) (Deublein and Steinhauser 2008). Among them, two primary conversion routes for producing biofuels from lignocellulosic biomass are biochemical (fermentation and digestion) and thermochemical (gasification and pyrolysis) processes as focal biomass conversion technologies (Figure 3). However, different kinds of biomass feedstocks have different defense mechanisms for protection against microbial and enzymatic attacks (Kallioinen 2014; Badgujar and Bhanage 2018). Direct and indirect factors that influence cellulose accessibility, in which accessible surface area is under the former one and the rest such as structural factors (cellulose crystallinity, DP, pore size) and chemical factors (acetyl groups, lignin content, hemicelluloses) are under the latter one (Hendriks and Zeeman 2009; Zhao et al. 2012). These factors can decline in overall enzymatic hydrolysis rates. In the production of fuels from lignocellulosic biomass, pretreatment is designated as a process by which cellulosic biomass is biodegradable by hydrolytic enzymes. However, it has also been reported that different pretreatment condition might affect the cellulase adsorption characteristics onto a substrate (Lu et al. 2002).

Furthermore, most potential cellulosic substrates using in biorefineries are heavily lignified, yet the digestibility of cellulose is directly related to lignin removal (Yang et al. 2011). It was reported that lignin plays a vital role in the adsorption of cellulases onto steam-pretreated hardwoods and act as a barrier to cellulase (Lu et al. 2002). Therefore, the choice for efficient and optimized pretreatment and hydrolysis processes with low-cost lignocellulosic biomass is required for the fermentation of carbohydrates in biomass to cost-effective production of alcoholic fuels. Both lignin and cellulose must be decomposed into glucose and xylose in biochemical conversion and H_2 and CO in thermochemical conversion (Mu et al. 2010). NREL designed both bio- and thermo-chemical conversion analysis using detailed Aspen Plus simulations, in which biochemical conversion analysis focuses on the production of fuels and products via sugars and other components derived from lignocellulosic biomass. On the other hand, thermochemical conversion applies to gasification or indirect liquefaction pathways that converts biomass-to-syngas for chemicals, fuels and power production through technoeconomic analysis (TEA) modeling. A concept of the combination of biochemical and thermochemical pathways provides a synergistic effect whereby the inherent advantages of each process are utilized. A general overview of the respective conversion processes is described in section 2.4, 2.5, and 2.6.

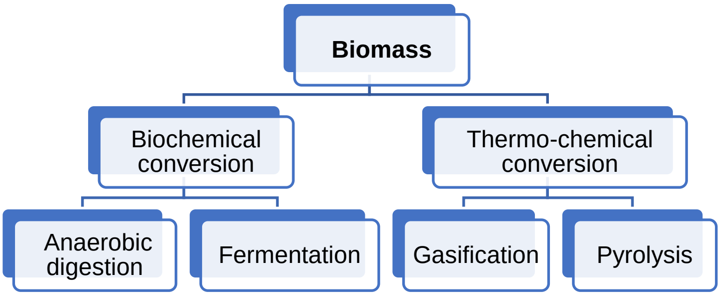


Figure 3. The main processes in the biomass conversion technologies for biofuel production.

2.4 Biochemical conversion technology

The US department of Energy defined biochemical processes as the use of living organisms or their products to convert organic material to fuels by both anaerobic and photosynthetic microorganisms to generate biofuels (Cantrell et al. 2008). In the process of biochemical conversion, biomass can be turned into different products, such as hydrogen, biomethane, acetone, butanol, ethanol, organic acids by selecting different microorganisms (Chen and Wang 2016). Two main products that reached to industrial scale for the bioenergy recovery from biomass are gaseous fuel as biomethane through anaerobic digestion (AD) and liquid fuel as bioethanol via alcoholic fermentation.

When considering the energy balance, biomass appears to be a very efficient source of biogas with 28.8 MJ/MJ compared to ethanol with 5 MJ/MJ (Deublein and Steinhauser 2008). However, it required high retention time (days) and most countries mainly used biogas for generation of heat and electricity, except Sweden used it as transport fuel. Gustafsson et al., (2020) reported that among IEA bioenergy task countries, Germany produced the highest the annual biogas production with around 120 TWh from >10,000 biogas plants in 2019 while the total biogas production was 692 GWh in Finland. If biomass is to be used for liquid fuel, ethanol has advantages over biogas due to its direct substitution to fossil fuels in the transport sector as well as its efficient alcoholic fermentation process (Petersson et al. 2007; Deublein and Steinhauser 2008). Therefore, ethanol is already produced on a high scale with targeted to 36.5 billion gallons by 2022 and it can be considered as an energy or a material product (Pardo-Planas et al. 2017).

The first biochemical design was reported for the cost competitiveness comparison between cellulosic ethanol, corn ethanol and petroleum-derived fuels in 2002 (Aden et al. 2002). The design was involved the feedstock handling, dilute acid pretreatment, enzymatic hydrolysis and co-fermentation after liquid and solid fraction and overliming process, and wastewater treatment unit (Figure 4). Azeotropic ethanol was produced from the fermented

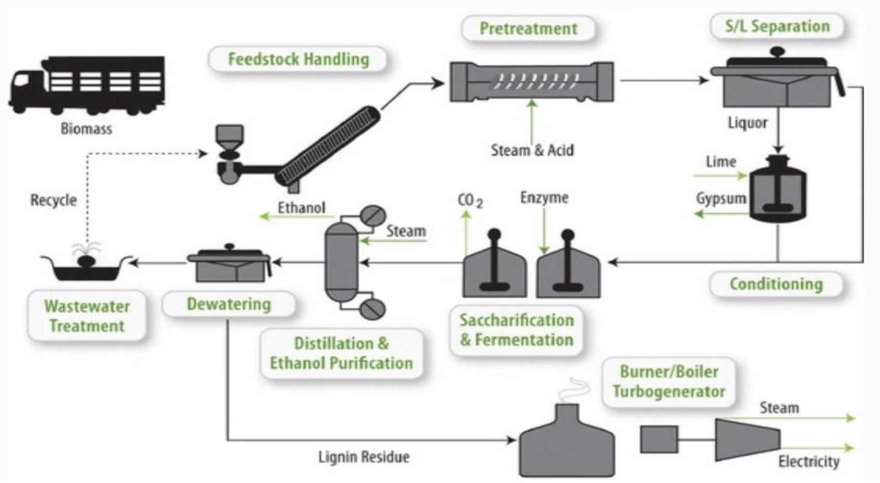


Figure 4. Bioethanol production from biochemical conversion process (According to Foust et al., 2009).

broth and unutilized lignin-rich residue was burnt to generate electricity selling as \$0.04/kWh and the treated water is also recycled back to the process (Foust et al. 2009). Technoeconomic analysis in NREL's design was enable prediction in the Minimum Ethanol Selling Price (MESP), for example, the selling price of corn ethanol and sugarcane ethanol were \$1.53/gal and \$1.14/gal in 2007\$ market price while TEA estimated were \$1.54/gal and \$1.29/gal in 2007\$ (Humbird et al. 2011).

The alcoholic fermentation involves various pretreatments, but the microorganisms in AD can feed on bigger substrate spectra than ethanol fermenting yeasts that pretreatment has not involved the same importance as in bioethanol production (Petersson et al. 2007; Braun et al. 2010). The difference between the microbial fermentation of biogas and bioethanol is that multi-strain mixed cultures induce biogas, while the latter is done by pure cultures (Braun et al. 2010). Nevertheless, the microorganism involvement in biogas production leads to the expansion of biochemical conversion technologies, for example, the fermentation of higher alcohols such as butanol and butane-diol. The main advantages of biochemical conversion technologies are the high selectivity and conversion efficiencies (Foust et al. 2009; Devarapalli and Atiyeh 2015). However, this technology has a drawback that lignin is not utilized, which reduces the potential ethanol yield that leads to the development of an indirect fermentation process. In a nutshell, the research area of the biochemical conversion process should be addressed to focus on conversion processes improvement and integrate with other technologies or produce high-value co-products to increase the economic feasibility of biofuels production.

2.4.1 Alcoholic fermentation

The alcoholic fermentation conversion involves five major stages, such as suitable biomass choice, effective pretreatment, cellulases and hemicellulases generation, hexose and pentose fermentation, and downstream processing (Menon and Rao 2012). In lignocellulosic biomass to biofuels conversion, there are three core stages, i.e., pretreatment (certain physical, chemical, or biological treatments), hydrolysis, and fermentation. The reason using various pretreatment methods in this process is a heterogeneous matrix of cellulosic biomass to reach ideal conversion effect, improve enzymatic hydrolysis yields (Daystar et al. 2015). After pretreated, cellulosic biomass is hydrolysed chemically or enzymatically to increase accessibility of C5-C6 sugars for microbial fermentation to produce biofuels (Devarapalli and Atiyeh 2015). The detailed of different pretreatment methods and literature on enzymatic hydrolysis have been described in sections 2.4.1.3 and 2.4.1.4.

2.4.1.1 Bioethanol

Cellulose and hemicellulose components of the lignocellulosic biomass is pretreated and enzymatically hydrolyzed to fermentable reducing sugars and are then fermented to ethanol by microbes via biochemical route. The major issues found in the lignocellulosic hydrolysates to ethanol are twofold: 1) the cellulose hydrolysate contains fermentable sugars with broad range of inhibitors, and 2) the hemicellulose hydrolysate contains hexoses and pentoses, and the latter is more difficult to ferment (Olsson and Hahn-Hägerdal 1996). Biomass is converted to ethanol through direct and indirect biological conversion processes.

The four major direct fermentation processes are SSF, SHF, SDCF, and CBP) while ethanol from syngas fermentation is an indirect fermentation (Kallioinen 2014; Devarapalli and Atiyeh 2015). In SHF, the enzyme production, saccharification, and fermentation are conducted in separate reactors at optimal conditions, but the accumulation of glucose and cellobiose hinder the cellulase (Lynd et al. 2002). Both hydrolysis and fermentation employ in the same reactor in SSF while glucose and xylose are co-fermented in the same reactor in SSCF (Devarapalli and Atiyeh 2015). SSCF overcame an issue in SSF, in which most microorganisms used for fermentation of glucose cannot utilize xylose. One single microorganism is applied for hydrolysis and fermentation steps in CBP (Lynd et al. 2002).

Fermentation can be performed as batch, fed batch and continuous operation modes. Batch fermentation is the most common used operation mode in fermentation, in which microorganisms are inoculated to a fixed volume of medium. The characteristics of batch process include initial high substrate concentration, a final high product concentration, and low risk of contamination, but low productivity with labour intensive (Olsson and Hahn-Hägerdal 1996; Yang 2015). Fed-batch fermentation is initiated with a low substrate concentration and microorganisms are inoculated and grown under batch mode for a certain amount of time, then more substrate is added to the fermenter when fermentation culture consumes the substrate to maintain the fermentation process (Li et al. 2011). Continuous mode can be controlled more easily than batch and fed-batch modes, and it is less labor intensive because fresh medium is continuously added to the fermenter; however, contamination is more serious in this operation due to long cultivation and sterility maintenance (Olsson and Hahn-Hägerdal, 1996). Li et al., (2011) reported that the batch mode provided the highest solvent yield in ABE fermentation (0.32 g/g) while the continuous mode gave the highest butanol (0.21 g/g), but the fed-batch mode was not recommended for solvent production.

The comparison of ethanol and butanol production from side-streams of agricultural and forestry are presented in Table 1. At present, commercial-scale bioethanol production from cellulosic feedstocks is derived from a biochemical pathway. One of the key advantages of using microorganisms in biochemical process is that it did not require high temperature. However, biochemical pathway suffers from an inherent disadvantage in that it cannot utilize lignin for the ethanol production, which reduces the potential ethanol yield (Pardo-Planas et al. 2017).

Table 1. Comparison of ethanol and butanol production from sugarcane bagasse, agricultural straws and side-streams of pulp and paper industry.

Substrate	Fermentation method	Strain	Ethanol yield (g/L)	Butanol yield (g/L)	References
Sugarcane bagasse	FBF	*C. acetobutylicum* GX01	nd	14.17	(Pang et al. 2016)
Sugarcane bagasse	CBP	*C. thermocellum* DSM 1237	0.86	nd	(Liu et al. 2020)
Barley straw	BF	*C. acetobutylicum* DSM 1731	nd	7.90	(Yang 2015)
Wheat straw	BF - SHF BF - SSF	*C. beijerinckii* P260	0.77 0.79	8.09 7.40	(Qureshi et al. 2008)

Wheat straw	SSCF	*Co-culture Saccharomyces cerevisiae and Pichia pastoris*	32.6	nd	(Zhang et al. 2017)
Kraft paper mill sludge	SSF	*S. cerevisiae* ATCC 200062	13.6	nd	(Kang et al. 2010)
	SSCF	*E. coli* ATCC 55124	15.0		
(I)Kraft paper sludge	FBF – SSF	*S. cerevisiae* MH 1000	34.2	nd	(Gottumukkala et al. 2016)
(II) Corrugated Recycled sludge	FBF – SSF	*S. cerevisiae* MH 1000	45.5		
Gas composition ($CO:H_2:CO_2$ 70:20:10)	Syngas fermentation	*C. carboxidivorans*	3.0	1.0	(Phillips et al. 2015)

FBF: Fed-Batch fermentation; BF: Batch fermentation; CBP: Consolidated bioprocessing; SHF: Separate hydrolysis and fermentation; SSF: Simultaneous saccharification and fermentation; SSCF: Simultaneous saccharification and co-fermentation; nd: no data available

2.4.1.2 ABE Fermentation

Acetone from ABE fermentation from different sugars and starches was mainly produced for military applications during the first World War (Karimi et al. 2015). However, this process lost its economic competitiveness for producing solvents owing to the rapid development of the petroleum industry in the last half of the twentieth century (Jones and Woods 1986). Butanol production is commonly produced from sugar-based feedstocks (sugarcane and sugar beet molasses), and starch-based feedstocks (corn and wheat) by Clostridium fermentation (Yang 2015). However, currently butanol is not produced from sugars and starches by microbial fermentation due to 60–80% of feedstock cost upon the total production cost (Pang et al. 2016). Using cheap and abundant lignocellulosic biomass as feedstock is an alternative solution for obtaining cost effective butanol production.

Butanol is biochemically produced by ABE fermentation with *Clostridium acetobutylicum* with ABE ratio 3:6:1 (Yang et al. 2013) and it has several significant advantages over ethanol. Two bacterial strains, *Clostridium acetobutylicum* and *Clostridium beijerinckii* are often used for ABE fermentation, which is a biphasic growth pattern that includes acidogenic and solventogenic phase (Li et al. 2011). Acetate, butyrate, hydrogen, CO_2 are produced by the solvent-producing *Clostridium* species during the exponential growth phase (acidogenic phase), and then the acids are reassimilated into acetone, butanol and ethanol production as the culture enters the stationary growth phase (solventogenic phase) (Jones and Woods 1986).

Aside from raw material, the major restrained factor in ABE fermentation for butanol production has been low productivity due to some problems such as heterofermentation, butanol toxicity to *Clostridium spp.* low cell density in the fermentation, formation of by-products, and lack of commercial bacteria (Tashiro et al. 2013; Yang 2015). In addition, the ethanol fermentation has a 10–30 times higher production rate than butanol from ABE fermentation (Jin et al. 2011). Thus, ethanol was gained attention over butanol during oil crisis in the 1970s and 1980s. Although ethanol has been extensively recognized as the most produced biofuel, its low hygroscopicity and low energy density faces a storage and distribution problem (Yang 2015). Therefore, thermochemical pathway (gasification and pyrolysis) emerges to achieve an economically viable ethanol production to convert lignin to raw material for the biofuels production via syngas fermentation.

The butanol yield varies depending on feedstocks, fermentation techniques, and processing method, but also the Clostridium spp (Yang 2015). Clostridium spp bacteria can produce butanol with high fermentation yield. Kolesinska et al., (2019) reviewed the productivity of butanol by using different clostridia with different feedstocks (Table 2). Many researchers have studied to improve the efficiency of lignin removal by developing an efficient pretreatment and enzymatic hydrolysis method for conversion of biomass into butanol. Pretreatment with alkaline, dilute acid, steam explosion and wet oxidation methods appear to generate inhibitors such as formic acid, furfural and HMF that must be detoxified prior to ABE fermentation. However, formic acid at a concertation below 0.4 g/L and furfural and HMF at concentration below 1.0 g/L are not toxic to ABE fermentation (Ezeji et al. 2007; Cho et al. 2012). Kuittinen et al., (2018) reported that the dilute acid prehydrolysate of S.schwerinii showed good fermentability in ABE fermentation without detoxification. Many in situ butanol recovery techniques, such as pervaporation, liquid-liquid extraction, gas striping, and adsorption with different operation mode such as batch, fed-batch or continuous fermentation, have been reported to enhance the efficiency of ABE fermentation. Lu et al., (2013) reported that batch fermentation with gas stripping for in situ product removal, butanol production from wood pulping hydrolysate was increased from 4.48 g/L to 13.46 g/L.

Table 2. Productivity of butanol by using different clostridia from different feedstocks (Adapted from Kolesinska et al., 2019)

Strain	**Carbon source**	**Productivity (g/L)**
C. beijerinckii	Soy molasses	8
C. beijerinckii BA101	Starch-based packing peanuts	18.9
C. acetobutylicum	Gelatinized sago starch	16
C. saccharoperbutylacetonicum N1-4	Cassava starch and chip hydrolysate	16.9
C. beijerinckii ATCC 55025	Wheat bran hydrolysate	8.8
C. beijerinckii	Wheat straw hydrolysate	12
C. saccharoperbutylacetonicum	Rice brans	7.7
C. acetobutylicum YM1	Pretreated deoiled rice bran	6.48
C. acetobutylicum	Fresh domestic wastes	3
C. pasteurianum	Microalgae Dunaliella sp. and glycerol	14.0–16.0
C. acetobutylicum DSM 792	Wasted vegetables	9.96–10.65

2.4.1.3 Pretreatment

Pretreatment is one of the most crucial steps in the biochemical process to convert the lignocellulosic biomass into biofuels or chemicals by fermentation. Pretreatment process is required prior to the enzymatic saccharification to convert polysaccharides to monosaccharides. The recalcitrant matrix of polymer is the main reason why plant biomass needs first to be pretreated prior to enzymatic hydrolysis and fermentation (Kallioinen 2014). The aim of the pretreatment is to open up the cell wall structure (by reducing the particle size, and by increasing the porosity and accessible surface area), separate lignin and hemicellulose from cellulose, and disrupt the crystallinity of cellulose, thus accessibility in cellulose and hemicellulose is better in enzymes or acids hydrolysis (Mosier et al. 2005; Zhao et al. 2012; Kallioinen 2014). From a commercial point of view, the choosing a right pretreatment is to get high product yields over total costs in the whole process including feedstock, pretreatment, hydrolysis, fermentation and downstream processing. Although there may never be a perfect pretreatment process, some expectations from a pretreatment have been identified as follows (Mosier et al. 2005; Kumar et al. 2009b; Saville 2011):

(i) Results in a high recovery of all carbohydrates, with a minimum of degradation products.
(ii) Avoids inhibitory to the subsequent hydrolysis and fermentation processes
(iii) Be cost-effective.
(iv) Preserves a highly digestible solids fraction (hemicellulose – pentose fractions) amenable to subsequent hydrolysis.
(v) Minimizes the need for pre- or post-processing, either in the form of mechanical size reduction or downstream detoxification.
(vi) Operates at a sufficiently high solids loading to avoid dilution of sugars and ethanol that would otherwise adversely affect downstream processing costs.
(vii) Use a wide range of lignocellulosic feedstocks.

The digestibility of cellulose is limited by structural, physicochemical, and compositional factors. Pretreatment is the process to alter those factors that the hydrolysis of the cellulose and hemicellulose fraction to monomeric sugars can be attained with greater yields. The various pretreatment methods has been developed and grouped into chemical, physical, biological and physico-chemical, electrical processes, and a combination of these (Kumar et al. 2009b). various pretreatments on lignocellulose degradation studied are acid pretreatment, organosolv pulping, steam explosion, AFEX, explosion, oxidation, and biological treatment (Jørgensen et al. 2007; Rovio et al. 2012). The physical form and size of the lignocellulosic material structure determine the pretreatment methods (Kucharska et al. 2018). For example, hemicellulose is easier to soluble than cellulose under milder reaction condition, especially in alkaline solution due to its low DP. The alkali pretreatment can remove lignin and improve the cellulose digestibility; however, the large amount of wastewater produced and the high cost of the alkali are drawbacks in its application (Rydholm 1965; Talebnia et al. 2010).

On the contrary, a wide range of lignocellulosic biomass was pretreated with dilute sulfuric acid pretreatment, resulting in cellulose swelling, hemicellulose solubility, and lignin alteration for subsequent enzymatic hydrolysis (Kumar et al. 2009b). Steam explosion and dilute acid pretreatment can generate the formation of furfural, HMF, weak organic acids and phenolic compounds, known inhibitors of microbial growth, enzymatic hydrolysis, and fermentation (Yang 2015). Detoxification prevented the formation of inhibitors and improve both cell growth and ethanol production, for example, treatment with laccase removed the phenolic compounds while anion exchange at pH 10 showed the most efficient method for removing those inhibitory compounds; however, it also resulted in loss of fermentable sugars (Larsson et al. 1999).

The efficient pretreatment can minimize use of expensive enzymes, for instance, only adding acid or alkali can remove lignin and hemicellulose at a reasonable cost (Wyman et al. 2005). However, acid hydrolysis is cheap and fast, but the acidic environment can form undesirable inhibitors due to the complicated several parameters such as substrate properties, acidity and the rate of decomposition of the products during hydrolysis (Taherzadeh and Karimi 2007). On the other hand, enzymatic hydrolysis is effective without yielding undesirable by-products, but hydrolysis is slow process and enzymes are expensive (Lee et al., 2019). However, lignin is often combusted to generate heat and electricity because it is not possible to break down further in the conversion process. (Mu et al., 2010). The enzymatic hydrolysis of the glucose yield of soybean hulls after acid, alkali, and hot extrusion pretreatments of soybean hulls is increased by 69.6%, 128.7%, and 132.2%, respectively (Yoo et al. 2011). Yang et al., (2002) reported that stem-exploded Douglas-fir wood chips was post-treated with alkaline peroxide, which removed 80 % of the lignin to improve the enzymatic conversion and lower the enzyme loading significantly that lead to further decrease the cost of ethanol production from softwoods.

Although several pretreatment methods have been applied over the years, pretreatment advances are still necessitated for cost-effectiveness with conventional fuels and chemical production (Wyman 1999). Therefore, many combined pretreatments, such as ammonia with lime and dilute sulfuric acid with pH control, have been reported (Wyman et al. 2005). In particular, alkaline wet oxidation pretreatment was typically applied to biomass having a low lignin content such as ryegrass, clover and wheat straw (Bjerre et al. 1996; Martín et al. 2008). Chen et al., (2011) studied the combined dilute acid and steam explosion pretreatment of rice straw exhibited in a lower level of inhibitors in the hydrolysate and a higher yield of xylose. Lu et al., (2011) and Salvachúa et al., (2011) reported that the microwave-assisted dilute sulfuric acid pretreatment of rape straw, resulted in 92.9% energy saving and fungal pretreatment combined with a mild alkali treatment of wheat straw, generated no inhibitors during the pretreatment, respectively. However, pretreatment has been considered as one of the most expensive steps in the conversion of biomass-to-ethanol with costs as high as 30¢/gallon ethanol produced and the total capital costs vary between 2 and 27% depending on the pretreatment method (Mosier et al. 2005; Kallioinen 2014). Ultimately, the choice of pretreatment is governed by the overall process costs and not just the pretreatment operation (Wyman et al. 2005; Humbird et al. 2011).

2.4.1.4 Enzymatic hydrolysis of lignocellulose

The hydrolysis process is the second step in the production of ethanol from lignocellulosic materials. Three major processes are dilute and concentrated acids, and enzymatic hydrolysis; however, the latter one is the most common method of producing bioethanol. Concentrated acid is normally applied at lower temperature and atmospheric pressure that requires longer retention times, but dilute acid is under both high temperature and pressure that leads to the fermentation interference due to the formation of toxic degradation products (Broder et al., 1995). The enzymatic hydrolysis method has shown distinct advantages over acid hydrolysis methods due to no corrosion issue, its mind process conditions and low formation of inhibitors that leads to the higher yields (Menon and Rao 2012). Although the use of enzymes for the hydrolysis of polysaccharides to monomeric sugars as an eco-friendly approach, there are disadvantages in the enzymatic hydrolysis such as high dosages, long hydrolysis time, and costs of enzymes.

Enzymes produced by a variety of microorganisms can degrade biomass-to-sugars, but the process require longer retention times, which is connected to the hydrolysis of hemicellulose (Kumar et al. 2009b). One of the most well characterized cellulase producer of biomass-degrading enzymes is the filamentous fungus *Trichoderma reesei* (Kallioinen 2014). Enzymatic hydrolysis involves cleaving the polymers of cellulose and hemicellulose using cellulytic enzymes (Zhang and Lynd 2004). Cellulose is degraded by hydrolytic cellulases while hemicellulose needs various enzymes, e.g. xylanases and mannanases, but lignin degradation is done by other enzymes, e.g. laccases, lignin peroxidases and manganese peroxidases (Kallioinen 2014). Cellulase refers to a mixture of enzymes (catalytic proteins) that includes: (1) endoglucanases (EG, endo-1,4-D-glucanohydrolase, EC 3.2.1.4.), which cleave randomly within the cellulose fiber, reducing polymer chain length; (2) exoglucanases or cellobiohydrolases (CBH, 1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91.), which attack the ends of highly crystalline cellulose fibers; and (3) β-glucosidase (EC 3.2.1.21), which hydrolyzes the small cellulose fragments (cellobiose, a glucose dimer) to glucose (Humbird et al. 2011).

The factors influencing saccharification can be divided into two: substrate-related and enzyme-related, in which the latter includes enzyme inactivation, products inhibition, and thermal instability of cellulases (Yang et al. 2011). The presence of lignin, hemicellulose, cellobiose, and glucose blocks enzyme accessibility and causes end-product inhibitions, thus reducing the efficiency of the hydrolysis process (Swain et al. 2019). Therefore, removing lignin before hydrolysis is advantageous to enhance cellulose accessibility and reduce nonproductive binding of enzymes, thus improving hydrolysis performance and less inhibitory to fermentation (Yang and Wyman 2006). Humbird et al., (2011) computed the enzyme cost contribution 12¢/gallon, approximate 16% of the ethanol production from the dilute acid pretreated corn stover.

The use of hemicellulases is required due to some insufficient pretreatment methods that left hemicelluloses in a substrate. A variety of enzymes is needed for the completion of hemicellulosic hydrolysis, i.e. Endo-1,4- β-xylanases (EC 3.2.1.8) solubilize xylan chain to oligomers whereas 1,4-β-xylosidases (EC 3.2.1.37) attack xylooligosaccharides from the non-reducing end and liberate xylose (Jørgensen et al. 2007). An endo-1,4-β-mannanases (EC 3.2.1.78) cleaves the galactoglucomannan main chain to produce oligosaccharides, in which β-glucosidases (EC 3.2.1.21), β-xylosidases (EC 3.2.1.37) and β-mannosidases (EC 3.2.1.25) are essential for the complete hydrolysis of hemicellulosic oligosaccharides to monosaccharides (Kallioinen 2014). A number of enzymes are needed due to the complex structure of hemicelluloses. For example, in wheat, xylanases and a number of α-L-arabinofuranosidases of various origin are needed in order to obtain efficient hydrolysis of wheat arabinoxylan because arabinofuranosyls can be both (1→2) and (1→3) linked to xyloses and also to doubly substituted xylose (Jørgensen et al. 2007).

Enzymatic digestion of lignocellulosic materials need synergistic action and many forms of enzyme synergism have been observed among cellulases including endoglucanase with exoglucanase, exoglucanase with exoglucanase, endoglucanase with endoglucanase, and exoglucanase or endoglucanase with b-glucosidase etc. (Yang et al. 2011). However, such synergism depends on enzyme sources and substrate features and core enzymes such as exo- and endo-cellulases cleave only cellulose that the assistance of other auxiliary enzymes such as xylanases and pectinases remarkably enhance the performance of cellulases and increases saccharification of pretreated hardwoods and softwoods and corn stover (Berlin et al. 2007; Jørgensen et al. 2007; Thite and Nerurkar 2015). Enzyme mixture optimization has been conducted for various substrates and the aim of the optimization is to hydrolyse lignocellulosic materials efficiently using lower enzyme dosages, and thus reduce the overall cost of biofuel production (Kallioinen 2014). The synergistic cooperation of cellulases and xylanase improved cellulose accessibility by removing the blocking effect of xylan and increasing fiber swelling and fiber porosity whereas the addition of cellulases with xylanase and surfactant, polyethylene glycol (PEG 4000) in the enzymatic hydrolysis produced fermentable sugars efficiently (glucose 86.9% and xylose 70.2%) (Hu et al. 2011; Yang et al. 2015). Many researchers have optimized enzyme hydrolysis by (i) on-site enzyme production that less needs for purification and stabilization of enzyme preparations, (ii) consolidated bioprocessing (CBP) where the fermenting microorganism produces the enzymes, (iii) synergism between enzyme and microbe, (iv) adsorption of cellulases and hemicellulases onto lignin, and (v) recycling of the enzymes (Berlin et al. 2007; Jørgensen et al. 2007; Banerjee et al. 2010).

2.4.2 Anaerobic digestion

In anaerobic digestion (AD), the specific microbial population converts the fermentable sugars to the gaseous compounds. AD involves the breakdown of biodegradable materials in the absence of oxygen and produces biogas, primarily methane (CH_4) and carbon dioxide (CO_2) by a community of anaerobic microorganisms (Cantrell et al. 2008). AD can accommodate a wide range of biomass resources such as organic fraction of municipal solid waste, domestic and commercial food waste, residues of agricultural and industrial processing, livestock manure, sewage sludge including biomass with high moisture content 80-90% to produce a gas with an energy content of about 20–40% of the lower heating value of the feedstock (McKendry 2002). In biogas production, a raw material with high carbohydrate content and nitrogen is suitable to increase gas production that usually 7–9% solids are inoculated with animal manure or MSW or fresh sludge from biogas plant (Petersson et al. 2007).The AD process occurs in three sequential phases, namely hydrolysis, fermentation, and methanogenesis. The microorganisms involved with each phase are metabolically dependent upon each other for survival (van Haandel and Lettinga 1994). In the hydrolysis stage, complex compounds such as proteins, carbohydrates and lipids are cracked into biomolecules such as amino acids, sugars, and long-chain fatty acids respectively by exoenzymes (hydrolase) (Deublein and Steinhauser 2008). Fermentative bacteria (acidogenic and acetogenic) convert the simple biomolecules into alcohols, acetic acid, volatile fatty acids, and H_2 and CO_2 gas mixture, then methanogens metabolized this gas mixture producing biogas comprising CH_4 (60–70%) and CO_2 (30–40%) (Cantrell et al. 2008).

One main disadvantage of AD is low concentration of biomass in the feed stream and low biogas productivity with high retention times (Lee et al. 2019). Petersson et al., (2007) reported that untreated winter rye, oilseed rape and faba bean straw produced low biogas yields for more than 50 days with 0.36, 0.42 and 0.44 m^3kg^-1 volatile solid (VS), respectively. A pretreatment of the substrates is assited to achieve shorter retention times and higher biogas yields in AD, for instance, the size reduction can improve degradation of lignocellulosic materials in hydrolysis process for biogas production (Braun et al. 2010). However, it could be more efficient if size reduction is combined with other pretreatments (Zhang et al. 1999). The alkali and acid pretreatment, pre-digestion, thermal, thermochemical (autoclave), ultrasonic, and ensilage of feed are found to have a positive effect on biogas yield from lignocellulose materials with cheap costs (Petersson et al. 2007). Steam explosion and thermal pretreatments are widely investigated for improving biogas production from forest residuals and wastes such as activated sludge, cattle manure or MSW (Menon and Rao 2012). Digestate, is a byproduct of the biogas production process, which consists of the remaining part of the feedstock originally fed into the digester once the gas is extracted, and therefore it contains water, nutrients and organic carbon that may be used as a bio-fertilizer. Mono and co-digestion for the production of power, heat and raw biogas to be upgraded to biomethane by removing CO_2 and hydrogen sulfide (H_2S) for the production of substitute natural gas (SNG) or compressed natural gas (CNG) or liquefied natural gas (LNG) give a contribution of about 8% (8.8 PJ) in Netherland (de Jong et al. 2011).

2.5 Thermochemical conversion technology

Thermochemical conversion provides a technology option for improving the bioenergy industry's economic viability by converting the maximum biomass resource utilization to produce biofuels (Phillips et al. 2007). Biochemical conversion technologies rely on enzymes and microbial cells to convert an intermediate mixed sugar to ethanol or butanol. Unlike the biochemical process, the thermochemical conversion technologies rely on a high-temperature and/or physical catalysts to break apart the bonds of organic matter to an intermediate gas or liquid, and reform these intermediates into a biofuel (Cantrell et al. 2008; Foust et al. 2009). Thermochemical conversion process has a number of other benefits and advantages: (1) small footprint; (2) utilizing a whole biomass including lignin; (3) low energy consumption; (4) no fugitives gas emission; (5) short processing time on the order of minutes; (6) a more comprehensive range of feedstocks and blend than biochemical conversion; and (7) produces reasonably high alcohol yields that lead to easy industrialization (Cantrell et al. 2008; Dutta et al. 2012; Li et al. 2017). However, this technology has not yet fully developed with respect to its commercial application.

Thermochemical conversion technologies has four main processes for the conversion of biomass into energy products: combustion, pyrolysis, gasification and liquefaction (Mofijur et al. 2019). Combustion is the oxidation of fuel, in which biomass can be completely burnt into heat, electricity or mechanical power in the presence of air (Mofijur et al. 2019). Air, oxygen or steam are used as a reaction medium in gasification to convert a dry or wet biomass into a combustible gas mixture, i.e., achieve a higher hydrogen-to-carbon (H/C) ratio, while combustion oxidizes carbon and hydrogen into water and CO_2, respectively (Cantrell et al. 2008; Basu 2010). However, the combustion product gas does not have useful heating value and its efficiency is only about 10% that leads to the substantial pollution (Jahirul et al. 2012). The two techniques that supply bio-oil or biocrude are pyrolysis and liquefaction. Pyrolysis is a thermal decomposition process in the absence of oxygen within a temperature range of 300 to 650 ^{o}C to convert the feedstock organic portion into a mixture of char and volatile gases (oxygenated hydrocarbons), which form a combustible pyrolytic oil or bio-oil (Cantrell et al. 2008; Basu 2010). This process partially removes carbon from the feed but does not add hydrogen and it is the first stage of both combustion and gasification process (Jahirul et al. 2012). Liquefaction process hydrolyzes the lignocellulosic components in biomass to convert into liquid fuels (bio-oil) at low temperature (250–350 °C) and elevated pressure (5–20 MPa) with or without catalyst in the presence of hydrogen (Cantrell et al. 2008). Liquefaction can be done through pyrolysis, gasification as well as through hydrothermal process. In hydrothermal liquefaction (HTL) process, biomass is converted into bio-oil by utilizing subcritical water (SCW) at temperatures (250 to 374 °C) with operating pressure from 4 to 22 MPa (Lee et al. 2019).

Choice of thermochemical conversion process depends upon the desired form of the energy, the type and quantity of biomass feedstock, economic conditions, environmental standards, and project specific factors (McKendry 2002). To evaluate cost-competitive commercial second-generation biofuel production, a techno-economic analysis (TEA) needs to be done (He and Zhang 2011). Particularly, TEA of indirect gasification was explored by researchers at the U.S. Department of Energy (Phillips et al. 2007; Foust et al. 2009; Swanson et al. 2010; Dutta et al. 2012). ASPEN Plus was used to model the indirect gasification and mixed alcohol synthesis, which illustrated in Figure 5 (Foust et al. 2009). This process produces syngas, which is primarily composed of CO, H_2, CO_2, CH_4, tars and water. Raw syngas primarily contains catalyst-fouling contaminants that must be removed before alcohol synthesis and the cleaned syngas is then converted to mixed alcohols, mainly ethanol and propanol through a fixed-bed molybdenum catalyst (Phillips et al., 2007). The unconverted syngas is recycled to the tar reformer while the uncondensed alcohols undergo distillation and purification to recover pure ethanol whereas methanol is recovered and recycled to the synthesis reactors to increase ethanol and higher alcohol yields (Foust et al., 2009).

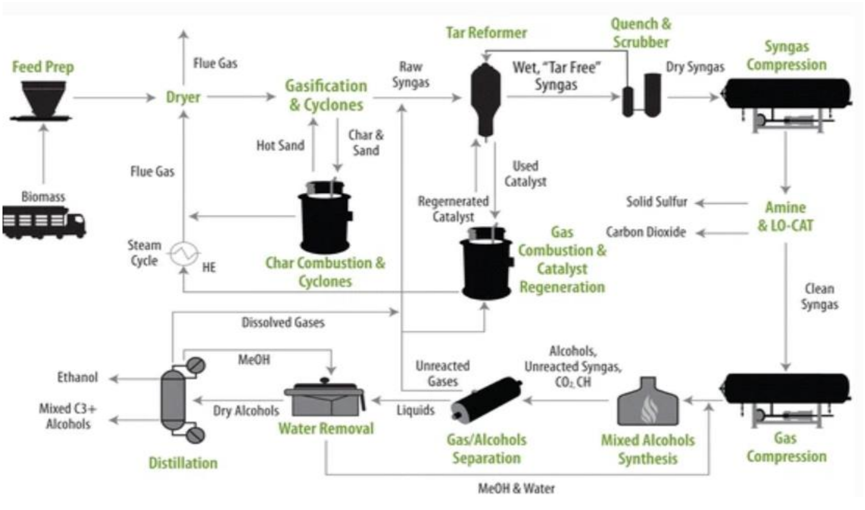


Figure 5. Bioethanol production via thermochemical conversion process (According to Foust et al., 2009).

Thermochemical conversion can use a wider range of feedstocks than biochemical conversion due to lignin in the biomass is not adversely affected; however, the MC of feedstock significantly affects alcohol yields (Daystar et al., 2015). Foust et al. (2009) estimated the MESP of wood chips feedstock was \$1.22/gal of ethanol and the higher alcohols could be sold as a co-product \$1.29/gal in 2007\$. However, the MESP of Southern pine \$2.05/gal was increased to \$2.47/gal in 2007\$ while using corn stover (Dutta et al., 2012).

2.5.1 Gasification

Gasification is a thermo-chemical conversion of a carbonaceous feedstock at high temperatures in in the presence of an oxidizing agent (also called a gasifying agent) to optimize the gas production. The feedstock (coal, petroleum, coke, biomass, etc.) is subjected to partial oxidation due to a lower concentration of oxygen than the stoichiometric requirement (Ciliberti et al. 2020). Biomass is preferable because it has insignificant content of N_2, S and ash, which releases lower emissions of nitrogen oxide, sulfur dioxide, and soot than conventional fossil fuels (Balat 2009). When the amount of oxygen exceeds a certain (stoichiometric) amount, the process moves from gasification to combustion to produce flue gas (Basu 2010). Flue gases are a mixture CO_2, water vapour, heavy metals, particulates, and acidic gases (Lettieri et al. 2010). The main product of gasification is a producer gas, which composed of combustible (Hydrogen, Methane and Carbon Monoxide) and non-combustible (Nitrogen, Carbon dioxide) gases (Balat et al. 2009). Lignocellulosic biomass can be gasified to produce clean fuel gases or synthesis gas (syngas), which is consisted mainly of H_2, CO and CO_2. Both producer gas and syngas are obtained from gasification process, but their final composition and characteristics are dependent on gasifying agent, the type of biomass, gasification process, gasifier type, residence time, temperature, pressure, equivalence ratio (ER), and catalyst used (Balat et al. 2009; Ciliberti et al. 2020). Gasification of biomass is generally presented the following reaction:

$$\text{Biomass} + O_2\ (\text{or } H_2O) \rightarrow CO, CO_2, H_2O, H_2, CH_4 + \text{other CHs} + \text{tar} + \text{char} + \text{ash}$$

A typical biomass gasification process involves four steps: oxidation, drying, pyrolysis, and reduction (Molino et al. 2016). The drying process strongly depends on the amount and thermodynamic state of water, and forms of water (bound water in cell walls and free water in voids of the wood) in biomass (Morf 2001). The typical moisture content of freshly cut wood ranges from 30 to 60%, and for some biomass, for example secondary pulp sludges, it can exceed 90% (Pöykiö et al. 2018). At temperature above 100 ^{o}C, the water is removed and converted into steam until the process continues to give off acetic acid, CO_2 and water between 200 – 280 ^{o}C (Demirbaş 2002). Pyrolysis is an essential prestep in a gasifier. Pyrolysis produces large quantities of solid tar and condensable gases, which can be broken down further into noncondensable gases containing CO, CO_2, H_2, CH_4 without major chemical reaction with air, gas and any other gasifying medium (Balat 2009; Basu 2010). The pyrolysis process may be represented by a generic reaction as:

$$\text{CnHmOp (Biomass)} \xrightarrow{\text{Heat}} \Sigma \text{ liquid CxHyOz} + \Sigma\text{gas Ca Hb Oc} + \text{H2O} + \text{C (char)}$$

Gasification involves the devolatilization (pyrolysis) and conversion of a heterogeneous supply of biomass feedstock in a presence of gasifying medium like steam and/or oxygen at high temperatures (800–1000 ºC) to produce synthesis gas (syngas) (Basu, 2010; Phillips et al., 2007). If oxygen is used as the gasifying agent, the conversion path moves toward an increase in carbon-based (CO and CO_2) while steam is used, the product gas results in a higher H/C ratio. The gasifying medium oxygen is more preferable than steam due to its higher heating value (12-28 MJ/Nm^3) compared to steam (10.18 MJ/Nm^3) (Basu 2010). There have been several thermochemical reactions: (i) water-gas shift reaction, (ii) carbonation (Boudouard reaction), (iii) oxidation, (iv) methanation, and (v) steam reforming, taken place in a gasifier simultaneously. The typical process that occur in biomass gasifier is illustrated in Figure 6.

The char gasification reaction plays a major role in the volume, design, and performance of gasifier because the rate of gasification of char is much slower than the rate of pyrolysis of the biomass that produces the char (Basu 2010). Therefore, the rate of the overall wood gasification process is determined by the kinetics of the heterogeneous gasification reactions (Balat 2009). Gasifiers are generally classified according to the fluidization regime (gas-solid contacting mode and gasifying medium) in the gasifier: (i) fixed or moving bed, (ii) fluidized bed, and (iii) entrained-flow bed. Balat et al. (2009) reported that most of the commercial gasifiers were 75% of downdraft fixed bed, 20% of fluidized bed, 2.5% of updraft fixed bed, and the rest 2.5% of the other designs. The fixed bed gasifiers are suitable for small- and medium-scale applications (< 10MW), while fluidized bed gasifiers are fit for large-scale applications, in particular, downdraft gasifiers are economically competitive could save $13,850 for 3000 h of operation compared to conventional LPG heating unit (Panwar et al. 2009; Basu 2010).

A technical challenge to gasification is tar formation, which are categorized into primary, secondary, and tertiary tars. The former are produced directly from the pyrolysis of cellulose, hemicellulose, and lignin while the latter are the result of several complex reactions by increasing temperature above 500 oC and 800 oC, respectively (Molino et al. 2016). But with the use of the catalytic reforming of char bed and some modification in gasifiers' design, the tar can be removed in the gasification process (Zeng et al. 2020).

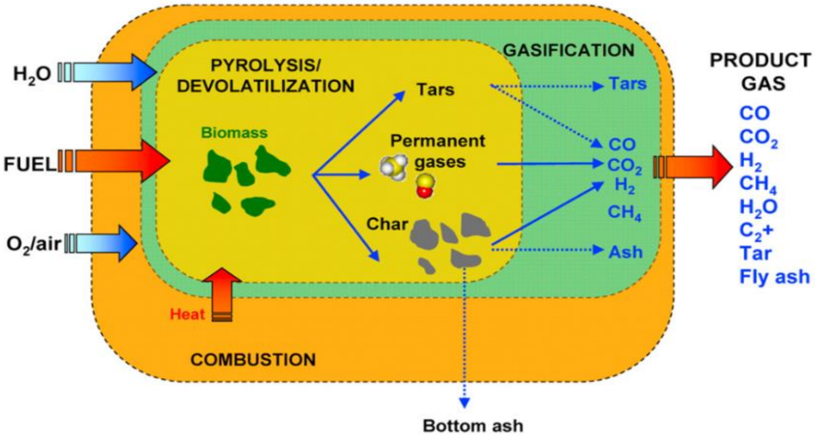


Figure 6. The gasification process (Adapted from Gómez-Barea and Leckner, 2010)

When olivine particles were used as a bed material, the gas yield increased by more than 50%, tar was reduced by 20 times and char was reduced by 30% as compared to using sand (Kumar et al. 2009a). When 10 wt% Fe-olivine catalyst was utilized in a pilot gasifier instead of olivine, increasing 40% and 88% of the gas yield and hydrogen, respectively, whereas reducing 46% and 46% of tar and methane content in the syngas, respectively (Heidenreich and Foscolo 2015). Tar has an advantage to increase the calorific value of the fuel during combustion of product gas (Kumar et al. 2009a). At the end of the entire process, two major fraction are left: the solid fraction such as char and ashes and the gaseous mixture such as syngas and a small amount of impurities, such as light hydrocarbons (ethane, ethylene, acetylene), hydrogen sulfide (H2S), sulfur dioxide (SO2), hydrogen chloride (HCl), nitrogen oxides (NOx), nitrogen (N2),and ammonia (NH3) (Ciliberti et al. 2020). Syngas is not only an energetic carrier for power production but also an intermediate product for liquid (bioethanol and biodiesel) and gaseous (biomethane) biofuel production (Molino et al. 2016). As syngas produced from biomass gasification has a low or absent impurities content, more research is required in order to increase the overall efficiency of gasification process to achieve an ideal syngas composition, thereby making the syngas fermentation process as efficient as possible (Ciliberti et al. 2020).

2.6 Integrated bio- and thermo-chemical technology

Lignocellulosic biomass can be converted into bioethanol by hydrolysis-fermentation (biochemical), gasification-synthesis (thermochemical), gasification-fermentation (integrated biochemical and thermochemical). Biochemical conversion has a high selectivity and conversion efficiencies while thermochemical technology has an ability to apply a wide range of feedstocks and technology robustness (Foust et al. 2009). The biomass-to-ethanol conversion efficiency is low since 25–30 wt.% biomass as lignin cannot be degradable in the hydrolysis-fermentation route (He and Zhang, 2011). In gasification-synthesis route, ethanol and higher alcohols are obtained via separation and purification processes after biomass is gasified into raw syngas, which is further reformed, cleaned, compressed, heated and converted into mixed alcohols (He and Zhang, 2011). Two kinds of gasification processes are classified depending on the method of heat supply: the direct gasification takes place the endothermic gasification reactions in the reactor while the indirect gasification of heat production occurs outside the reactor (Aranda et al. 2014). Both direct gasification (autothermal – one gasifier) and indirect gasification (allothermal - two gasifiers) can produce nitrogen-free syngas, which is favorable for clean combustion technology and the synthesis of liquid fuels and chemicals (Prabowo et al. 2014). However, the product gas from indirect gasification is suitable for synthesis applications after proper cleaning and upgrading without the need for an expensive air separation unit because it allows high feedstock conversion and also better control and process optimization that produces a high value gas which contains compounds such as CH_4, C_2-C_4 gases (including ethylene and acetylene), benzene, toluene, xylene, and tar (Liakakou et al. 2021).

The gasification-synthesis route has many advantages because syngas can be directly used in heat and power production as well as a platform to focus on transportation fuel production such as bioethanol, mixed alcohols, biodiesel; hydrogen, synthetic natural gas

(SNG), methanol and dimethyl ether (DME) (Molino et al. 2016; Ciliberti et al. 2020). However, commercialization of catalytic synthesis of ethanol from syngas, despite a few pilot plants ranging from 0.3 to 67 MW is built and operated, has not been succeeded due to the huge cost in the syngas cleaning (He and Zhang 2011). Key issues in technology development of several advanced biofuels production are (i) strategies for reducing capital requirements to reach commercial scale, (ii) improvement in conversion efficiency and (iii) to be widely deployed. These strategies have to integrate the use of core technology components such as (hemi-) cellulose to sugar conversion in chemical industry and tar-free syngas production (IEA, 2011). Therefore, the different advantages from both technologies has been taken for the microbial conversion of syngas (Table 3).

The integrated gasification-syngas fermentation process, in which biomass is first gasified to produce intermediate syngas, which is then converted to alcohols and organic acids by the Wood-Ljungdahl metabolic pathway of acetogenic microorganisms as biocatalysts (Ljungdahl et al., 1965). *Clostridium ljungdahlii* has been used for the syngas fermentation due to its most well-characterized strains and used broadly as a model for acetogenic bacteria in fermentation experiments (Liakakou et al. 2021). The techno-economic assessment of production of ethanol from lignocellulosic biomass from both biochemical and thermochemical processes has been previously designed, simulated and optimized mainly with ASPEN Plus software by many researchers from NREL (Phillips et al. 2007; Swanson et al. 2010; Humbird et al. 2011; Dutta et al. 2012).

Table 3. Advantages, disadvantages, and yield ranges of bioethanol per dry tonnes of feedstock from biochemical and thermochemical processes (Speight 2011; Heidenreich and Foscolo 2015).

Process	**Advantage**	**Dis-advantage**	**Bioethanol yields (L dry-t^{-1})**		**Energy content (MJL^{-1})**	**Energy yields (GJ t^{-1})**	
			L	H	LHV	L	H
Biochemical (Enzymatic hydrolysis fermentation)	(1) high selectivity and conversion efficiencies (2) high sugar yield (3) optimal conditions (4) no corrosion problems	(1) cannot utilize lignin (2) long reaction time (3) limited to one or few products (4) require additional microbes and enzymes for more products	110	300	21.1	2.3	6.3
Thermo-chemical (Gasification)	(1) short processing time (2) utilize a whole biomass and tolerant for almost any biomass (3) nearly complete biomass conversion (4) high process efficiencies and high-quality syngas	(1) high investment costs (2) high pressure and temperature resistant materials (3) high energy requirement (4) sulphur and tar yields	120	160	21.1	2.5	3.4

L: Low; H: High; LHV: Lower heating value

The major contributions to the production costs are from biomass feedstock and syngas cleaning in the indirect gasification process that a biomass-to-ethanol plant should be built around 200 MW as the biomass input, which gives the ethanol production cost €0.33/L (€2011) (He and Zhang 2011). However, commercialization of both catalytic synthesis of ethanol from syngas and gasification syngas-fermentation are still in an early stage of development.

3. AIM OF THE STUDY AND EXPERIMENTAL DESIGN

The overall aim of the study was to investigate the biofuel production from primary and secondary biomass feedstocks through biochemical, thermochemical, and combined bio- and thermo-chemical conversion technologies. The study was conducted under three platforms (biogas, sugars and syngas) with two kinds of carbon-based feedstock (lake bottom biomass and side-streams from pulp and paper mill process) for the production of bioethanol, biobutanol and biogas as the prime products (Figure 7).

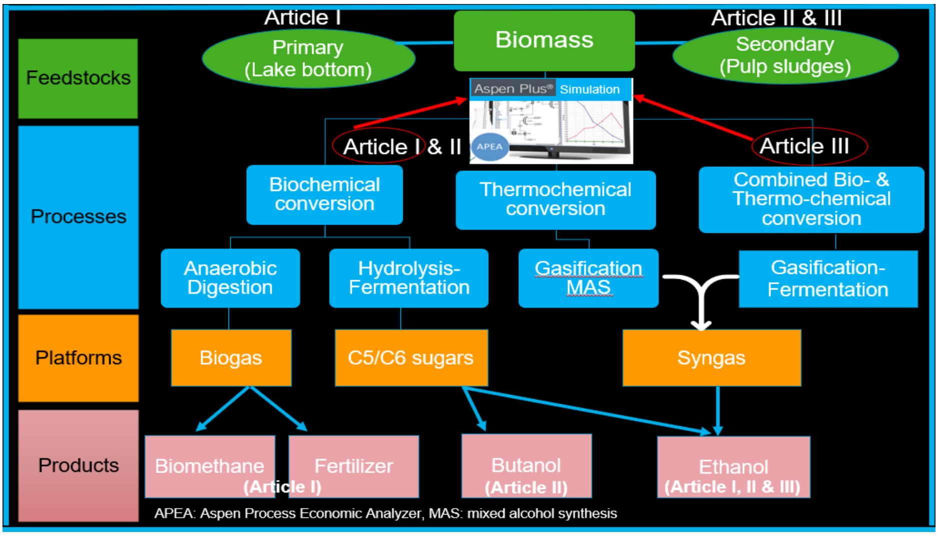
The potential biomethane and bioethanol production from mesotrophic and eutrophic lake bottom biomass (Article I) and bioethanol production from primary pulp sludge (Article III) was modeled by Aspen Plus software. The economic feasibility of these feedstocks utilization was evaluated by an Aspen Process Economic Analyzer (Articles I and III). The rejects from kraft pulping (PI), primary and secondary sludges (PII and PIII) were tested for ABE fermentation (Article II).

More specifically, the aims were:

(i) to study the suitability of mesotrophic and eutrophic lake bottom materials – both potential non-food lignocellulosic feedstocks in Finland – for bioethanol production through saccharification and fermentation, and gasification and mixed alcohol synthesis processes as well as biomethane production via anaerobic digestion coproduced with the process residues as fertilizer (Article I).

(ii) to analyze the biobutanol production of three side-streams (PI, PII and PIII) from different manufacturing process of kraft and mechanical pulping through ABE fermentation using *Clostridium acetobutylicum* (Article II).

(iii) to characterize and compare the chemical composition of the eight side-streams (PI – PVIII) from different pulping process, and to investigate the potential bioethanol production from the primary sludge (PII) through integrated gasification-syngas fermentation (Article III).



APEA: Aspen Process Economic Analyzer, MAS: mixed alcohol synthesis

Figure 7. Overall framework for the present study on energy-driven biorefinery from lake bottom biomass and PPMS through biochemical and thermochemical and the combination of bio- and thermo-chemical conversion processes.

4. MATERIALS AND METHODS

4.1 Primary and secondary biomass feedstocks used in this research

The ML and EL lake bottom materials were collected from two locations: Lake Likokanta and Lake Kutunjärvi in eastern Finland (Article I). They contained a heterogeneous mixture of above- and below-ground lake bottom substances such as sediments, woody debris, straws and rhizomes of reed, and other locally found aquatic plants (Table 4). Eight samples (PI - PVIII) were received from pulp and paper and board mills in Finland (Articles II and III). Butanol production via ABE fermentation using *C. acetobutylicum* from three side-streams (PI, PII and PIII) was investigated in Article II. The studied pulp samples (PI – PVIII) were from different stages of chemical and mechanical pulping processes such as pre-hydrolysis stage, zero fiber, primary sludge, secondary sludge, bark sludge, biosludge, and mixed primary and secondary sludge (Table 4). The ML and EL lake bottom materials and primary sludge (PII) from the kraft pulping were evaluated for bioethanol production, modeled by Aspen Plus® simulation (Articles I and III). All studied samples were kept in sealed containers at 5°C prior to the analysis.

Table 4. Origin of the studied samples evaluated in this research.

Samples	Process stage	Sample type and contents
ML[a]	Nil	dead biomass of lake bottom such as stems, straw and rhizomes of the common reed, common club-rush and Scirpus spp. mixed with inorganic sediments, such as humus, sand and clay
EL[a]	Nil	Woody debris, straws and rhizomes of reed and a variety of dead and fresh local aquatic plant species
PI	Kraft pulping, rejects from pre-hydrolysis stage	Residues from wood handling, bark residues, fiber materials, wood knots
PII[b]	Kraft pulping, primary sludge	Fiber residues, cooking chemicals, fillers, traces etc.
PIII	Mechanical pulping, surplus wood from the pulp industry	Zero fiber (the stage before fiber sedimentation)
PIV	Mechanical pulping, secondary sludge from wastewater treatment	Fiber residues, biological wastewater treatment chemicals, microorganisms etc.
PV	Kraft pulping, wood debarking stage	Softwood bark sludge
PVI	Kraft pulping, primary sludge	Fiber residues, fillers, cooking chemicals, traces etc.
PVII	Kraft pulping, secondary sludge from wastewater treatment	Fiber residues, biological wastewater treatment chemicals, microorganisms etc.
PVIII	Kraft pulping, mixed sludge from primary and secondary sludges	Fiber residues, wastewater treatment chemicals, microorganisms etc.

Feedstocks used in the Aspen Plus modeling in [a] Article I and [b] Article III.

4.2 Chemical analysis

Dry matter (DM), total solids and ash contents of the studied samples (both lake bottom biomass and pulp sludges) was determined according to the procedure of NREL (Sluiter et al. 2008a, b) (Articles I, II and III). All studied samples were oven-dried (105 °C) before being analyzed. The loss-on-ignition (LOI) as organic matter content was performed according to Kuokkanen et al. (2008) (Articles I, II and III). The pH of the lake bottom samples was measured by a Lab 860 (SCHOTT) glass electrode pH meter (Article I). The elemental combustion method (infrared gas analyzer: TC-L series, Shimadzu's ECO, USA) and the Kjeldahl method (Kjeldahl 1883) were used for total carbon (Total C%) and total nitrogen (Total N %), respectively, for lake bottom biomass (Article I). The digital titration method (Titronic®universal, SI Analytics GmbH, Germany) and alkalinity strips (Hach Company, USA) were used for alkalinity measurement.

Gas chromatography-mass spectroscopy (GC/MS, 8040 series; Shimadzu, Japan) was used for volatile fatty acids (VFA) for the fresh lake bottom samples. The elemental analyses for lake bottom biomass and pulp sludges (Articles I and II) were measured by a thermo electron spectrometer (model ICP-OES-IRIS Intrepid II XSP, Thermo Fisher Scientific, Waltham, MA, USA). A 600 MHz Bruker nuclear magnetic resonance (^{1}H NMR) spectroscopy (Bruker Prodigy TCI 600 S3 H&F-C/N-D-05 Z, Ettlingen, Germany) was used for the analysis of liquid samples of pulp sludge (Article II) such as sugars, solvents and other compounds (acetic acid, formic acid, lactic acid, butyric acid, 5-hydroxymethylfurfural — HMF, furfural) (Yang et al. 2015). Before NMR analysis, all untreated PPMS samples were centrifuged at

5000 g for 10 min to collect the supernatant. In Articles I, II and III, the chemical composition of lake bottom biomass and PPMS was determined according to Hayes (2012).

4.3 Dilute acid pretreatment and enzymatic hydrolysis of PPMS

In Article II, the pulp side-stream from kraft pulping pre-hydrolysis stage (PI) was pretreated with water, 0.1% and 0.2% dilute sulfuric acid at 180 °C and 200 °C for 10, 20, 30, 45, and 60 min. Two kinds of preparation was involved before dilute acid pretreatment: one was washed with 10% w/v DM content in 200 mL of distilled water while the other was unwashed. Both washed and unwashed studied samples (PI, PII and PIII) were pretreated under the efficient condition, 0.2% sulfuric acid at 180°C for 10 min (Article II, Figure S1). The mixture separation was done by using 125 mm diameter Whatman 589/l filter paper. The carbohydrates and degradation products of the pretreated liquid hydrolysate were investigated, whereas the solid fractions were washed and analyzed for enzymatic hydrolysis with 7% DM loading for 48 h.

All studied samples (PI, PII and PIII) in Article II were hydrolysed under four preparations: (i) untreated (UN) as control, (ii) washed-unpretreated (W), (iii) unwashed-pretreated (UWP), and (iv) washed-pretreated (WP). Enzymatic hydrolysis was done by a commercial cellulase preparation (CEL) Celluclast 1.5 L (Sigma-Aldrich, Saint Louis, MO, USA) (10 filter paper units (FPUs)/g of DM), β-glucosidase (Novozyme 188, Novozymes A/S, Bagsværd, Denmark) (200 nkat/g DM) and endo-1,4-b-xylanase (XYL) from *Trichoderma longibrachiatum* (2g/100g DM) after 48 h of incubation at 50 °C with an agitation speed of 200 rpm. The dinitrosalicylic acid (DNS) method described by Miller (1959) was used for reducing sugar analysis after conducting enzymatic hydrolysis. A nuclear magnetic resonance (NMR) spectroscopy was used for the analysis of sugar and acid concentrations.

4.4 Preparation for ABE fermentation

Clostridium acetobutylicum DSM 1731 was obtained from the German collection of microorganisms and cell cultures (DSMZ, Braunschweig, Germany) and was anaerobically inoculated in 50 mL of Reinforced Clostridial Medium (RCM) for 14 to 16 h (Hirsch and Grinsted 1954). The stock culture was incubated for 16 h at 37 °C in a Memmert incubator (Memmert, Germany). The P2 medium was described by Yang (2015) with a filter-sterilized buffer was prepared by dissolving 10 g/L of yeast extract with 220 g/L ammonium acetate ($NH_4CH_3CO_2$); 50 g/L each of dipotassium phosphate (K_2HPO_4) and monopotassium phosphate (KH_2PO_4); 20 g/L magnesium sulfate heptahydrate ($MgSO_4.7H_2O$); 1 g/L each of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$), manganese sulfate monohydrate ($MnSO_4.H_2O$) and sodium chloride (NaCl); 0.1 g/L each of para-aminobenzoic acid and thiamin, and 0.001 g/L biotin. The medium and bottles were autoclaved at 121 °C for 15 min and left at room temperature for cooling. The enzymatic hydrolysate of studied side-streams were dissolved in a 100 mL serum bottles with 30 mL of a mixture solution that contained RCM and P2 medium. The ABE fermentation process was started by the anaerobically transfer of 2 mL of *C. acetobutylicum* culture into the inoculum which contained 1 mL of actively growing culture with 50 mL of a sterilized prepared fermentation P2 medium supplemented with 30 g/L of glucose in a 125 mL screw capped Pyrex bottle and 10 g/L of yeast extract as described by Yang (2015). Approximately 2 µL of sample was drawn using a syringe at 0, 24, 48, 72, 96 h. All experiments were performed in duplicate.

4.5 Modelling of primary and secondary biomass feedstocks potential for biofuel production

Three models were developed for the potential biogas and bioethanol production from the utilization of lake bottom biomass through three bioenergy conversion processes: (i) anaerobic digestion; (ii) saccharification and fermentation; (iii) gasification and mixed alcohol synthesis, using commercial Aspen Plus software. The calculations of the fermentation and digestion processes including the condensing and alcohol separation unit were measured by the non-random-two-liquid (NRTL) property method. The IDEAL property method was used in the gasification process, such as drying, gasification, and reforming units. Gas cleaning and alcohol synthesis units were modeled by ELECNRTL and SRK methods, respectively. Henry components were calculated to estimate non-condensable gases in the liquid phase. In Article III, primary sludge (PII) from the kraft pulping was selected among eight different side-streams (PI–PVIII) from Finnish pulp and paper and board mills, as a representative substrate for ethanol production. The integrated gasification syngas fermentation technological process was modeled by Aspen Plus® V9 simulation software. The process flow contains gasification combined with char combustion, syngas purification, syngas fermentation, ethanol purification, nd dehydration without the wastewater treatment unit in this research. The main process flow diagram and mass balances of the process were described in more detail in Figures 2-4 in Article I and Figure 1 in Article III.

4.6 Economic evaluation of biofuel production processes

In Articles I and III, the techno-economic parameters of anaerobic digestion, saccharification and fermentation, and indirect gasification and mixed alcohol synthesis, and integrated gasification-syngas fermentation were evaluated by an Aspen Process Economic Analyzer (APEA)with the assumption that the feedstock according to Table 5. In Article III, APEA software uses a volumetric model to calculate costs and provides more reliable estimates than the scaling factor method of estimation. Capital expenditures cost (CAPEX), which includes direct and indirect costs, OPEX, and the main techno-economic parameters, were estimated over a biofuel plant lifetime of 20 years. Contingency was added as 18% of the total direct and indirect costs. The main financial assumptions are utilities and raw material costs, along with operating costs that include operating charges (25 %), biofuel plant overheads (50%), and general and administrative expenses (8 %). Escalation parameters were also considered for project capital, products, raw materials, utilities, and operating and maintenance labor.

Table 5. Economic parameter in biofuel production from the proposed biochemical, thermochemical and combined bio- and thermo-chemical technologies.

Parameters	**Article I**			**Article III**
Process	Anaerobic digestion	Saccharification and fermentation	Indirect gasification mixed alcohol synthesis	Gasification-Syngas-fermentation
Feedstock	Mesotrophic and eutrophic lake bottom biomass			Primary Sludge PII
Plant size (dry tonnes/day)	2000	2000	2000	72600 dry tonnes/year
Operation (h/year)	8410			8000
Plant life (year)	20			20
Parameters did not include in the model	Water process treatment and waste disposal such as ash, lignin, sulfur, char, digestate			Nutrient demand and pH regulation
Products	Methane	Ethanol	Butanol	Ethanol

The economic profitability of the modeled process was evaluated for different amounts of feedstock, namely 50,000, 100,000, 2000,000 and 300,000 tonnes (wb) annually at an ethanol price range of €0.2–1.0/L (Article III, Figures S1-S4). In addition, the effect of government subsidy on ethanol production (€150/t) and the impact of a gate fee from €5 to €25 on the economic profitability of pulp sludge for gasification-syngas fermentation was investigated under a different tax rate of 20, 25, 30 and 35%, respectively.

4.7 Calculation

In Article I, the higher heating value (HHV) was evaluated according to the correlation equation (eq. 1) described in Channiwala and Parikh (2002). The lower heating value (LHV) was then calculated according to the equation by Hakkila (1989) (eq. 2). The Buswell equation (Buswell and Hatfield 1936) (eq. 3) was used for theoretical biogas yield using ultimate analysis data.

$$\text{HHV (MJ/Kg)} = 0.3491C + 1.1783H + 0.1005S - 0.1034O - 0.0151N - 0.0211A \text{ --------- } (1)$$

where HHV is the higher heating value (MJ/Kg), *C, H, S, O, N* and *A* are the mass fractions of carbon, hydrogen, sulphur, oxygen, nitrogen and ash content in the studied mesotrophic (ML) and eutrophic (EL) lake bottom biomass on a dry mass basis.

$$\text{LHV (MJ/Kg)} = \text{HHV} - 0.2205 \times \text{H} \text{ --- } (2)$$

where LHV is the lower heating value (MJ/Kg) and H is the % hydrogen content (by dry mass) of the feedstock.

$$C_cH_hO_oN_nS_s + 1/4\,(4c-h-2o+3n+2s)\,H_2O \rightarrow 1/8\,(4c-h+2o+3n+2s)\,CO_2 + 1/8\,(4c+h-2o-3n-2s)\,CH_4 + nNH_3 + sH_2S \text{ --- } (3)$$

where *c*, *h*, *o*, *n*, and *s* represent the molar proportions of mass fractions of elements *C, H, O, N* and *S* in the organic fraction of the biomass.

5. RESULTS AND DISCUSSION

5.1 Characterization and chemical compostions of the studied feedstocks

Lignocellulosic biomass is composed of cellulose, hemicellulose, lignin and a small fraction of inorganic matter and the relative chemical composition vary in each plant that the degree of biomass decomposition and recalcitrance depends on its composition during pyrolysis and hydrolysis, respectively, (Ahorsu et al. 2018). Research into potential biomass evaluation includes characterization, utilization, processing, production, conversion efficiency improvement, economic profitability and feasibility studies of the feedstocks. The aim of characterization is to understand the chemical behaviour of the potential biomass feedstocks because several characteristics such as moisture content (MC), ash content, heating value and chemical compositions affect the performance of biofuel. In order to predict the potential utilization of lake bottom biomass, the chemical composition, such as ash and moisture contents, and the major organic elements (C, H, O, S and N) were investigated (Table 6). The very high ash content in ML (79.6%) was unfavorable for biofuel production, but the results of the inorganic analysis indicate that the ash of both ML and EL biomass can be used as fertilizer in forestry, agriculture and landscaping (Article I, Table 5).

In the pulp and paper industry, MC in pulp sludge depends on the stage of pulping process, from which pulp waste is collected. The MC of all studied samples (PI– PVIII) contains a high-ranged between 70% and 80% of moisture (Table 6) that can cause additional heat consumption in drying stage gasification. Therefore, water vaporization is the only treatment method available to pretreat the feedstock. The ash content of almost all the examined pulp samples exhibited an ash content of 4–8% (Table 6), which conformed to the 6% and 9% ash contents reported for generated sludges from paper mill and kraft pulping sludge by Méndez et al. (2009) and Gottumukkala et al. (2016). Moreover, the ash content was inversely correlated with heating values and carbon content that the greatest ash content (23.52%) observed in secondary sludge PVII showed the lowest HHV (17.14 MJ/kg) and carbon content (44.68%) (Table 6). The carbon content in both EL biomass (52.3%) and all studied pulp samples, PI–PVIII (44–58 %), was in agreement with the carbon content of switchgrass (49.7%) and pine chips (52.8%), sewage sludge (50.9%) and pulp and paper mill sludges (29–55 %) (Scott et al. 1995; Ptasinski 2016).

The EL biomass has a high sugar concentrations (23.6%) compared to ML (6.9%), and thus the former one is potential for the theoretical ethanol yield potential (Table 6). The structural composition of the pulp and paper mill sludges vary moderately from one plant to another, depending on the type of feedstocks, wastewater cleaning technique applied, and freshwater consumption (Kuokkanen et al. 2008). For example, the kraft pulping rejects (PI) had the highest sugar content (70.9%) and the lowest lignin (19.1%) content that it was found to be the best choice for biobutanol production (Article II, Table 2). However, the side-streams from kraft pulping process (PV, PVII and PVIII) contained low glucan contents

Table 6. Chemical composition of the studied materials used in this research.

Article I Lake bottom biomass			**Articles II and III Pulp and paper manufacturing process side-streams**								**Average values of pulp samples**
Parameter	ML	EL	P I	P II	P III	P IV	P V	P VI	P VII	P VIII	
(%)											
Moisture content (% dry matter)	22.2	56.9	77.30	75.80	87.70	90.00	78.00	66.59	82.17	71.00	78.6
Lost-on-ignition	14.5	93.5	94.81	92.92	92.89	95.72	94.13	91.05	76.48	91.99	91.2
Ash content	79.6	5.1	5.19	7.08	7.11	4.28	5.87	8.95	23.52	8.01	8.8
Glucan	54.0	57.3	56.54	43.72	39.16	42.18	16.21	40.58	2.43	29.87	33.8
Xylan	31.5	16.4	6.82	4.01	6.26	8.45	2.48	6.95	0.43	5.09	5.1
Mannan	2.3	8.1	5.52	2.67	8.17	7.11	3.25	7.58	0.85	5.38	5.1
Arabinan	5.9	7.7	0.90	0.58	0.89	0.81	1.71	0.77	0.29	0.83	0.8
Galactan	5.1	8.6	1.11	0.99	1.71	1.54	1.41	1.36	0.87	1.26	1.3
Rhamnan	1.2	2.0	0.03	0.34	0.29	0.21	0.30	0.13	0.46	0.23	0.2
Total sugars	6.9	23.6	70.91	52.32	56.48	60.31	27.77	57.36	5.33	42.66	46.6
Klason Lignin	8.2	46.8	19.10	19.15	24.87	24.18	37.62	22.42	36.05	31.31	26.8
ASL	0.7	1.8	0.51	2.63	0.81	0.84	1.07	0.63	4.15	1.36	1.5
Extractives	1.7	12.8	7.50	10.05	7.71	10.19	26.70	8.55	9.98	9.26	11.2
Carbon	10.3	52.3	48.70	47.44	48.78	51.09	57.94	47.62	44.68	50.62	49.6
Hydrogen	1.2	5.8	6.14	6.39	6.07	6.45	6.51	5.98	5.21	6.25	6.1
Nitrogen	0.5	2.3	0.05	2.57	0.56	0.64	1.12	0.39	5.29	1.62	1.5
Sulphur	0.2	0.4	0.43	0.73	0.18	0.33	0.09	0.56	2.57	0.63	0.7
Oxygen	6.4	33.7	39.48	35.80	37.31	37.20	28.48	36.51	18.74	32.87	33.3
HHV (MJ/kg)	2.9	20.2	19.48	19.23	19.40	20.40	22.31	18.95	17.14	20.01	19.6
LHV (MJ/kg)	2.6	18.9	18.13	17.83	18.06	18.98	20.88	17.63	16.00	18.64	18.3
Syngas (dry gas) (kg/h)	nd	nd	1116.31	1214.83	595.60	499.96	1086.67	1572.00	800.81	1420.65	1038.4

nd - not determined.

(2.43–29.87%) while other kraft pulp sludges (PI, PII and PVI) and mechanical pulp sludges (PIII and PIV) were broadly similar in sugar content (glucan 39.16–56.54%) (Table 6). The major inorganic and organic components in pulp sludges are heavy metals from wood, kaolinite and CaCO3, organic binders, cellulose fiber, and lignin (Kuokkanen et al. 2008). The high concentration of CaCO3 in the pulp sludges can influence the enzymatic hydrolysis, but can improve in ABE fermentation (Gottumukkala et al., 2016; Yang et al., 2013). The extractives in all studied samples were low content because they were removed during pulp and paper mill processes such as pre-hydrolysis, and primary and secondary clarifiers. The very high LOI values in the studied paper mill sludges are reasonable because the samples remain rich in polymeric carbohydrates, lignin, starch, latex and carboxymethylcellulose that derived from the pulping process (Pöykiö et al. 2018). These contents are favourable effects on the physical, chemical and microbiological properties of soil that can apply in composting and land applications (Kuokkanen et al. 2008). The ML biomass contained lower organic matter (14.5%) than the EL biomass (93.5%) due to a visibly higher sediment content in the ML biomass.

The PII and PIII samples showed higher inorganic concentrations than the PI sample whereas the harmful elements such as As, Cd, Cr, Cu, Hg, Ni, Pb, Zn concentrations in the PI and PIII samples were lower than the maximum allowed heavy metal contents for Finnish fertilizer products (MMM (24/2011) 2013) (Article II, Table 3). The PI and PII samples were collected from the kraft pulping final process that the elevated S and Na concentrations in PI and N and P concentrations in PII were found in the residue. The butanol production from PI sample could be potential due to its high sugar, low ash content, low lignin, and low inorganic element concentrations. Among eight pulp side-streams (PI-PVIII), low amount of syngas, high ash content and extremely high moisture content produced from the PIII, PIV, and PVII pulp sludge confirm the unsuitability of these samples for the gasification process. However, we assumed that different pulp sludges could be mixed in the future due to minor variations in the chemical composition of PI, PII, PV, PVI, and PVIII pulp side-streams. In order to account for the possibility of side-stream mixing, the average reference side-stream should be used in the gasification-syngas fermentation process. Although PVI showed the highest amount of syngas and the lowest MC, PII was the closest to the average values of the syngas amount, organic matter, moisture and ash contents, 1038.4 kg/h, 91.2%, 78.6%, and 8.8%, respectively (Table 6). Therefore, the primary sludge, PII, was selected for the bioethanol plant model and to estimate the economic feasibility of the integrated gasification syngas-fermentation (Article III).

5.2 Biomethane production from lake bottom biomass and its ash residue utilization

The mesotrophic and eutrophic lake biomass were evaluated for the potential biogas production through anaerobic digestion, which was computed by Aspen Plus modelling. The key factors that affect biogas production were total solids, volatile fatty acids (VFA), pH, alkalinity and C:N ratio (Article I, Figure 2). The biomass from EL had a high C:N ratio (19.9/1) compared to ML (15.7/1), consistent with the earlier finding, in which the optimum yield of biogas was in the range of C:N ratio 16:1–25:1 (Deublein and Steinhauser 2008). The Ripley ratio (VFA/alkalinity) indicates the determination of the stability of the anaerobic digestion process, for example, when the Ripley ratio lower than 0.4, the anaerobic digester is functioning stably while the digester significant instability will take place at values ≥ 0.8 (Callaghan et al. 2002). The studied biomass had a high Ripley ratio (4.6 in EL and 1.6 in ML), which suggests significant instability and indicates non-digestion at the beginning of the anaerobic digester system. The pH of ML (5.5) and EL (4.2) bottom biomass did not meet the optimum range of pH 6.8–7.2 for biogas production (Gerardi 2003).

The nature and amount of lignin–carbohydrate complexes (LCC) linkages and lignin substructures affect the efficiency of pulping, hydrolysis, and digestibility of biomass (Tarasov et al. 2018). However, the main digestible components in the lake bottom biomass are carbohydrates and thus lignin is omitted while considering its conversion to biogas in this research. Thermal pretreatment (Thermo-Druck-Hydrolysis technique) was applied at the beginning of the anaerobic digestion process due to the high lignin content in the lake bottom biomass. A retention time of 25 days was applied in the digestion process under the mesophilic temperature range with minimum destruction of 70%, assuming that the digester worked optimally (pH, mixing, sufficient amount of nutrients, etc.).

The ML and EL biomass had biogas yield of 38.9 mL/g volatile solid (VS) and 136.6 mL/g VS, respectively (Article I, Table 3). This correlates with the previous studied by Tibebu (2015), where biogas production from a co-substrate of ML and EL bottom biomass with straw/horse manure was 157.8 mL/g VS and 182.2 mL/g VS respectively after a 25-day anaerobic digestion experiment. The ML material yielded a low biogas yield, caused by low VS with high total solids, low C:N ratio value, and indigestible lignin content compared to the EL biomass. Co-digestion with N-rich materials such as lime mud from paper making, waste eggshells, calcium carbonate ($CaCO_3$), and sodium bicarbonate ($NaHCO_3$) would help to adjust alkalinity and pH stability in the anaerobic digestion process (Chen et al. 2015). Inorganic element contents including harmful elements (mg kg^{-1}) in the ML and EL biomass and their ash were measured for analyses of the digestate, which can be applied as fertilizer. The heavy metal elements concentration in the ash samples in both the ML and EL biomass were below detection limits (Article I, Table 5). This results indicate that the process residue of both EL and ML biomass can be used for soil improvements and fertilizer in forestry.

5.3 Dilute sulfuric acid pretreatment of PPMS

Mendes et al., (2009) reported that it is important to consider the impact of a pre-hydrolysis stage on the kraft process in addition to evaluating the efficiency of the hydrolysis and fermentation processes. Therefore, the residue of pre-hydrolysis kraft pulping (PI) was selected for pretreatment with water, 0.1% and 0.2% H_2SO_4 due to its promising chemical composition, high total sugar content and its original pulping process stage. The pretreatment with water, at high temperature (200 ^{o}C) and during long pretreated time (30, 45, and 40 min) experienced to produce the fermentation inhibitors, such as furfural, HMF, acetic acid, and formic acid. The efficient pretreatment was 0.2% H_2SO_4 at 180 ^{o}C for 10 min in this study. In order to test the pretreatment and hydrolysis efficiency, samples with water washing and non-washing steps were prepared prior to dilute acid pretreatment and enzymatic hydrolysis.

An elevated amount of reducing sugars was found in the unwashed PI prehydrolysate (330 mg g$^{\mathbf{-1}}$) compared to that of PI washed sample (242.5 mg g$^{\mathbf{-1}}$), whereas both unwashed and washed PII and PIII prehydrolysate samples did not have sugars (Article II, Table 4). This finding showed that water washing did not affect sugar recovery in PII and PIII prehydrolysate samples while the conversion of hemicellulose to monomeric sugars was not occurred during dilute acid pretreatment, instead HMF, furfural, and formic acid were formed. However, the monosaccharide sugars (0.04 mg g^{-1} glucose, 10.75 mg g^{-1} xylose, 5.07 mg g^{-1} arabinose, and 0.17 mg g^{-1} mannose) were found in the washed PI prehydrolysate sample (Article II, Table 4). Pulp and paper mill side-streams as a substrate for saccharification has an additional advantage because the manufacturing process can disrupt crystallinity of cellulose during papermaking. However, the high ash content (>50%) in PPMS can harm saccharification, and thus deashing and water washing are applied to enhance glucan digestibility and eliminate ash, inorganic substances and cooking chemical residues from the pulping process (He et al. 2014; Guan et al. 2016; Huang et al. 2016). In contrast, the high ash content in PPMS can positively impact in saccharification and biofuels production, for instance, primary sludge with *Saccharomyces cerevisiae* gave the highest ethanol yield (Mendes et al. 2016) while paper mill sludge with $CaCO_3$ increased sugar utilization and butanol production using *Clostridia* strain (Yang et al. 2013; Gottumukkala et al. 2016).

5.4 Enzymatic hydrolysis of PPMS

For aiming to the total saccharification of hemicellulosic and cellulosic fractions to the fermentable sugars, it is important that the chosen pretreatment sufficiently alters cellulosic fraction for enzymatic hydrolysis (Kuittinen et al. 2018). However, the studied samples (PI, PII and PIII) were from different pulping process that the operation equipment used in the mill could be considered as pre-existing pretreatment and most of the hemicelluloses had already been extracted. For example, the water pre-hydrolysis of birch wood resulted in the removal of the hemicelluloses (predominantly xylan) since the cellulose and lignin yields remained rather similar under the pre-hydrolysis stage. (Borrega et al. 2018). Precisely, the rejects from pre-hydrolysis stage (PI) was preliminarily tested with water washing and non-washing under a wide range of DM loadings (2 – 10%) for 48 h. The sugar concentration in the washed-pretreated (WP) PI sample resulted in 34.7 g/L at 7% DM loading in comparison with 35.5 g/L at 10% DM loading in that of sample.

All samples (PI, PII and PIII) in this study were enzymatically hydrolysed with 7% DM loading under four conditions, such as UN, W, UWP and WP as described earlier in section 4.3. The synergistic interaction between cellulase (endoglucanases and cellobiohydrolases) and hemicellulases (xylanases and mannanases) can improve the hydrolysis without increasing enzyme loading (Hu et al. 2011). The unwashed-pretreated PI hydrolysate achieved the highest glucose concentration (6.94 g/L) at 48 h under cellulase and xylanase synergism (CEL+XYL) (Article II, Figure S2). It was apparent that all the untreated hydrolysate of PI, PII and PIII samples (UN) could produce sugar concentrations without dilute acid pretreatment. However, the enhancement resulting from the dilute acid pretreated samples (UWP and WP) was higher sugar concentration than untreated samples (Figure 8). As expected, the enzymatic hydrolysate of the unwashed-pretreated PI side-stream (PI-UWP) (0.68 g/g) resulted a greater sugar concentration than the washed-pretreated PI side-stream (PI-WP) (0.57 g/g) (Figure 8).

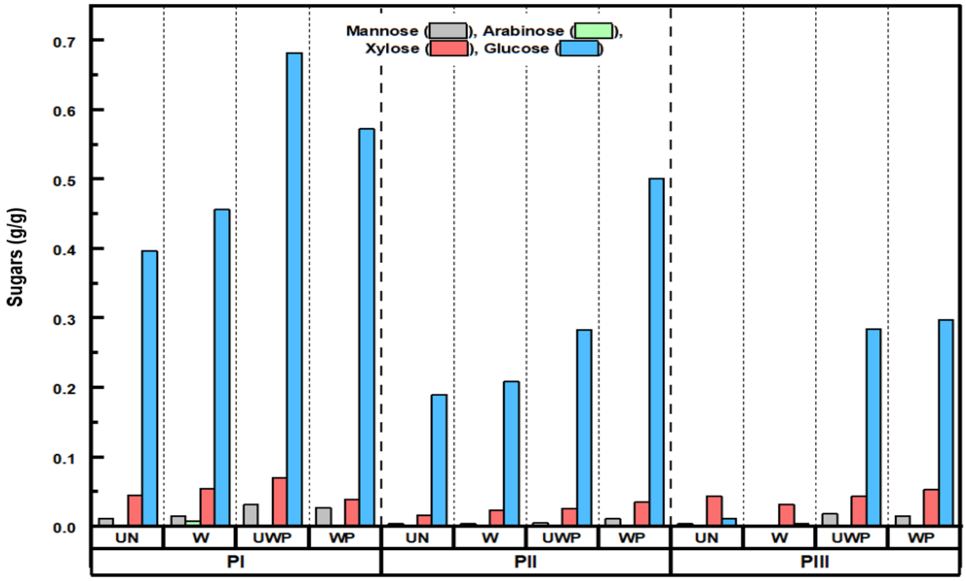


Figure 8. Concentrations of sugars from enzymatic hydrolysis of PPMS (PI, PII and PIII): untreated as control (UN), washed-unpretreated (W), unwashed-pretreated (UWP) and washed-pretreated (WP) (Adapted from Article II).

This finding indicates that the effective saccharification was found in the unwashed-pretreated PI (PI-UWP) because water washing prior to dilute acid pretreatment can remove soluble carbohydrates and, therefore, decrease the cellulosic sugar yield, left in the pulp and paper mill processes. However, the PII and PIII samples did not result in similar improvement in hydrolysis, and both required prewashing before dilute acid pretreatment and saccharification. Since the unwashed-pretreated PI sample (UWP) had the highest glucose yield, the ABE fermentation had consequently conducted for all unwashed hydrolysed samples in this research.

5.5 Butanol production from PPMS via ABE fermentation

The PI, PII and PIII samples from different pulping process stages, were currently unutilized and potential sugar-contained, were studied to produce butanol via ABE fermentation (Article II, Figure 1 and Table 1). *Clostridium acetobutylicum* and other *Clostridium* species are able to produce acetone, hydrogen, ethanol and butanol lignocellulosic components (Oh et al. 2009; Aristilde et al. 2015). Previous reports imply that *C. acetobutylicum* can metabolize either glucose or xylose individually, but xylose metabolism is severely inhibited when glucose is available (Ounine et al. 1985; Aristilde et al. 2015). Jiang et al., (2014) reported that glucose is a preferable sugar in ABE fermentation compared to other reducing sugars due to its high efficiency in sugar-specific mechanism. To investigate the studied side-streams' fermentability by glucose uptake at different concentrations, a similar concentration of glucose (30 g/L) was added into the unwashed hydrolyzed (UWP) PI, PII and PIII samples, although they had a different reducing sugar contents of 65.8 g/L, 33.5 g/L, and 38.5 g/L, respectively.

During 72 h fermentation, the PI side-stream produced 5.1 g/L of butanol at 24 h increased to a maximum yield 8.96 g/L, while butanol productions of PII and PIII was steadily increased to 4.38 g/L and 4.91 g/L, respectively (Figure 9). The reason of low fermentation rates in PII and PIII was that butyric acid and acetic acid were produced more at the beginning of the fermentation, for example, the greatest amount 2.55 g/L of butyric acid was found after 24 h fermentation (Article II, Figure 4B). Probably, PII experienced potential "acid flash" phenomenon because glucose uptake in PII ceased 24 h and > 28% of the glucose remained unconsumed that the fermentation was finished earlier than expected. This phenomenon causes a weak fermentation in the solventogenic phase due to low pH and excessive acids (Maddox et al. 2000; Yang et al. 2013). Although the solventogenic phase was observed, the glucose and mannose, and xylose uptake ceased after 24 h and 48 h in the PIII sample, with a plateau of 3.48 g/L of butyric acid at 72 h (Article II, Figure 4C).

At the end of fermentation, the PI and PII samples contained unconsumed xylose. The *C. acetobytylicum* DSM 1731 utilized 2.42 g/L (PI) and 0.81 g/L (PII) of xylose after 72 h fermentation. This is conformed to the previous study by Pang et al. (2016), who reported that 11 g/L of xylose remained unconsumed (using *C. acetobytylicum* GX01) after 72 h fermentation. Based on the ABE fermentation yield results, the unwashed hydrolyzed PI had 12.76 g/L of butanol, while unwashed hydrolysate PII and PIII resulted 5.20 g/L and 6.32 g/L, respectively. The PI result is compatible with a previous study done by Guan et al., (2016) in terms of enzyme loading, solid loading, and ABE yield (Table 7). Therefore, the utilization of Finnish PPMS is considered integration; it is typical that the residues from one stage of production can be utilized in another one. For example, autohydrolysis may also be used in combination with kraft pulping to produce co-products such as ethanol besides pulp (Mendes et al., 2009) as well as energy can be produced by burning black liquor from the pulp making process, and biosludge from wastewater treatment plants (Nurmesniemi et al., 2007).

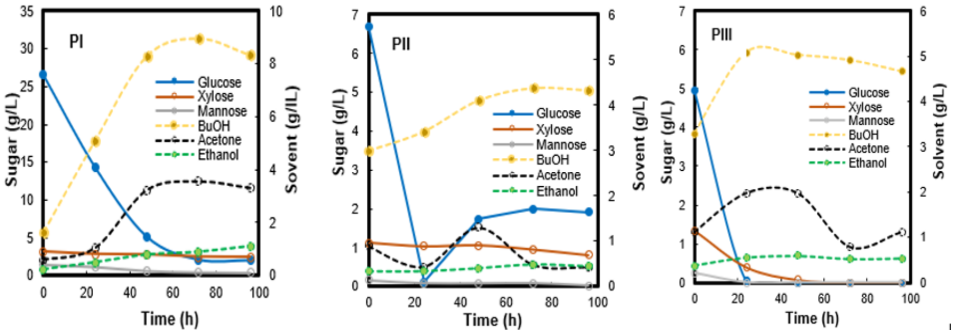


Figure 9. ABE fermentation by C. acetobutylicum DSM 1731 using enzymatic hydrolysate of PI, PII and PIII PPMS for 96 h (Adapted from Article II).

Table 7. ABE fermentation comparison between this study and other studies.

Feedstock	Pretreatment & hydrolysis	Strain	Method	BuOH (g/L)	ABE (g/L)	References
PI	0.2% H_2SO_4, 180 °C, 10 min and enzymatic hydrolysis	*C.acetobutylicum* DSM 1731	Batch fermentation	8.33	12.76	This study
PII				4.33	5.20	
PIII				4.66	6.32	
Paper mill sludge	15% NaOH, 140 °C, 60 min, enzymatic hydrolysis	*C. sporogenes* NCIM 2337	Batch fermentation	nd	0.559	(Gogoi *et al.* 2018)
Recycled kraft paper mill sludge	No pretreatment and enzymatic hydrolysis	*C.acetobutylicum* ATCC-824	Simutaneous saccharification fermentation	6.8 - 9.7	10.6 17.1	(Guan et al. 2016)
Wood pulp hydrolysate	Alkaline extraction followed by dilute acid hydrolysis	*C. beijerinckii* ATCC 51743	Batch fermentation, Activated charcoal detoxification	6.27	8.98	(Lu et al. 2013)

5.6 Bioethanol potential from lake bottom biomass via fermentation and gasification processes

The fermentation-derived ethanol production from sugar, starch or lignocellulosic biomass has been researched for decades. Sugar and starch-based feedstocks are predominant at the commercial level. Simultaneous saccharification and fermentation (SSF) process is a favored option because it improved hydrolysis rates, yields, and concentrations of ethanol with less capital investment compared to competing processes. In addition, the continuous removal of the sugars by the yeasts reduces the end-product inhibition of the enzyme complex. The key step in bioethanol production from fermentation is to degrade the lignocellulosic material to convert sugars whereas indirect gasification allows high fuel conversion and process optimization that converts a mixture of dry feedstock and steam to syngas and char. Furthermore, the syngas and flue gas are not mixed, which means the producer gas is not diluted with N_2 coming from the air used for combustion, and thus, is suitable for alcohol synthesis applications (Aranda et al. 2014). However, the high lignin and low fiber content of lake bottom biomass may limit the formation of fermentable sugars and affect the conversion efficiency of biomass to biofuel. For this purpose, two models of Aspen Plus software were evaluated the potential utilization of ML and EL biomass for the bioethanol production.

The biochemical process used in this research was co-current diluted sulfuric acid pretreatment of lake bottom biomass, followed by saccharification of the remaining cellulose, and then fermentation of the resulting glucose and xylose to ethanol. The model involved some unit operations such as hot water treatment, acid hydrolyzer, neutralizer, enzymatic hydrolyzer, fermenter, flash separator and filter. The treatment by hot water caused swelling of biomass and hydrolysis by diluted sulfuric acid leads to the hemicellulose decomposition to monomeric xylose with conversion of 90% and 5% loss to degradation products according to Humbird et al. (2011). After enzymatic hydrolysis, the hydrolysate containing monomeric sugars fermented by *Zymomonas mobilis* bacteria. This modified strain of *Z. mobilis* can simultaneously ferment glucose and xylose to ethanol, assuming all of the available glucose is converted to ethanol. According to Hamelinck et al., (2005), modified *Z. mobilis* bacteria produce ethanol from glucose, xylose and other monomeric sugars (mannose, galactose, arabinose) with conversion rates of 92%, 85% and 90% respectively. After separation and filtration, the ethanol containing fermentation broth was fed into two rectification columns, where >98.9% of ethanol was recovered. Final dehydration of bioethanol to fuel grade is performed with molecular sieves. Since bioethanol yield strongly depends on the holocellulose fraction in the biomass, the lower amount of holocellulose in ML biomass (6.9%) was clearly seen in the extremely low ethanol yield of 40 L t^{-1} compared to that of EL biomass (23.6%) with 137 L t^{-1} of dry EL biomass (Article I, Table3).

When modelling with commercial Aspen Plus software for theoretical bioethanol yields estimated by indirect gasification and mixed alcohol synthesis process, a high moisture content observed in the EL biomass led to higher energy consumption at the feed drying stage compared to the ML biomass. The ash and moisture contents significantly affect the gasification process that the technological process started with the drying of biomass to a moisture content of 5 wt.%. The dried feed was converted to gases, tar, char, and ash via indirect gasification. A fluidized bed gasifier was used in the process, where circulating hot sand was the heat supply and steam was a fluidized medium. In the process, feed preparation, gasification, gas cleanup and conditioning, alcohol synthesis and alcohol separation units were involved. The main reactions of the mixed alcohol synthesis, corresponding conversions and yields of gasification products were defined based on the calculation of Phillips et al., (2007). More detailed descriptions of syngas conversion to alcohols can be found in Article I. As the ash content in ML (79.6 %) would be unfavorable for gasification, resulted in an extremely low ethanol yield of 57.1 L/t (dry matter) for ML biomass whereas the ethanol yields of ML biomass from mixed alcohol synthesis after gasification was 244.5 L/t, which was comparable with ethanol yields 265 – 492 L/t from cellulosic stock, wood and MSW feedstocks (Article I, Table 3).

5.7 Bioethanol potential from primary sludge via gasification-syngas fermentation process

Hydrolysis of fermentation of lignocellulose is much more complex than just fermentation of sugar and starch. Many researchers study bioethanol production from pulp and paper mill sludge by fermentation. Primary sludge without pretreatment achieved 48.9% of ethanol yield in SSF (Mendes et al. 2016), whereas biomass to ethanol conversion with combined pretreatment processes can bring the ethanol efficiency to 48% on an HHV basis (Hamelinck et al. 2005). However, the biomass-to-ethanol conversion efficiency in the hydrolysis-fermentation route is fairly low since lignin cannot be broken down, and hydrolysis of cellulose and hemicellulose depends on choice of pretreatment, and the development of cheaper and more efficient enzymes and microorganisms (He and Zhang 2011).

Unlike fermentation, all components of the biomass including lignin are converted to syngas in gasification and the overall energy efficiency in direct and indirect gasification has 39% and 47%, respectively, on an HHV basis (Phillips et al. 2007; Dutta and Phillips 2009).

Recently, concepts for process integration and combination are developed for better gas quality and purity, higher process efficiencies, and lower investment costs (Heidenreich and Foscolo 2015). Biomass gasification integrated with syngas fermentation is a promising model of second-generation biorefining. In syngas fermentation, the fermentation efficiency to ethanol increased with microbial preference of carbon substrate as high as 55-71.9% (Ramachandriya et al. 2016), whereas the commercial LanzaTech process resulted 51.6% in carbon conversion efficiency (Liakakou et al. 2021). Therefore, an integrated gasification-syngas fermentation plant for ethanol production from primary sludge (PII) was modeled by Aspen Plus® V9 simulation software, which was the focus of this research (Article III).

The pulp, paper and board industry produces a substantial amount of side-streams such as rejects, bark, de-inked sludge, paper mill sludge (fiber sludge), zero fiber (the stage before fiber sedimentation), debarking wood waste, black liquor, green liquor dregs, lime muds, chemical flocculation sludge, primary sludge (fiber sludge), secondary sludge (biosludge), and tertiary sludge (Bajpai, 2015; Gottumukkala et al., 2016; Lahtinen, 2017; Pöykiö et al., 2018). In the pulp and paper industry, kraft pulp mill is the primary candidate that produced > 95% of the chemical pulps while total generated sludge (fiber sludge, biosludge, and primary sludge) accounted for 14.1% of total generated side-stream (Branco et al. 2019; Hassan et al. 2019). The main side-streams of paper and board mills are paper mill sludge (fiber sludge) and primary sludge, in which the latter generated average 6.8% compared to 24.5% of the total generated sludge (Hassan et al. 2019). The primary sludge contains cellulose fibers as the principal organic component including fillers, pitch, lignin, ash and inert solids from the chemical recovery and is commonly used to mix with secondary sludge to enhance the dewatering properties (Ochoa de Alda 2008). However, primary sludge is categorized as a low-value side-stream because its status of utilization is < 30% and mostly disposal in landscaping (Hassan et al. 2019). Otherwise, the fibrous primary sludge has a significantly better heat value if incinerated for combined heat and power generation without the secondary biosludge (Saastamoinen 2019). In considering the utilization of primary sludge as a value-added product, it could be a valuable resource on its own and it can therefore be explored by potential bioethanol production using integrated gasification-syngas fermentation in this research.

Syngas fermentation is an indirect fermentation process because biomass is first converted into syngas through gasification and, it is not fed directly into the fermenter using several microorganisms to produce fuels and chemicals (Devarapalli and Atiyeh, 2015). Acetogens are the most widely used anaerobic microbes through the Wood–Ljungdahl metabolic pathway which allows them to produce acetate and ethanol as the main end products, but also other acids and chemicals, such as acetic acid, butyric acid, hexanoic acid, isopropanol, butanol, hexanol, 2,3-butanediol, acetone, caproate, and lactate (Ljungdahl et al. 1965; Ramachandriya et al. 2016; Sun et al. 2019; Ciliberti et al. 2020). Furthermore, acetogens involved in syngas fermentation are able to operate under flexible $CO:H_2:CO_2$ ratios; thus, syngas reforming is not required to match fermentation conditions; however, traces of condensable volatiles (tar) present in the syngas may lead to intoxication of the acetogens and, therefore, must be removed (Ramachandriya et al. 2016; Chowdhury et al. 2019). We used *Clostridium ljungdahlii* in the model because it is the most studied acetogen for the conversion of syngas to ethanol (Köpke et al. 2010).

The feedstock input was started by 50 000 tonnes of wet primary sludge (PII) gradually increased to 100,000, 200,000, and 300,000 tonnes of annual feedstock. Clearly, the amount of syngas produced and, consequently, the yield of ethanol, are directly dependent on the level of feedstock inputs. Drying is a critical issue in primary sludge utilization in the gasification process that the combustion of char was used as a source of heat for direct drying technique producing the flue gases as the heat supply in the model. However, the amount of produced flue gases was insufficient to achieve the target moisture content (30-60%) of primary sludge (PII). Primary sludge has high fiber and low ash (< 30% w/w) contents that the sludge pretreatment technologies such as pre-dewatering (thickening and conditioning) and mechanical dewatering methods could apply before drying to improve the gasification efficiency (Bajpai 2015). This model enabled to produce 99.6 wt% ethanol purity from wet primary sludge 300,000 tonnes/year and resulted production for about 3011 kg/h (3816.43 L/h and 24 090 tonnes/year) of anhydrous bioethanol, which is equivalent to 0.332 kg of ethanol per 1 kg of dry biomass (Article III). In comparison, the potential bioethanol yields in the integrated gasification-syngas fermentation from garden waste and switchgrass have been reported to be 0.217 kg/kg of dry biomass and 0.292 kg/kg of dry biomass, respectively (Pardo-Planas et al. 2017; Safarian et al. 2020).

5.8 Economic evaluation of bioethanol production

The economic analysis was investigated to evaluate the possible utilization of new biomass source (lake bottom material in Article I) and forest industrial side-streams (primary sludge in Article III) for the generation of biofuels that could provide considerable economic advantages in the bioeconomy. Lignocellulosic biomass can be converted into bioethanol by gasification-synthesis, hydrolysis-fermentation, and gasification-fermentation. In hydrolysis-fermentation process, the costs of ethanol production are highly sensitive to the delivered feedstock cost, operating scale, feedstock handling and preparation, choice and costs of feedstocks, optimal utilization of feedstock, and pretreatment methods as well as biomass sugar composition (Hamelinck et al. 2005). Besides, the enzyme hydrolysis step remains as a major techno-economic bottleneck in lignocellulose biomass-to-ethanol in hydrolysis-fermentation route (Menon and Rao, 2012). In the gasification-synthesis route, nearly complete biomass conversion into mixed alcohols from which ethanol and higher alcohols are obtained; however, commercialization of catalytic synthesis of ethanol from syngas is under development (He and Zhang 2011). To overcome these barriers, the development of the integrated indirect gasification with syngas-fermentation process needs to be considered to advance the cost-effective conversion of second-generation bioethanol production. Therefore, LanzaTech Inc. built two commercial-scale syngas fermentation pilot plants from biomass gasification using agricultural waste and MSW in 2018 (Ciliberti et al. 2020; Liakakou et al. 2021).

In Articles I and III, techno-economic evaluation was conducted by Aspen Process Economic Analyzer (APEA) in terms of ethanol yield, suitability of the biomass feedstock, capital and operating costs, utility cost, synthesis selectivity, carbon and CO conversion efficiencies, and Ethanol Selling Price (ESP). Estimated ethanol sales was €600 tonne^{-1}, whereas the propanol and higher alcohol mixture (€480 tonne^{-1}) was calculated as 80 % of the ethanol price. Techno-economic estimation showed that the profitability index on the bioethanol production from gasification and mixed alcohol synthesis in both ML (1.066) and EL biomass (1.094) were almost similar as the major contributions to the production cost were syngas cleaning and separation of alcohols in this process (Table 8). In contrast, the IRR of the EL biomass (42.3%) was greater feasible compare to ML biomass (3.9%) in saccharification and fermentation process (Table 8).

The most profitable process was the bioethanol production from EL biomass by saccharification and fermentation, and thus EL was a potential non-food lignocellulosic biomass source for the biorefining industry. The main aim of the second-generation ethanol production is cost-competitive with first-generation ethanol price to allow it to compete in the fuel market. Several researchers published techno-economic analysis for bioethanol production from enzymatic hydrolysis fermentation (Humbird et al., 2011; Piccolo and Bezzo, 2009), indirect gasification-mixed alcohol catalytic conversions (Dutta and Phillips, 2009; He et al., 2013; He and Zhang, 2011; Phillips et al., 2007), but techno-economic analysis for integrated gasification-syngas fermentation process is scarcely reported (Phillips et al. 2017). Piccolo and Bezzo (2009) revealed that the cost of ethanol production from the integrated gasification-syngas fermentation was €1.20/L (€1.40/L; €2019) compared to €0.80/L (€1.0; €2019) ethanol selling price from enzymatic hydrolysis fermentation, as shown in Table 9. In the EU, the average price of a liter of gasoline (with taxes) in 2020 was about €1.37 and is expected to increase to €1.57 in 2030 (Navas-Anguita et al. 2019). Kraft pulping is the dominant process for converting wood chips into pulp fibers, and Finland is the second highest producer of kraft pulp in Europe, with a market share of 30% (Koreneff et al. 2019). The amount of feedstock input is critical in regard to the economic feasibility of bioethanol production – an increase in feedstock amount leads to a decrease in ethanol selling price.

As a result, bioethanol production from pulp sludge requires the co-location of an existing pulp and paper mill that could provide the required amount of feed material. An alternative synergistic approach is to implement biofuel supporting policies and regulations, either a bioenergy support subsidy and fiscal incentives in the form of tax reliefs by the government, or a change in the cost-effective plant policy, such as the imposition of a gate fee. In this study, both the effect of government subsidies on ethanol production and the impact of a gate fee policy were evaluated at a constant IRR of 15% under a different tax rate of 20, 25, 30, and 35%, respectively.

Table 8. Techno-economic analysis of bioethanol production from lake bottom biomass.

	Ethanol yield from saccharification and fermentation		**Ethanol Yield from gasification and mixed alcohol synthesis**	
	ML	EL	ML	EL
Ethanol yield, L tonne^{-1}	40.0	137.0	57.1	244.5
Total capital cost, M€	16.8	16.3	29.5	83.3
Total operating cost, M€/year	18.4	32.0	27.4	113.0
Total raw materials cost, M€/year	12.5	25.7	15.1	78.3
Total utility cost, M€/year	2.7	2.2	8.2	22.4
Total product sales, M€/year	13.3	45.7	20.6	89.0
Internal Rate of Return (IRR), %	3.9	42.3	5.3	6.7
Payout period (PO), year	45.5	4.9	40.3	35.4
Profitability index	1.023	1.115	1.066	1.094

ML: Mesotrophic lake bottom biomass; EL: Eutrophic lake bottom biomass

The favourable effect of a gate fee has been already reported, where a gate cost of $74/t (€65.8/t; €2019) for corn stover in China produced an ethanol price of $2.86/gal (€2.38/gal; €2019), which was much lower than the bioethanol market selling price of $3.45/gal (€3.07/gal; €2019) (Zhao et al. 2015). The commercialization of the gasification-syngas fermentation can be performed by either subsidy support at €150/t of ethanol or a gate fee of €20/t of feedstock. Both of them can give more attractive capture in IRR of 15%, however, the implementation of a gate fee is an entirely realistic and practical scenario. An ethanol subsidy support of €150/t led to the ethanol price €0.61–0.71/L while a gate fee of €20/t of feedstock achieved an ethanol selling price €0.60–0.70/L for 300,000 tonnes of primary sludge (Table 9). Thus, our findings indicate that the potential of kraft primary sludge for bioethanol production should be considered by implementing a viable gate fee or a bioenergy subsidy through integrated gasification-syngas fermentation process.

Table 9. Comparison of techno-economic analyses of the biochemical, thermochemical, and combined bio- and thermo- chemical processes; all values in 2019 Euro, with the depreciation rate set accordingly.

Parameters	**Humbird et al., 2011**	**He and Zhang, 2011**	**Dutta et al., 2012**	**Piccolo and Bezzo, 2009**	**Article III**
Process	Enzymatic hydrolysis fermentation	Indirect gasification	Indirect gasification	Gasification-syngas fermentation	Gasification-syngas fermentation
Feedstock	Corn stover	Wood chips	Southern pine wood	Hardwood chips	Kraft primary sludge (PII)
Feedstock cost (€/ dry ton)	56.2	64.25	57.9	71.6	0 [b]
Moisture content (wt %)	20.0	50.0	35	47.4	75.8
Plant size (dry metric tonnes per day)	2000	~ 2105	2000	2030 [a]	72,600 (dry ton per year)
Plant life (years)	30	20	30	15	20
Operation (h/year)	8410	8405	8410	8406	8000
Total capital investment (M€)	406.6	113.0	485.7	588.4	94.5 [c]
Internal rate of return (%)	10	10	10	7 – 10	15
Ethanol yield	79 gal/ dry ton (330 [a] L/mT)	22102 kg/h	83.8 gal /dry ton	282 L/ dry ton	3011 kg/h (24 099.5 ton/year)
ESP (€/L)	0.60 [a]	0.37	0.50	1.40	0.60–0.70 [d] 0.61–0.71[e]

ESP: Ethanol selling price; [a] estimated by Phillips et al., (2017); [b] assumed co-location with a Kraft pulp mill; [C] calculated without wastewater treatment cost, utility production, and ash disposal, [d] with gate fee, [e] with subsidy.

6. CONCLUSION AND RECOMMENDATION FOR FUTURE STUDIES

Alternative non-food biomass and forest industrial side-streams for biorefineries, we studied the biofuel production from lake bottom biomass and PPMS through biochemical, thermochemical and integrated bio-thermo-chemical conversion technologies. The composition of the lake bottom biomass depends on the origin of the biomass and varies between wood and non-wood sources because it is a heterogeneous mixture of carbonaceous organic materials that include plant biomass, humus, and sediment. Eutrophic lake bottom biomass (EL) was shown to have a high concentration of total sugars that gave higher biogas and ethanol yields than mesotrophic lake bottom biomass (ML). Since bioethanol yield strongly relied on the holocellulose fraction in the biomass, the extremely low ethanol yield of 40 L/t of dry ML biomass was resulted, compared to 137 L/t of dry EL biomass. The high ash content in ML (79.6%) was unfavorable for bioethanol production; 57.1 L/t (dry matter) in contract to a greater ethanol yield of 244.5 L/t (dry matter) from the gasification of the EL biomass. However, the elemental analysis results indicated that the ash of both EL and ML lake bottom biomass could be used as fertilizer in forestry, agriculture and landscaping. Although the most beneficial process was the bioethanol from fermentation of EL biomass, the utilization of EL biomass in gasification required cheaper techniques for alcohols separation and syngas cleaning. The synergies effect and anaerobic co-digestion of EL biomass with other industrial waste would be interesting options for future studies.

Dilute acid 0.2% H_2SO_4 at 180 ºC for 10 min was an efficient pretreatment method in ABE fermentation. The lowest ash and lignin contents, and the highest sugar content in PI sample found to be a prospective substrate for butanol production. No sugar recovery in the PII and PIII prehydrolysate samples could be concluded that water washing did not affect before pretreatment and hydrolysis. A higher sugar concentration in PI unwashed-pretreated (UWP) enzymatic hydrolysate than that of the washed-pretreated (WP) sample indicated that prewashing could remove soluble carbohydrates and, resulting in the highest butanol and ABE yields 8.33 g/L and 12.7 g/L, respectively. Since PI, PII and PIII have a different reducing sugar contents, the different amount of glucose should initially be added to the hydrolyzed samples in the fermentation medium as well as the comparison of washed hydrolyzed and unwashed hydrolyzed samples should be investigated for ABE fermentation under pH control in future studies. Due to minor variations in the chemical composition of PI, PII, PV, PVI, and PVIII side-streams, they can be mixed and effectively utilized in an integrated process, for example, the hydrothermal liquefaction (HTL) process by utilizing subcritical water (SCW) of wet sludge for bio-oil production or adding ABE operations unit would be installed close to a kraft pulp mill.

When the highest flowrate of wet biomass 300,000 tonnes/year (72600 dry tonnes) of primary sludge was utilized, the current model of the integrated gasification-syngas fermentation process produced as 24,090 tonnes/year of ethanol (about 3011 kg/h of anhydrous bioethanol), which is equivalent to 0.332 kg of ethanol per 1 kg of dry biomass. The key indicators that impact the gasification-syngas fermentation, such as ash content, moisture content and drying occurred inside the gasifier should be further considered. In future model development, the process of combustion could be designed to burn out unreacted syngas and waste streams from the wastewater treatment unit, thereby increasing heat production in biofuel plants at less expenses. The economic viability of ethanol production from primary sludge can be achieved either by a gate fee of €20/t of feedstock or subsidy support at €150/t of ethanol, where are likely to be effective in offering a compatible ethanol selling price between €0.60–0.70/L and €0.61–0.71/L, respectively, for 300,00 tonnes of wet primary sludge under different tax rates.

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