VALUE ADDED BIOPRODUCTS FROM POTATO PEEL WASTE

A Dissertation

Presented in Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

with a

Major in Environmental Science

in the

College of Graduate Studies

University of Idaho

by

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May, 2014

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Abstract

Potato peel waste (PPW) is a group of incidental materials (peeling, trimming, cutting, and washing wastes) generated during potato processing, which exhibits a significant management and environmental issue. PPW samples collected from potato processing plants were fully characterized and converted into value added bioproducts through biological and thermochemical processes in this project. For the first time, lactic acid was found as the dominant product of anaerobic fermentation of PPW with undefined mixed cultures. The batch fermentation was studied under different pretreatment processes, enzymatic hydrolysis, temperature and solids loading conditions. The maximum yields of lactic acid, acetic acid, and ethanol were respectively, 0.22, 0.06, and 0.05 g g^{-1}, and the highest lactic acid concentration of 14.7 g L^{-1} was obtained from bioreactor with initial solid loading of 60 g L^{-1} at 35°C without additional enzymes. Similar fermentation broth compositions were obtained with seed cultures from different municipal wastewater treatment plants, and the Illumina sequencing revealed that the microbial community structures of three seed cultures were different but unique microbial community structures were formed with more than 96% of genus *Lactobacillus* in all three fermenters, as compared to <0.1% in seed cultures. The results of sequencing batch reactor (SBR) experience showed that the decreasing retention time and solids content increased lactic acid production, and a good overall lactic acid yield and productivity (138.0 mg g^{-1} d^{-1}) was achieved in a 3.5 L upscale SBR reactor, and high purity (91%) of aqueous lactic acid (50% w/w) was obtained through activated carbon adsorption, ion-exchange and vacuum condensation, and quantified and confirmed by HPLC and NMR.

The un-reacted PPW fermentation residue (PPW-FR) was further utilized for bio-oil and bio-char production with pyrolysis at 450°C and the results showed that a significant amount of hydrocarbon compounds (alkanes and alkenes) in bio-oil with similar aliphatic compounds as transport fuels and the bio-char exhibited better butane adsorption performances and exhibited potential use as an adsorbent. The processes described in this dissertation demonstrated an integrated lab-scale utilization of PPW, which can be used as references for industrial applications.

Acknowledgements

I would like to present this dissertation to my parents and family.

I would like to express my gratitude to my major professor Dr. Armando McDonald for his fatherly guidance, support and mentoring. I would like to acknowledge my committee members Drs. Erik Coats, Kerry Huber, and Steven Shook for their time and contribution to this research. I also would like to acknowledge the J.R. Simplot Company for providing financial support for my research and learning activities, and the help from Steve Vernon, Duane Evans, and Dr. Jeff Bohlscheid.

I would like to thank the technical assistance across the University from Drs. Larry Forney, Karol Gliniewicz, Helena Mendes-Soares, and Matthew Settles (Department of Biological Sciences and the Institute for Bioinformatics and Evolutionary Studies), Nick Guho, Andrea Hanson, and Edmond Stowe (Department of Civil Engineering), Drs. Jean'ne M. Shreeve, Alexander Blumenfeld, and Chunlin He (Department of Chemistry). I also would like to thank the faculty and staff from Department of Forest, Rangeland and Fire Sciences and Environmental Science & Water Resources Program, especially Dr. Tom Gorman, Dr. Jan Boll, Chris Dixon, Brenda Haener, Debbie Rigby, Jena Gram and Cece Connors.

Special thanks to the Renewable Material Program, the people and the laboratory.

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Chapter 1 Introduction

1.1 Background

Potatoes (*Solanum tuberosum L.*) are one of important stable food crops and major sources of starch for human consumption (Schieber and Saldana, 2009). Potatoes as the world's fourth most important food crop behind corn, rice, and wheat, have experienced a steady growth globally over the last two decades especially in developing counties (FAO, 2014). China is ranked the largest potato producer with an estimation of 88,290,500 tonnes harvested in 2011, followed by India (42,339,400 tonnes), Russia (32,681,470 tonnes), Ukraine (24,248,000 tonnes), and United States (19,488,460 tonnes) (FAO, 2014). The majority of potato are used to for table consumption of fresh potato, however, this is undergoing significant change into frozen French fries, chips and shoestrings, canned foods, and other value-added products over the past several decades with the increasing of fast food industries (Lin et al., 2001; Schieber and Saldana, 2009). At present, approximate 60% of potato harvest in United States is manufactured to processed products as shown in Table 1.1 (NPC, 2012).

Potato peel waste (PPW) is a group of incidental materials (peeling, trimming, cutting, and washing wastes) generated during potato processing. The peeling loss caused by different procedures applied such as steam, abrasive, or lye peeling ranges from 6 to 10% (Mader et al., 2009). In combination with other trimming, cutting, and washing losses, the total PPW generated in potato processing plants can reach 15 to 40% depending on the final products (Schieber et al., 2001). PPW is considered as zero value waste from

potato processing plants, has caused considerable environmental concern and management problems (Arapoglou et al., 2010). PPW has been applied as fertilizer by agricultural land disposal or used for livestock feed, which were regarded as the best management practices for the potato processing industry (Nelson, 2010; Smith, 1986). PPW can also be a potential resource for the production of chemicals based on its significant levels of carbohydrates, protein, and bioactive compounds (Camire et al., 1997; Schieber and Saldana, 2009). The objective of this review is to summarize the available literature on PPW and its utilization to bioproducts and bioenergy, and to further explore value added from by-products of potato processing industry with integrated, environmental-friendly, and economic favorable solutions.

Table 1.1 The utilization of potatoes in United States 2008-2010 (NPC, 2012).

Utilization items	Production (10^6 kg)		
	2008	2009	2010
Total production	21,086	21,978	20,539
Table stock	5,555	5,910	5,457
Processing	12,875	12,999	12,520
Chips and shoestrings	2,590	2,162	2,778
Dehydrated	2,065	2,260	1,734
Frozen French fries	6,814	7,041	6,887
Other frozen products	992	1,067	679
Canned products	105	101	84
Other canned products (hash, stews, soups)	40	38	35
Starch, flour, and other	269	330	323
Livestock feed sales	41	332	30
Seed sales	1,062	1,027	1,048
Other non-sales (use on self-farms)	210	230	214
Shrinkage and loss	1,343	1,480	1,270

1.2 Structure and chemical composition analysis

1.2.1 Morphology study

The cross section and outer cell layers of potato tuber is illustrated in Figure 1.1. The skin or periderm with the associated parts in surface such as lenticels, eyes, and the bud and stem ends, the ring of vascular bundles (also known as the xylem), parenchyma tissue and the medullar rays and medulla (also known as the pith) are four clearly distinguishable areas (FAO, 1999; Grommers and Krogt, 2009). The skin or periderm is a ring of six to ten suberized cell layers, where its formation usually begins at the stem end of young tuber with the new-formed cells (phellem) constitute the periderm and become suberized (Grommers and Krogt, 2009). The parenchyma tissue is the major part of potato tuber with starch grains (or granules) as reserve material, which is comprised of cortex cells and perimedullary zone (FAO, 1999).

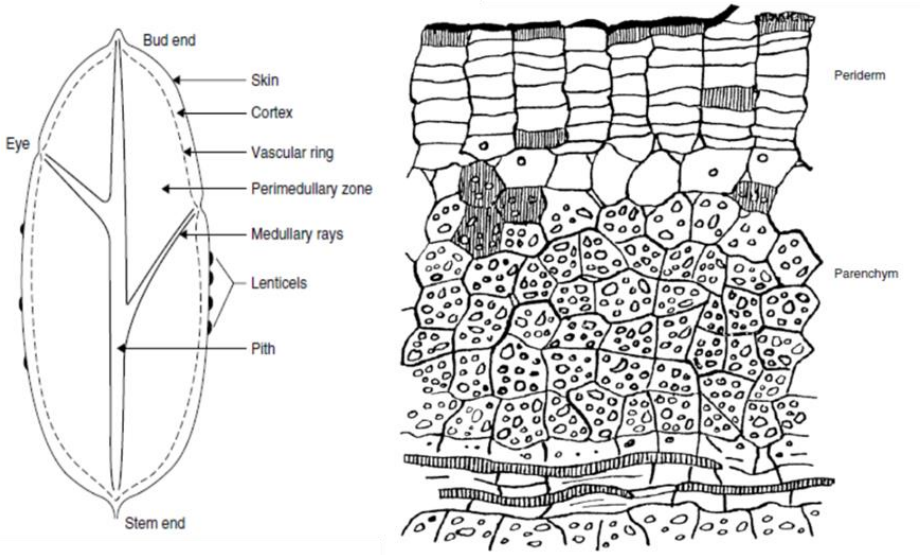

Figure 1.1 Cross section and outer cell layers of potato tuber (Grommers and Krogt, 2009).

The potato cell walls contain mainly microfibrillae of cellulose and pectic substances with average thickness of 1 μm, which are linked together by the middle lamella. The middle lamellae consist mainly of pectin, and are often interrupted to form intercellular spaces (Grommers and Krogt, 2009). Generally, potato is a swollen stem mainly composed of water, and the chemical composition of potato varies and is influenced by variety, environment and farming practices (FAO, 1999). A general profile of chemical compositions of potato and PPW is listed in Table 1.2.

Table 1.2 Chemical compositions of potato and PPW (Camire, 1997; FAO, 2008).

Constituents	Potato (% wet basis)	PPW (% dry basis)
Moisture	72-75	-
Starch	16-20	21-52
Dietary fiber	1-1.8	25-61
Protein	2-2.5	16-19
Fat	0.1-0.2	0.6-1.1
Ash	0.9-1.4	6-7.7

1.2.2 Starch and dietary fiber

The potato starch granules range in size from 10 to 100 μm in diameter (Hoover, 2001), which consists of two polysaccharides (amylose and amylopectin) composed exclusively of glucose (Figure 1.2). Amylose is a linear polysaccharide made up of α-D-(1,4)-glucopyranosyl units and is a component of potato starch. While amylopectin is the main and highly branched component in potato starch (70-80% by weight) consisting of both α-D-(1,4)-linkages and α-D-(1,6)-branches (Bertoft and Blennow, 2009). Amylose has a weight average molecular weight (M_w) ranging between 0.2 and 3.9×10^6 g mol^{-1} and a number average degree of polymerization (DP_n) ranging between 840 and 21,800. While amylopectin has a M_w of 60.9×10^6 g mol^{-1} and a DP_n of 11,200 (Bertoft and Blennow,

2009). Potato starch has many superior applications as compared to other cereal starches due to its resistance to enzymatic degradation (Sun et al., 2006).

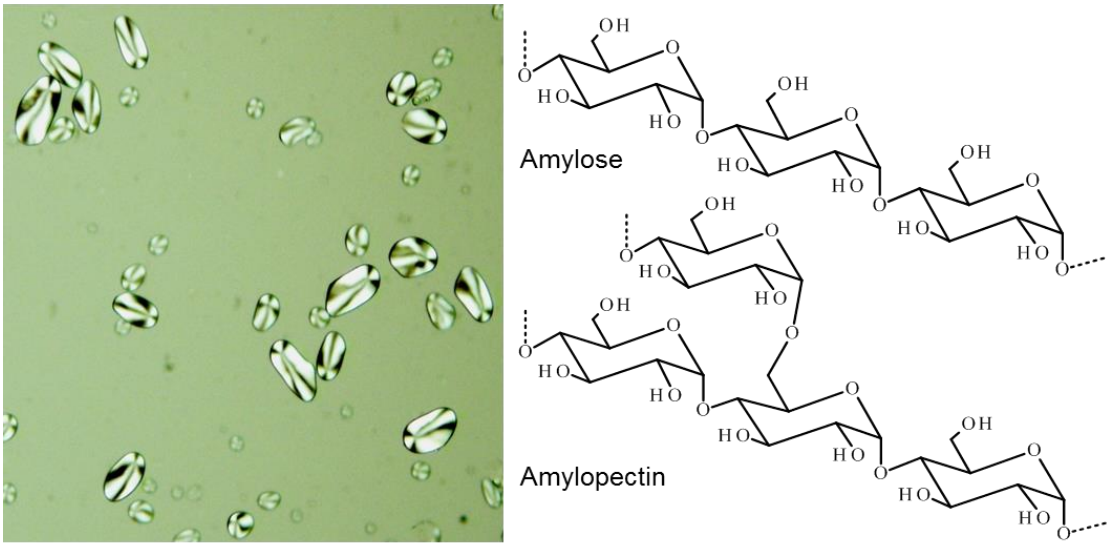

Figure 1.2 Polarized optical micrograph of potato starch granules (left) and partial structures of linear amylose (top right) and branched amylopectin (bottom right).

Dietary fiber is a complex mixture of carbohydrates in plant materials that resistant to enzymatic digestion, which consists of cellulose, hemicellulose, lignin, oligosaccharides, pectin, gum, and waxes (Dhingra et al., 2012). Polysaccharides are the main components of potato cell walls which include mainly cellulose (~30%), pectin (56%), xyloglucan (~11%), heteromannans (~3%) and heteroxylans (<3%) (Oomen et al., 2003). Figure 1.3 shows a polarized optical micrograph of potato cell walls and idealized structures of cellulose and pectin. Cellulose is composed of linear β-D-(1,4)-glucopyranosyl units with an extended, ribbon-like conformation, where an interwoven network of cellulose microfibrils (4-6 nm in diameter) are formed in potato cell walls (Harris, 2009). Pectic polysaccharides are the most abundant polysaccharides in potato cell walls, which have

very complex structures and are usually composed of the domains of homogalacturonan, rhamnogalacturonan and xylogalacturonan (Harris, 2009). These dietary fiber polysaccharides and starch in potato tuber and peel are excellent carbon sources which have been used to produce organic compounds via fermentation (Arapoglou et al., 2010; Barnett et al., 1999; Liu et al., 2013).

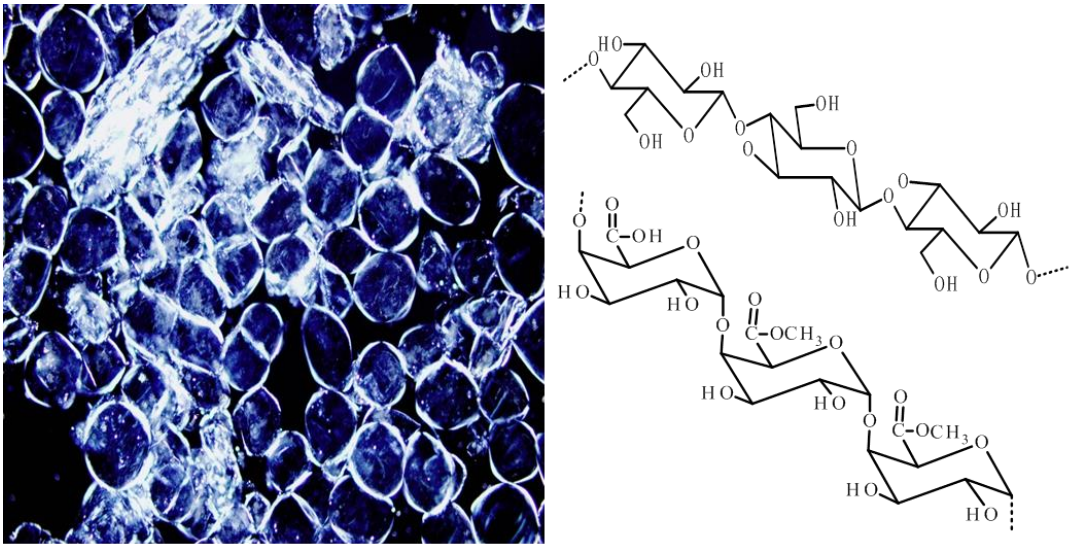

Figure 1.3 Polarized optical micrograph of potato cell walls (left) and idealized partial structures of cellulose (top right) and pectic polysaccharide (bottom right).

1.2.3 Protein, lipids and minerals

Protein is an important component in potato tuber that involved in energy metabolism, protein destination, and storage or disease/defense responses, which is also a main byproduct of starch manufacture industry (Karenlampi and White, 2009). Potato proteins have been classified into three groups: glycoprotein with size at 40,000 g mol^{-1}, complex proteins with size at 22,000 g mol^{-1}, and proteinase inhibitors, in which patatin was found to be the most abundant accounting for up to 40% of total soluble proteins in potato

(Ralet and Gueguen, 2000). Lipids are only small fraction of potato components that dominate with phospholipids and galactolipids (Klaus et al., 2004). In addition, the predominant fatty acid is linoleic acid (about 50% of total fatty acids), followed by α-linolenic acid, and palmitic aicd (Dobson et al., 2004; Karenlampi and White, 2009). Potatoes are also an excellent source of mineral elements for humans required (Karenlampi and White, 2009). A study by Mahmood et al. (1998) showed that PPW contained approximately 5.5% ash (minerals) with predominant of potassium (3.7%), followed by calcium (0.6%), phosphate (0.4%), and other 11 minerals. All these minerals combined with organic nitrogen generated from protein hydrolysis provide sufficient nutrients for growth of microorganisms, and can significantly reduce the nutrition supply for the biological conversion of PPW into value added bioproducts.

1.2.4 Suberin

Suberin is a natural biopolymer that is a component of endodermis of primary roots and periderm of mature roots and bark (Turber et al., 2013). This complex biopolyester is aromatic-aliphatic cross-linked and thick lamellae-like three-dimensional structure, which is highly resistant to enzymatic and chemical depolymerization (Ferreira et al., 2012; Mattinen et al., 2009). Figure 1.4 shows a proposed structure of potato suberin adapted from Bernards (2002). The suberin monomers are crossed-linked through mainly ester bonds of glycerol units, where the aliphatic domain consists of even numbered long chain units (C_{16}-C_{26}) of alkanoic acids, alkanols, α,ω-alkanedioic acids, and ω-hydroxyalkanoic acids and the aromatic domain contains mainly hydroxycinnamic acids and small amount of *p*-coumaryl, coniferyl, and sinapyl alcohols (Ferreira et al., 2012; Lopes et al., 2000).

Suberin accounts for approximately 25% of the constituents in lipid-free potato peel cell wall (Graca and Pereira, 2000). The suberin monomer composition is determined after a $NaOCH_3$ methanolysis to afford glycerol (27%), α,ω-diacids (40%), ω-hydroxyacids (18%), alkanoic acids (10%), alkan-1-ols (3%), and aromatic hydroxycinnamyl monomers (<1%) (Graca and Pereira, 2000). Due to the distinct composition profile, suberin has been regarded as a valuable renewable resource for the preparation of novel hydrophobic materials to replace petroleum based aliphatic polyesters (Sousa et al., 2011).

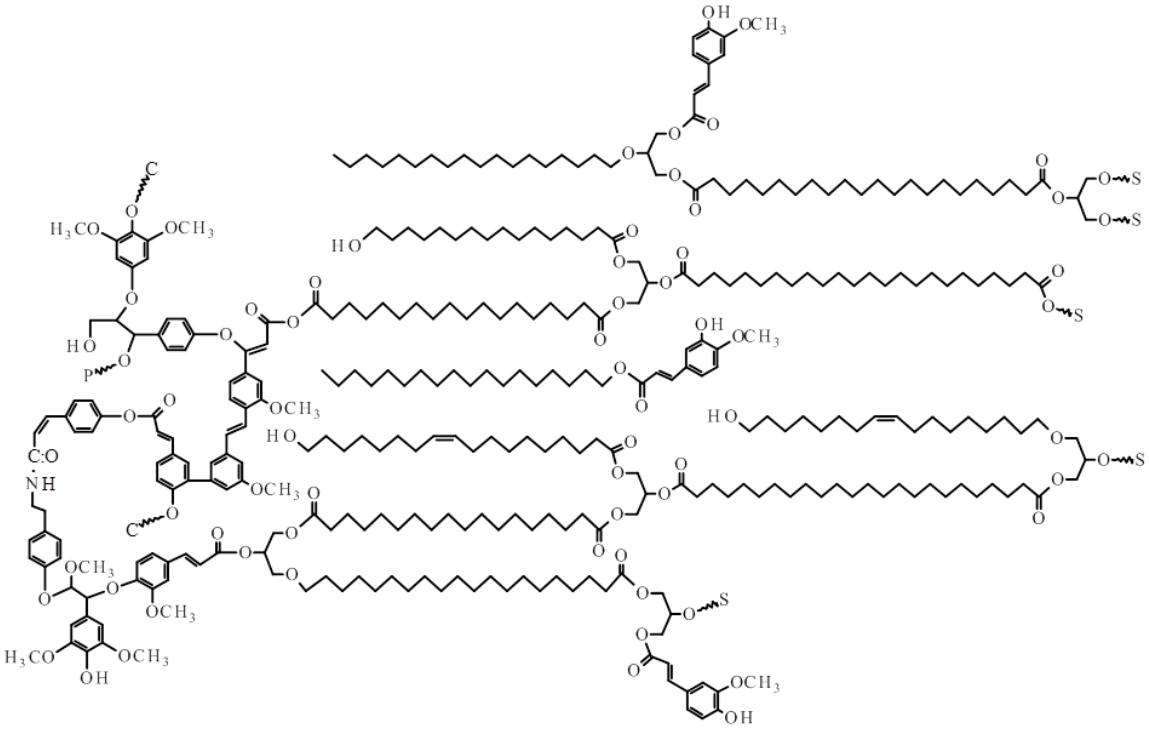

Figure 1.4 Hypothetical structure of potato suberin.

1.2.5 Bioactive compounds

Phenolics and glycoalkaloids are two important classes of bioactive compounds found in

potatoes (Mader et al., 2009). About 50% of these bioactive compounds are located in the potato peel and adjoining tissues (Friedman, 1997). The phenolic compounds are thought to play a major role in antioxidant activity of potato peels (Wu et al., 2012). The phenolic compounds found are generally (i) phenolic acids with C_6-C_1 and C_6-C_3 structure such as caffeic acid, coumaric acid, ferulic acid, protocatechuic acid, gallic acid, salicylic acid, and vanillic acid and (ii) flavonoids with C_6-C_3-C_6 backbone such as chlorogenic acid (Schieber and Saldana, 2009). Glycoalkaloids are also present in peel and are plant secondary metabolites and include α-chaconine and α-solanine. These compounds are known to influence the flavor of potato flesh and are regarded to be toxic to microorganisms, viruses, insects, and animals in high concentration (Mader et al., 2009; Schieber and Saldana, 2009). The chemical structure of common phenolic and glycoalkaloids compounds in potato are shown in Figure 1.5. The total bioactive compound concentration in potato peels were reported to range from 1.3 to 27 mg g^{-1} depending on potato variety and peeling methods (Al-Weshahy et al., 2009; Mader et al., 2009; Wu et al., 2012). As a natural antioxidant, potato peel extracts have exhibited superior free radical scavenging ability as compared to synthetic antioxidants (butylated hydroxyanisole and butylated hydroxytoluene) to suppress lipid oxidation and has been used as lamb meat and soy bean oil preservatives (Aia-ur-Rehman et al., 2004; Kanatt et al., 2005).

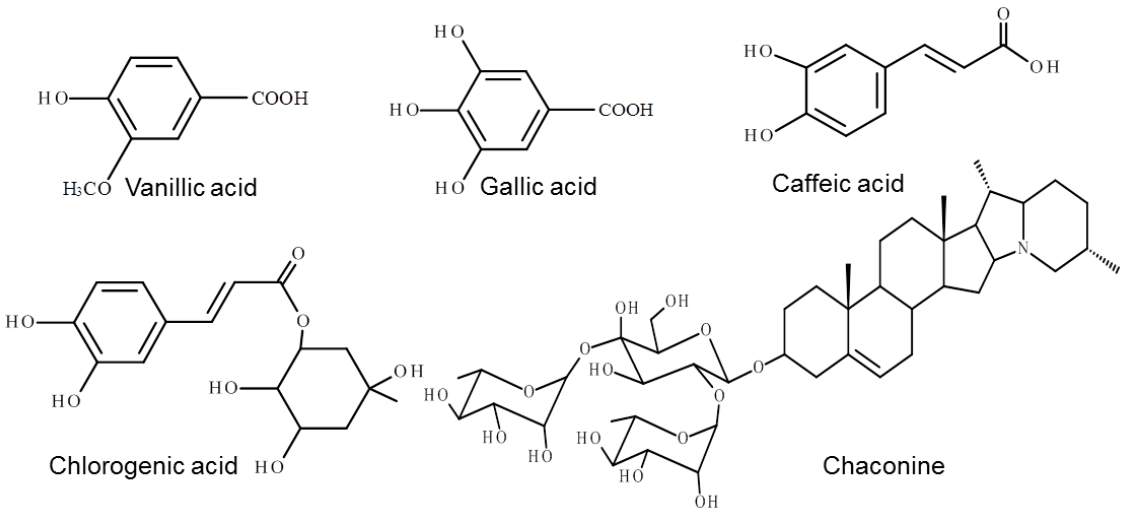

Figure 1.5 Chemical structures of bioactive compounds found in potato peel.

1.3 Waste management and utilization

Conventional waste management strategies for PPW are focused on direct physical elimination of organic pollutants, such as cropland composting application and feed for ruminant animals (Nelson, 2010). However, potential environmental issues associated with PPW land application and water drainage and contamination are a growing concern. Therefore, alternative uses of food processing waste streams for value added bioproducts and bioenergy promises a solution for waste management. Generally there are three different pathways to utilize these organic wastes for value addition as illustrated in Figure 1.6.

Extraction techniques by various organic solvents have been used to obtain lipids, protein, and other valuable bioactive compounds from plant derived materials (Wang et al., 2013). Biological conversion of waste, using various microorganisms, is employed to produce ethanol, H_2, biogas, carboxylic acids (lactic acid, succinic acids, etc.), industrial

polysaccharides and polyesters. Sugars (glucose, xylose, etc.) are normally the intermediate compounds for these biological processes which can be derived from cellulose, hemicellulose, and starch by acidic or enzymatic hydrolysis. In addition, studies were found that lactic acid can be produced directly from starch by some microorganisms (John et al., 2009).

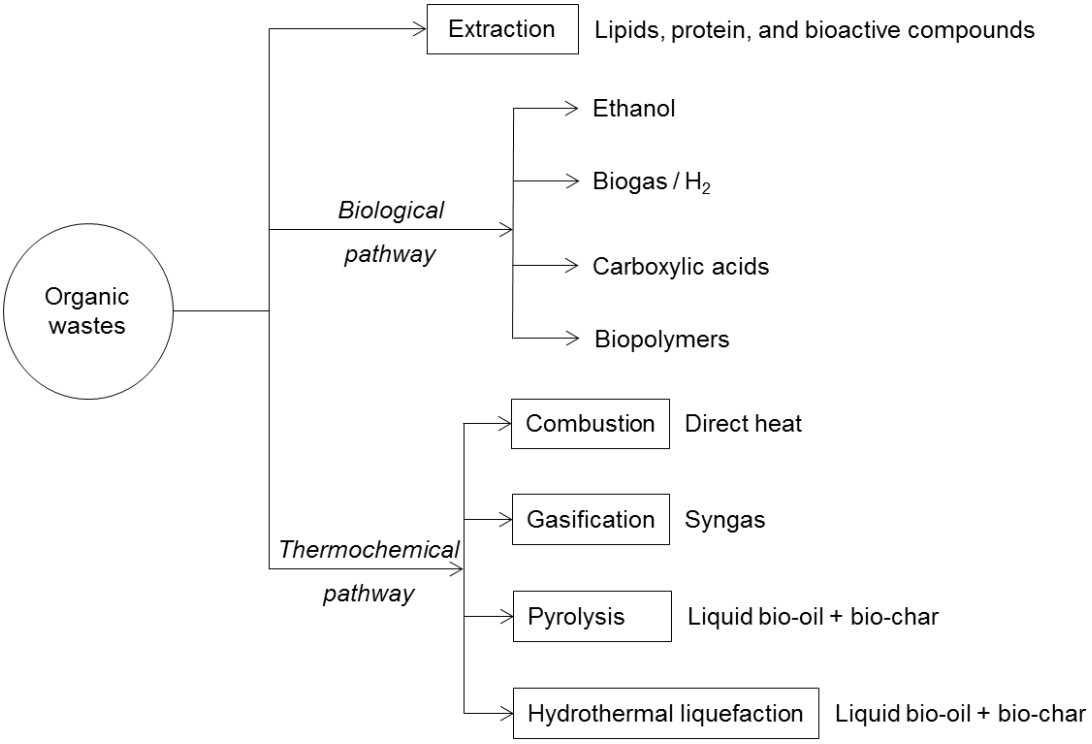

Figure 1.6 Available pathways to utilize organic wastes for value added bioproducts and bioenergy.

Thermochemical conversion involves high temperature chemical reforming processes to cleave bonds of organic matter (thermal cracking) to produce smaller molecular fragments. These processes include combustion, gasification, pyrolysis, and hydrothermal liquefaction. The products of thermochemical conversion are direct heat energy, synthesis gas (syngas), liquid bio-oil, and bio-char and the yield of each product is dependent on

the particular process and feed stock used (Cantrell et al., 2008). Moisture content is a critical factor for utilization, where high moisture content waste materials are suitable for fermentation, anaerobic digestion (AD), and hydrothermal liquefaction, while low moisture wastes are suitable for combustion, gasification, and pyrolysis (Wang et al., 2013). An integrated waste management and utilization strategy should consider the physical extraction to identify high value phytochemicals, followed by biological conversion and thermochemical conversion to make value addition.

1.4 Extraction of phenolic antioxidants from PPW

The extraction of natural phenolic antioxidants from PPW and other plant materials usually involves various solvent systems, and the extractable compounds and extraction yields are highly dependent on the applied solvents and extraction methods (Goli et al., 2005). Water and aqueous mixtures of methanol, ethanol, and acetone are common solvents have been used to extract phenolic compounds from potato peels (Schieber and Saldana, 2009). The aqueous extracts of potato peels contain a number of antioxidant compounds that exhibited free radical scavenging activities in various assays under *in vitro* conditions (Singh and Rajini, 2004). Al-Weshahy and Rao (2009) estimated polyphenolic content and antioxidant properties of peel samples from six potatoes varieties using methanol as solvent, where significant differences on the total phenolic compounds (1.27-3.31 mg g^{-1}) and antioxidant potential were observed. Mohdaly et al. (2010) studied the effects of solvent type on phenolic extraction from potato peels and found that methanol gave the highest yield at 2.91 mg g^{-1}followed by ethanol (2.74 mg g^{-1}), acetone (2.39 mg g^{-1}), hexane (1.12 mg g^{-1}), diethyl ether (1.12 mg g^{-1}), and petroleum

ether (1.08 mg g^{-1}).

The conventional solid-liquid extraction methods such as maceration, Soxhlet extraction, and reflux have been reported to recover phenolic compounds from potato peels (Al-Weshahy and Rao, 2009; Mohdaly et al., 2010; Schieber and Saldana, 2009). However, these traditional techniques usually require long extraction times and high solvent usage, and these combined can degrade heat-labile compounds (Singh et al., 2011). Several modern techniques are recently being used, such as microwave assisted extraction (MAE) and subcritical fluid extraction (SFE), which offer short extraction times, lower solvent consumption, and high selectivity towards target compounds (Brusotti et al., 2014; Singh et al., 2011). In MAE microwave energy is applied to heat the sample and solvent to promote product dissolution (Brusotti et al., 2014). SFE, also known as pressurized liquid extraction (PLE), uses applied solvent pressure to promote the material disruption to aid in the extraction of compounds (Singh and Saldana, 2011; Wijngaard et al., 2012).

Singh et al. (2011) studied the phenolic antioxidants extraction from potato peels by MAE and concluded that methanol concentration and extraction time played significant roles in the extraction of individual phenolics, where a maximum of 3.94 mg g^{-1} phenolics was obtained. A study by Wu et al. (2012) further confirmed that MAE is more efficient method than traditional refluxing extraction (60% ethanol, 80°C, 2 min, and solid-to-solvent ration of 1:40) to obtain total phenolic yields in potato tuber (1.4 mg g^{-1}) and PPW (10.3 mg g^{-1}) samples. Wijngaard et al. (2012) used response surface methodology to optimize solid-liquid extraction and SFE on polyphenols extraction from industrially generated PPW, and found that the SFE did not enhance the maximum yields

in comparison to solid-liquid extraction but reduced the reaction time from 22 to 5 min and solvent consumption from 75 to 70%. Alternatively, hot water extraction (180^{o}C, 30 min) was proven to be an effective method to extract phenolic compounds from PPW (0.82 mg g^{-1}, wet basis) without the need of organic solvents (Singh and Saldana, 2011).

1.5 Bioproducts production from PPW via biological conversion

1.5.1 PPW hydrolysis

In PPW polysaccharides (cellulose, pectin and starch) are the most abundant components but need to be hydrolyzed to sugars (monosaccharides) to be in a useful form and then to be converted biologically to alcohols and acids. Enzymatic hydrolysis is one of most popular methods to hydrolyze polysaccharides to their respective monosaccharides. For example, the enzymes α-amylase and amyloglucosidase is used catalyze the hydrolysis of glycosidic linkages of starch into malto-oligosaccharides and glucose, while cellulase can hydrolyze cellulose into cello-oligosaccharides and glucose but at a slower rate. Camire and Camire (1994) studied enzymatic starch hydrolysis of extruded PPW and found that starch can be completely hydrolyzed to glucose with amyloglucosidase at 55°C in 2 h. Acid hydrolysis is an alternative approach to hydrolyze polysaccharides, especially cellulose and hemicellulose in lignocellulosic substrates. Lenihan et al. (2010) used 10% phosphoric acid at 135°C for 8 min to decompose potato peels and obtained a high conversion of sugars (82.5% theoretical sugar yield). Bhattacharyya et al. (2013) employed sonication to enhance sugar production by 30% after a dilute sulfuric acid (0.5-1%) hydrolysis of PPW. In most scenarios the hydrolysis process was combined with a

biological conversion step to generate alcohols and acids with low levels of inhibitors (Sun and Cheng, 2002).

1.5.2 Carboxylic acids production

Carboxylic acids are a group of weak organic acids (RCO_2H) and can be broken down into the following classes: short chain fatty acids ($C \leq 6$), long chain fatty acids ($C > 6$), α-hydroxy acids (with a hydroxyl group on the adjacent carbon, such as lactic acid, glyceric acid), dicarboxylic acids (with two carboxylic acid functional groups, such as succinic acid, adipic acid), tricarboxylic acids (with three carboxylic acid functional groups, such as citric acid), amino acids, keto acids (pyruvic acid), and aromatic carboxylic acids (benzoic acid). Figure 1.7 gives a metabolism pathway of partial carboxylic acids production from glucose via fermentation (Koutinas et al., 2014). The short chain fatty acids, also called volatile fatty acids (VFAs), comprises of acetic acid, propionic acid, butyric acid, valeric acid, and caproic acid. These VFAs are the main organic products of primary fermentation reactions and are also the intermediate compounds for AD to produce CH_4 (Agler et al., 2011). VFAs are valuable chemical precursors to produce alkanes as fuels. In order to maximize the VFAs production, iodoform as methane inhibitor was used to cut down the pathway of VFAs into CH_4 (Chan and Holtzapple, 2003; Domke et al., 2004). The effects of pH on VFAs production in AD of sewage sludge were also investigated, where improved VFAs production and reduced CH_4 were observed at pH 10 (Zheng et al., 2013). Another study by Bengtsson et al. (2008) found that the VFAs composition can be controlled through pH manipulation. A variety of organic materials such as sewage sludge, paper waste, and food waste have

been investigated to produce VFAs (Chan and Holtzapple, 2003; Domke et al., 2004; Hong and Haiyun, 2010; Zheng et al., 2013). Limited references were found on VFAs production by undefined mixed cultures from PPW. Parawira et al., (2004a) studied VFAs production from PPW under mesophilic condition where 420 mg g^{-1} acetic acid, 310 mg g^{-1} butyric acid, 140 mg g^{-1} propionic acid and 90 mg g^{-1} caproic acid were obtained.

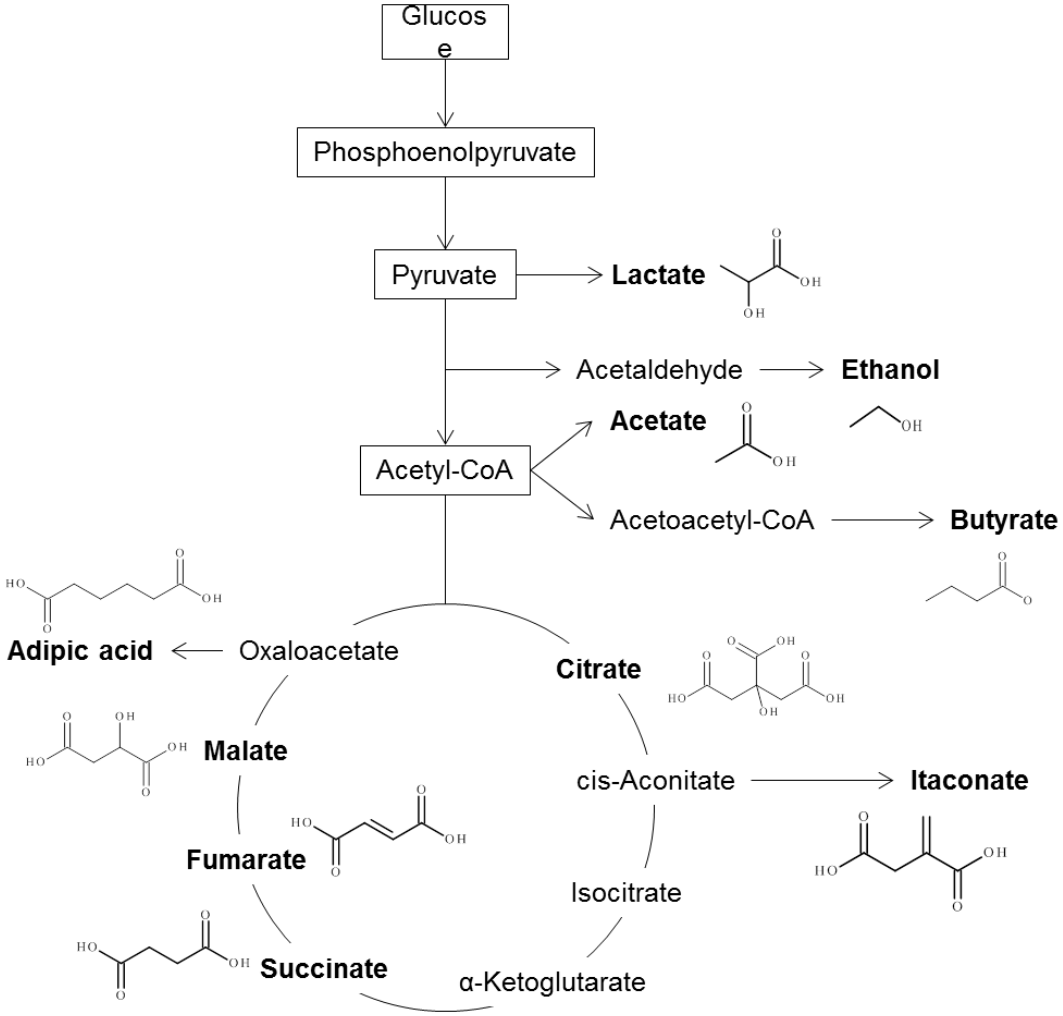

Figure 1.7 Metabolic pathway of carboxylic acids production via fermentation.

The use of undefined mixed cultures originating from the wastewater treatment fields has

become an attractive method for producing chemicals (fatty acids) and bioenergy (CH_4 and H_2) through microbial selection and bioprocess manipulation (Kleerebezem and van Loosdrecht, 2007). However, only a narrow range of fatty acids can be generated and this approach cannot yield significant amount of other carboxylic acids such as citric acid, gluconic acids, succinic acid, and lactic acid (Agler et al., 2011; Kleerebezem and van Loosdrecht, 2007). These carboxylic acids have been produced by pure cultures from different organic wastes. Imandi et al. (2008) used a statistical experimental design to optimize citric acid production (0.2 g g^{-1}) from pineapple waste fermentation with *Yarrowia lipolytica* NCIM3598. Gluconic acid was biosynthesized at a yield of 82 g L^{-1} by solid state fermentation of sugarcane molasses with tea waste using *Aspergillus niger* ARNU-4 (Sharma et al., 2008). Zhang et al. (2013) was able to produce succinic acid (32 g L^{-1}) in high purity from bakery waste using a simultaneous hydrolysis and fungal autolysis approach. However, PPW has only been used to produce lactic acid and ethanol with pure cultures.

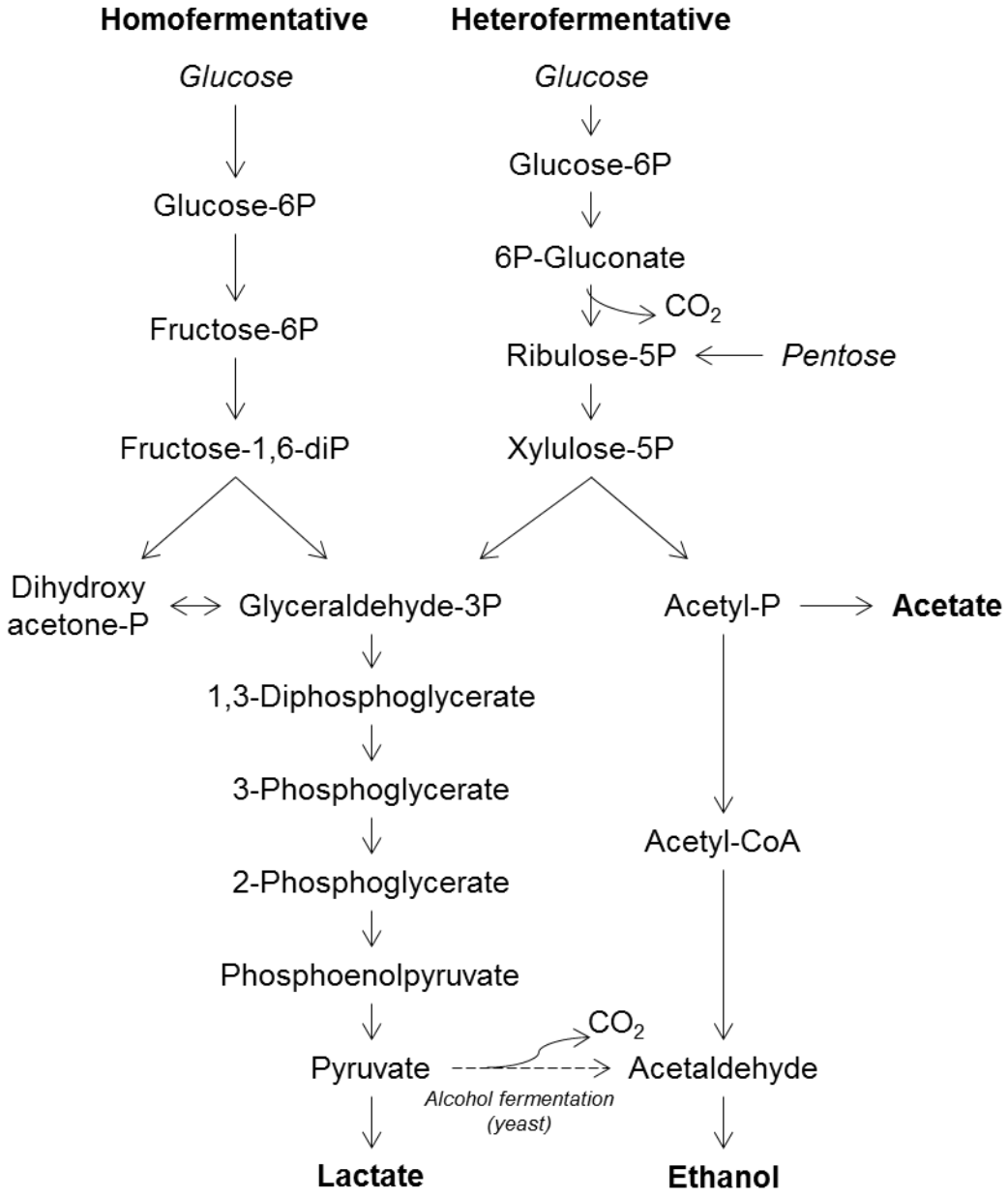

Figure 1.8 Metabolic pathways of lactic acid fermentation from glucose.

Lactic acid (2-hydroxypropionic acid), can be manufactured either by chemical synthesis from petrochemicals or carbohydrate fermentation with bacteria and fungi. Currently nearly all lactic acid manufacture is based on carbohydrate fermentation (Datta and Henry, 2006). Biotechnological production of lactic acid can be classified into two

pathways as shown in Figure 1.8, where homo lactic acid fermentation catalyze glucose exclusively into lactic acid via the Embden-Meyerhof-Parnas (EMP) pathway and the hetero lactic acid fermentation metabolizes glucose into lactic acid, CO_2, acetic acid and ethanol (Wee et al., 2006). Chatterjee et al. (1997) studied lactic acid production by direct fermentation of waste potato mash with amylase-producing strain *Lactobacillus cellobiosus*, using $CaCO_3$ to neutralize acidity, and was able to achieve 50% conversion of starch to lactic acid in 48 h. Liu et al. (2005) used pure fungal culture (*Rhizopus oryzae* NRRL 395) to produce lactic acid from cull potato and was able to obtain a 10% conversion. The role of nitrogen on the lactic acid production from waste potato starch by *Rhizopus arrhizus* WEBL 0501 was studied by Zhang et al. (2007) and found that a yield of 91% was achieved by addition of 0.909 g L^{-1} ammonium nitrate. The importance of lactic acid as a commodity chemical is due to the fact it is widely used in food, pharmaceutical, cosmetic and industrial applications, especially for producing the bioplastic, polylactic acid (PLA).

1.5.3 Biopolymer biosynthesis

Polyhydroxyalkanoates (PHAs) and extracellular polysaccharides (EPSs) are two groups of bacterial polymers that can be synthesized from various carbon sources. Figure 1.9 gives the metabolic pathways involved in the synthesis of PHAs and EPSs from glucose (Harutoshi, 2013; Pena et al., 2011). PHAs are biodegradable polyesters accumulated as carbon/energy storage in bacteria under nutrient limited fermentation condition (van Loosdrecht and Salehizadeh, 2004). Polyhydroxubutyrate (PHB) and polyhydroxybutyrate-co-3-hydroxyvalerate (PHB-co-3HV) are two most common PHAs

and have been studied extensively for the past 80 years (Keshavarz and Roy, 2010). The industrial productions of PHAs have been successfully demonstrated and most of current researches focused on the applications as new biodegradable plastics (Chen, 2009). One study on PHA production from potato waste has been documented by Rusendi and Sheppard (1995). They used PPW enzymatic hydrolysates to produce fermentable sugars which were converted to PHB (5 g L^{-1} or 77% biomass) with *Alcaligenes eutrophus*.

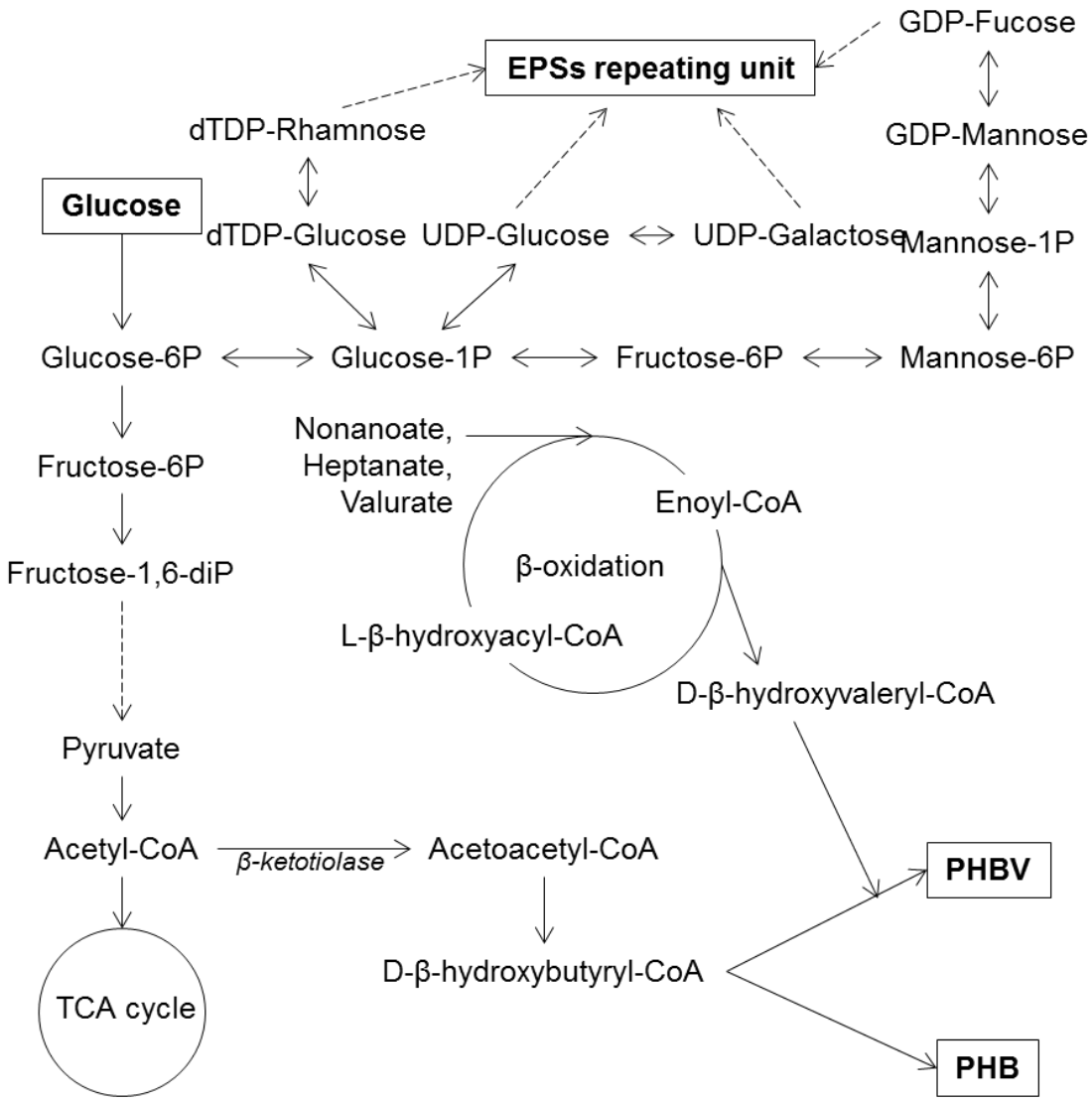

Figure 1.9 Metabolic pathways involved in the biosynthesis of PHAs and EPSs.

EPSs are bacterial polysaccharides that are synthesized either extracellularly or intracellularly and then transported to extracellular environment as macromolecules such as xanthan, gellan, alginate, cellulose, pullulans and many more (Freitas et al., 2011). Pullulan is a mixed α-1,4- and α-1,6-linked glucan biosynthesized by *Aureobasidium pullulans* (Roukas, 1998). Barnett et al. (1999) studied pullulan production from PPW hydrolysates with *A. pullulans* NRRLY-6220 and found that hydrolyzates produced by β-amylase had high EPSs and pullulan yields. A later study by Goksungur et al. (2011) used response surface methodology to optimize the production of pullulans (incubation time, initial substrate concentration, and initial pH) using hydrolyzed PPW starch. Xanthan is also an important industrial polysaccharide, consisting of a pentasaccharide repeat of glucose, mannose, and glucuronic acid (2:2:1), and is used in applications such as food thickening. Xanthan has been biosynthesized by *Xanthomonas campestris* using PPW as feedstock (Bilanovic et al., 2011).

1.6 Conversion of PPW into energy products

1.6.1 Ethanol fermentation

Ethanol is a renewable biofuel that has been widely used as partial gasoline replacement in order to reduce the dependence on fossil fuels (Sun and Cheng, 2002). Ethanol can be produced directly from sugars by yeasts or bacteria. Sugars can be obtained from starchy or lignocellulosic resources through separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) processes (Lin and Tanaka, 2006). The metabolic pathway of ethanol production is also illustrated in Figure 1.8, where

pyruvate can be converted into acetaldehyde and then ethanol plus the release of CO_2 (1 mole of glucose is converted into 2 mole of ethanol and 2 mole of CO_2 under alcohol fermentation). Ethanol has been commercially produced successfully from corn, sugarcane, and wheat in large scale plants (Gnansounou and Dauriat, 2005). Most current research activities have focused on the utilization of lignocellulosic materials from forestry and crop residues and food processing wastes. However, some of these substrates require a pretreatment to improve sugar yields. Pretreatment methods include: (i) physical (mechanical refining and pyrolysis), (ii) thermophysicochemical (steam explosion, ammonia fiber explosion and CO_2 explosion), (iii) chemical (ozonolysis, acid or alkaline hydrolysis, oxidative delignification and organosolv process), and (iv) biological (rot fungi and enzymes) to breakdown, disrupt, or solubilize lignin, cellulose and hemicellulose components in order to improve sugars recovery (Kumar et al., 2009; Sun and Cheng, 2002).

Ethanol production from PPW has been implemented in two ethanol plants with capacities of 11-15 million liters in Idaho a decade ago (Mann et al., 2002). Generally, PPW is dispersed and partially hydrolyzed using enzymes followed by ethanol fermentation (Yamada et al., 2009). Arapoglou et al. (2010) studied the ethanol production from PPW using separate enzymatic hydrolysis and fermentation stages and respectively, resulted in 92.5% conversion to reducing sugars (18.5 g L^{-1}) and ethanol (7.6 g L^{-1}) with *Saccharomyces cereviciae var. bayanus*. *Clostridium acetobutylicum* DSM 1731 has been reported to produce a mixture of acetone, butanol and ethanol (ABE) from PPW (Grobben et al., 1993). Utilization of PPW for ethanol production has been

demonstrated; however, the small scale of potato waste operations, as compared to corn conversion plants, makes this an uneconomic endeavor.

1.6.2 Anaerobic digestion (AD)

AD is a biological process to break down organic material, such as carbohydrates, protein, and lipids into intermediate VFAs and terminal biogas compounds (chiefly CH_4 and CO_2) by microorganisms. This practice has been widely used for stabilizing organic wastes and energy production from municipal sewage sludge, animal manure, and agricultural and food industries. The AD process offers several significant advantages for waste management including effective pathogen removal, minimal odor emission, and fewer by-products produced (Ward et al., 2008). As shown in Figure 1.10, the AD process occurs in four distinct steps: 1) *hydrolysis* of complex polymers (carbohydrates, fats, and proteins) into simple monomers (sugars, fatty acids, and amino acids) by hydrolytic enzymes; 2) *acidogenesis* of sugars and amino acids to produce VFAs, acetic acid, H_2 and CO_2 together with organic compounds that act as both electron donors and acceptors; 3) *acetogenesis* of VFAs to produce acetic acid, H_2 and CO_2; and 4) *methanogenesis* to produce CH_4 and CO_2 (Gujer and Zehnder, 1983; Parkin and Owen, 1986).

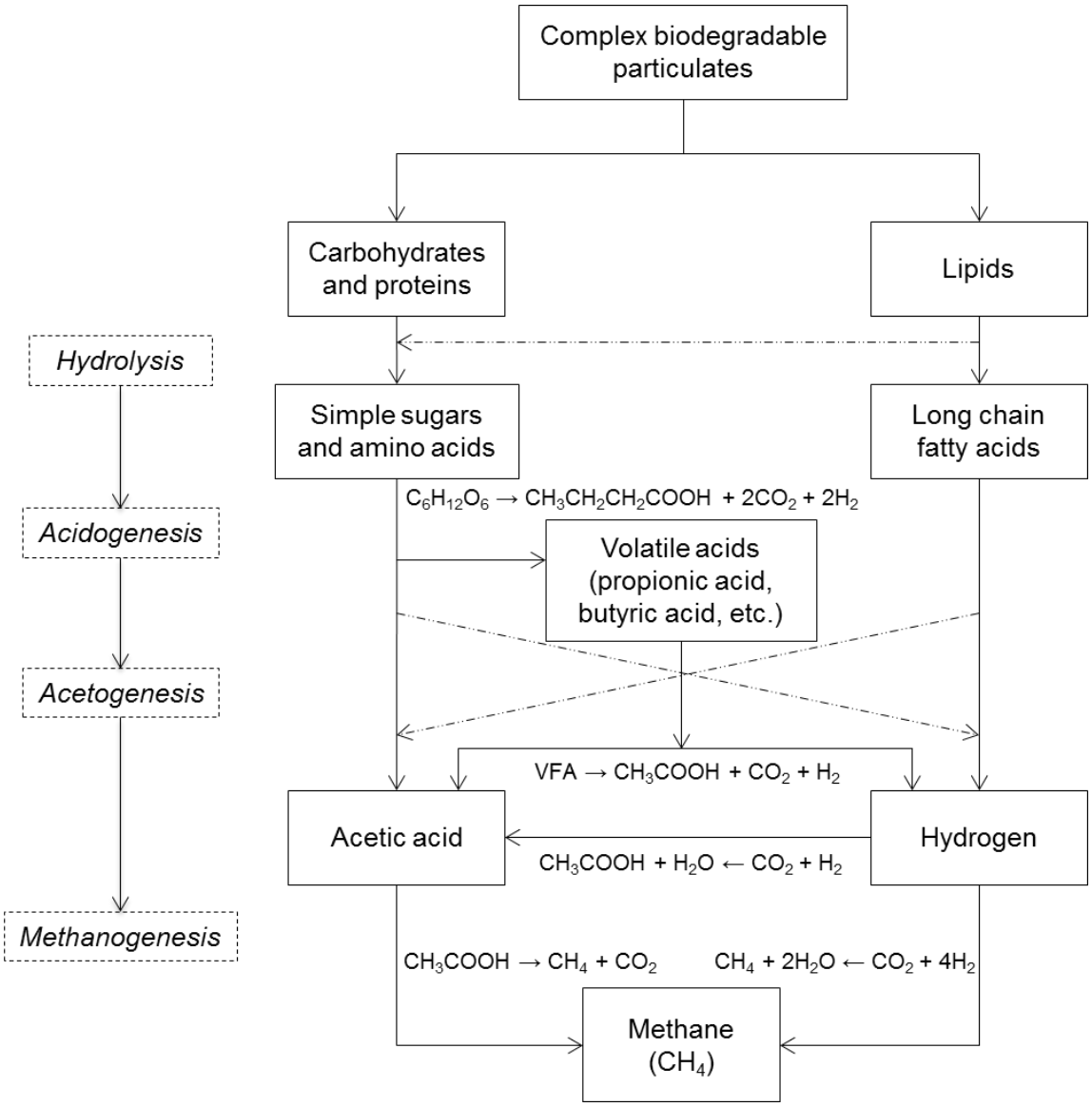

Figure 1.10 Schematic diagram of methane formation in an AD.

The AD of PPW and other organic materials can take place at both mesophilic temperatures (30-40°C) and thermophilic temperatures (50-60°C), where the biogas production and microbial structures at the two temperature conditions are quite different (Ward et al., 2008). Two-stage AD has several advantages over the conventional one-stage process, where the first stage serves as hydrolysis and acidification processes followed by the methanogensis stage in order to improve the overall efficiency (Demirer

and Chen, 2005; Parawira et al., 2008; Parawira et al., 2005). Parawira et al. (2007) compared the CH_4 generation from PPW with two-stage digesters of mesophilic-mesophilic, mesophilic-thermophilic, thermophilic-thermophilic configurations, and the results showed that mesophilic conditions had higher CH_4 yields and longer reaction time compared with thermophilic conditions. Co-digestion of different organic materials can improve the CH_4 production by adjusting the C/N ratio (Ward et al., 2008). The co-digestion of PPW and sugar beet leaves gave up to 60% higher CH_4 yield as compared to the digestion of PPW or sugar beet leaves alone (Parawira et al., 2008; Parawira et al., 2004b). The co-production of CH_4 and H_2 from PPW was also studied in two-stage AD with controlled pH at 5.5 and 7 for the first stage H_2 production (68 L kg^{-1} TS) and second stage CH_4 production (225 L kg^{-1} TS) (Zhu et al., 2008).

1.6.3 Pyrolysis

Pyrolysis is a thermochemical conversion process to thermally crack organic material in the absence of oxygen (Mohan et al., 2006). The pyrolysis process can be either slow or fast pyrolysis, where the latter is operated at high heating and transfer rates at 425-550°C with short vapor residence times and rapid cooling of the vapor and aerosol components to obtain relative high yields of liquid bio-oil (Bridgwater et al., 1999; Mohan et al., 2006). A wide range of biomass materials such as wood, agricultural and forestry residues, grasses, and algae have been evaluated using pyrolysis to produce fuels for heat and power generation or upgraded into transport fuels (Czernik and Bridgwater, 2004; Mohan et al., 2006). Onal et al. (2011) studied the pyrolysis of PPW in static, N_2, and steam atmospheres and found that the highest bio-oil yield (41%) was obtained in steam

atmosphere as compared to 27% in N_2. Co-pyrolysis of PPW and high density polyethylene produced bio-oils of improved quality and quantity than PPW alone (Onal et al., 2012). The pyrolyzed PPW bio-oils exhibited similar H/C ratios and aliphatic sub-fractions with currently utilized transport fuels (Onal et al., 2012; 2011). The produced bio-chars were also evaluated as adsorbents which showed excellent adsorption capacities to heavy metals and phenolic derivatives (Moreno-Pirajan and Giraldo, 2012; 2011).

1.7 Miscellaneous uses

In addition to the above studies, PPW has also been utilized into various products. A series of proteins and enzymes have been produced from PPW by pure cultures fermentation (dos Santos et al., 2012; Gelinas and Barrette, 2007; Shukla and Kar, 2006). Kang and Min (2010) developed edible films from potato peel with the additives glycerol and soy lecithin using high-pressure homogenization, irradiation, and ultrasonic treatments. Tammineni et al. (2013) added oregano oil into PPW to make edible films and showed that the oregano oil reduced the film strength and increased the water vapor permeability, and exhibited antimicrobial potential as coatings on seafood and other food products to inhibit bacterial growth of *Listeria monocytogenes*. Cellulose nano-crystals were produced from PPW and had a longer fiber length than cotton derived nanocrystals (Chen et al. 2012). Furthermore, the cellulose nano-crystals were used as reinforcement and a vapor barrier additive in films. Direct use of PPW as a low cost adsorbent were studied by Prasad and Abdullah (2009) and Samarghandy et al. (2011) and showed that Fe (II) ions and reactive black 5 were able to be removed in aqueous solutions.

1.8 Objectives

The aims of this dissertation are to examine approaches for the utilization of PPW generated from potato processing plants to value added bioproducts through biological and/or thermochemical processes. By using this strategy the biomass can be selectively converted into sugars, carboxylic acids, alcohols, and CH_4 while minimizing waste. Currently, undefined mixed cultures are mainly used to produce CH_4 by AD and VFAs by acidogenesis fermentation. However, organic acids such as lactic acid have not been produced using this approach. Therefore, the goal of this project is to better utilize and manage PPW to produce products and mitigate waste generation.

Figure 1.11 shows the overall structure of this dissertation which is focused on obtaining value added bioproducts from PPW. Chapter 1 is a general introduction and review of potato and PPW structure and chemistry and their utilization. Chapter 2 covers the production and optimization of the value added chemicals (lactic acid, acetic acid and ethanol) by batch fermentation using undefined mixed cultures (activated sludge). Chapter 3 examined the lactic acid production by anaerobic fermentation using a sequencing batch bioreactor inoculated with undefined mixed cultures and investigated the robustness and resilience of microbial communities. Chapter 4 covers the optimization of lactic acid production using an anaerobic fermentation sequencing batch bioreactors. Chapter 5 gave a detailed chemical and thermal characterization of PPW and PPW-fermentation residue (PPW-FR) in order to utilize this fermentation co-product. Chapter 6 covers preliminary work on the thermochemical conversion of PPW and PPW-FR by pyrolysis into a bio-oil and bio-char. Chapter 7 summarizes the conclusions and

potential further work beyond this dissertation.

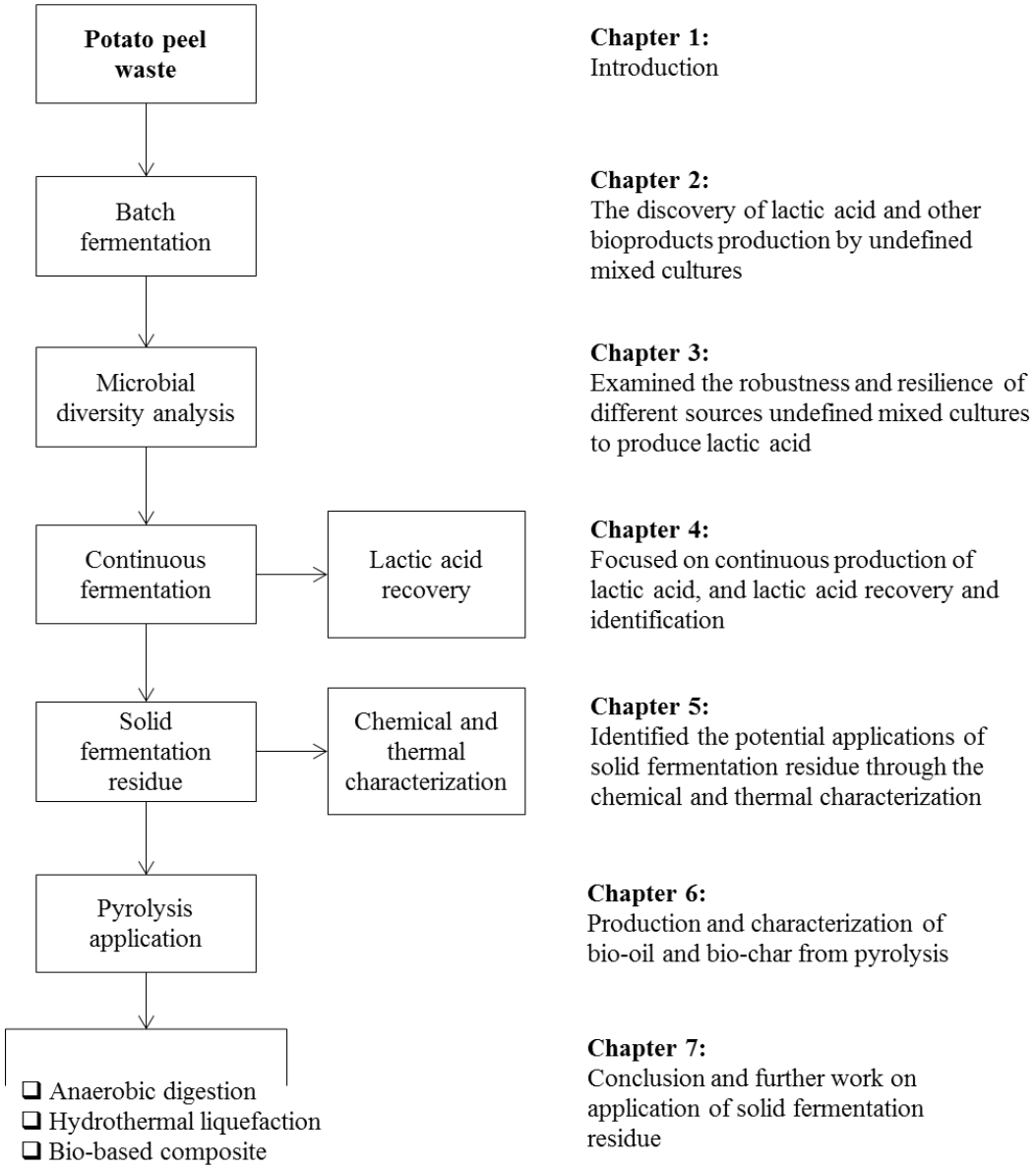

Figure 1.11 Schematic diagram showing the overview and integration of the chapters of this dissertation.

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Chapter 2 Fermentation of potato peel waste with undefined mixed cultures

2.1 Abstract

Potato peel waste (PPW) as zero value byproduct generated from food processing plant contains a large quantity of starch, non-starch polysaccharide, lignin, protein, and lipid. PPW as one promising carbon source can be managed and utilized to value added bioproducts through a simple fermentation process using undefined mixed cultures inoculated from wastewater treatment plant sludge. A series of non-pH controlled batch fermentations under different conditions such as pretreatment process, enzymatic hydrolysis, temperature, and solids loading were studied. Lactic acid (LA) was the major product, followed by acetic acid (AA) and ethanol under fermentation conditions without the presence of added hydrolytic enzymes. The maximum yields of LA, AA, and ethanol were respectively, 0.22 g g^{-1}, 0.06 g g^{-1}, and 0.05 g g^{-1}. The highest LA concentration of 14.7 g L^{-1} was obtained from a bioreactor with initial solids loading of 60 g L^{-1} at 35°C.

2.2 Introduction

Potatoes are a major crop for the US Pacific Northwest (PNW; Idaho, Washington and Oregon); 1.26×10^{10} kg were harvested with the total value of 1.9 billion U.S. dollar in 2011. Approximately 60% of harvested potatoes were manufactured for French fries, canned foods, starch and flour products in food processing plants while the remaining 40% are unprocessed whole potatoes (NPC, 2012). The processing of potatoes generates a significant waste stream. The typical steam peeling losses are approximately 8%, which produced a loss of about 1.01×10^{9} kg potato peel waste (PPW) in the PNW in 2011. PPW

generated from the processing plants is conventionally considered a zero value waste (Arapoglou et al., 2010). In Idaho, most of the PPW is currently used for local cattle feed.

The main chemical constituents of PPW are starch, non-starch polysaccharide (pectin, cellulose and hemicelluloses), lignin, protein, lipid, and ash (Camire et al., 1997). This chemical composition provides a good source of carbon for conversion into bioproducts. In this regard, and with an increasing worldwide energy demand, volatile fossil fuel prices, and environmental sustainability awareness, carbohydrate waste streams such as PPW (Jeon et al., 2008; Mars et al., 2010) are promising alternative carbon resources to make biofuels and chemicals via biochemical conversion of sugars using a carboxylate platform (Chang et al., 2010; Holtzapple and Granda, 2009). With such a platform, sugars can be converted to various organic compounds by acid or enzymatic hydrolysis, glycolysis, acidogenesis, and acetogenesis (Agler et al., 2011); proceeding to complete biodegradation generates methane (CH_4). While CH_4 as the final product of anaerobic methanogenesis is a renewable form of natural gas, the economic feasibility of anaerobic digestion (AD) makes the biogas less favorable than other intermediate chemicals such as lactic acid (LA), ethanol, and volatile fatty acids (VFAs) (Voegele, 2011). However, combining AD with a portfolio of bioproducts from a waste stream could improve the economic feasibility of such ventures.

Specifically focusing on LA, conventional industrial LA production starts with glucose derived from starch or lignocellulosic biomass by separate hydrolysis and fermentation or simultaneous saccharification and fermentation in the presence of enzymes and pure culture (Ou et al., 2011; Yamada et al., 2009). However, a single microbial strain only

functions in a narrow range of substrates and operating conditions (Lin et al., 2011; Zhan et al., 2003). Although using known two or more mixed cultures can minimize this limitation and potentially widen the scope of usable carbohydrate materials to some extent (Cui et al., 2011; John et al., 2007), other restrictions such as sterile conditions, certain microbe concentration, pH, nutrients, and temperature are also critical for fermentation productivity (Cock and Stouvenel, 2006; Zhang et al., 2007). Using undefined mixed cultures can handle the complex conditions with the highest microbial diversity, great adaption, and self-evolution abilities. Mixed microbial consortia have been applied to complex substrates in the environmental sector to break down solids and purify wastewater under aerobic and anaerobic conditions for more than a century (Agler et al., 2011; Wanger et al., 2002). More recently, the production of VFAs by anaerobic fermentation with undefined mixed cultures from waste and wastewater has been studied (Bengtsson et al., 2008). However, to the authors' best knowledge there is no paper published that produced LA from PPW using an undefined mixed microbial culture.

In this study we explored the possibility of PPW fermentation with undefined mixed culture, and analyzed the influences of different bioreactor operational factors (PPW gelatinization, addition of hydrolytic enzymes, temperature, and PPW solid content) on the production of LA. This simple and novel approach can potentially maximize the value of PPW and further broaden the utilization of carbohydrate waste.

2.3 Materials and methods

2.3.1 Potato peel waste

PPW samples were obtained from a regional potato processing plant (JR Simplot Company, Nampa, ID) and frozen (-20°C) before use. Carbohydrate analysis was performed by high performance liquid chromatography (HPLC) on the 2-stage acid hydrolysates (ASTM E 1758-01). Starch was estimated by enzymatic-HPLC assay (Delgado et al., 2009). The lignin and suberin components in potato cell walls are mixed together and the measurement of this complex was determined by acid insoluble and acid soluble fractions according to ASTM D 1106-96 and Schoening and Johansson (1965) methods, respectively. Lipid content was determined by Soxhlet extraction with CH_2Cl_2 according to ASTM D 1108-96. Protein was estimated by multiplying total nitrogen (N) by 6.25. Ash was determined by dry oxidation at 600°C. The chemical compositional results are listed in Table 2.1.

Table 2.1 Chemical composition of potato peel waste (PPW).

Parameters	Dry weight (%)
Carbohydrate	63.2±4.2
Starch	34.3±2.7
Protein (N_{tot} 6.25)	17.1±0.3
Lipids	1.2±0.0
Lignin and suberin complex	
Acid soluble	6.2±0.2
Acid insoluble	4.1±0.0
Ash	9.6±0.1

2.3.2 Fermenter set-up

Air-locked glass bioreactors with a working volume of 800 mL were used for batch

fermentation experiments. The temperature was controlled by a heated water-jacket, and at the start of each test all bioreactors were inoculated with 2% activated sludge from the Moscow, Idaho municipal wastewater treatment plant and fed with PPW.

Four bioreactor operating factors were considered in this study: (i) PPW pre-gelatinization (boiled for 30 min at 100°C), (ii) addition of hydrolytic enzymes (6 u g^{-1} mixed *α-amylase* and *glucoamylase* (Sigma, USA) and 30 u g^{-1} *cellulase* (Fisher Scientific)), (iii) reactor temperature (20, 35 and 50°C), and (iv) solids loading (20, 40, and 60 g L^{-1}). Operating factors (i) and (ii) were evaluated in the first round of experiments, followed by (iii) and (iv) sequentially. Bioreactors were operated for 12 d until no significant changes in organic acid concentration were observed. The experiments were performed in duplicate. The bioreactor labeling was as follows: (A) un-gelatinized PPW, (B) gelatinized PPW, (C) un-gelatinized PPW and hydrolytic enzymes, and (D) gelatinized PPW and hydrolytic enzymes.

2.2.3 Analytical methods

LA, acetic acid (AA), and ethanol were quantified through ion exclusion HPLC analysis, using a Rezex ROA organic acid column (7.8 mm x 30 cm, Phenomenex, Torrance, CA) and a Waters HPLC (Waters, Milford, MA) equipped with differential refractive index detector (ERC-5710, ERMA), on elution with 0.005 N aqueous sulfuric acid (0.5 mL min^{-1}) at 65°C. Sugars were quantified by HPLC using two Rezex RPM columns in series (7.8 mm x 30 cm, Phenomenex, Torrance, CA) and a Waters HPLC (Waters, Milford, MA) equipped with differential refractive index detector (Waters model 2414), on elution

with water (0.5 mL min^{-1}) at 85°C. DO and pH were measured with Orion-3-Star DO/pH portable meter (Thermo Fisher Scientific Inc., Waltham, MA). Electrospray ionization-mass spectrometry (ESI-MS) was performed on a LCQ-Deca instrument (ThermoFinnigan, San Jose, CA).

2.2.4 Calculation

LA yield was calculated as the amount of LA production per unit of PPW used as:

$Y_{LA} = (C\text{-}C_0) \times V/M \times 100\%$

where, Y_{LA} is the LA yield (g g^{-1}), C is actual LA concentration (g L^{-1}), C_0 is initial LA concentration (g L^{-1}), V is bioreactor working volume (L), and M is the initial mass of solid in bioreactor (g).

2.2.5 Statistical analysis

All the measurements were tested in duplicates and the average values were reported and analyzed with SAS 9.3 (SAS Inc., Cary, NC, USA) under the analysis of variance (ANOVA).

2.4 Results and discussion

2.4.1 Effect of gelatinization and enzymatic hydrolysis

A 2×2 factorial experiment using 40 g L^{-1} PPW batch fermentation at 20°C was performed regarding the operating factors of (i) with and without gelatinization process and (ii) with and without addition of hydrolytic enzymes to assess the effects of starch gelatinization and addition of enzymes (Figure 2.1). LA, AA, and ethanol were the three

major products synthesized by batch fermentation. The identity of LA was also confirmed in the fermentation broth by positive ion ($[M+H]^+$ = m/z 91) and negative ion ($[M-H]^-$ = m/z 89) ESI-MS. Without the presence of hydrolytic enzymes, bioreactors A and B had similar trends of high LA content and low AA and ethanol production levels. The bioreactors A and B have significantly different fermentation results, and it was observed that gelatinized PPW fermentation in bioreactor B had a higher conversion yield than the un-gelatinized PPW fermentation bioreactor A. The concentrations of LA and AA for bioreactor B were, respectively, 9.4 g L^{-1} and 5.3 g L^{-1} (yields of 0.20 g g^{-1} and 0.11 g g^{-1}) on the 9^{th} day and were 20% and 18% higher than bioreactor A. This result can be explained as the gelatinization process made starch more accessible for bioconversion (Basel et al., 2006). The composition of the three main products changed and the conversion increased with the addition of hydrolytic enzymes in bioreactors C and D, which produced higher LA and ethanol levels relative to AA. The gelatinized PPW bioreactor D had a slightly higher LA concentration of 7.3 g L^{-1} (yield of 0.15 g g^{-1}) than the un-gelatinized PPW bioreactor C of 6.5 g L^{-1} (yield of 0.13 g g^{-1}) on the 3^{rd} day, but they were not significantly different. Both bioreactors (D and C) produced the same concentration of ethanol at 6.0 g L^{-1} (yield of 0.13 g g^{-1}) and AA at 2.6 g L^{-1} (yield of 0.07 g g^{-1}).

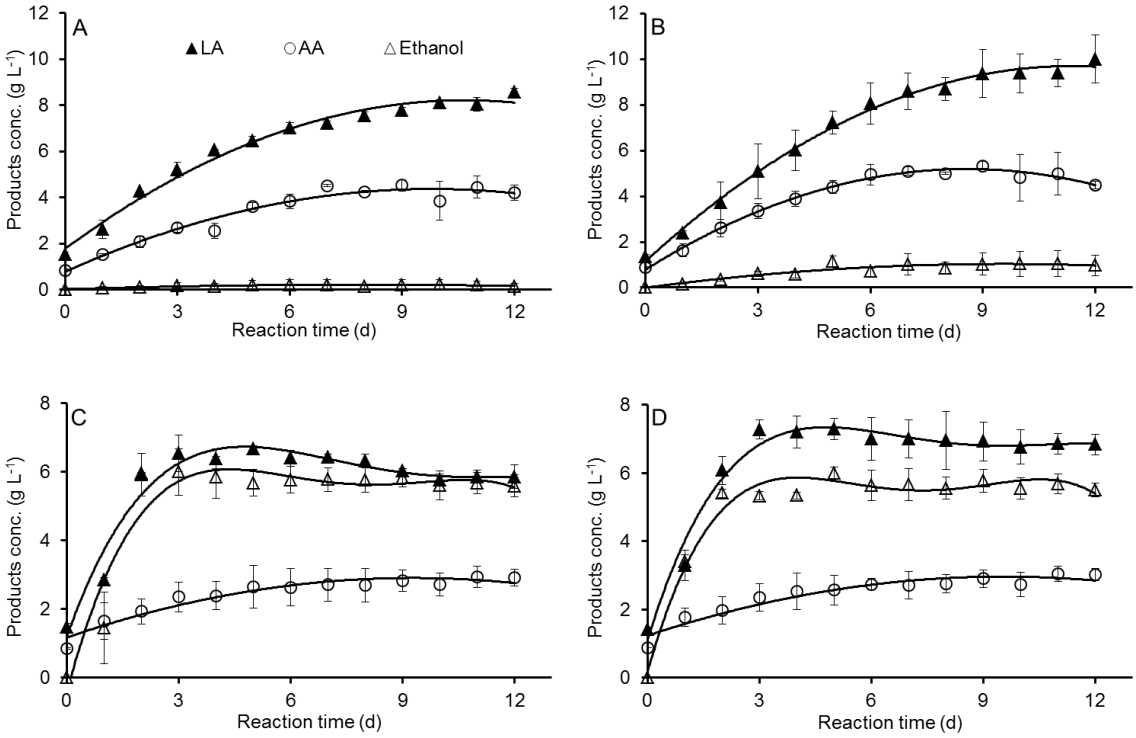

Figure 2.1 Influence of starch gelatinization and enzymatic hydrolysis on batch fermentation to LA (▲), AA (○) and ethanol (Δ) at 20°C for: (A) un-gelatinized PPW; (B) gelatinized PPW; (C) un-gelatinized PPW and hydrolytic enzymes; and (D) gelatinized PPW and hydrolytic enzymes.

The effect of enzymatic hydrolysis was different with PPW gelatinization, which not only increased carbohydrate conversion, but also changed the fermentation product composition. Bioreactors C and D had the peak values of LA, ethanol, and AA after 2-3 days. The glucose concentration for bioreactors C and D increased by 15-20 g L^{-1} at the first 6 h and reduced to almost zero on the 2nd day (data not show), because sugars were readily converted to ethanol and LA (Arapoglou et al., 2010; Ou et al., 2011). While only <1 g L^{-1} glucose contents were observed in bioreactors A and B. Microorganisms that can produce LA include lactic acid bacteria (such as *Lactobacillus* sp.) and filamentous fungi

(such as *Rhizopus* sp.), which can convert 1 g glucose into 1 g LA by homolactic fermentation pathway or 0.5 g LA, 0.26 g ethanol and 0.24 g CO_2 by heterolactic fermentation pathway (Wee et al., 2006) and the commercial LA production is based on pure culture of homolactic fermentation (Zhang et al., 2007). Lactic acid fermentation is more efficient than alcoholic fermentation (theoretically 0.51 g ethanol and 0.49 g CO_2 from 1 g glucose). In addition, LA has a higher market price at \$1.35 kg^{-1} than ethanol at \$0.7 kg^{-1} (van Haveren et al., 2008). Based on the products composition of the fermentation bioreactor B, we can infer that >80% LA was generated by homolactic fermentation (Wee et al. 2006) if assumed that heterolactic and not alcoholic fermentation was the only reason of ethanol production.

2.4.2 Effect of temperature

Since the fermentation without the presence of starch and cellulose hydrolytic enzymes in bioreactor B was able to produce more LA at 20°C than the other bioreactors (A, C and D), and a further assessment on the mesophilic (35°C) and thermophilic (50°C) conditions were conducted (Figure 2.2). A new set of experiments were conducted at 35°C and still resulted in LA, AA, and ethanol as the main products formed. Temperature has been shown to be an important factor in organics conversion (Cock and Stouvenel, 2006; Parawira et al., 2004). Unlike the gradual increase of LA and AA at 20°C, the bioreactor at 35°C showed a higher LA production ability, which reached 8.2 g L^{-1} (yield of 0.18 g g^{-1}) on the 2^{nd} day and the highest concentration of 10.0 g L^{-1} (yield of 0.22 g g^{-1}) on day 7. In contrast, for LA only 3.7 g L^{-1} (yield of 0.06 g g^{-1}) on the 2^{nd} day and 8.6 g L^{-1} (yield of 0.18 g g^{-1}) on the 7^{th} day were observed for the bioreactor operating at 20°C.

At 35°C the bioreactor produced a lower AA content of 2 g L^{-1} and higher ethanol concentration of 2 g L^{-1} than at 20°C. This finding was different to that reported by Torijia et al. (2003) which observed an increase in AA and a decrease in ethanol contents with an increase in temperature from 15 to 35°C with pure cultures. The trends of AA and ethanol production of the 35°C bioreactor also indicated >80% reaction had been completed in the first two days. LA production at 35°C was shown to be approximately 3 times higher than at 20°C (based on LA yield on the 2nd day).

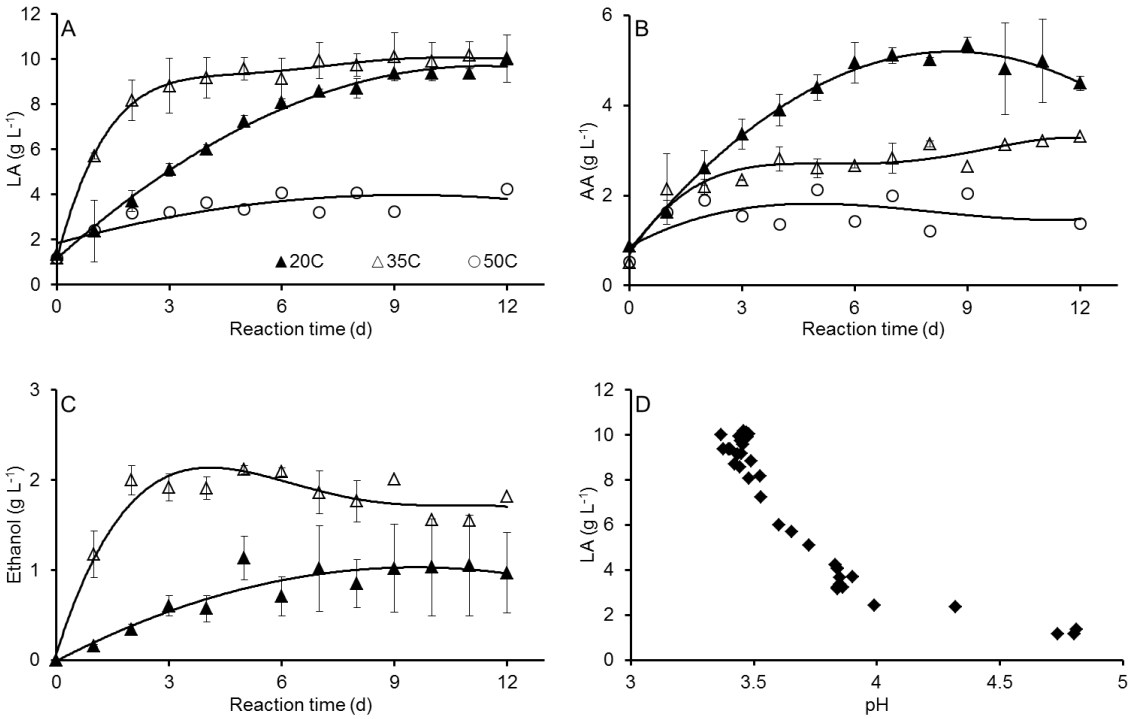

Figure 2.2 Influence of temperature (▲20, Δ 35, and ○ 50°C) on gelatinized PPW fermentation to produce (A) LA; (B) AA; (C) ethanol; and (D) scatter plot between LA produced and pH of three bioreactors.

Fermentation at 50°C was shown to decrease LA (4.0 g L^{-1}) and AA (2.0 g L^{-1}) production at day 2 and no ethanol was detected. This observation can be explained as

some of LA functional microorganisms here might be not suited to thermophilic conditions (Zhang, et al., 2007). The relation between produced LA and pH for the three bioreactors is shown in Figure 1D. It was shown that with an increasing LA concentration from 1.1 g L^{-1} to 10.0 g L^{-1}, the pH decreased from 4.8 to 3.4, which was around the pKa of LA (3.86). This correlation makes pH a good indicator of the LA fermentation in this system (Bengtsson et al., 2008).

2.4.3 Effect of solids loading

Solids loading is another important parameter associated with effective fermentation. Building on the studies discussed previously, the effect of solids loading at 20, 40 and 60 g L^{-1} on gelatinized PPW were evaluated at 35°C; the results are given in Table 2. The maximum concentrations for LA, AA, and ethanol obtained from the higher solids loading (60 g L^{-1}) were 14.7 g L^{-1}, 4.0 g L^{-1}, and 3.2 g L^{-1}, respectively. LA contributed to approximately 67% (w/w) of the total synthesized organic compounds, and all three bioreactors reached a maximum LA yield of 0.22 g g^{-1}, which appeared on the 4^{th}, 7^{th}, and 8^{th} day, respectively for reactors at 20, 40 and 60 g L^{-1} solids loading (Table 2.2). This phenomenon was also reported on volatile fatty acids production (Parawira et al., 2004), which can be explained as substrate inhibition and a long process needed for microorganisms to consume more food carbohydrates. The maximum total organic compounds (LA, AA and ethanol) yield reached 0.37-0.39 g g^{-1}.

Table 2.2 Yields of gelatinized PPW fermentation products with different solids loading at 35°C.

Solids loading ($g\ L^{-1}$)	Products conc. on the 2nd day ($g\ L^{-1}$)				Maximum LA conc. ($g\ L^{-1}$)				
	LA	AA	Ethanol	Y_{LA} ($g\ g^{-1}$)	LA	AA	Ethanol	Time (d)[a]	Y_{LA} ($g\ g^{-1}$)
20	4.5	1.3	1.0	0.18	5.3	1.4	1.1	4	0.22
40	8.2	2.2	2.0	0.18	10.0	2.8	1.9	7	0.22
60	10.6	2.6	2.7	0.15	14.7	4.0	3.2	8	0.22

[a] Time at which LA concentration reached maximum values.

A model adopted from sugar and other organic compounds production by acid hydrolysis of sorghum straw and enzymatic hydrolysis of starch (Delgado et al., 2009; Vazquez et al., 2007) was modified and applied in this study to predict the LA production from PPW and expressed as:

$$C = C_0 + C_p \times (1 - e^{-kt})$$

where, C is the actual LA concentration ($g\ L^{-1}$), C_0 is the initial LA concentration ($g\ L^{-1}$), C_p is the LA production potential ($g\ L^{-1}$), k is the rate of LA produced (d^{-1}), and t is the reaction time (d).

Figure 2.3 shows the comparison of model prediction and experimental data of LA concentration with time for the fermentation of PPW at solids loading of 20, 40, and 60 g L^{-1} at 35°C. The values of k were 1.1, 0.72, and 0.55 d^{-1} respectively, and values of C_p were 4.3, 8.9, and 13.1 g L^{-1} respectively which were close to the experimental values. The statistical parameters showed a good agreement between experimental and predicted data ($r^2 > 0.98$).

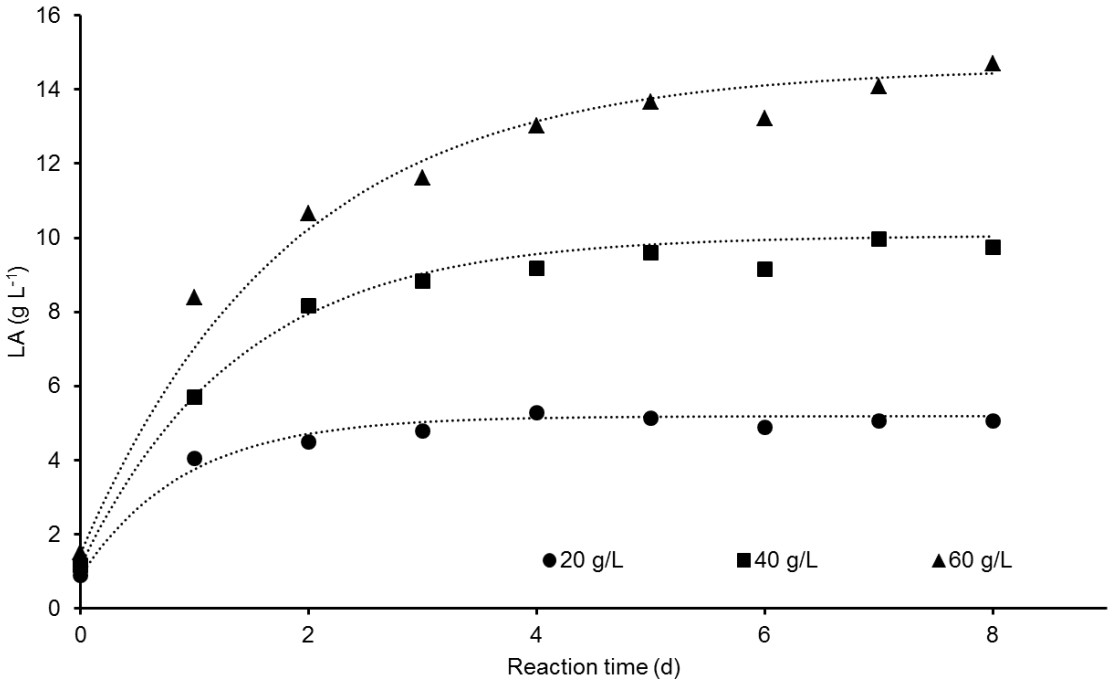

Figure 2.3 Experimental data points and predicted dependence (dotted lines) of LA production by fermentation of gelatinized PPW at different solids loading (20, 40, and 60 g L^{-1}) at 35°C.

2.5 Conclusion

This study successfully developed a simple fermentation process using an undefined mixed microbial culture to convert PPW to mainly LA without pH control and sterile conditions. The maximum yields of LA, AA, and ethanol were respectively, 0.22, 0.06, and 0.05 g g^{-1}, and the highest LA concentration of 14.7 g L^{-1} was obtained from bioreactor with initial solid loading of 60 g L^{-1} at 35°C. This approach can potentially lower the cost of LA production since it is using a zero value waste stream, without the need of using a refined sugar feedstock, and sterile bioreactors with pure cultures.

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Chapter 3 Comparative analysis of microbial community of novel lactic acid fermentation inoculated with different undefined mixed cultures

3.1 Abstract

Three undefined mixed cultures (activated sludge) from different municipal wastewater treatment plants were used as inoculation starters in a novel lactic acid fermentation process fed with potato peel waste (PPW). Anaerobic sequencing batch fermenters were run under near identical conditions to produce predominantly lactic acid. Illumina sequencing was used to examine the 16S rRNA genes of bacteria in the three seed cultures and fermenters. Results showed that the structure of microbial communities of three seed cultures were different. All three fermented biomass samples had unique community structure with more than 96% of genus *Lactobacillus*, as compared to <0.1% in seed cultures. The species-level taxonomic assignments of *Lactobacillus* sp. exhibited different taxa distributions in three fermented biomass samples. Results of this study suggest the structure of microbial communities in lactic acid fermentation of PPW with undefined mixed cultures were robust and resilient, which opens the door to engineer on microbial utilization of carbohydrate waste streams to produce lactic acid.

3.2 Introduction

Lactic acid is an important industrial chemical with various applications in food, pharmaceutical, textile, leather, and other chemical industries, especially for the biodegradable plastic, polylactic acid (PLA), production (John et al., 2007). Currently, nearly all the lactic acid manufactured is based on carbohydrate fermentation (Datta and

Henry, 2006), where pure strains of lactic acid producing microorganisms such as *Lactobacillus* sp. and *Aspergillus* sp. are usually employed. Within these lactic acid producing microorganisms, lactic acid bacteria (LAB) are a prominent group of microaerophilic, Gram-positive, non-spring, non-respiring cocci or rods, and acid tolerant bacteria that can ferment hexose sugars to produce lactic acid (Axellson, 2004). The core genera of LAB comprise *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*, which belong to the phylum of Firmicutes, class Bacilli and order Lactobacillales (Stiles and Holzapfel, 1997). Lactic acid production using LAB species can be classified into two pathways, where the homofermentative LAB catabolize glucose exclusively into lactic acid via the Embden-Meyerhof-Parnas pathway and the heterofermentative LAB usually metabolize glucose into lactic acid, CO_2, acetic acid and ethanol (Wee et al., 2006).

A relative high yield and optically pure lactic acid usually can be biosynthesized through pure culture fermentation. Some operational restrictions such as sterile condition, microbe concentration, pH, nutrients, and temperature are also critical to fermentation productivity (Zhang et al., 2007). Recently, the production of value added carboxylic acids and methane have been widely studied using undefined mixed cultures (activated sludge) from municipal wastewater treatment plants (Agler et al., 2012; Coats et al., 2013; Forrest et al., 2012; Werner et al., 2011). Activated sludge, also known as sewage sludge, is a highly complex biological mixture with bacteria as the dominant group, but also contains eukaryotes, archaea and viruses as determined by conventional molecular

techniques and next-generation sequencing methods (Ju et al., 2013; Wang X. et al., 2012; Zhang et al., 2012). These studies showed high microbial diversity and revealed an abundance of microbial resources. These microbial resources can be used as inoculation starters in biological conversion processes for specific organic compound production through feeding and operational parameter adjustments (Bengtsson et al., 2008).

Previously we have demonstrated the production of lactic acid from potato peel waste (PPW) with undefined mixed cultures by batch fermentation. In this updated study, we collected activated sludge samples from three municipal wastewater treatment plants located in different geographic regions of the United States as inoculation starters to examine the robustness and resilience of microbial communities in this novel lactic acid fermentation process. We characterized the chemical properties of three fermentation broths and analyzed the structures of microbial communities of seed cultures and fermented biomass samples using Illumina sequencing technology. The objective of this study was to address the following two questions. (i) Do different inoculation starters affect the lactic acid production from PPW? and (ii) Will the microbial community form into a unique and homogeneous one after fermentation?

3.3 Materials and methods

3.3.1 Feedstock, mixed cultures and fermentation

The PPW feedstock used in this experiment was obtained from a potato processing plant of JR Simplot Company (Caldwell, Idaho) and stored at -20°C in Ziploc bags. Detailed chemical composition of this feedstock was conducted according to the analytical

methods described previously, and the results are listed in Table 3.1. The mixed cultures (activated sludge samples) used in fermentation experiments were taken from an aeration tank (Moscow, Idaho) and return activated sludge tank (Boise, Idaho and West Lafayette, Indiana) of municipal wastewater treatment plants. Table 3.2 lists the basic profile of three municipal wastewater treatment plants and the inocula sources. Collected fresh sludge samples were kept chilled in plastic bottles and couriered overnight. The received samples were divided into two portions: one portion was used immediately for the fermentation experiments and the other portion was kept at -20°C for genomic DNA extraction and analysis.

Table 3.1 Chemical composition of potato peel waste.

Parameters	Dry weight (%)
Starch	16.8
Cellulose	7.8
Hemicellulose	14.7
Protein	25.4
Lipids	2.0
Lignin and suberin	21.6
Ash	11.1

The fermentation experiments were conducted in three independent glass vessels with working volume of 800 mL, maintained at 35°C, and sealed with a fermenting air-lock. The bioreactors were inoculated with 5% seeds from municipal wastewater treatment plants (Boise, Moscow, and West Lafayette respectively labeled as Reactor B0, M0, and W0) and fed daily with the same amount of gelatinized PPW (placed in a boiling water bath for 30 min and cooled) to maintain the feeding solid loading of 30 g L^{-1} and solid/hydraulic retention time (SRT/HRT) of 2 days. The reactors were stirred and cycled daily and ran for at least 12 days (6 SRT/HRT cycles) to ensure steady state. After that

the fermentation broth and biomass samples for each bioreactor (labeled B1, M1, and W1) was collected in three successive days and the mixed biomass samples were stored at -20°C for further DNA analysis.

Table 3.2 Profiles of three municipal wastewater treatment plants.

Code	Location	Process configuration	Flow rate (10^3 m^3 d^{-1})	Sampling point	Sampling time
B	Boise, ID	Nitrifying activated sludge	53.8	return activated sludge tank	6/11/2013
M	Moscow, ID	Hybrid A2/O process with oxidation ditch	11.4	aeration tank	6/12/2013
W	West Lafayette, IN	Nitrifying activated sludge	24.6	return activated sludge tank	6/18/2013

3.3.2 Characterization of fermentation broth

The fermentation broth samples were centrifuged at 12,000 rpm for 5 min and the clear supernatants were analyzed according to the methods described below: pH was measured with Orion-3-Star DO/pH portable meter (Thermo Fisher Scientific Inc., Waltham, MA). Ammonium nitrogen (NH_4-N), total phosphorous (TP), and chemical oxygen demand (COD) were determined respectively, according to standard methods (APAH et al, 1998) using the Nessler method 4500-NH_3, ascorbic acid method 4500-P, and 5220-D with Hach high-range COD kits using Beckman D640 spectrophotometer (Beckman Instruments, Fullerton, CA).

Lactic acid, succinic acid and glucose were quantified by high-performance liquid chromatography (HPLC) using a Rezex ROA organic acid column (Φ7.8 mm x 30 cm, Phenomenex, Torrance, CA) with a Waters HPLC pump and differential refractive index detector (ERC-5710, ERMA, Japan) on elution with 0.005 N H_2SO_4 (0.5 mL min^{-1}) at

65°C. Alcohols and volatile fatty acids (VFAs) in the broth were quantified by gas chromatography (GC) on Agilent 6890 instrument (Agilent Inc., Palo Alto, CA, USA) with a flame ionization detector and Alltech-Heliflex-AT™ Wax capillary column (Φ 0.32 mm × 30 m, Grace Davison Discovery Sciences, Deerfield, IL, USA) at 150°C. The injector and detector temperature were operated at 210°C with He as carrier gas, and all the samples were acidified to pH 2 with HCl prior to injection.

The D- and L- lactic acid ratio was determined by the method of Inoue et al. (2007) with minor changes: clear supernatant samples (1 mL) were acidified to pH 2 with HCl and extracted with ethyl acetate (4 mL). A mixed solution of extract and (-)-menthol solution (100 µL) in ethyl acetate were evaporated to remove the ethyl acetate under a stream of nitrogen at 37°C for menthylation. Toluene (200 µL) and acetyl chloride (100 µL) were added to the residue, and the mixture was then heated at 100°C for 1 h. The excess reagents were removed under a stream of nitrogen and the residue was dissolved in chloroform for GC-MS analysis (Polaris Q, ThermoQuest, San Jose, CA, USA) equipped with a ZB-1 capillary column (30 m × 0.25 mm Ø, Phenomenex, Torrance, CA, USA) with a temperature program of 160-250°C (5 min) at a rate of 10°C min^{-1} and 250-320°C at a rate of 30°C min^{-1}.

3.3.3 DNA extraction

The three seed cultures (B0, M0, and W0) from municipal wastewater treatment plants and three fermented biomass samples (B1, M1, and W1) in fermentation reactors were thawed on ice and vortexed independently to ensure that a homogeneous solution was

obtained. 250 μL of each sample was gently mixed with 100 μL of lytic enzyme cocktail (50 μL lysozyme 500 kU mL^{-1}, 6 μL mutanolysin 25 kU mL^{-1}, 4 μL lysostaphin 3000 kU mL^{-1}, and 41 μL mixture of 10 mM Tris-HCl and 50 mM EDTA pH 8.0) and incubated at 37°C for 1 h in a dry heat block. All samples were then transferred to a bead beating tubes contains 750 mg of zirconia-silica beads (Φ 0.1 mm) and placed in Mini-BeadBeater-96 at room temperature for 1 min at 2100 rpm followed by a brief centrifugation. The isolation of bacterial genomic DNA was performed with a QIAamp DNA Mini kit (Qiagen Inc., USA) according to the manufacturer's protocol. The isolated DNA was examined with 1% agarose gel stained with ethidium bromide in 0.5x TAE buffer and quantified with QuantiFluor dsDNA kit (Promega, Inc.) on Turner TBS-380 mini-fluorometer (Turner BioSystems, USA) and verified with Agilent DNA 1000 kit on Agilent Bioanalyzer 2100 according to manufacturer's recommendations.

3.3.4 PCR amplification and Illumina sequencing

The bacterial 16S rRNA gene was amplified using primers that flanked the variable regions 1 and 3 (Escherichia coli positions 27F-534R). Generations of sample amplicons were performed using a double round of PCR and dual indexing on the Illumina MiSeq. The first round of PCR extracts the targeted V1-V3 regions. The second round of PCR attaches the sample barcode and sequencing adapters. The PCR primer sequences are described as 27F primer (27F-YM1: ACACTGACGACATGGTTCTACAGT**AGAGTTTGATCCTGGCTCAG**; 27F-YM2: ACACTGACGACATGGTTCTACA***C***GT**AGAGTTTGATCATGGCTCAG**; 27F-YM3: ACACTGACGACATGGTTCTACA***AC***GT**AGAGTTTGATTCTGGCTCAG**;

27F-YM4:

ACACTGACGACATGGTTCTACA***AC***GT**AGAGTTTGATTCTGGCTCAG**; 27F-Bif: ACACTGACGACATGGTTCTACA***GTAC***GT**AGGGTTCGATTCTGGCTCAG**; 27F-Bor:

ACACTGACGACATGGTTCTACA***CGTAC***GT**AGAGTTTGATCCTGGCTTAG**; 27F-Chl:

ACACTGACGACATGGTTCTACA***ACGTAC***GT**AGAATTTGATCTTGGTTCAG**), 534R primer (534R-1:

TACGGTAGCAGAGACTTGGTCTCC**ATTACCGCGGCTGCTGG**; 534R-2:

TACGGTAGCAGAGACTTGGTCT***G***CC**ATTACCGCGGCTGCTGG**; 534R-3:

TACGGTAGCAGAGACTTGGTCT***TG***CC**ATTACCGCGGCTGCTGG**; 534R-4:

TACGGTAGCAGAGACTTGGTCT***ATG***CC**ATTACCGCGGCTGCTGG**; 534R-5:

TACGGTAGCAGAGACTTGGTCT***CATG***CC**ATTACCGCGGCTGCTGG**; 534R-6:

TACGGTAGCAGAGACTTGGTCT***TCATG***CC**ATTACCGCGGCTGCTGG**; 534R-7:

TACGGTAGCAGAGACTTGGTCT***ATCATG***CC**ATTACCGCGGCTGCTGG**) and adapter primers (P7-CS2:

CAAGCAGAAGACGGCATACGAGAT*NNNNNNNN*TACGGTAGCAGAGACTTGGTCT; P5-CS1:

AATGATACGGCGACCACCGAGATCTACAC*NNNNNNNN*ACACTGACGACATGGTTCTACA), where the underlined sequences are universal sequences CS1 (ACACTGACGACATGGTTCTACA) and CS2 (TACGGTAGCAGAGACTTGGTCT). Bold Sequences denote the universal 16S rRNA primers 27F and 534R, and seven different 27F primer sequences were used in order to capture the greatest number of taxa.

The bold, italized bases were added to the template specific primers in order to introduce variability of base calls during Illumina sequencing. The adapter primers included the Illumina specific sequences P7 and P5 for dual indexing where the unique sequence barcodes were denoted by the 8 italicized N's. This allowed us to sequence the amplicons from many samples simultaneously using relatively few barcoded adapter primers, and afterwards assign each sequence to the sample they were obtained from (40 P7-CS2 primers and 40 P5-CS1 primers enable 800 unique samples identifiers).

The concentrations of amplicons were determined using a picogreen assay and a Fluorometer (SpectraMax GeminiXPS 96-well plate reader) and then pooled in equal amounts (~100 ng) into a single tube. The amplicon pool was then cleaned to remove short undesirable fragments from the following procedure. First the pool is size selected using AMPure beads (Beckman Coulter), the product was then ran on a 1% gel, gel cut and column purified (Qiagen MinElute PCR purification kit), and size selected again with AMPure beads. To determine the final quality we PCR amplified the resulting amplicon pool with Illumina adaptor specific primers and ran the PCR product on a DNA 1000 chip for the Agilent 2100 Bioanalyzer. The final amplicon pool was deemed acceptable only if no short fragments were identified after PCR, otherwise the procedure was repeated again. The cleaned amplicon pool is then quantified using the KAPA 454 library quantification kit (KAPA Biosciences) and the Applied Biosystems StepOne plus real-time PCR system. Finally, sequences were obtained using an Illumina MiSeq paired-end 300bp protocol (Illumina, Inc., San Diego, CA).

3.3.5 Sequence analysis and taxonomic classification.

Raw DNA sequence reads from the Illumina MiSeq were demultiplexed and classified in the following manner. The custom python application dbcAmplicons (https://github.com/msettles/dbcAmplicons) was used to identify and assign reads by both expected barcode and primer sequences. Barcodes were allowed to have at most 1 mismatch (hamming distance) and primers were allowed to have at most 4 mismatches (Levenshtein distance) as long as the final 4 bases of the primer matched the target sequence perfectly. Reads were then trimmed of their primer sequence and merged into a single amplicon sequence using the application flash (Magoc and Salzberg, 2011). Finally, the RDP Bayesian classifier was used to assign sequences to phylotypes (Wang et al., 2007). Reads were assigned to the first RDP taxonomic level with a bootstrap score ≥50.

The reads categorized as belonging to the genus *Lactobacillus* were used in further analysis to identify which species of this genus were present in the samples. We used blastn in BLAST+ (Camacho et al., 2009) to compare each read to a database composed of 16S rRNA gene sequences longer than 1000 bp belonging to the genus *Lactobacillus* (downloaded from NCBI in February 2014). The identity of the different species reported as a first hit for each read was recorded, the number of reads assigned to each of the taxa counted, and their relative abundance in the different samples calculated.

3.3.6 Data analysis

The diversity indices of Shannon-Wiener, Simpson, and Inverse of Simpson, principal

component analysis (PCA), and heat map illustration were performed using R (i386 v3.0.3; http://www.r-project.org/).

3.4 Results and discussion

3.4.1 Lactic acid fermentation

All three sequencing batch fermentations inoculated with different undefined mixed cultures had similar conversions to organic acids and alcohols and generated comparable chemical compositions as given in Table 3.3. The pH values were measured at about 3.9 as would be expected because the majority of organic compounds produced were carboxylic acids. The fermentation of PPW also released quantities of ammonia N (152-165 mg L^{-1}) and total P (72-80 mg L^{-1}), which provided sufficient nutrients for the biological conversion. The total organic compounds in the fermentation broths can be denoted as COD and the values were in the range of 14.4-14.8 g L^{-1}, in which 72-81% of them were identified and quantified as known carboxylic acids, alcohols, and glucose. Lactic acid was the most abundant carboxylic acid in all three fermentation broths at concentration of 5.6-6.4 g L^{-1}, followed by acetic acid (1.1-1.6 g L^{-1}), succinic acid (0.4 g L^{-1}), and minor C_3 to C_5 VFAs (<0.2 g L^{-1}). Ethanol was the third most abundant compound in the fermentation broths at a concentration of 0.9-1.2 g L^{-1}. Other known compounds such as glucose (0.2 g L^{-1}) and propanol (<0.2 g L^{-1}) were also detected. Lactic acid comprised of 61-70% of total known organic compounds and was a racemic mixture of L/D enantiomers. All the three fermentations exhibited selectivity for lactic acid production and generated similar lactic acid yields (0.18-0.21 g g^{-1} PPW fed). The

similarities of the three fermentation broths suggested that the end-products of fermentation were determined by feeding and operating parameters. The results are similar to other studies (Forrest et al., 2012), in which VFAs were produced from recycled office paper waste materials with different undefined mixed cultures.

Table 3.3 Fermentation results with three different undefined mixed cultures.

Fermentation broth	B1	M1	W1
pH	3.90	3.93	3.88
NH_4-N (mg L^{-1})	164.7 ± 20.4	162.3 ± 21.2	151.5 ± 13.4
TP (mg L^{-1})	72.2 ± 3.2	80.4 ± 2.2	76.4 ± 4.8
COD (mg L^{-1})	14404 ± 198	14787 ± 113	14417 ± 233
Lactic acid (g L^{-1})	6.15 ± 0.17	5.55 ± 0.04	6.41 ± 0.27
Glucose (g L^{-1})	0.16 ± 0.02	0.15 ± 0.01	0.15 ± 0.01
Ethanol (g L^{-1})	0.89 ± 0.08	1.18 ± 0.05	1.18 ± 0.04
Propanol (g L^{-1})	0.02 ± 0.00	0.16 ± 0.06	0.05 ± 0.01
Succinic acid (g L^{-1})	0.37 ± 0.04	0.36 ± 0.04	0.42 ± 0.03
Acetic acid (g L^{-1})	1.14 ± 0.05	1.55 ± 0.15	1.38 ± 0.05
Propionic acid (g L^{-1})	0.07 ± 0.02	0.10 ± 0.01	0.09 ± 0.00
Butyric acid (g L^{-1})	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Valeric acid (g L^{-1})	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Yield of lactic acid (g g^{-1} PPW fed)	0.204	0.184	0.212
L/D ratio of lactic acid	51:49	49:51	53:47

3.4.2 Sequencing data and microbial diversity

Three seed samples collected from different municipal wastewater treatment plants (B0, M0, and W0) and three from the fermentation reactors (B1, M1, and W1) were analyzed. A total of 444,036 high quality sequences were obtained from the raw data after Illumina sequencing. The sequence information and the microbial diversity indices are given in Table 3.4. The seed cultures (B0, M0, and W0) had a high number of operational taxonomic units (OTUs) at about 531-616, while the number of OTUs of the fermented biomass samples (B1, M1, and W1) were determined at only 39 to 86. The results were similar to kimchi fermentation (Jung et al., 2012; Park et al., 2012), in which the number

of OTUs decreased during fermentation.

Table 3.4 Diversity indices from six biomass samples.

Diversity index	B0	B1	M0	M1	W0	W1
No. of reads	71507	60854	71564	57206	86823	96082
OTU	531	84	590	39	616	86
Shannon-Wiener	3.94	0.17	4.27	0.15	4.56	0.17
Simpson's	0.95	0.06	0.97	0.05	0.98	0.06
Inverse of Simpson's	18.52	1.06	36.06	1.05	52.38	1.06

The Shannon-Wiener's diversity index reflects both the species richness and evenness of a community. Results showed that the Shannon-Wiener's indices of the three seed cultures were quite different where the W0 had the highest diversity at 4.56, followed by M0 at 4.27 and B0 at 3.94. The microbial communities changed during the fermentation process, and decreased values (0.15-0.17) of Shannon-Wiener's indices for fermented biomass samples were observed. The Simpson's indices of seed cultures and fermented biomass samples were determined respectively, at 0.95-0.98 and 0.05-0.06. The Inverse of Simpson's index shared the same trend with Shannon-Wiener's index. Significant differences in microbial diversity between inoculated seed cultures and fermented biomass samples were observed in our study, in concordance with other reports (Agler et al., 2012; Forrest et al., 2012). These results suggest that seed cultures have relatively higher richness and evenness than the microbial communities present after the fermentation process.

The microbial communities of these samples were analyzed by PCA and the results are shown in Figure 3.1. The results showed that the seed cultures were separated from each other. The fermented biomass samples were shown to have similar microbial

communities and fall into one cluster, which was significantly different from the seed samples. The PCA results indicated that fermentation process reconstructed the microbial communities and formed a relatively homogenous group. Results in other studies (Werner et al., 2011) also revealed that the formation of unique microbial communities were possible in different anaerobic digestion systems.

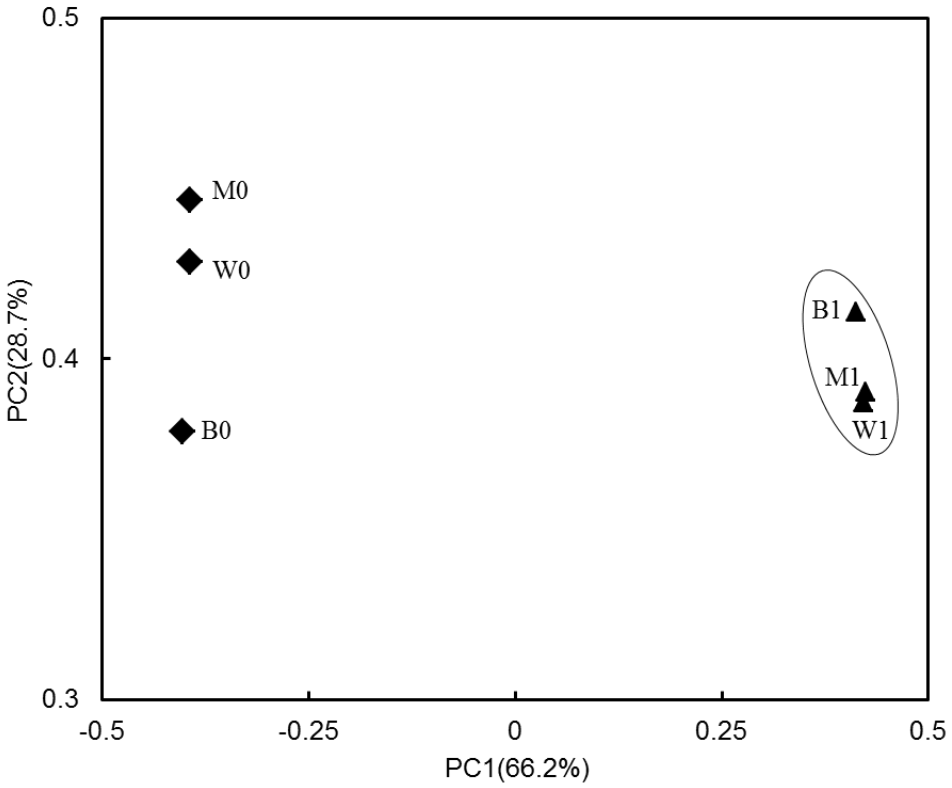

Figure 3.1 Principal component analysis (PCA) of six biomass samples based on the relative abundance of bacterial communities.

3.4.3 Microbial composition of seed cultures

The seed cultures from the three different wastewater treatment plants had increasing unclassified sequencing portions in the total community at various taxonomic ranks

(phylum: 1-2%, class: 2-6%, order: 8-10%, family: 22-24%, and genus: 29-36%), which was also revealed in other literature (Sanapareddy et al., 2009). The relative abundance of OTUs at the phylum level varied across the different samples and the structure of microbial community at the phylum level were different. Figure 3.2 shows that Proteobacteria was the most abundant phylum in B0 and W0, which was consistent with the results of bacterial communities in freshwater, intertidal wetland, and marine sediments (Wang Y. et al., 2012) and activated sludge (Zhang et al., 2012). W0 had the highest Proteobacteria abundance, accounting for 68% of total effective bacterial sequences, followed by B0 (49%) and M0 (37%). However the most dominant phylum in M0 was identified as Actinobacteria, and the abundance of Actinobacteria in B0, M0, and W0, respectively, were quantified at 43, 40 and 11% of total effective bacterial sequences. The other phyla present included Chloroflexi (1-7%), Bacteroidetes (1-7%), Firmicutes (2-5%), Planctomycetes (1-3%), and TM7 (1-2%).

Within Proteobacteria, α-Proteobacteria was the dominant class, accounting for 49-68% in all three seed cultures, followed by β-Proteobacteria (13-25%) and γ-Proteobacteria (2-20%). This observation was similar to what was observed in studies on activated sludge using microarray analysis (Xia et al., 2010). Other studies using 454 pyrosequencing (Wang X. et al., 2012; Zhang et al., 2012) generated different results with β-Proteobacteria as the most abundant within the Proteobacteria. A total 9 taxa were identified within the α-Proteobacteria. Rhizobiales was the dominant group with a range of 29-41% in all three samples, followed by Hyphomicrobium (14-31%) and Rhodobacter (7-22%). The other 6 detected groups with low abundance were

Amaricoccus (1-16%), Rhodobacteraceae (7-11%), Sphingomonas (0-11%), Roseicyclus (0-9%), Sphingomonadaceae (1-6%), and Mesorhizobium (1-7%). The phylum Actinobacteria was dominated by the class Actinobacteria and order Actinomycetales in all three seed cultures, however, the most abundant families were different and identified as Mycobacteriaceae (60%), Intrasporangiaceae (33%), and Mycobacteriaceae (37%) in B0, M0, and W0, respectively. The microbial composition of activated sludge can vary greatly with source, temperature, pH, organic loading, and other operational parameters (Zhang et al., 2012). Our findings in this study suggested that the structures of microbial communities of activated sludge in different wastewater treatment plants were different.

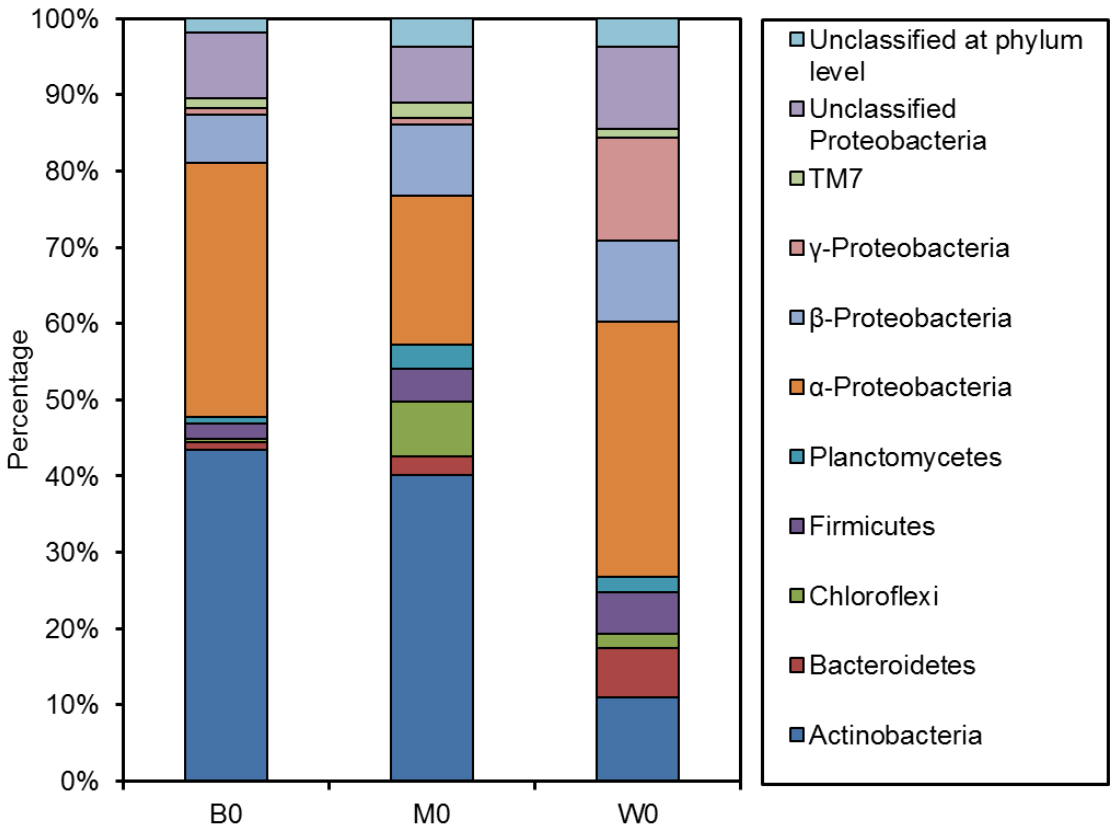

Figure 3.2 Abundances of different phyla and classes in Proteobacteria in the three seed samples. Taxa represented occurred at >1% abundance in at least one samples.

3.4.4 Comparison of genus-level microbial communities

Fermentation of PPW resulted in simplification of microbial communities as revealed by the sequencing results. *Lactobacillus* became the most dominant genus as a result of PPW fermentation, accounting for >96% of total effective bacterial sequences in all three fermenters. In order to compare the difference of seed cultures and fermented biomass samples, the genus profiles were analyzed. Within the 654 assigned genera, only 7 were shared by all six samples, which belong to four phyla of Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. These shared genera were observed with 3-5% and >98% of all classified sequences in seed cultures and fermented biomass samples, respectively. The homogeneous microbial population was also observed in other studies on fermented food products (Humblot and Guyot, 2009; Jung et al., 2012).

The top 10 most abundant genera in each sample (a total 38 genera for six samples) were selected and compared with their abundance in other samples, as shown in Figure 3.3.The most abundant genera in the seed samples were different (*Mycobacterium* (18.2%) in B0, *Tetrasphaera* (8.5%) in M0, and *Rhizobacter* (3.5%) in W0). This was similar to previous studies using Illumina sequencing on activated sludge (Ju et al., 2013). While, only low levels (<0.1%) of these dominant genera were found in the fermented biomass samples. In the fermented biomass samples *Lactobacillus* was the dominant genus (B1 at 96.7%, M1 at 97.3%, and W1 at 96.7%), followed by *Paralactobacillus* (0.4-1.0%) and other minor genera (<0.1%) such as *Streptococcus*, *Acetobacter*, and *Bifidobaterium*. The presence of these genera was not a surprise because the majority of fermentation broths contained predominantly lactic acid with minor alcohols and other fatty acids.

Lactobacillus was also found to be the dominant genus (>70%) in other studies (Nie et al., 2013), in which the acetic acid fermentation process was employed to make vinegar. To the authors' best knowledge, this is the first time that lactic acid and *Lactobacillus* were found as the most dominant fermentation products and genera in a potato fermentation process with undefined mixed cultures (activated sludge).

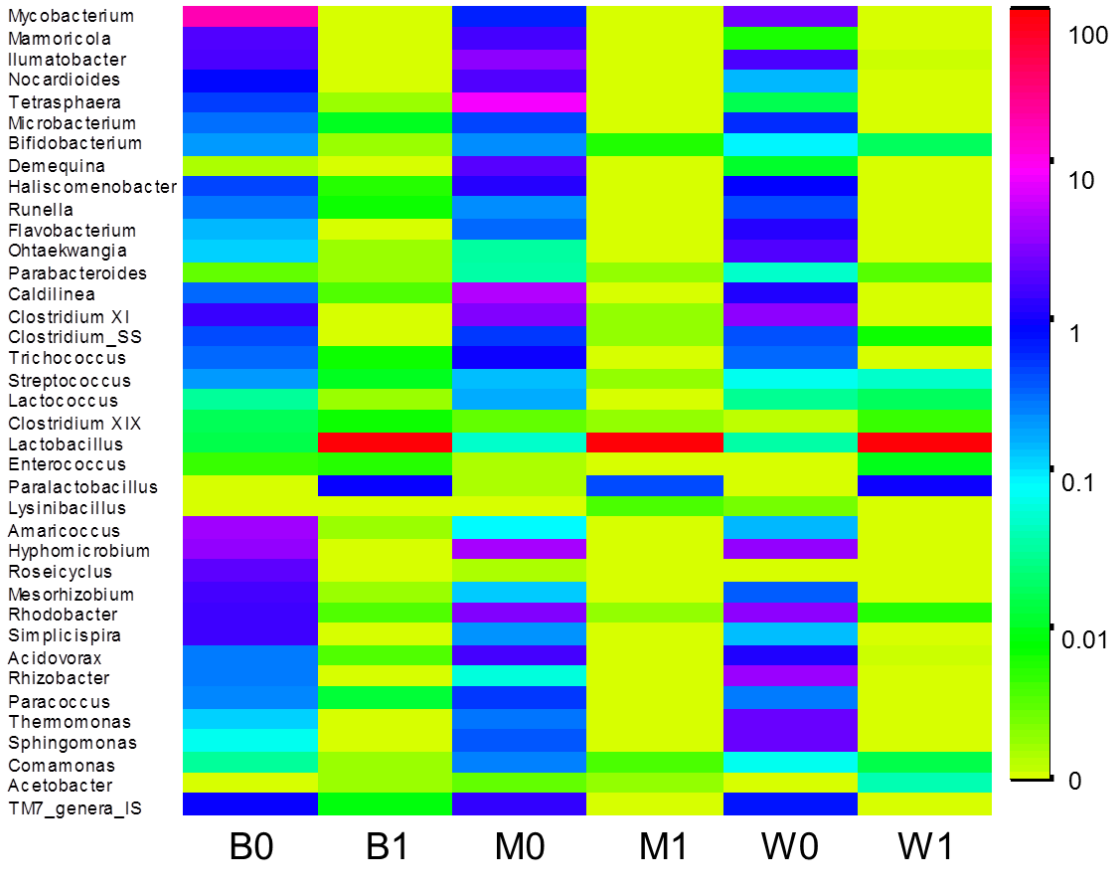

Figure 3.3 Heat map of top 10 genera in each sample. The 10 most abundant genera in each sample were selected (a total of 38 samples were selected for all 6 samples) and the abundances were compared to those in other samples. The color intensity in each cell shows the percentage of genus in a sample.

3.4.5 Genus *Lactobacillus*

The lactobacilli belong to the phylum Firmicutes, class Bacilli, order Lactobacillales, and family Lactobacillaceae, a major part the LAB group. Species-level taxonomic assignments of *Lactobacillus* sp. were conducted in order to further investigate the microbial communities of three fermented biomass samples. A total of 334,870 effective sequencing reads of lactobacilli in samples B1, M1, and W1 were obtained and a total of 87 species-level taxa were observed. The relative abundance of different species (>1%) varied across different samples and the results are shown in Figure 4.4. The *Lactobacillus* sp. G24 was the most abundant OTU found in B1 (44%) and W1 (46%), while only 20% was found in M1. The dominant species in M1 was *L. gasseri* (34%), which was found to be at <1% in B1 and W1. The species with abundance >10% were *L. mucosae* in B1 (21%) and W1 (22%), *L. amylovorus* in B1 (17%), *L. panis* in sample M1 (14%), *L. amylolyticus* in W1 (11%), and *L. delbrueckii* in M1 (10%). Other species with abundance <10% included *L. secaliphilus (1%)*, *L. reuteri* (0.3-2%), and *L. fermentum* (2-5%) in all three fermented biomass samples. The species-level differences among three fermented biomass samples could be due to differences in the inocula. In addition, lactobacilli are known for their characteristics to produce lactic acid and inhibit and/or kill the growth of other microbes by producing antimicrobial agents (Dalie et al., 2010), which can explain the limited microbial diversity.

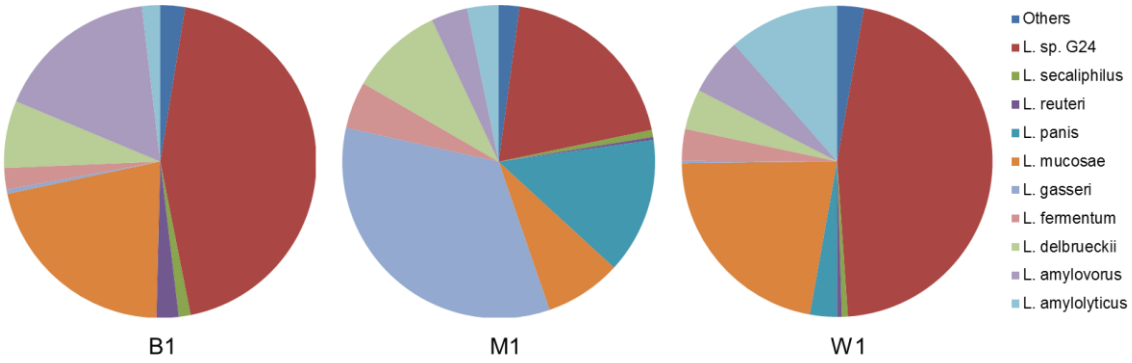

Figure 3.4 Abundances of different species-level *Lactobacillus* in the three fermented biomass samples. Taxa represented occurred at >1% abundance in at least one samples.

3.5 Conclusion

Three undefined mixed cultures (activated sludge) from different municipal wastewater treatment plants were used as inoculation starters to ferment PPW into predominantly lactic acid. Illumina sequencing results revealed that the microbial community structures of three seed cultures were different. Unique microbial community structures were formed with more than 96% of genus *Lactobacillus* in all three fermenters, as compared to <0.1% in seed cultures. In addition, differences of *Lactobacillus* sp. among three fermented biomass samples were also observed with species-level taxonomic assignments. These findings provide alternative solution for utilization of PPW and other carbohydrate wastes, and show the robustness and resilience of microbial communities in lactic acid fermentation of PPW with undefined mixed cultures. Finally, our work demonstrates for the first time that lactic acid and *Lactobacillus* species were found to be the most dominant fermentation product and genus in a potato fermentation process with undefined mixed cultures (activated sludge).

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Chapter 4 Lactic acid production from potato peel waste by anaerobic sequencing batch fermentation

4.1 Abstract

Lactic acid (LA) is an important industrial feedstock for producing polylactic acid (PLA), a biodegradable plastic alternative to petrochemical derived plastics. Currently nearly all LA production in industries is produced by pure cultures fermentation from food and non-food carbohydrate or waste stream feed-stock, however, the operational cost for this mode can be high. This paper presents an alternative to LA production from potato peel waste (PPW) by anaerobic fermentation in a sequencing batch reactor (SBR) inoculated with undefined mixed culture seeds from municipal wastewater treatment plant. With the aim of maximizing LA yield and productivity, a series of SBR reactors were employed and operated in 0.8 L and 3.5 L scales. The overall LA production yield of 0.12-0.25 g g^{-1} PPW and highest productivity of 138.0 mg g^{-1} d^{-1} were achieved. This study showed a potential for making value added bioproducts from PPW.

4.2 Introduction

Lactic acid (LA) is a useful organic acid widely used in food, pharmaceutical, cosmetic and industrial applications, especially for making polylactic acid (PLA). PLA is a bioplastic can be synthesized from LA by condensation polymerization, to provide an alternative thermoplastic to traditional petrochemical derived plastics (Wang et al., 2009). The global market for LA is experiencing steady growth recently with its various industrial applications, especially the high speed development and commercialization of

LA based bioplastics driven by rising oil price and strict government regulations and consumers demand to green products. LA can be manufactured either by chemical synthesis from petrochemicals or carbohydrates fermentation with bacteria and fungi, in which the latter one is the most popular technology studied and currently nearly all LA production is based on carbohydrate fermentation in industries (Datta and Henry, 2006).

Glucose and other simple sugars are ideal substrates for LA production, where these substrates can be harvested by hydrolysis of natural polysaccharides from agricultural crops such as wheat, corn and potato, and the feedstock usually represents 30-40% of total operational cost for LA production (Zhang et al., 2007), which makes using inexpensive carbohydrate materials attractive (Alonso et al., 2010; Li et al., 2012; Sreenath et al., 2001; Zhao et al., 2009). However, operational restrictions such as the selection of inoculum, sterile condition, pH, temperature and nutrients are also critical to fermentation process (Zhang et al., 2007). Some efficiency and economic issues for LA production are still existent and current research efforts are focused on looking at alternative fermentation technologies (Reddy et al., 2008).

Potato is the world's fourth most important crop behind corn, rice and wheat, and has experienced steady worldwide growth in the last two decades as a stable food crop and major starch source (FAO, 2014). In North America approximate 60% of potato production was processed for French fries, can foods, starch and flour products in potato processing plants (NPC, 2012), which generated a significant amount of potato peel waste (PPW) during the peeling and cutting procedures. This kind of waste stream contains a large quantity of carbohydrate especially starch, can be easily digested by

microorganisms for value added bioproducts production.

In practice, most PPW from the potato processing industry was used for local livestock feed (Nelson, 2010). Parawira et al. (2004) evaluated the feasibility of volatile fatty acids (VFAs) production during anaerobic mesophilic digestion of PPW where 19 g L^{-1} total VFAs were obtained. Methane generation from PPW under mesophilic and thermophilic conditions was also studied by Parawira et al. (2007). The results showed that a higher methane yield of 0.49 L g^{-1} chemical oxygen demand (COD) was obtained under mesophilic conditions than that of thermophilic conditions (0.41 L g^{-1} COD) in methanogenic reactors. Arapoglou et al. (2010) studied ethanol production from high starch content PPW by enzymatic hydrolysis and fermentation, and a maximum of 7.58 g L^{-1} ethanol was obtained with two steps of versatile enzymatic hydrolysis and a follow-up anaerobic fermentation with *Saccharomyces cereviciae var. bayanus* at pH 5.0 and 32°C for 2 days. The production of LA from PPW was also studied by using identified single or mixed cultures such as *Lactobacillus var.* and *Rhizupus var.* (Afifi, 2011; Liu et al., 2005; Zhang et al., 2007), which has been regarded as one promising, efficient and environmental friendly solution because starch can be converted into LA directly with no greenhouse gas CO_2 emission during the fermentation process.

Our previous batch fermentation study has proved that LA can be produced using undefined mixed cultures inoculated from wastewater treatment plant under an optimal temperature of 35°C without the assistance of enzymatic hydrolysis pretreatment. In this updated research, the continuous production of LA by anaerobic fermentation in sequencing batch reactors (SBR) were conducted to further assess the potential of

commercialization of this technology.

4.3 Materials and methods

4.3.1 Materials

PPW samples used in this study were collected from two different potato processing plants of JR Simplot Company (Nampa and Caldwell, Idaho). The collected PPW samples were frozen at -20°C in plastic containers before use. Freeze-dried samples were prepared for chemical composition analysis. Fresh mixed cultures used for fermentation was collected from a local municipal wastewater treatment plant (Moscow, Idaho).

4.3.2 Reactor operation and experimental design

Two different sized fermenters were operated in this study. Small scale fermentations used 1 L glass bottles fitted with an air-lock were used as SBR reactors, and 40 mL mixed culture was added to the reactors at the start of operation. The reactors were magnetically stirred (500 rpm) and temperature controlled at 35°C with a heated water-jacket. PPW sample from the Nampa plant was gelatinized (boiled for 30 min and cooled) prior to use. A design of experiments (DOE) based on a 2^3 full factorial central composite design (CCD) were conducted with three retention time (RT) levels of 2, 3, and 4 days and three feeding solid content (SC) levels of 30, 40, and 50 g L^{-1}. The mixed solution was drawn off and fed with the same volume of gelatinized PPW slurry once a day according to the RT and SC (for example, RT of 2 d and SC of 30 g L^{-1} reactor, 400 mL mixture was removed and same volume of gelatinized PPW with SC of 30 g L^{-1} was

added daily) which generated a solid loading rate (SLR) range from 7.5 to 25 g d^{-1} L^{-1}. Sampling and analysis were conducted after at least 3 cycles of RT to ensure steady state conditions of the bioreactors were obtained. A total of thirteen conditions composed of 4 cubic points and 4 axial points and 5 replicates at the center point were conducted. The experimental data were processed using Design Expert 8.0 software (Stat-Ease, Inc., MN, USA).

The larger scale fermentation study used a 3.5 L HDPE reactor equipped with mechanical stirring (300 rpm) and temperature control (35°C). PPW sample at a SC of 80 g L^{-1} from the Caldwell plant was used. The fermenter was fed either un-gelatinized or gelatinized PPW according to the operating parameter settings for a total of 40 d. Four conditions were assessed: (i) RT1.5, fed daily with un-gelatinized PPW at a RT of 1.5 d for 8 d; (ii) RT2, fed daily with un-gelatinized PPW at a RT of 2 d for 10 d; (iii) RT1.5G, fed daily with gelatinized PPW at a RT of 1.5 d for 14 d; (iv) RT1G, fed twice a day with gelatinized PPW at a RT of 1 d for 8 d. The fermentation broth was sampled and analyzed according to methods described below. To ensure a steady state condition only data obtained after 5 cycles of each stage (at least 3 observations) were processed and analyzed by SAS v9.3 software for the Tukey's HSD test at a probability level of p = 0.05.

4.3.3 Recovery of LA

The recovery of LA from the large scale fermentation broth was conducted according to Lin et al. (2010) with slight modification. The fermentation broth was centrifuged (7000

rpm for 1 h) and the supernatant was filtered through glass fiber paper to separate the solid residue. The brown filtrate was mixed with 5% activated carbon (Sigma-Aldrich) for 2 h to remove protein, color and cell debris, and the mixture was clarified by centrifugation and filtration. The colorless solution was then passed through an ion-exchange resin column (Amberlite IR $120H^+$, Φ 2 cm x 40 cm) at 5 mL min^{-1} to protonate the acids. The eluent (pH 2) was vacuum distilled at 70-80°C, filtered (0.45 μm nylon), to obtain a concentrated solution of LA.

4.3.4 Analytical techniques

Chemical analysis of solid materials was carried out as previously described: starch and polysaccharides were determined by enzymatic or 2-stage acid hydrolysis followed by high performance liquid chromatography (HPLC) assay; lignin and suberin complex was calculated by the solid residue loss of 2-stage acid hydrolysis; protein was estimated by multiplying total N by 6.25; lipids was determined by Soxhlet extraction with CH_2Cl_2; and ash was estimated by dry oxidation at 600°C. The fermentation broth samples were analyzed after centrifugation (12,000 rpm, 5 min) and filtration (0.45 μm nylon). Soluble COD tests were determined according to method 5220-D of Standard Methods (APHA et al., 1998) using Hach high-range kits with a heating block and Beckman DU640 spectrophotometer. LA, glucose and succinic acid were quantified by ion exclusion HPLC using a Rezex ROA organic acid column (Φ7.8 mm x 30 cm, Phenomenex, Torrance, CA), Waters HPLC pump and differential refractive index detector (ERC-5710, ERMA, Japan) on elution with 0.005 N H_2SO_4 (0.5 mL min^{-1}) at 65°C. Sugars were quantified by HPLC using two Rezex RPM columns in series (Φ 7.8 mm x 30 cm,

Phenomenex, Torrance, CA) with differential refractive index detection (Waters model 2414) on elution with water (0.5 mL min^{-1}) at 85°C. VFAs and alcohols were measured using a Hewlett-Packard (Palo Alto, CA, USA) 6890 Series gas chromatography (GC) with a flame ionization detector and Alltech-Heliflex- AT™ Wax capillary column (Φ0.32 mm x 30 m, Grace Davison Discovery Sciences, Deerfield, IL, USA) at 150°C. The injector and detector temperature were operated at 210°C, and He as carrier gas. Samples were acidified to pH 2 with HCl prior to injection. Recovered LA was dissolved in D_2O (0.5 mL) in a 5-mm NMR tube to acquire ^{1}H and ^{13}C spectra on a 300 MHz Bruker AVANCE 300 instrument. Duplicate tests were conducted in this study.

4.4 Results and discussion

4.4.1 PPW composition

Table 4.1 gives the values of chemical composition of PPW (dry basis). Highest carbohydrate contents observed were 39.9% (19.4% for starch) and 51.3% (27.4% for starch) respectively, in Nampa and Caldwell PPW samples. The lignin and suberin complex, and protein contents were respectively, at 29.2% and 22.4% in Nampa sample, and 16.0% and 22.8% in Caldwell sample. The soluble liquid fraction from PPW had COD contents of 250 and 156 mg g^{-1} PPW respectively, in Nampa and Caldwell samples, which contained mainly mono- and oligo-saccharides plus and some organic acids. Therefore, the soluble liquid fraction contained a significant amount of easily digestible sugars (carbon sources) along with solid carbohydrates suitable for fermentation.

Table 4.1 Chemical composition of PPW from Nampa and Caldwell processing plants.

Composition	Dry weight (%)	
	Nampa	Caldwell
Carbohydrate	39.9	51.3
Starch	19.4	27.4
Lignin and suberin	29.2	16.0
Protein	22.4	22.8
Lipids	2.6	2.1
Ash	8.5	11.0
Soluble COD*	250	156

* unit as mg g^{-1} PPW (dry basis).

4.4.2 Small scale fermentation

A statistical DOE approach using CCD response surface methodology (RSM) was employed in this part to determine the correlation between LA production (concentration and yield) and the two influencing factors (RT and SC). A second order polynomial equation was used to describe as:

$$Z = \alpha_0 + \alpha_1 \times \chi_1 + \alpha_2 \times \chi_2 + \alpha_{11} \times \chi_1^2 + \alpha_{22} \times \chi_2^2 + \alpha_{12} \times \chi_1 \times \chi_1,$$

where, Z was the predicted LA concentration and/or yield, α_0 was a constant, α_1 and α_2 were the linear coefficients, α_{11} and α_{22} were the quadratic coefficients, α_{12} was the interaction coefficient, χ_1 and χ_2 were coded independent variables. The ANOVA for response surface quadratic model of LA concentration showed that interaction was not significant (P-value = 0.9905) that the LA concentration was not impacted by the interaction of RT and SC. Table 4.2 listed the ANOVA table for a reduced quadratic model without the effect of interaction. The Model F-value of 31.51 implied the model was significant and there was only a 0.01% chance that a "Model F-value" this large could occur due to noise. Both RT and SC were effective variables with P-values of <0.0001 and 0.0004 respectively, which indicated that LA concentration was highly

dependent on RT and SC. The model for LA yield shared same trend in that the LA yield was highly dependent on RT and SC individually but less impacted by their interaction.

Table 4.2 Analysis of variance for LA concentration shown as reduced quadratic model coefficients for the centered and scaled variables.

Source	Sum of squares	DF	Mean square	F value	P-value (Prob>F)	Coefficient	
Model	30.44	4	7.61	31.51	<0.0001	Intercept	+7.07
χ_1	8.46	1	8.46	35.02	0.0004	α_1	-1.03
χ_2	17.23	1	17.23	71.34	<0.0001	α_2	+1.47
χ_1^2	1.09	1	1.09	4.52	0.0661	α_1^2	+0.40
χ_2^2	4.13	1	4.13	17.10	0.0033	α_2^2	+0.77
Residual	1.93	7	0.24				
Lack of fit	1.87	3	0.47	29.43	0.0032		
Pure error	0.064	4	0.016				
Corrected total	32.37	12					

As shown in Figure 4.1 (left), LA concentration increased with increasing SC, as expected, since the feedstock was the only carbon source for LA biosynthesis. Similar results were observed with pure culture fermentation in the literature (Liu et al., 2005). Although, decreasing RT also increased the LA concentration, the significance was less than the influence of SC. The highest LA concentration of 11.10 g L^{-1} was obtained at RT 3 d and SC 54.14 g L^{-1}. The lowest LA concentration of 6.05 g L^{-1} was observed at RT 4.14 d and SC 40 g L^{-1}. However, as illustrated in Figure 4.1 (right), LA production yield increased with decreasing of RT and SC, and the model result showed that RT worked as a more critical factor than SC. The LA yield was shown to increase from 0.12 g g^{-1} (RT 4.41 d and SC 40 g L^{-1}) to 0.25 g g^{-1} (RT 2 d and SC 30 g L^{-1}), which also led a productivity of 28.2 and 124.9 mg g^{-1} d^{-1}. This optimal yield was higher than previous batch fermentation studies despite a significant lower carbohydrate content of the feedstock used. This finding may be due to the continuous fermentation mode was able to

remove end products and possible inhibitors on a regular basis (Ou et al., 2011). However, with a longer RT the LA yield decreased, which can be explained that LA became a carbon source for microorganisms to be further converted to acetate, propionate, CO_2, and CH_4 by secondary fermentation (Agler et al., 2011).

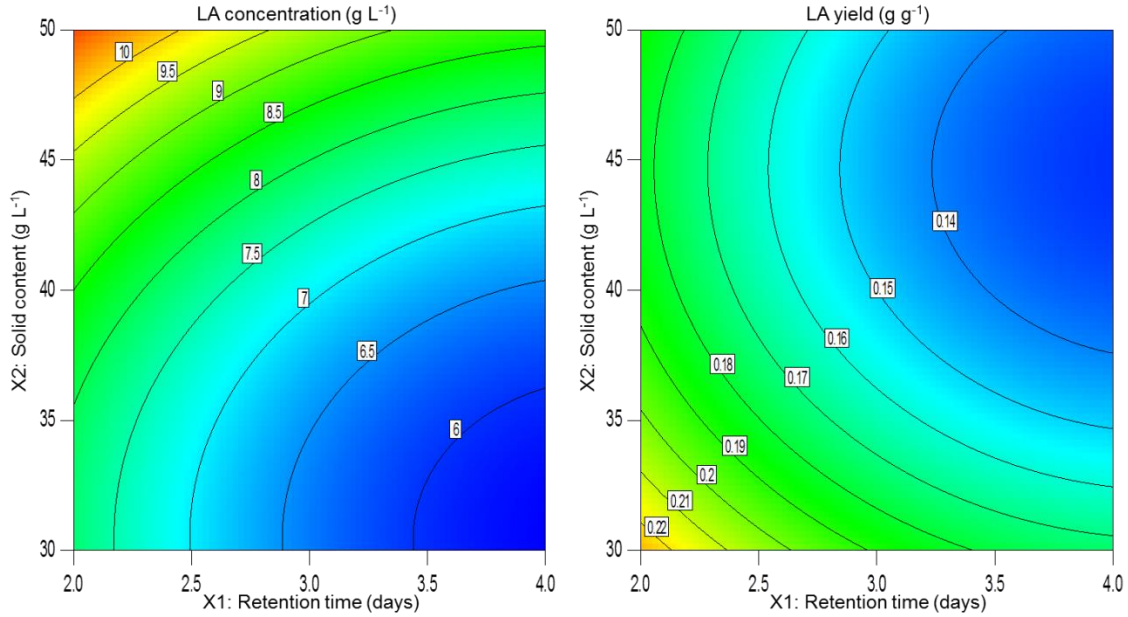

Figure 4.1 Contour plot of effects of interactive RT and SC on LA concentration (left) and yield (right).

4.4.3 Large scale fermentation

As discussed above, LA production was highly affected by RT and SC. In order to establish the influence of these factors as well as its adaption to upscale fermentation, a 3.5 L reactor with mechanical stirring was carried out to investigate LA and other organic compounds production with a RT of 1 to 2 d and constant SC of 80 g L^{-1}. Stage 1 (RT1.5) and stage 2 (RT2) were fed un-gelatinized PPW while stage 3 (RT1.5G) and stage 4 (RT1G) were fed gelatinized PPW which was based on our earlier batch fermentation

studies. Figure 4.2 shows the two main products in the fermentation broth, LA and acetic acid (AA), over the four reactor conditions (stages) used. With an increase of RT from 1.5 to 2 d in stage 1 and stage 2, the LA concentration was similar at about 8.6 L^{-1} while the AA concentration increased from 3.8 to 5.0 g L^{-1}. Similar trends were observed in stage 3 and stage 4 where LA concentration was similar at about 11.0 g L^{-1} however, the AA concentration decreased from 6.8 to 4.8 g L^{-1} with the decrease in RT from 1.5 to 1 d. According to the Tukey's HSD tests, the RT changes from either 1.5 to 2 d (in stage 1 and 2) or 1.5 to 1 d (in stage 3 and 4) did not introduce significant difference in LA production, while LA concentration was highly influenced by the gelatinization pretreatment process. The highest LA yield of 0.14 g g^{-1} was obtained in stage 4 which was lower than the result using the small scale fermentation process (0.12-0.25 g g^{-1}). This result corresponded to the highest productivity of 138.0 mg g^{-1} d^{-1}.

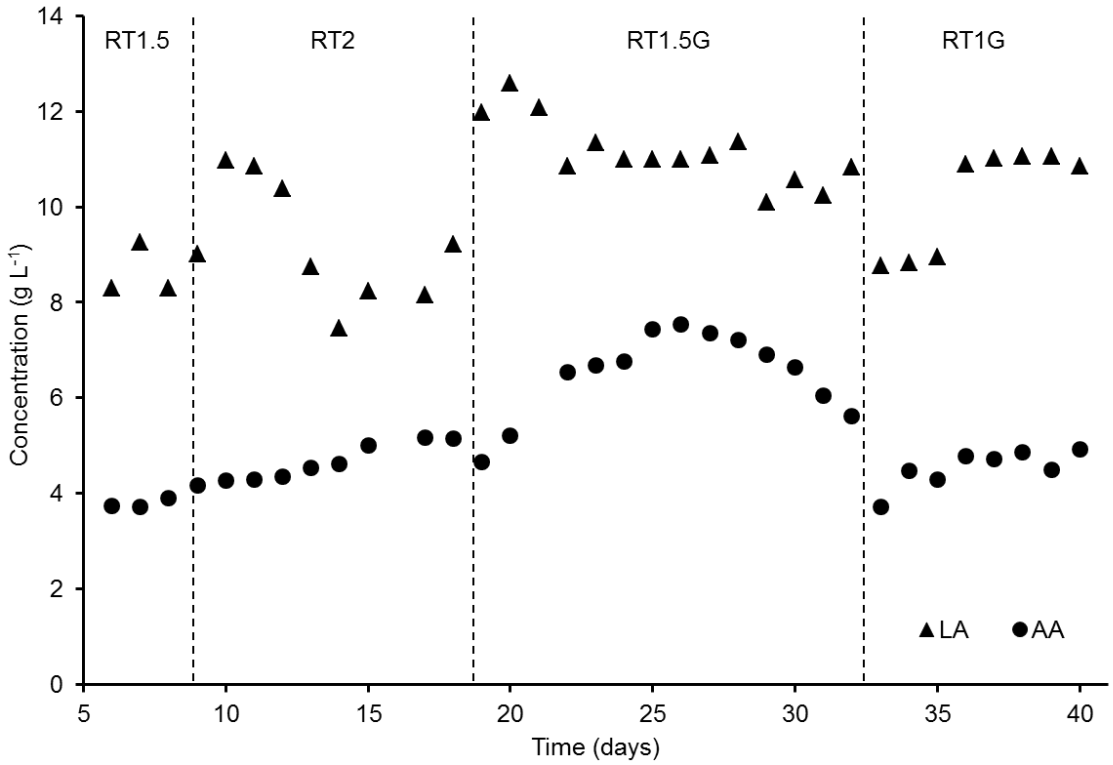

Figure 4.2 Large scale PPW fermentation trial showing LA (▲) and AA (●) concentration with running time under four different operating conditions.

Figure 4.3 shows the overall fraction of organic compounds in fermentation broth of the four stages. LA accounted for 54-64% of the total known organic compounds, where the highest level of 64% was found in stage 4 (RT1G). Similar to the small scale fermentation, higher LA fractions were observed in lower RT conditions. AA was the second most abundant fermentation product and accounted for an average of 27-35%, followed by minor by-products (8-14%) of ethanol, propanol, propionic acid, butyric acid, succinic acid, and glucose. The presence of these alcohols and organic acids by-products in the fermentation broth were not unexpected, which were also observed from the LA production by pure culture fermentation (Ou et al., 2011).

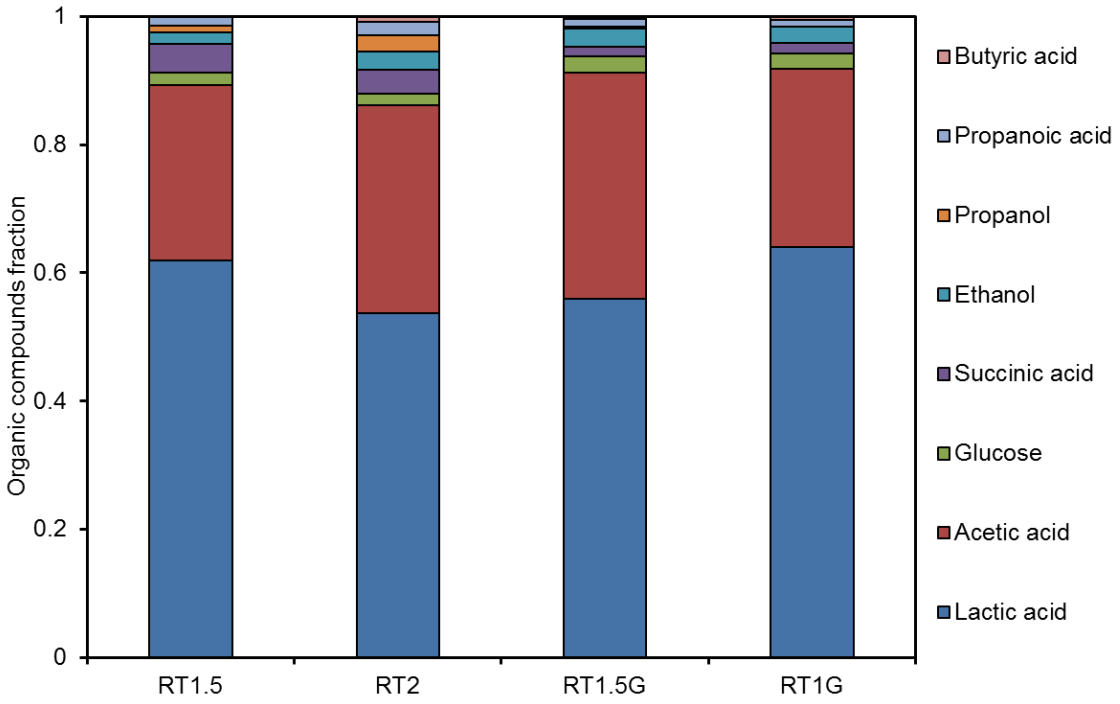

Figure 4.3 Fraction of products produced in the large scale PPW fermentation trial operating at four different conditions.

4.4.4 LA recovery

An integrated recovery method using cation-exchange and vacuum distillation was employed to isolate and purify LA from the large scale mixed fermentation broths. This method was preferable than reactive extraction since no new organic impurities will be introduced, and this approach has been applied in LA isolation as well as for other organic acids (Ataei and Vasheghani-Farahani, 2008; Lin et al., 2010). An average of 50% w/w aqueous LA (91% purity) with yield about 70% was obtained and quantified by HPLC, which is comparable to other studies on succinic acid recovery from fermentation broth (Zhang et al., 2013). The identity of LA was confirmed by NMR spectroscopy (data not shown). The ^{1}H NMR spectrum of recovered LA showed signals corresponding to

HOD (4.79 ppm in ^{1}H) and LA (3.92 ppm for CH and 0.95 ppm for CH_3), and the ^{13}C NMR spectrum showed peaks at 177.6 ppm for C=O, 65.5 ppm for CH and 18.4 for CH_3) which were assigned to LA (Brown, 2008). No other signals were observed associated with organic impurities in the spectra and this was supported by HPLC analysis.

4.5 Conclusion

LA was produced successfully and recovered by anaerobic fermentation of PPW with undefined mixed cultures in SBR bioreactors. Decreasing RT and SC increased LA production with a good overall LA production yield and highest productivity (138.0 mg g^{-1} d^{-1}) was achieved. This approach of converting food waste using mixed microbial cultures can potentially lower operational costs for LA production than traditional pure culture fermentation, which showed a potential for making value added bioproducts from PPW and other waste biomass. Continuous efforts should be focused on a scale-up production and how to separate and purify LA more efficiently.

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Chapter 5 Chemical and thermal characterization of potato peel waste and its fermentation residue as potential resources for biofuel and bioproducts production

5.1 Abstract

The growing demand for renewable fuels has driven the interest in utilization of alternative waste materials such as food processing waste. Potato peel waste (PPW) is an organic rich waste stream generated in potato processing plants which contains a significant amount of fermentable carbohydrate (starch). Fermentation of PPW yielded about 60% un-reacted PPW fermentation residue (PPW-FR). The PPW and PPW-FR were characterized by a combination of Fourier transform infrared (FTIR) and solution state nuclear magnetic resonance (NMR) spectroscopies, gas chromatography mass spectrometry (GC-MS) and thermogravimetric analysis (TGA) to quantify changes in the chemical and thermal properties before and after fermentation. Fermentation of PPW resulted in primarily removal of starch and concentrating cellulose, lignin, suberin, protein, and lipids in PPW-FR. The PPW-FR could be converted into crude biofuel and bioproducts via thermochemical conversion.

5.2 Introduction

Potatoes are the world's fourth largest crop behind corn, rice, and wheat and have experienced a steady growth over the last two decades as a stable food crop and a major source of starch (FAO, 2014). According to data from the National Potato Council (NPC, 2012), United States (U.S.) ranks the 5th of potato producing countries with a production over 21.7 billion kg in 2011. The Pacific Northwest produces >60% of the U.S potato

production of which 50-60% of the harvest converted to French fries, can foods, starch, and other potato products. A typical potato processing plant can generate 6-10% potato peel waste (PPW) from peeling process (Mader et al., 2009), and other defect removal, trimming and cutting processes can generate an additional 15% waste. This combined waste stream can pose a significant waste management effort and therefore knowing this waste's composition will facilitate its utilization.

The composition of steam PPW was determined by Camire et al. (1997) and shown to contain starch (25%), non-starch polysaccharide (30%), lignin and suberin complex (20%), protein (18%), lipids (1%), and ash (6%). The lipid fraction was shown to be comprised of long chain fatty acids, triglycerides, alcohols, sterols, sterol esters, and phenolics (Dobson et al., 2004). Suberin, a rubbery polyester material composed of polyaromatic and polyaliphatic domains, has been found in the cell walls of potatoes (Graca and Pereira, 2000). Lignin, a three-dimensional polymer made up of phenylpropane units, has also been detected in plant cell wall (Bunzel et al., 2011).

PPW has been utilized in a variety of applications and traditionally has been used for local animal feed (Afifi, 2011; Arapoglou et al., 2010; Camire, et al., 1997; Liu et al., 2005; Parawira et al., 2007; Wijngaard et al., 2012). Suberin and lignin fractions have been pressure extracted from PPW to obtain value added antioxidants (Wijngaard et al., 2012). While, fermentation of PPW starch has been employed to generate alcohols and organic acids. Arapoglou et al. (2010) converted PPW by sequential enzymatic hydrolysis and fermentation using *Saccharomyces cereviciae* to yield ethanol. Lactic acid has also been produced from PPW using pure cultures of *Lactobacillus var.* or *Rhizupus*

var (Afifi, 2011; Liu et al., 2005). Recently, PPW has been directly converted to primarily lactic acid using undefined mixed microbial culture fermentation (in previous chapter). However, a significant amount of un-reacted solid PPW fermentation residue (PPW-FR) was obtained (Arapoglou et al., 2010). This PPW-FR waste will also need to be managed. Another popular option for waste management of PPW is by anaerobic digestion (AD) to produce CH_4 and electricity (Coats et al., 2013). However, AD systems require large digesters based on the slow methanogenic bacterial conversion of biomass to CH_4 and unpleasant odor (namely H_2S) generation (Coats et al., 2013). Work by Kang and Min (2010) has used PPW as a novel substrate for making bio-based flexible films for packaging applications with good barrier properties.

Currently, food crops such as corn and soybean are the two predominant biomass resources widely used for liquid bioethanol and biodiesel production worldwide (Manuel, 2007). However, the controversy between food and fuel also promoted the utilization of other non-eligible biomass materials, such as agricultural and forestry residues (Soria and McDonald, 2012), animal manure (Coats et al., 2013), algae (Chakraborty et al., 2012), and food processing wastes (Wang et al., 2013). PPW and PPW-FR are promising carbon rich waste resources for producing value added biofuels and bioproducts. Onal et al. (2011) used steam pyrolysis of PPW (at 500°C) to generate >25% bio-oil and contained a significant amounts of aliphatics that could be used in transportation fuels. Biochar from pyrolyzed PPW, another coproduct, was shown to remove heavy metals from aqueous solutions (Moreno-Pirajan and Giraldo, 2012). These approaches to utilize PPW will minimize waste streams, reduce the carbon footprint, mitigate greenhouse gases and

generate a range of bioproducts.

The aim of this study is to characterize the chemical and thermal properties of PPW and PPW-FR in order to target possible processes for conversions into biofuel and bioproducts. The composition was determined by a combination of Fourier transform infrared (FTIR) and solution state nuclear magnetic resonance (NMR) spectroscopies and selective degradations followed by gas chromatography mass spectrometry (GC-MS) analyses. Thermogravimetric analysis (TGA) was employed to determine the thermal degradation (pyrolysis) kinetics. The results of this study can be used in future work to evaluate the biofuel and bioproducts production from PPW and PPW-FR.

5.3 Materials and methods

5.3.1 Sample preparation

The PPW (*Russet Burbank*) sample was collected from a potato processing plant (J.R. Simplot Company, Nampa, ID) in May 2012 and stored frozen and a portion freeze dried for subsequent analysis. Hybrid poplar (*Populus deltoides x Populus tremuloides*) sample was obtained from Greenwood Resources (Portland, OR) and Wiley milled to <0.5mm particle size. The fermentation process to make ethanol, acetic acid and lactic acid from PPW was described (in previous chapter). A 1 L glass bottle with an air-lock was used as reactor, operated in sequencing batch mode with moderate mixing speed and at 35°C. The fermenter was inoculated with 5% mixed microbial cultures (sewage sludge from Moscow wastewater treatment plant, Idaho) only at the first day running. Gelatinized PPW (boiled for 30 min and cooled down) was fed daily and same amount of mixed

slurry was drawn off and a retention time of 2 days and solid loading of 40 g L^{-1} were kept. Sampling was conducted after 4 weeks running to obtain a steady state, and the PPW-FR was then recovered by centrifugation and freeze dried for subsequent analyses. A detailed characterization flow process is shown in Figure 5.1. All the results in this study were calculated on dry weight basis biomass and measured in triplicate.

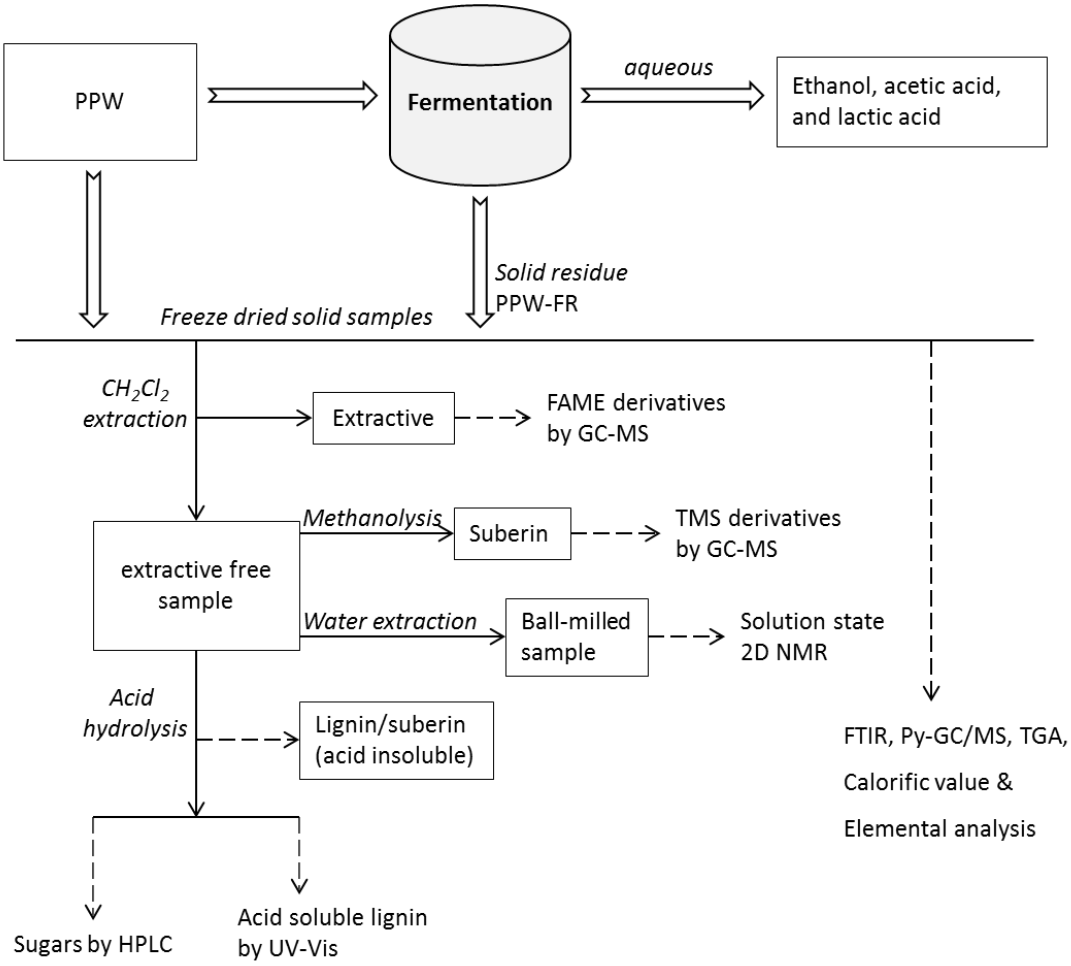

Figure 5.1 Schematic diagram of PPW and PPW-FR sample generation and analytical procedures.

5.3.2 Compositional and calorific analyses

Freeze dried samples (4-5 g) were Soxhlet extracted with CH_2Cl_2 (150 mL) for 16 h according to ASTM D 1108-96 and the extractives (lipids) were determined gravimetrically. The lignin and suberin complex was divided into acid insoluble and acid soluble factions and the analyses were performed on extractives free samples according to ASTM D 1106-96 and Schoening and Johansson (1965), respectively. Carbohydrate analysis was performed on the 2-stage acid-hydrolyzates according to ASTM E 1758-01. More specifically, dried sample (200 mg) was incubated in 72% H_2SO_4 (2 mL) for 1 h at 30°C, then diluted into 4% H_2SO_4, and subjected to a secondary hydrolysis in an autoclave (117 KPa and 121°C) for 30 min. The hydrolyzate was filtered to obtain acid insoluble residues content gravimetrically. The hydrolysis filtrate was made up to 250 mL and an aliquot portion taken to determine acid soluble fraction content at 205 nm using an absorption coefficient of 110 L g^{-1} cm^{-1} (Beckman DU640 spectrometer). An aliquot portion of the hydrolyzate (5 mL) was transferred to a centrifuge tube to which internal standard (inositol, 1 mL, 0.5 mg mL^{-1}) and $PbCO_3$ (0.16 g) were added, mixed well, centrifuged, and the supernatant (4 mL) deionized (column of Amberlite IR-120 H^+ (0.5 mL) and Amberlite IRA35 OH^- (0.5 mL) resins) and filtered (0.45 µm) into HPLC vials. Monosaccharides were the quantified by HPLC using two RPM columns in series (7.8 mm × 30 cm, Phenomenex) at 85°C equipped with differential refractive index detector (Waters Associates model 2414) on elution with 0.5 mL min^{-1} water. Starch content was estimated by enzymatic hydrolysis assay according to AACC method 76-11 using glucoamylase (Sigma-Aldrich) and the sugars analyzed by HPLC. More

specifically, sample (0.50 g) was dissolved in water (25 mL) and heated to 135°C for 1 h, cooled, acetate buffer (pH 4.8, 2.5 mL) and glucoamylase (5 mL, 150 IU) added to the solution, incubated at 55°C for 2 h and analyzed by HPLC.

5.3.3 GC-MS analysis of CH_2Cl_2 extractives and suberin

CH_2Cl_2 extract (5.0 mg) was weighed into a 5 mL reacti-vial^{TM} to which 2 mL of $CH_3OH/H_2SO_4/CHCl_3$ (1.7:0.3:2.0 v/v/v) was added, and the mixture was heated for 90 min at 90°C to form fatty acid methyl ester (FAME) derivatives (Osman et al., 2012). $CHCl_3$ contained 1-napthalaneacetic acid as an internal standard (50 µg mL^{-1}). To the cooled mixture water (1 mL) was added, vigorously shaken, centrifuged, the $CHCl_3$ layer removed, dried through anhydrous sodium sulfate, and transferred to a GC vial. The prepared FAME derivatives were analyzed by $GC\text{-}MS_{EI}$ (FOCUS-ISQ, ThermoScientific); temperature profile: 40°C (1 min) to 320°C at 5°C min^{-1} with injector temperature of 320°C, mass transfer line temperature of 325°C and ion source temperature of 240°C; GC capillary column: RTx-5MS (30 m, 0.25 mm Ø, 0.25 µm film thickness, Restek). The eluted compounds were identified with authentic standards (C12, C14, C16 and C18) and by spectral matching with the National Institute of Standards and Technology (NIST) 2008 mass spectral library. The abundance of each compound was referenced against the area of internal standard.

Suberin analysis was performed on samples by $NaOCH_3$-catalyzed methanolysis and subsequent GC-MS analysis (Graca and Pereira, 2000). CH_2Cl_2 extractive free sample (500 mg) was refluxed in freshly prepared 12 mmol L^{-1} $NaOCH_3$ in dry CH_3OH (50 mL)

for 3 h. The reaction mixture was filtered (glass fiber paper) and the residue washed with CH_3OH (25 mL) and $CHCl_3$ (25 mL). The filtrate (1 mL) in a GC vial was concentrated to dryness to which CH_2Cl_2 (containing anthracene as internal standard (50 µg mL^{-1}, 1 mL)), N,O-bis (trimethylsilyl)-trifluoro-acetamide containing 1% trimethylchlorosilane (50 µL) and pyridine (50 µL) were added and then heated to 70°C for 30 min. Suberin derived trimethylsilyl (TMS) derivatives were analyzed by GC-MS_{EI} (Focus-ISQ, ThermoScientific)); temperature profile: 40°C (1 min) to 300°C (20 min) at 5°C min^{-1} with injector temperature of 320°C, mass transfer line temperature of 300°C and ion source temperature of 220°C; GC capillary column: RTx-5MS (30 m, 0.25 mm Ø, 0.25 µm film thickness, Restek). The eluted compounds were identified with authentic standards, literature, and by spectral matching with the NIST 2008 mass spectral library, and the abundance of each compound was referenced against the area of internal standard.

5.3.4 Spectroscopy

FTIR spectroscopic analysis was performed using an Avatar 370 spectrophotometer (Thermo Nicolet) with an attenuated total reflection (ATR) probe (ZnSe crystal). The spectra were ATR and baseline corrected using Omnic v7.0 software.

^{13}C-^{1}H HSQC NMR spectroscopy was performed on a 500 MHz Bruker AVANCE 500 spectrometer. CH_2Cl_2 extractive free samples (10 g) were extracted with water overnight, the solid residue freeze-dried and ball-milled in a porcelain jar (0.5 L) for 14 d. The ball-milled samples (40 mg) were dissolved in dimethylsulfoxide (DMSO)-d_6 (1 mL) with the

aid of sonication and analyzed according to Kim et al (2008).

5.3.5 Thermogravimetric analysis

TGA was performed on samples (4-6 mg) using TGA-7 (Perkin-Elmer) instrument using a temperature program of 30 to 900°C at a heating rate of 5, 10, 20, 30, 40 K min^{-1} under N_2 (30 mL min^{-1}). TGA and differential thermogravimetry (DTG) data were analyzed using Pyris v8 software. Activation energy (E) and pre-exponential factor (A) were calculated according to ASTM E 1641-07. The decomposition kinetics was assumed to be first-order to determine E and A by the following equations:

$$E = -\left(\frac{R}{b}\right) \times \frac{\Delta(\log\beta)}{\Delta(1/T)}$$

$$A = -\left(\frac{\beta'}{E_r}\right) \times R \times \ln(1-\alpha) \times 10^{a}$$

where, R is gas constant 8.314 J mol^{-1} K^{-1}, b is the approximate derivative given by ASTM E 1641 numerical integration constants, β is the heating rate K min^{-1}, β' is the heating rate nearest the midpoint of the experimental heating rates K min^{-1}, T is the temperature (K) at constant conversion, E_r is the refined value of Arrhenius E, α is the conversion value of decomposition, and a is the approximation integral taken from ASTM E 1641 of numerical integration constants.

5.3.6 Py-GC/MS Analysis

Samples (< 0.1 mg) were injected into a quartz capillary tube and pyrolyzed at 500°C in an inert atmosphere (He, 0.125 MPa) using a Pyrojector II unit (SGE Analytical Science) coupled to a GC-MS_{EI} (FOCUS-ISQ, ThermoScientific) instrument. The compounds

were separated on a RTx-5ms (30 m x 0.25 mm Ø, 0.25 μm film thickness, Restek) capillary column with a temperature program of 50 to 250°C (10 min) at 5°C min^{-1} with injector temperature of 255°C, mass transfer line temperature of 255°C and ion source temperature of 240°C. The eluted compounds were identified with NIST 2008 library matching and by their mass spectra, and the relative abundance of each derived compound was calculated using CO_2 as internal standard (Osman et al., 2012; Phillai et al., 2011).

5.4 Results and discussion

5.4.1 Chemical, elemental and calorific values analyses

PPW and PPW-FR compositions, elemental contents, and calorific values are given in Table 5.1. The PPW contained approximately 17% starch and 22% non-starch polysaccharides. It was observed that most of the starch was consumed and converted to ethanol, acetic acid and lactic acid during fermentation leaving 2% residual starch and 20% non-starch polysaccharides in the PPW-FR. Lignin and suberin complex content increased during fermentation from 22% to 37% with a concomitant removal of carbohydrate. The extractives (lipids and secondary metabolites) comprise of fats, waxes, long-chain fatty acids and alcohols, accounting for 2 and 8% respectively in PPW and PPW-FR. After fermentation extractives accumulated in PPW-FR, which is in accordance with the distillers dried grains from ethanol fermentation (Belyea et al., 2004).

A decrease of ash content from 11% to 7% was observed and could be attributable to solubilization. Elemental composition analysis showed a significant difference between

the PPW-FR and PPW. The C and H content increased while the O content decreased after fermentation due to the removal of carbohydrates. The C and H contents in the PPW-FR were similar with woody biomass but higher than herbaceous biomass (wheat straw, corn stover and switchgrass) (Sannigrahi et al., 2010). A 10% increase in calorific value was observed after fermentation (19.2 MJ kg^{-1}) and this is consistent with the high C/H ratio (Sannigrahi and Ragauskas, 2011) and comparable to hardwoods (Sannigrahi et al., 2010). These findings suggest that the PPW-FR could be a promising biomass resource for biofuel production.

Table 5.1 Compositional, elemental, and calorific values analyses of PPW and PPW-FR.

Component	PPW	PPW-FR
carbohydrate		
starch	16.83 ± 0.52	2.07 ± 0.13
non-starch glucan	7.75 ± 0.68	7.79 ± 0.31
galactan	6.91 ± 0.10	5.31 ± 0.14
mannan	0.75 ± 0.07	1.35 ± 0.07
xylan	3.52 ± 0.21	1.47 ± 0.10
arabinan	3.53 ± 0.06	4.44 ± 0.08
holocellulose	22.46 ± 0.79	20.36 ± 0.08
lignin and suberin		
acid insoluble	15.94 ± 0.04	30.31 ± 0.20
acid soluble	5.70 ± 0.20	6.68 ± 0.00
CH_2Cl_2 extractive	1.98 ± 0.15	7.68 ± 0.00
ash	11.05 ± 0.07	7.47 ± 0.00
C	43.78±0.15	47.81±0.01
H	5.96±0.12	6.41±0.04
N	4.06±0.01	4.02±0.08
O	46.21±0.28	41.78±0.05
calorific value	17.37±0.38	19.19±0.03

* unit for calorific value in MJ kg^{-1}, other in % dry weight basis.

5.4.2 Compositional analysis of extractives and suberin

The CH_2Cl_2 extracts were converted to their FAME derivatives and analyzed by GC/MS, in which total 17 compounds ranged from C12 (lauric acid) to C30 (melissic acid) were

identified (Table 5.2 and Figure 5.2). The total biomass lipid content increased from 7.5 to 14.3 mg g^{-1} after fermentation. Linoleic (C18:2, 39%), palmitic (C16:0, 18%), and linolenic (C18:3, 16%) acids were the main components in PPW and in agreement with Dobson et al. (2004). The levels of linoleic, palmitic and linolenic acids increased respectively by 29.7%, 70.8, and 84.3% upon fermentation. Other minor (<10%) fatty acids were also found: lauric (C12:0), myristic (C14:0), pentadecanoic (C15:0), heptadecenoic (C17:0), stearic (C18:0), eicosanoic (C20:0), heneicosanoic (C21:0), docosanoic (C22:0), tricosanoic (C23:0), tetracosanoic (C24:0), hexacosanoic (C26:0) acids, montanic acid (C28:0), nonacosylic acid (C29:0), and melissic acid (C30:0). Dobson et al. (2004) quantified lipids, as FAMEs, in two potato species from C14 to C24 and also detected odd numbered (C15:0, C17:0, C21:0 and C23:0) fatty acids. The total 17 fatty acids increased by 90% after fermentation, and the increasing lipids could be produced by microorganisms during fermentation (Liu et al., 2012).

Table 5.2 FAME derivatives of PPW and PPW-FR extracts.

No.	Compound	RT (min)	M^+ (m/z)	PPW (mg g^{-1})	PPW-FR (mg g^{-1})
1	lauric acid (C12:0)	22.59	214	0.00	0.01
2	myristic acid (C14:0)	27.07	242	0.02	0.03
3	pentadecanoic acid (C15:0)	29.15	256	0.02	0.02
4	palmitic acid (C16:0)	31.13	270	1.37	2.34
5	heptadecenoic acid (C17:0)	33.04	284	0.01	0.02
6	linoleic acid (C18:2)	34.31	294	2.93	3.80
7	linolenic acid (C18:3)	34.43	296	1.21	2.23
8	stearic acid (C18:0)	34.86	298	0.25	0.38
9	eicosanoic acid (C20:0)	38.27	326	0.06	0.10
10	heneicosanoic acid (C21:0)	39.89	340	0.02	0.03
11	docosanoic acid (C22:0)	41.42	354	0.09	0.14
12	tricosanoic acid (C23:0)	42.91	368	0.01	0.02
13	tetracosanoic acid (C24:0)	44.34	282	0.20	0.28
14	hexacosanoic acid (C26:0)	47.07	410	0.28	0.58
15	montanic acid (C28:0)	49.61	438	0.67	2.61
16	nonacosylic acid (C29:0)	50.82	452	0.14	0.60
17	melissic acid (C30:0)	51.99	466	0.26	1.15
	total			7.54	14.33

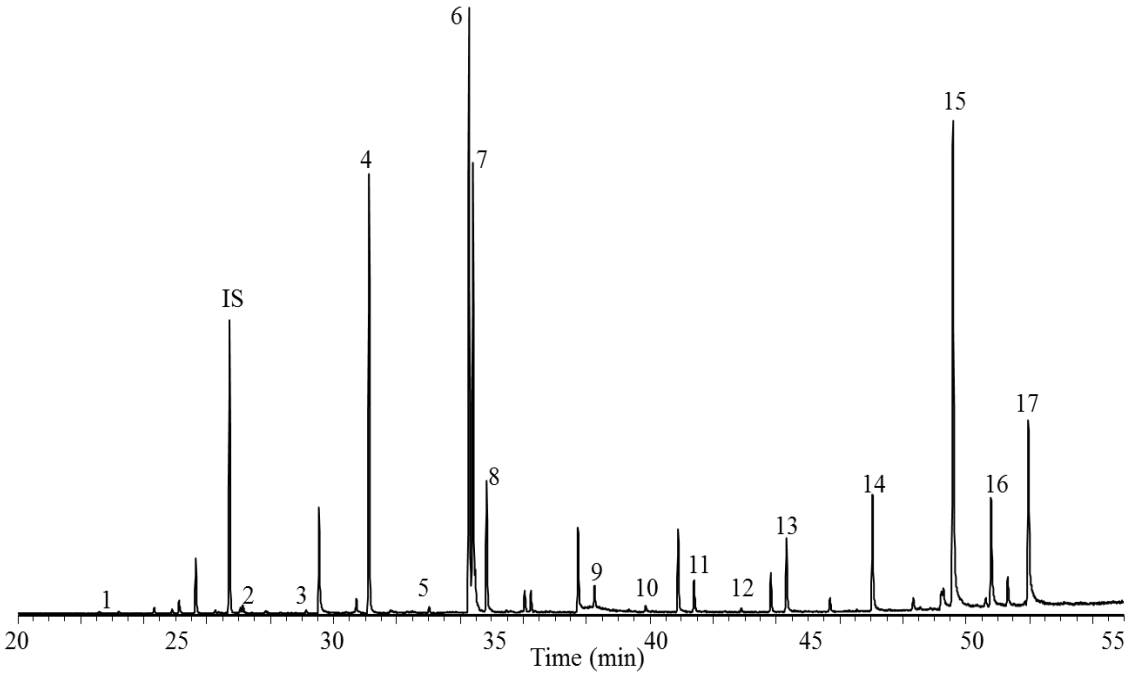

Figure 5.2 Total ion chromatogram of FAME derivatives of CH_2Cl_2 extract of PPW-FR (IS, 1-naptheneacetic acid; 17 peaks identified).

We determined the abundances of lignin and suberin complex gravimetrically in the previous section at 22 and 37% respectively, for PPW and PPW-FR. Suberin is regarded as a major constituent of potato cell walls (Graca and Pereira, 2000), a further analysis to obtain a better content value and composition was necessary. Figure 5.3 shows the composition of suberin and was comprised of mainly glycerol, alkanoic acids, α,ω-diacids, and ω-hydroxyacids, as well as minor amounts of alkan-1-ols, hydroxycinnamic acids, and amino acids. Similar suberin constituents were also identified by other researchers (Mattinen et al., 2009; Wang et al., 2010). Glycerol was the most abundant component in suberin at 48%, a cross-linker of the aliphatic and aromatic domains, and content is consistent with the literature (Moire et al., 1999). Suberin content was shown

to increase in PPW after fermentation from 58 to 166 mg g^{-1} as starch levels decreased.

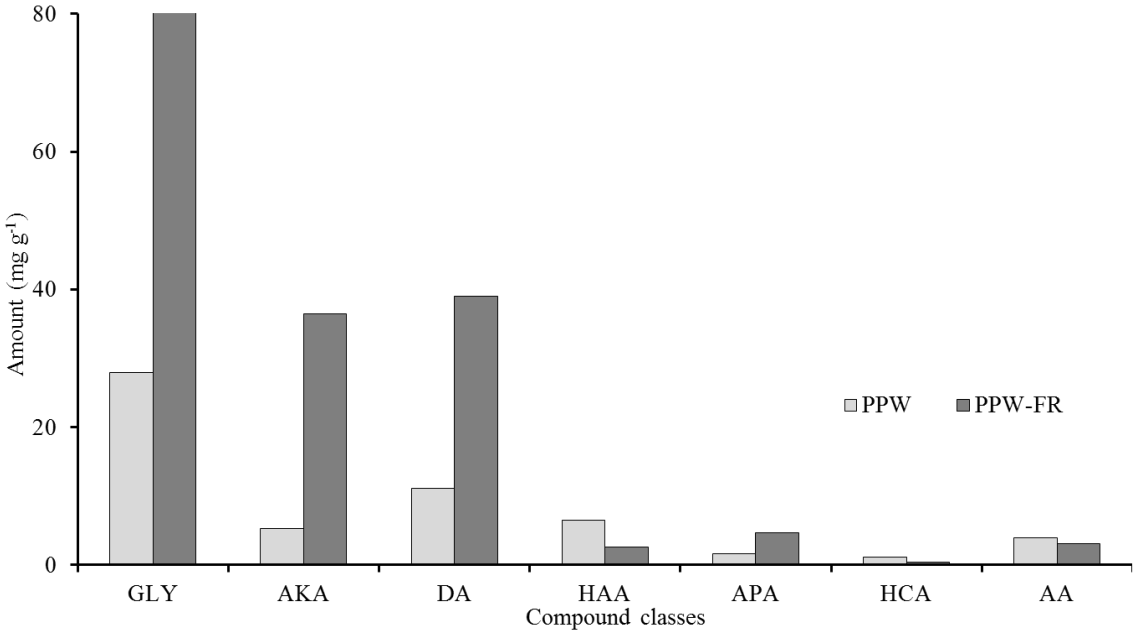

Figure 5.3 Levels of suberin derived compound classes present in PPW and PPW-FR by methanolysis (GLY, glycerol; AKA, alkanoic acids; DA, diacids; HAA, hydroxyalkanoic acids; APA, aliphatic alcohols; HCA, hydrocinnamic acids; AA, amino acids).

5.4.3 Spectroscopic analysis

FTIR spectroscopy was further used to analyze functional group changes of PPW and PPW-FR and their spectra are shown in Figure 5.4. The main characteristics were attributed to the presence of cellulose/starch, hemicellulose, lignin-suberin complex, and protein. A strong hydrogen bonded O-H stretching band (Soria and McDonald, 2012) at 3329 cm^{-1}, C-H (CH_3, CH_2) stretching vibration bands at 2974, 2915, and 2849 cm^{-1} were observed for PPW, whereas similar characteristic bands appeared at 3339 and 2917 and 2850 cm^{-1}, respectively for PPW-FR. Absorption ester bands for fatty acid, hydroxy fatty acid, and diacids in lipids and suberin polymers (Mattinen et al., 2009) were detected at

1735 cm^{-1} (C=O) and 1238 cm^{-1} (-C-O-C) in PPW-FR. However, these bands were detected, at low intensity, in PPW at 1733 and 1255 cm^{-1} and reflective of lower lipid and suberin contents. These results are in agreement with the compositional analysis mentioned above. A distinct amide I C=O stretching band (Schilling et al., 2004), for protein, was detected at 1609 cm^{-1} in PPW, whereas the band was shifted to 1628 cm^{-1} in PPW-FR. Other functional groups associated with proteins were observed and overlapped with carbohydrate and lignin bands at 3640~3040 cm^{-1} for N-H stretching and 1600~1500 cm^{-1} for amide II C-N stretching and N-H bonding absorptions (Zarrinbakhsh et al., 2013).

In general, the absorption bands from 1600 to 1400 cm^{-1} derived from C=C vibration of aromatic ring were assigned to lignin (Mattinen et al., 2008). Three distinct bands at 1515, 1452, and 1418 cm^{-1} were detected in PPW-FR, while only two bands at 1514 and 1408 cm^{-1} and a shoulder around 1450 cm^{-1}were detected in PPW. A clear absorption band at 1370 cm^{-1} denoted as aliphatic C-H stretching (Singh et al., 2005) in CH_3 or phenolic OH was observed only in PPW-FR. The band at 1314 cm^{-1} was assigned to C-H vibration. Finally, a strong band ranging from 1200 to 900 cm^{-1} was assigned to -C-O-C- vibration of pyranose sugar rings (carbohydrates) in both PPW and PPW-FR samples.

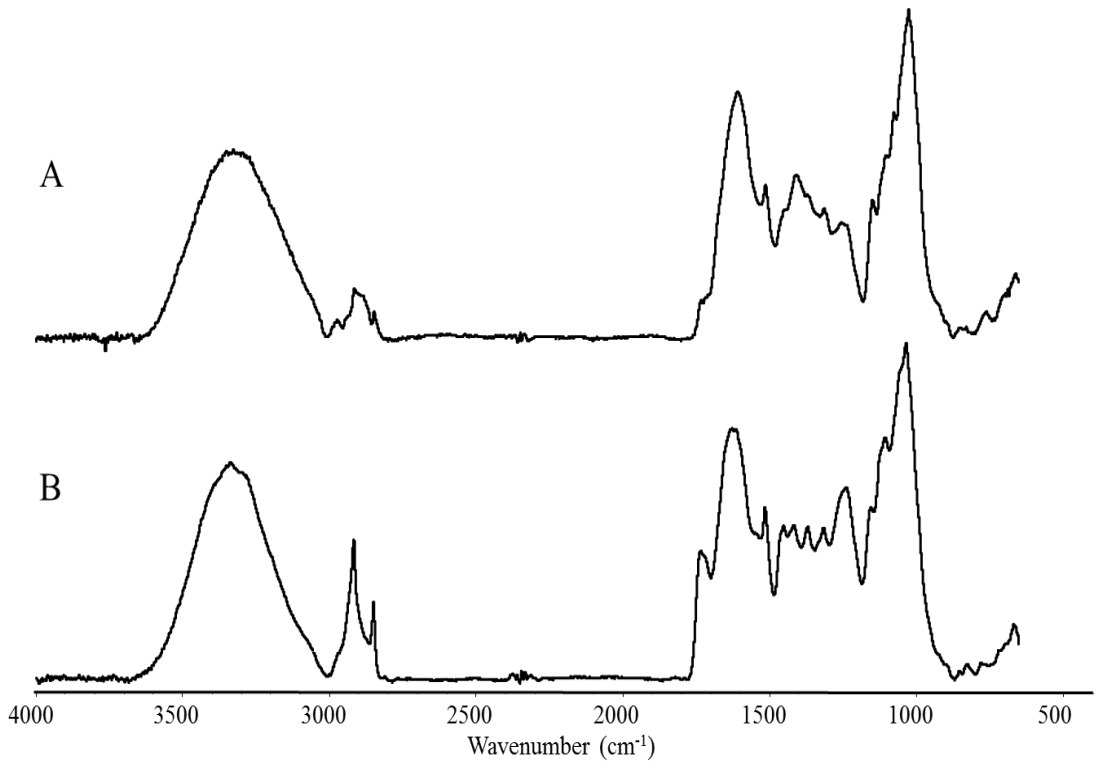

Figure 5.4 FTIR spectra of PPW (A) and PPW-FR (B).

2D-NMR spectroscopy was employed to non-destructively analyze PPW and PPW-FR by fully solubilizing the samples in DMSO following the approach by Kim et al. (2008) and Lu and Ralph (2011) which were developed for woody biomass. This approach was trialed to assess its suitability to characterize PPW. Figure 5.5 shows the 2D HSQC ^{13}C-^{1}H correlation spectra of ball-milled hybrid poplar, PPW and PPW-FR samples. Most of ^{1}H-^{13}C correlations in the δ_C/δ_H 50-120/2.5-5.5 ppm region belong to polysaccharides and the δ_C/δ_H 100-150/6-8 ppm region is contributed by aromatic components. Hybrid poplar as a model lignocellulosic substrate exhibited significant cellulose, hemicellulose, and lignin signals, where the anomeric C/H correlation of cellulose and xylan appear at δ_C/δ_H 103/4.4 ppm for (1→4)-β-D-Glc*p*, 104/4.2ppm for (1→6)-β-D-Glc*p*, and 102/4.3 ppm for (1→4)-β-D-Xyl*p* respectively. Correlations of lignin appear at δ_C/δ_H 104/6.7 ppm for C_2-

H_2 and C_6-H_6 in syringyl units, 111/7.0, 115/6.7 and 119/6.8 ppm for C_2-H_2, C_5-H_5, and C_6-H_6 in guaiacyl unit, respectively. Other clusters of C/H correlations for polysaccharides and lignin were also observed and the results are in accordance with Lu and Ralph (2011). In this research, non-derivatized solution state 2D NMR was first introduced to analyze potato peel samples (Figure 5.5) and the NMR spectra of PPW and PPW-FR samples were quite different than hybrid poplar. The clusters of ^{1}H-^{13}C correlations were associated with polysaccharides, suberin and lignin. The methyl signal appears at δ_C/δ_H 21/2.1 ppm for acetyl group in polysaccharides (Kim et al., 2008), aliphatic methylenic groups (-CH_2-) appears at δ_C/δ_H 29/1.4 ppm for suberin components (Wang et al., 2010), C6 appears at δ_C/δ_H 61/3.8 ppm for C_6-H_6 in cellulose and C_γ-H_γ in β-O-4 substructures, C5 and C4 appears at δ_C/δ_H 73-74/3.6-3.8 ppm for C_5-H_5 and C_4-H_4 correlation in cellulose, and anomeric C1 appears at δ_C/δ_H 100/4.9-5.3 ppm for anomeric C/H of (1→4)-α-D-Glc*p* and (1→6)-α-D-Glc*p* in starch (Cheng and Neiss, 2012; Kim et al., 2008; Lu and Ralph, 2011) were present in both PPW and PPW-FR. Signals attributable to lignin at δ_C/δ_H 56/3.6-3.9 ppm (methoxyl group) and δ_C/δ_H 115/6.5-6.8 ppm (C_5-H_5 correlation for guaiacyl) were observed only in the PPW-FR, which are in agreement with FTIR and Py-GC/MS data. Unfortunately, due to the poor spectral dispersion in the NMR spectra of PPW and PPW-FR limited chemical information was obtained. This may be due to the complex nature of PPW samples (containing protein, suberin, and lipids) as compared to hybrid poplar. Therefore, this approach to analyzed PPW directly by NMR was not successful and may require further optimization.

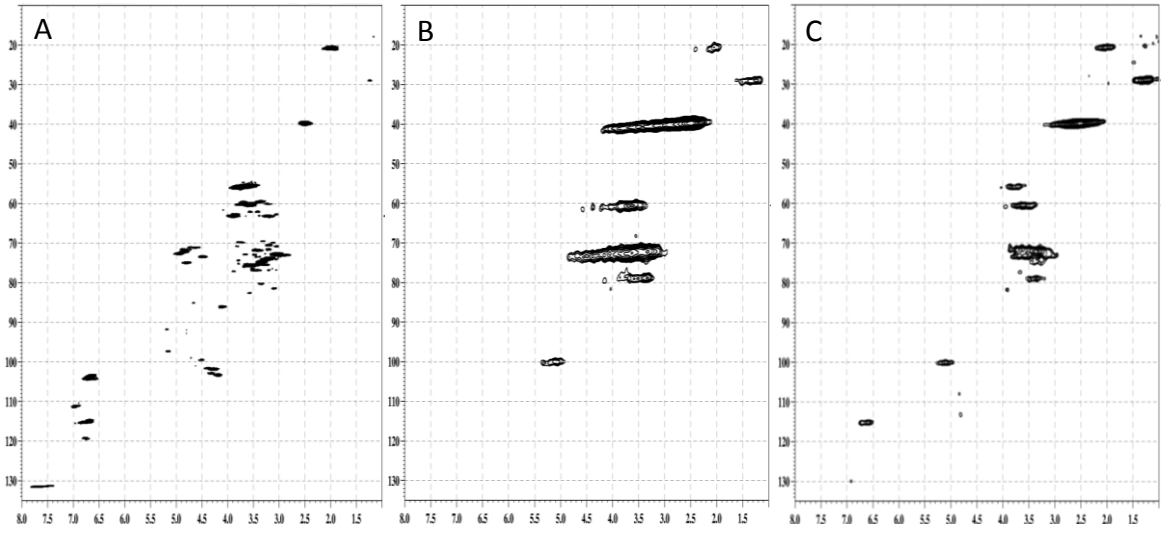

Figure 5.5 HSQC ^{1}H-^{13}C NMR spectra of (A) Hybrid Poplar, (B) PPW, and (C) PPW-FR in DMSO-d_6.

5.4.4 TGA/DTG analysis

Figure 5.6 shows the TGA and DTG thermograms for PPW and PPW-FR samples. Thermal degradation of biomass can be classified into four regions: (i) dewatering at 50-130°C; (ii) evaporation of volatile compounds due to degradation 130-220°C; (iii) thermolysis/pyrolysis of polymers (depolymerization) at 220-460°C; and (iv) char formation at >460°C (Gao et al., 2012; Wei et al., 2013). Thermal decomposition of the following main constituents occurs at: 280-350°C for starch; 315-400°C for cellulose, 220-315ºC for hemicelluloses; starts at 250°C for lignin and 290ºC for suberin. Two distinct peaks were observed from the DTG thermograms at 279°C and 423°C for PPW and 285°C and 457°C for PPW-FR, respectively. In addition, less char/ash was seen in the PPW-FR than PPW, which is in accordance with ash content determination. The E before 70% α of samples at 5% conversion intervals were calculated based on ASTM

methods. Generally, the E values for PPW and PPW-FR were similar, where decreased trends were observed in both samples after 45% conversion. However, slightly lower values were found in PPW-FR than PPW after α reached 45%. The average E and A values were respectively, 214 kJ mol^{-1} and 9.18×10^{24} for PPW and 209 kJ mol^{-1} and 1.65×10^{22} for PPW-FR. Similar values of E and A for wheat straw sample were observed at 260 kJ mol^{-1} and 2.00×10^{22} (Gao et al., 2012). The lower E in PPW-FR indicates that less energy is required for thermal decomposition and suitable for pyrolysis (Meszaros et al., 2004).

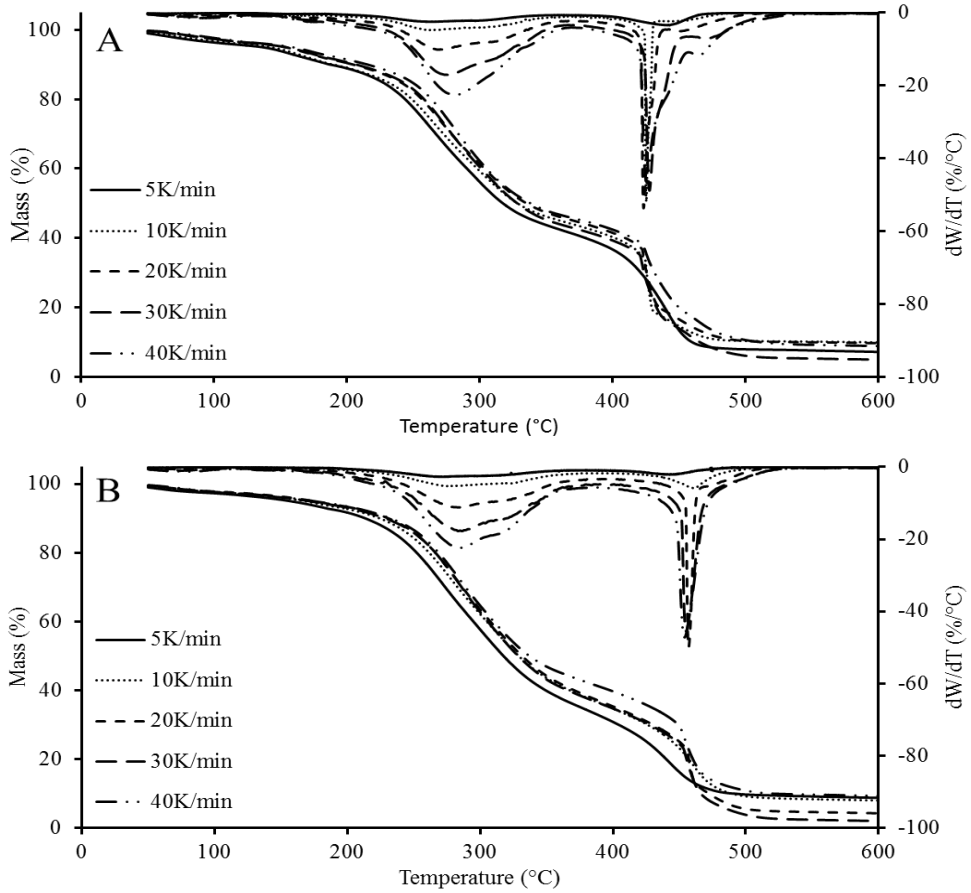

Figure 5.6 TGA and DTG thermograms of PPW (A) and PPW-FR (B) at different heating rates (5 to 40 K min^{-1}).

5.4.5 Py-GC/MS analysis

Py-GC/MS was used as tool to rapidly analyze biomass samples and assess potential pyrolysis bio-oil products. Py-GC/MS products of PPW and PPW-FR at 500°C are listed in Table 5.3. 51 pyrolysis products were identified with the main product being CO_2 followed by acetic acid (14.0% for PPW and 7.0 % for PPW-FR), acetic anhydride (7.9% and 5.1%), hexadecenoic acid (5.5% and 5.5%), and phenol (5.3% and 5.7%). A range of phenolic compounds, including guaiacyl and syringyl derivatives, were observed totaling at 26.7% in PPW and 35.1% in PPW-FR. Long chain fatty acids and alkane were derived from lipid and suberin decomposition (Osman et al., 2012; Turner et al., 2013) and the relative percentage increased from 22.0% into 27.8% after fermentation. The carbohydrate derived compounds decreased from 45.3% into 31.1% from PPW during fermentation. Nitrogen containing compounds (such as pyrrole, methylpyrrole, acetamide, and indolizine) derived from protein were also identified with similar relative percentages in both PPW and PPW-FR samples, which is in agreement with the C, H, N analyses.

Table 5.3 Compounds released by Py-GC/MS of PPW and PPW-FR.

No.	Compound	RT (min)	M^+ (m/z)	Formula	Relative abundance (%)	
					PPW	PPW-FR
1	acetaldehyde	1.36	44	C_2H_4O	2.89	2.17
2	acetic acid	1.66	60	$C_2H_4O_2$	14.00	7.02
3	acetic anhydride	2.05	102	$C_4H_6O_3$	7.92	5.13
4	pyrrole	2.98	67	C_4H_5N	2.37	2.12
5	toluene	3.12	92	C_7H_8	2.80	2.53
6	acetamide	3.27	59	C_2H_5NO	0.45	1.39
7	2,3-butanediol	3.47	90	$C_4H_{10}O_2$	2.09	0.00
8	2-oxo-3-cyclopentene-1-acetaldehyde	4.27	124	$C_7H_8O_2$	1.72	1.70
9	3-methylpyrrole	4.38	81	C_5H_7N	1.20	0.60
10	3-furylmethnol	4.71	98	$C_5H_6O_2$	1.01	0.76
11	6-hydroxy-hexan-2-one	5.01	116	$C_6H_{12}O_2$	0.92	1.54
12	1,2,3-Cyclopentanetriol	5.72	118	$C_5H_{10}O_3$	0.24	0.20
13	2-methyl-2-cyclopentenone	5.85	96	C_6H_8O	0.72	0.48
14	2(5H)-furanone	6.06	84	$C_4H_4O_2$	2.03	1.38
15	1,2-cyclopentanedione	6.30	98	$C_5H_6O_2$	1.76	2.11
16	1-methyl-1-cyclopenten-3-one	7.33	96	C_6H_8O	1.39	0.64
17	4-methyl-2(5H)-furanone	7.74	98	$C_5H_6O_2$	0.57	0.71
18	phenol	7.83	94	C_6H_6O	5.28	5.74
19	3-methyl-1, 2-cyclopentanedione	9.04	112	$C_6H_8O_2$	3.19	2.83
20	2-methyl-phenol	9.83	108	C_7H_8O	2.10	1.61
21	3-methyl-phenol	10.44	108	C_7H_8O	3.32	3.87
22	guaiacol	10.76	124	$C_7H_8O_2$	3.21	5.34
23	3-ethyl-2-hydroxy-2-cyclopenten-1-one	11.62	126	$C_7H_{10}O_2$	1.57	1.09
24	2,3-dimethyl-phenol	13.01	122	$C_8H_{10}O$	2.54	2.39
25	3-ethyl-phenol	13.09	122	$C_8H_{10}O$	2.24	2.23
26	4-methyl-guaiacol	13.68	138	$C_8H_{10}O_2$	0.82	1.45
27	1,4:3,6-dianhydro-hexopyranose	14.17	144	$C_6H_8O_4$	0.50	0.87
28	coumaran	14.49	120	C_8H_8O	1.45	2.83
29	2-ethyl-methyl-phenol	14.96	136	$C_9H_{12}O_2$	0.91	0.90
30	4-ethyl-guaiacol	16.03	152	$C_9H_{12}O_2$	0.76	1.42
31	1,4-benzenediol	16.23	110	$C_6H_6O_2$	0.29	0.61
32	indolizine	16.47	117	C_8H_7N	1.99	1.79
33	4-vinyl-guaiacol	16.98	150	$C_9H_{10}O_2$	2.73	4.97
34	syringol	17.97	154	$C_8H_{10}O_3$	0.27	0.52
35	propioguaiacone	20.44	180	$C_{10}H_{12}O_3$	0.72	1.26
36	α-hydroxydodecanoic acid	21.34	216	$C_{12}H_{24}O_3$	0.57	0.53
37	tetradecanoic acid	27.54	228	$C_{14}H_{28}O_2$	0.95	1.28
38	pentadecanoic acid	29.54	242	$C_{15}H_{30}O_2$	0.90	0.83
39	hexadecenoic acid	31.11	254	$C_{16}H_{30}O_2$	0.95	0.88
40	hexadecanoic acid	31.47	256	$C_{16}H_{32}O_2$	5.53	5.51
41	2,6,10-trimethyltetradecane	33.91	240	$C_{17}H_{36}$	1.03	1.25
42	octadecyonic acid	34.61	280	$C_{18}H_{32}O_2$	1.40	1.69
43	oxacycloheptadec-8-en-2-one	34.91	252	$C_{16}H_{28}O_2$	1.80	3.83
44	eicosane	37.35	282	$C_{20}H_{42}$	1.67	2.40
45	vaccenic acid	38.88	282	$C_{18}H_{34}O_2$	2.05	2.81
46	heneicosene	40.43	295	$C_{21}H_{42}$	1.05	1.17
47	2-methyleicosane	40.51	296	$C_{21}H_{44}$	1.34	2.19
48	eicosenoic aicd	41.95	310	$C_{20}H_{38}O_2$	0.84	0.98
49	2-methyl-eicosane	43.64	296	$C_{21}H_{44}$	1.00	1.80
50	erucic acid	45.55	338	$C_{22}H_{42}O_2$	0.92	0.67

5.5 Conclusion

In this study, we have performed a comprehensive chemical and thermal characterization of PPW and PPW-FR to obtain information to better utilize this resource for bioproducts. A significant increase of lignin/suberin, C/H contents, and calorific value was observed in PPW-FR as a result of fermentation. Results were further verified by FTIR and NMR analyses. Thermal characterization by TGA and Py-GC/MS analyses indicated that PPW-FR required less energy for thermal conversion processes, such as pyrolysis, and more high value chemical compounds could be recovered. The chemical and thermal properties of PPW-FR, and to a lesser extent PPW, make it a promising renewable biomass for biofuel and bioproducts production.

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Chapter 6 Production and characterization of bio-oil and bio-char from pyrolysis of potato wastes

6.1 Abstract

Bio-oil and bio-char were produced from potato peel waste (PPW) and PPW fermentation residue (PPW-FR) using a lab-scale Auger pyrolyzer at 450°C. Proximate and ultimate analyses of the feedstock materials PPW and PPW-FR were determined. The bio-oils were fractionated and analyzed by gas chromatography mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), and electrospray ionization mass spectrometry (ESI-MS). The bio-chars were characterized by Fourier transform infrared spectroscopy (FTIR) and butane activity (BA) measurement. The PPW and PPW-FR bio-oils had a relatively high level of lipid and suberin derived compounds as compared hybrid poplar (HP) pyrolysis bio-oil. The BA measurement also suggested the PPW and PPW-FR bio-chars have better adsorption performances than HP bio-char.

6.2 Introduction

The United States produced 21.7 million tons of potatoes in 2011, of which about 50-60% is converted in French Fries and other products (NPC, 2012). The process to make these potato products generates 6-10% potato peel waste (PPW) depending on the final products and this waste stream is a significant waste management issue (Schieber et al., 2001). However, this waste is also a promising resource for large scale production of bio-based fuels and chemicals (Arapoglou et al., 2010). Biological conversion of PPW to ethanol and other chemicals cannot utilize all the components of PPW, which inevitably

leaves behind a significant amount of un-reacted solid PPW fermentation residue (PPW-FR) and has the potential to be used for fuel.

With the main purpose of reducing the national reliance to petroleum import and strength the energy independence and security, the Energy Independence and Security Act of 2007 mandates the use of 36 billion gallon per year renewable fuels by 2022 including conventional biofuels, advanced biofuels, cellulosic biofuels, biomass-based biodiesel (Picou and Boldor, 2012). Biomass is considered as one of most promising and alternative renewable resources with high potential to reduce the national reliance to fossil fuels (Yanik et al., 2007). However, not all biomass materials with suitable physical and thermochemical properties can be utilized due to the availability and logistical constrains (Caputo et al., 2005). Many efforts have focused on exploring cheap, abundant, and centralized biomass resources for energy production (Kim et al., 2013).

Pyrolysis is one of most promising thermal decomposition technologies to convert biomass materials into bio-oil, bio-char, and gases at about 450-500°C in the absence of oxygen (Mohan et al., 2006). Woody biomass derived pyrolysis bio-oil contains a complex mixture of water, phenols, alcohols, ethers, esters, aldehydes, ketones, sugars, and carboxylic acids (Strahan et al., 2011). This bio-oil can be used directly as a fuel for heat and power generation, or as intermediate feedstock for various chemicals and liquid transport fuels production (Ingram et al., 2008). Bio-char is a co-product of pyrolysis comprises mainly stable aromatic forms of organic carbon (Sohi et al., 2010). Bio-char is currently being used as a soil amendment and to sequester carbon in the soil (Mitchell et al., 2013). Furthermore, bio-char can also be used as an efficient adsorbent for

environmental contaminant removal (Moreno-Pirajan and Giraldo, 2012; 2011). A variety of biomass materials such as wood, grasses, agricultural and forestry residue, animal waste, and sewage sludge have been carried out for pyrolysis application (Cantrell et al., 2008; Ingram et al., 2008; Mohan et al., 2006).

Several research activities on the pyrolysis of PPW have been reported and Onal et al. (2011) compared the bio-oil production from PPW pyrolysis under static (25% yield), N_2 (27% yield), and steam atmosphere (41% yield). Characterization of aliphatic rich bio-oils revealed that PPW is a promising biomass candidate for bio-oil production (Onal et al., 2012; 2011). The physical and chemical properties of bio-char from pyrolysis of PPW were studied and the adsorption capacities to heavy metals and phenolic derivatives were also evaluated and results indicated a promising application as adsorbent (Moreno-Pirajan and Giraldo, 2012; 2011). However, these studies only focused on bio-oil or bio-char analyses, a comprehensive characterization including both bio-oil and bio-char is necessary. Residual lignocellulosic biomass after fermentation and corn distillers dried grains residues have been utilized for value added bio-oil production by pyrolysis (Giuntoli et al., 2011; Wang et al., 2012). However, there is no study mentioned the pyrolysis of PPW-FR.

In this study, biomass samples PPW and PPW-FR were pyrolyzed in a lab-scale Auger reactor. Bio-oils and bio-chars from the pyrolysis of PPW and PPW-FR were characterized by gas chromatography mass spectrometry (GC-MS), electrospray ionization mass spectrometry (ESI-MS), and by Fourier transform infrared spectroscopy (FTIR) and compared with those generated from hybrid poplar.

6.3 Materials and methods

6.3.1 Feedstock and pyrolysis procedure

The PPW (*Russet Burbank*) sample was collected from a potato processing plant (J.R. Simplot Company, Nampa, ID). The PPW-FR was recovered by centrifugation from a 1L anaerobic sequencing batch reactor which primarily utilized PPW into ethanol, acetic acid and lactic acid under mesophilic (35°C) fermentation. The hybrid poplar (HP, *Populus deltoides x Populus tremuloides*) sample was obtained from Greenwood Resources (Portland, OR). All three feedstock samples were dried for 48 h at 50°C and grounded in a Wiley mill to <1 mm particle size screen prior to use. The proximate analysis (ash content, fixed carbon (FC), and volatile matter (VM)) of these samples was performed according to ASTM E870-82. The calorific values for pressed dried pellet samples (5.5 mm x 6.3 mm Ø) were determined by bomb calorimetry (Model 1261, Parr Instruments) according to ASTM D 5865-04. The C, H, and N analysis (O by difference) were determined on a CE-440 elemental analyzer (EAIExeter Analytical) and results are listed in Table 1. Thermogravimetric analysis (TGA) was performed on a Perkin Elmer TGA-7 instrument (50-900°C at 20°K min^{-1} under N_2 at 30 mL min^{-1}).

Pyrolysis was conducted at 0.5 kg h^{-1} feeding rate in an Auger reactor (Φ 5 cm × 90 cm) at 450°C with N_2 purge (6 L min^{-1}). The auger speed was adjusted to 1 rpm to obtain an 8 s resident time. Vapors were condensed through an ice-water cooled tube and shell condensing system and bio-oil was recovered. Bio-char was collected at the end of reactor.

6.3.2 Bio-oil fractionation

The fractionation method for bio-oil characterization was based on procedures according to Sipila et al. (1998) as shown in Figure 6.1. Bio-oil (5.0 g) was vigorously mixed with water (10 g) for 15 min and centrifuged for 30 min at 5000 rpm to obtain a water-soluble (WS) supernatant fraction and water-insoluble (WI) bottom fraction. The bio-oil WS fraction was further extracted by adding 4 volume of diethyl ether to obtain ether-soluble (ES) and aqueous (AQ) fractions.

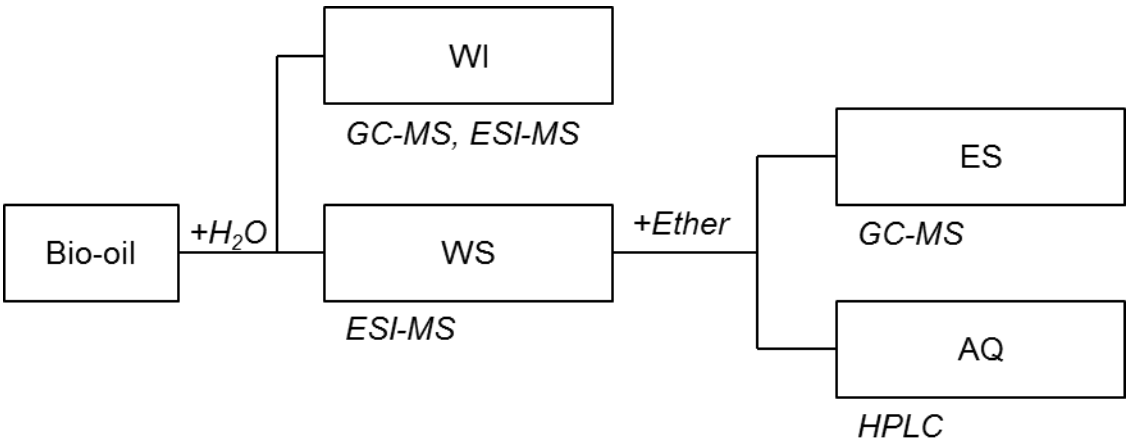

Figure 6.1 A schematic diagram showing the fractionation and characterization of the bio-oil.

6.3.3 Bio-oil characterization

The pH was measured with Orion-3-Star DO/pH portable meter (Thermo Fisher Scientific Inc., Waltham, MA). GC-MS (FOCUS-ISQ, Thermoscientific) was used to analyze the bio-oil fractions (1 mg in $CHCl_3$ (1 mL) containing anthrancene (5 µg mL^{-1}) as internal standard), where the separation was achieved using a RTx-5MS capillary column (30 m × 0.25 mm Ø, Restek) using a temperature program of 40°C (1 min) to

320°C at 5°C min^{-1}. Compounds were identified using known standards, mass spectral library matching (National Institute of Standards and Technology (NIST) 2008) and by their mass spectra. The AQ fractions were analyzed by high performance liquid chromatography (HPLC) using a Rezex ROA organic acid column (7.8 mm × 30 cm, Phenomenex) and a Waters HPLC (Waters, Milford, MA) equipped with a differential refractive index detector (ERC-5710, ERMA), on elution with 0.005 N aqueous sulfuric acid (0.5 mL min^{-1}) at 65°C. The bio-oil fractions, dissolved in methanol (1 mg mL^{-1}) were directly analyzed by positive ion ESI-MS (m/z 50-2000) on a LCQ-Deca LC-MS instrument (Thermoquest, San Jose, CA) at a flow rate of 10 µL min^{-1}. Data analysis was based on the calculation of number average molar mass (M_n) as $M_n = \sum M_i N_i / \sum N_i$ and weight average molar mass (M_w) as $M_w = \sum M_i^2 N_i / \sum M_i N_i$ with M_i as m/z and N_i as intensity of ions (Osman et al., 2012).

6.3.4 Bio-char characterization

Bio-char samples were oven dried at 105°C for 24 h before analysis. FTIR spectra were acquired on an Avatar 370 (ThermoNicolet) spectrophotometer in the attenuated total reflection (ATR) mode (ZnSe). The spectra were ATR and baseline corrected using Omnic V9 software and lignin condensation indices (CI) were calculated according to Soria and McDonald (2012), where the sum of all spectra minima intensity between 1,500 and 1,050 cm^{-1} was divided by the sum of all spectra maxima intensity between 1,600 and 1,030 cm^{-1}. The butane activity (BA) was determined according to ASTM D5742-95 where dry bio-char (16.7 mL, of known weight) placed into the sample tube flushed with butane (250 mL min^{-1}) until a constant weight gain was obtained at 25°C.

The BA was determined gravimetrically from butane weight gain. Activated charcoal (plant cell culture grade, Sigma-Aldrich) was used as a reference standard. The proximate analysis and calorific values of bio-char samples were determined according the methods described above.

6.4 Results and discussion

6.4.1 Biomass properties and pyrolysis products

The proximate (ash, FC, VM) and ultimate (C, H, N) analyses of three biomass samples used for this study are presented in Table 6.1. Major differences can be observed among the biomass samples. The FC's were much higher for both PPW and PPW-FR (13-14%) than the HP sample, which also results in HP having a high VM than PPW and PPW-FR samples. These results are in accordance with reference data (Demirbas, 2004). Higher ash contents were found in PPW and PPW-FR samples than that of HP. The PPW and PPW-FR samples have high N contents (4%), as expected, because these are a tuberous plant derived (Vassilev et al., 2010). The PPW-FR sample had the highest C content at about 48%. The calorific value for PPW-FR sample was 10% higher than it is for PPW sample, which is close to the measured 19.6 MJ kg^{-1} of HP sample.

Table 6.1 Proximate and ultimate analyses of three feedstock samples.

	HP	PPW	PPW-FR
Proximate analysis (%)			
Ash	0.8	9.3	6.5
VM	89.4	76.5	79.8
FC	9.8	14.2	13.4
Calorific value (MJ kg^{-1})	19.6	17.4	19.2
Ultimate analysis (%)			
Carbon	46.7	43.8	47.8
Hydrogen	6.1	6.0	6.4
Nitrogen	0.4	4.1	4.0
Oxygen	46.8	46.2	41.8

TGA showed that the thermal degradation onset temperatures for PPW and PPW-FR were below 450°C (Figure 6.2), and therefore the pyrolysis reaction at 450°C would be a suitable temperature to conduct this experiment. The bio-oil and bio-char yields as well as the properties of bio-oils from the three selected pyrolysis reactions are given in Table 6.2. HP pyrolysis produced the highest bio-oil yield of 40% and lowest char yield of 15%, while PPW and PPW-FR generated bio-oil at 23 and 26% and bio-char at 31 and 32%, respectively. There was a considerable amount of unrecovered tar residue accumulated on the transfer tube condenser which could account for the low bio-oil yields. The bio-oil and bio-char yields are partially dependent on heat transfer rate and reactor designs (Ingram et al., 2008), and in this experiment study we did not attempt to optimize the pyrolysis productivity. The yields reported here were comparable to those reported by Onal et al. (2011) for PPW which can be improved through process optimization. The bio-oil generated from the pyrolysis of PPW and PPW-FR samples were high at pH >8 as compared to pH 3.6 for HP bio-oil. This significant difference in pH can be explained by the high N content in PPW and PPW-FR samples, which can be released during pyrolysis as amines (Mullen and Boateng, 2011).

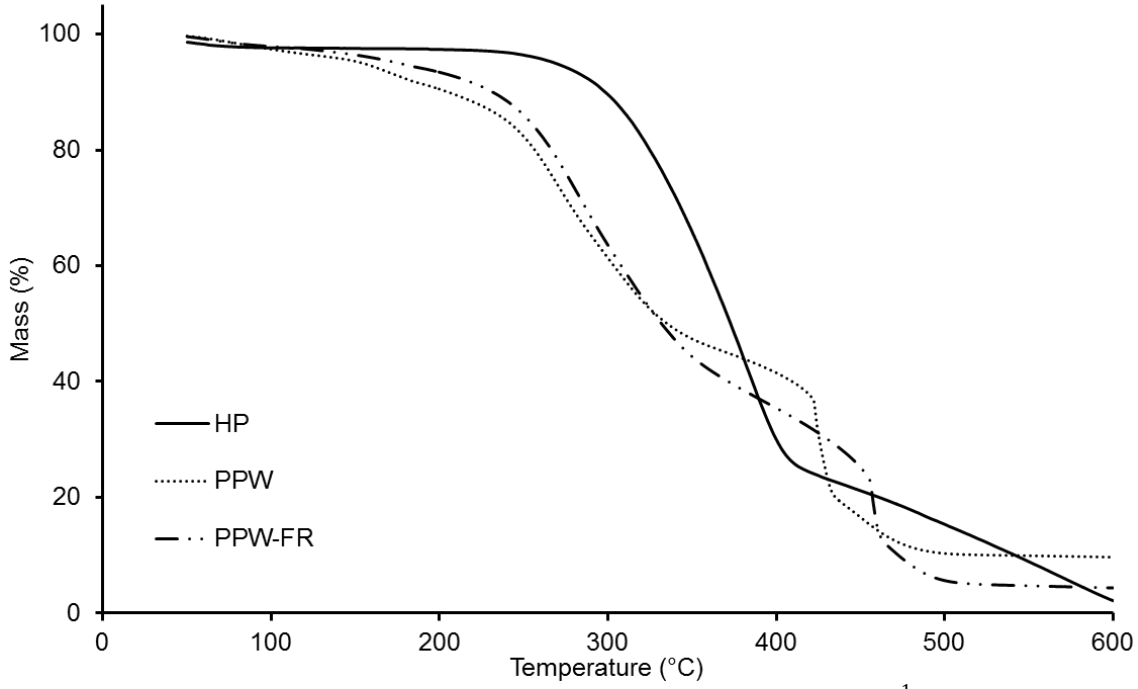

Figure 6.2 TGA thermograms of HP, PPW and PPW-FR at 20 K min^{-1} heating rate.

Table 6.2 Pyrolysis yields and properties of bio-oil produced from HP, PPW, and PPW-FR.

	HP	PPW	PPW-FR
Feedstock moisture (%)	4.3	4.8	5.5
Bio-oil yield (%)	40.1	22.7	25.6
Bio-char yield (%)	15.2	30.5	32.2
Bio-oil pH	3.57	8.61	8.01
Bio-oil fractionation			
WI (%)	34.7	13.2	22.8
WS (%)	65.3	86.8	77.2

6.4.2 GC-MS and HPLC analysis of bio-oil compounds

The proportion of WI fraction varied from 13 to 35% for the bio-oils produced, while the highest proportion was obtained from the pyrolysis of PPW-FR (Table 6.2). Generally, the WI fraction consists mainly of pyrolytic lignin (Ba et al., 2004), while in this study the lipids and suberin derivatives were found more prevalent in this WI bio-oil fraction

from PPW and PPW-FR. GC-MS analysis of the WI fractions are shown in Figure 6.3 and the compound identity and composition given in Table 6.3. The bio-oil WI fraction from HP contained mainly lignin derivatives (89%) with the major compounds being phenol (27%), isoeugenol (14.4%) and guaiacol (6.7%). A total of 34 compounds were identified from PPW and PPW-FR bio-oil WI fractions, of which about 53-55% were lignin derivatives with phenol and guaiacol being present at >10%. Lipids and suberin derivatives such as long chain fatty acids, alcohols, alkanes, and alkenes were present in high quantity in the PPW (33%) and PPW-FR (40%) pyrolysis bio-oils and consistent with the literature (Onal et al., 2011). The existence of such hydrocarbons has advantages for the PPW and PPW-FR bio-oils than HP bio-oil because these can be easily converted to renewable diesel (Li et al., 2012). Oxacycloheptadec-8-en-2-one was found in WI bio-oil from PPW (18.4%) and PPW-FR (15.8%) and this compound has appeared in pyrolytic vegetable oil waste (Xu et al., 2011).

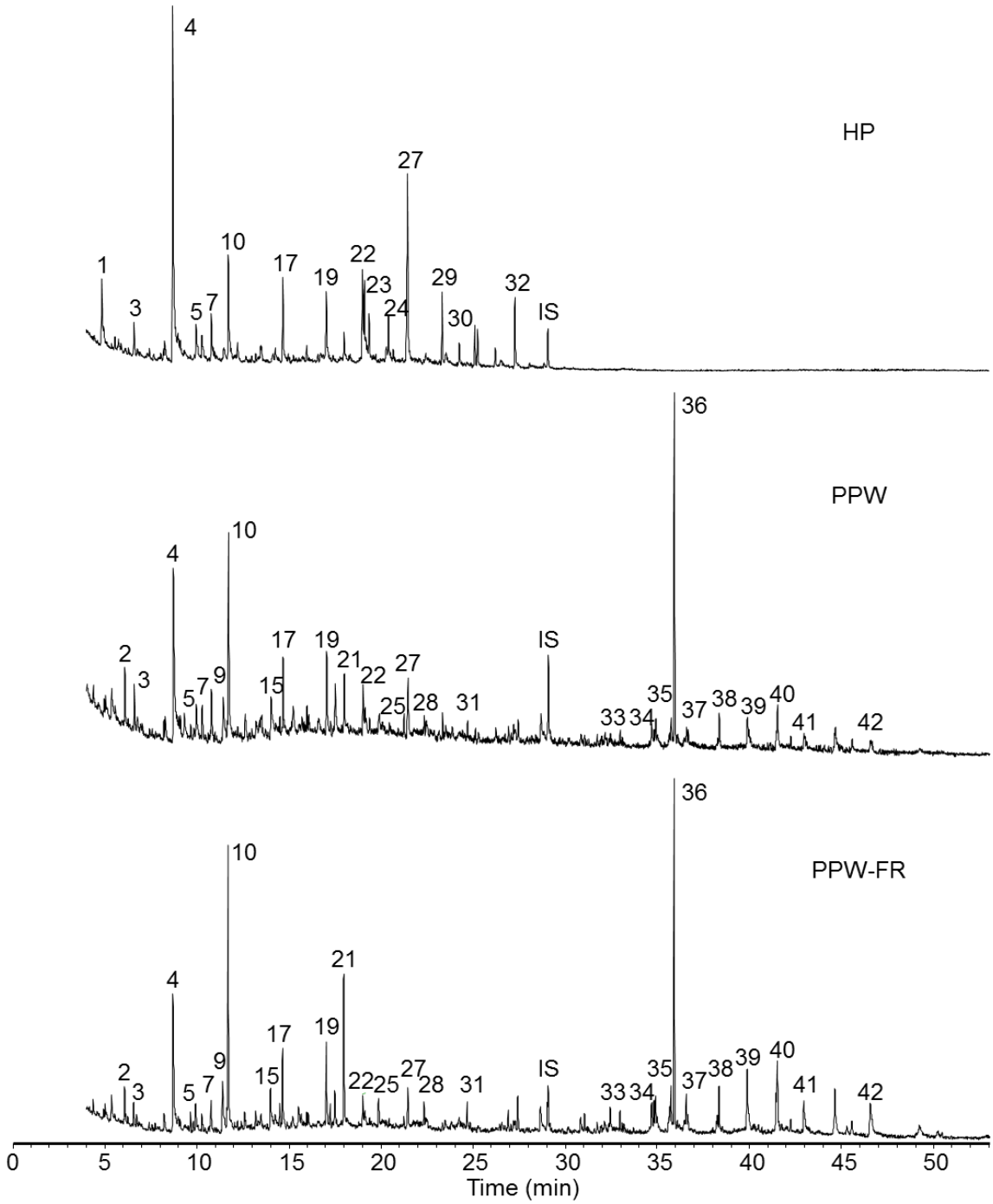

Figure 6.3 Total ion chromatograms of HP, PPW, and PPW-FR pyrolysis bio-oils WI fractions.

Table 6.3 GC-MS analysis results of HP, PPW, and PPW-FR bio-oils WI fractions.

No.	RT (min)	Compound	Formula	m/z	Conc. (% of WI bio-oils)		
					HP	PPW	PPW-FR
1	4.82	2-oxo-3-cyclopentene-1-acetaldehyde	$C_7H_8O_2$	124	3.78		
2	6.10	1,3,5,7-cyclooctatetraene	C_8H_8	104		1.93	0.76
3	6.62	2-methyl-2-cyclopenten-1-one	C_6H_8O	96	1.83	1.99	1.65
4	8.77	phenol	C_6H_6O	94	27.20	14.01	10.50
5	9.95	3-methyl-1, 2-cyclopentanedione	$C_6H_8O_2$	112	3.34	1.82	0.82
6	10.34	2,3-dimethyl-2-cyclopenten-1-one	$C_7H_{10}O$	110	1.36	1.70	0.79
7	10.84	2-methylphenol	C_7H_8O	108	3.17	2.60	2.37
8	10.88	3-phenoxy-1,2-propanediol	$C_9H_{12}O_3$	168	0.57		
9	11.41	3-methylphenol	C_7H_8O	108		3.63	3.37
10	11.74	guaiacol	$C_7H_8O_2$	124	6.68	11.77	9.84
12	12.21	2,6-dimethylphenol	$C_8H_{10}O$	122	1.09		
13	12.61	3-ethyl-2-hydroxy-2-cyclopenten-1-one	$C_7H_{10}O_2$	126		1.33	0.61
14	13.47	2,3-dimethylphenol	$C_8H_{10}O$	122	1.46		
15	14.01	3-ethylphenol	$C_8H_{10}O$	122		2.50	3.82
16	14.26	6-methylguaiacol	$C_8H_{10}O_2$	138	0.57		
17	14.72	4-methylguaiacol	$C_8H_{10}O_2$	138	5.01	3.96	2.62
18	15.99	3-ethyl-5-methylphenol	$C_9H_{12}O$	136	0.90	0.98	0.46
19	17.06	4-ethyl-guaiacol	$C_9H_{12}O_2$	152	4.99	4.79	6.01
20	17.50	indolizine	C_8H_7N	117		3.33	1.32
21	17.98	vinyl-guaiacol	$C_9H_{10}O_2$	150	1.63	3.49	7.22
22	19.06	syringol	$C_8H_{10}O_3$	154	5.89	2.71	0.86
23	19.11	eugenol	$C_{10}H_{12}O_2$	164	2.77		
24	19.36	propyl-guaiacol	$C_{10}H_{14}O_2$	166	2.25	0.61	0.34
25	19.85	10-pentadecen-1-ol	$C_{15}H_{30}O$	242		1.13	4.28
26	20.45	2-methoxy-5-propenylphenol	$C_{10}H_{12}O_2$	164	2.10	0.53	0.39
27	21.48	isoeugenol	$C_{10}H_{12}O_2$	164	14.36	3.10	5.26
28	22.33	9-tetradecenoic acid	$C_{14}H_{26}O_2$	226		0.77	0.80
29	23.32	3,4,5-trimethoxytoluene	$C_{10}H_{14}O_3$	182	3.57		
30	24.26	3,5-dimethoxyacetophenone	$C_{10}H_{12}O_3$	180	1.30		
31	24.67	1-hexadecanol	$C_{16}H_{34}O$	242		0.64	1.50
32	27.28	4-allyl-2,6-dimethoxyphenol	$C_{11}H_{14}O_3$	194	4.17		
33	32.43	pentadecanoic acid	$C_{15}H_{30}O_2$	242		0.43	1.18
34	34.80	3-nonadecene	$C_{19}H_{38}$	266		0.61	1.23
35	34.90	2-methyl-1-hexadecanol	$C_{17}H_{36}O$	258		1.16	1.82
36	35.91	oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	236		18.40	15.76
37	36.57	9-nonadene	$C_{19}H_{38}$	266		0.78	0.69
38	38.34	2-methyloctadecane	$C_{19}H_{40}$	268		1.62	2.08
39	39.87	2-methylhexadecan-1-ol	$C_{17}H_{36}O$	258		2.47	4.03
40	41.50	6-methyloctadecane	$C_{19}H_{40}$	268		3.22	2.45
41	42.94	10-heneicosene	$C_{21}H_{42}$	294		1.06	1.51
42	46.55	9-hexacosene	$C_{26}H_{52}$	364		0.90	3.66

Table 6.4 GC-MS and HPLC analyses results of HP, PPW, and PPW-FR bio-oils WS fractions.

No.	RT (min)	Compound	Formula	m/z	Conc. (% of WS bio-oils)		
					HP	PPW	PPW-FR
GC-MS							
1	4.78	furfural	$C_5H_4O_2$	96	5.21		
2	5.28	2-furfuryl alcohol	$C_5H_6O_2$	98		11.39	8.50
3	5.66	1-acetyloxy-2-propanon	$C_5H_8O_3$	116	2.03		
4	6.54	2-methyl-2-cyclopenten-1-one	C_6H_6O	94	1.68	3.70	3.71
5	6.71	2-furylmethylketone	$C_6H_6O_2$	110	0.46	1.83	1.68
6	6.81	butyrolactone	$C_4H_6O_2$	86	0.46		1.08
7	8.15	5-methyl-2-furaldehyde	$C_6H_6O_2$	110	0.75		
8	8.21	1-methyl-1-cyclopenten-3-one	C_6H_8O	96	1.30	4.08	3.60
9	8.67	phenol	C_6H_6O	94	30.93	10.37	7.00
10	9.91	3-methyl-1, 2-cyclopentanedione	$C_6H_8O_2$	112	6.19	6.63	5.75
12	10.25	2,3-dimethyl-2-cyclopenten-1-one	$C_7H_{10}O$	110	0.72	2.29	1.88
13	10.77	2-methylphenol	C_7H_8O	108	2.06	2.00	1.37
14	11.46	3-methylphenol	C_7H_8O	108	0.57	1.04	0.48
15	11.53	2-ethyl-3,6-dimethylpyrazine	$C_8H_{12}N_2$	136		1.21	0.50
16	11.70	guaiacol	$C_7H_8O_2$	124	5.40	9.01	14.22
17	12.67	3-ethyl-2-hydroxy-2-cyclopenten-1-one	$C_7H_{10}O_2$	126	0.60	1.74	1.64
18	14.67	methyl-guaiacol	$C_8H_{10}O_2$	138	1.95	1.53	1.84
19	15.17	2-undecenoic acid	$C_{11}H_{20}O_2$	184		0.98	0.70
20	16.65	3-methoxy-1,2-benzenediol	$C_7H_8O_3$	140	0.61		
21	17.04	4-ethylguaiacol	$C_9H_{12}O_2$	152	0.67	0.51	1.01
22	18.99	syringol	$C_8H_{10}O_3$	154	4.98	3.04	2.63
23	21.42	isoeugenol	$C_{10}H_{12}O_2$	164	2.45	1.05	1.07
24	23.35	3,4,5-trimethoxytoluene	$C_{10}H_{14}O_3$	182	0.67	0.08	0.15
HPLC							
1	18.90	levoglucosan	$C_6H_{10}O_5$	162	1.48	7.23	7.43
2	20.40	formic acid	CH_2O_2	46	4.83	5.40	5.86
3	22.20	acetic acid	$C_2H_4O_2$	60	19.85	18.73	21.17
4	25.80	propanoic acid	$C_3H_6O_2$	74	4.15	6.16	6.73

The WS fractions comprised about 65-87% of the bio-oils and the highest proportion was obtained from pyrolysis of PPW. Partitioning the WS compounds into ether, then the (ES) and (AQ) fractions helped to simplify the mixtures and identify compounds respectively by GC-MS and HPLC (Table 6.4). WS compounds are mainly derived from the decomposition of carbohydrates (Mullen and Boateng, 2011) which include acids, alcohols, ketones, aldehydes, sugars, and phenolics (Mullen and Boateng, 2011; Sipila et al., 1998).The WS fraction in PPW and PPW-FR bio-oils comprised 65-66%

carbohydrate derivatives such as acetic acid, propionic acid, formic acid, levoglucosan, and furfuryl alcohol, while about 31-32% lignin derivatives were also measured with phenol and guaiacol as the most abundant compounds, in compared with the 48% carbohydrate derivatives and 51% lignin derivatives in WS fraction of HP bio-oil. These pyrolysis WS (or AQ) fractions can be utilized by fermentation to ethanol and lipids (Lian et al., 2010).

6.4.3 ESI-MS analysis of bio-oil

The limitation of GC-MS results is only volatile and semi-volatile compounds can be analyzed (Soria and McDonald, 2012) which accounts for only portion of the bio-oil. ESI-MS has been successfully used to characterize pyrolysis bio-oil (Alsbou et al., 2014; Zhou et al., 2013) and hydrothermal treated algae (Chakraborty et al., 2012) in order to obtain the average molar mass (M_n and M_w) of bio-oil samples. Figure 6.4 shows the ESI-MS of WI and WS bio-oil samples and their molar mass distribution. The results clearly show that the bio-oils were a complex mixture of monomeric and oligomeric compounds. For the PPW and PPW-FR bio-oil WS samples the major protonated species ($[M+H]^+$) at m/z 97, 111, 125, 127, 139, 153, 163, 181 and 325 were tentatively assigned to furfural, benzenediol or methylfurfural, guaiacol, hydroxymethylfurfural, methylguauacol, ethylguauacol, levoglcuosan, coniferylalcohol, and cellobiosan, respectively (Osman et al., 2012). The HP bio-oil WS fraction contained additional $[M+H]^+$ ions, to those mentioned above, at m/z 167 and 197 which were tentatively assigned to propylguaiacol and propylsyringol, respectively. The bio-oil WI samples ESI-MS were more complex and shown to contain similar $[M+H]^+$ characteristic ions to the

WS samples. The M_n and M_w values determined by positive ion ESI-MS and are given in Table 6.5. The spectra of HP bio-oil were similar with other research on positive ion ESI-MS analysis of forest residue bio-oil (Alsbou et al., 2014), where the M_w values for WI and WS HP bio-oils were 285, and 226 g mol^{-1}, respectively. The M_w for the WI fractions of PPW and PPW-FR bio-oils were respectively, at 243 and 245 g mol^{-1}, while the M_w for the WS fractions were 211 and 224 g mol^{-1}.

Table 6.5 Molar mass data of HP, PPW, and PPW-FR bio-oil WS and WI fractions determined by ESI-MS.

Pyrolysis	WI		WS	
	M_n	M_w	M_n	M_w
HP	232	285	190	226
PPW	210	243	181	211
PPW-FR	209	245	185	224

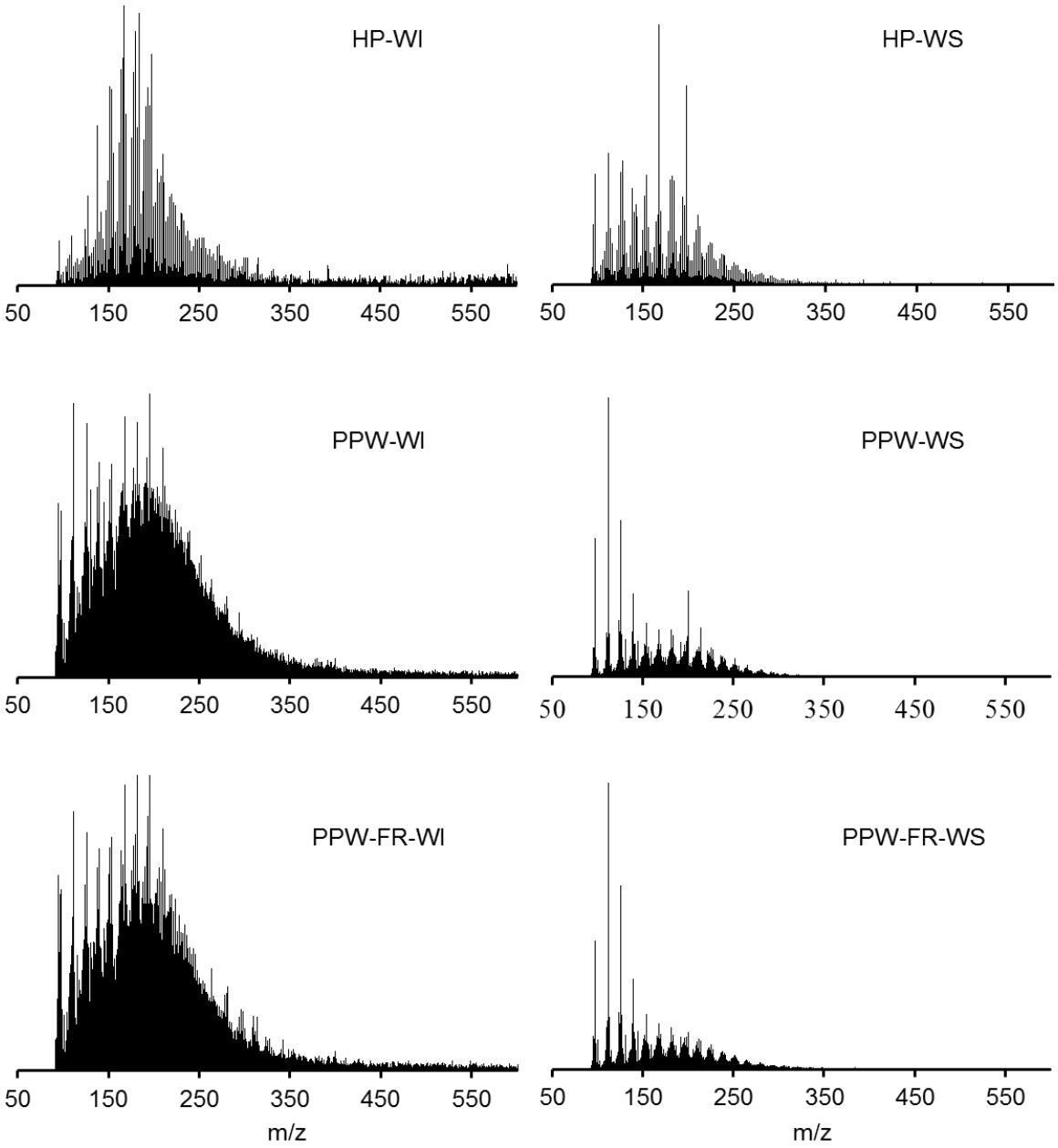

Figure 6.4 Positive ion ESI-MS of WI and WS fractions of bio-oils from pyrolysis of HP, PPW, and PPW-FR

6.4.4 Bio-char characterization

The bio-char samples were analyzed by FTIR spectroscopy (Figure 6.5), and the band

assignments are listed in Table 6.6. The H-bonded O-H stretching vibration at 3379-3337 cm^{-1} and aliphatic bands C-H (CH_3, CH_2) stretching vibration at 2962-2851 cm^{-1} were observed for all samples (Faix, 1992; Wei et al., 2013). The O-H stretching band indicates that the bio-char samples contain significant levels of hydroxyl groups. A conjugated (Hibbert's) ketone C=O band at 1699 cm^{-1} was derived from lignin demethoxylation of aromatic methoxy groups or β-O-4 cleavage (Shabaka and Nada et al., 1990) and only appeared in HP bio-char. The absorption bands between 1600-1500 cm^{-1} and 1449-1397 cm^{-1} were assigned to C-C/C=C skeletal vibrations of aromatic rings of lignin, and CH_2 scissor and aromatic ring vibration, respectively (Mattinen et al., 2008; Soria and McDonald, 2012). An absorption band of aliphatic C-H in CH_3 or phenolic OH bending (Sharma et al., 2004) was observed at 1371 and 1379 cm^{-1} for bio-char samples from pyrolysis HP and PPW-FR, respectively. The band at 1315-1308 cm^{-1} was attributed to a C-H stretching vibration. The band at 1262-1246 cm^{-1} was assigned to C-C and C-O stretching in guaiacyl units. The syringyl groups showed C-C and C-O stretching bands at 1116-1108 cm^{-1} (Faix, 1992; Soria and McDonald, 2012). Furthermore, C-O-C vibration, C-OH stretching, and C-OH and O-CH_3 stretching bands were, respectively observed at 1160, 1056, and 1033 cm^{-1}in only the HP bio-char. Bio-char produced from biomass pyrolysis is mainly comprised of polycondensed lignin structures and partial un-reacted carbohydrates. The level of lignin cross-linking can be determined by CI values. The CIs for bio-chars from HP, PPW, and PPW-FR were respectively, 0.81, 0.73, and 0.73. A higher CI value for HP bio-char was due to more of a condensed structure than that of PPW and PPW-FR (Faix, 1992), and these results were comparable to the reference data (Soria and McDonald, 2012).

Table 6.6 FTIR spectral band assignments for HP, PPW and PPW-FR bio-char samples.

Band assignment	Band frequency (cm^{-1})		
	HP	PPW	PPW-FR
O-H stretching vibration	3337	3373	3379
C-H (CH_3, CH_2) stretching vibration	2929	2919, 2851	2921, 2852
Ketone HC=O stretching vibration	1699		
Aromatic C-C/C=C stretching	1592, 1512	1563	1565,
CH_2 scissor and aromatic ring vibration	1449, 1421	1397	1434, 1401
O-H or C-H bending	1371		1379
C-H stretching	1314	1308	1315
C-C and C-O stretching in guaiacyl	1246	1256	1262
C-O stretching and H-bonded	1160		
C-OH stretching and C-H in-plane deformation in syringyl	1108	1115	1115
C-OH stretching	1056		
C-OH and O-CH_3 stretching	1033		

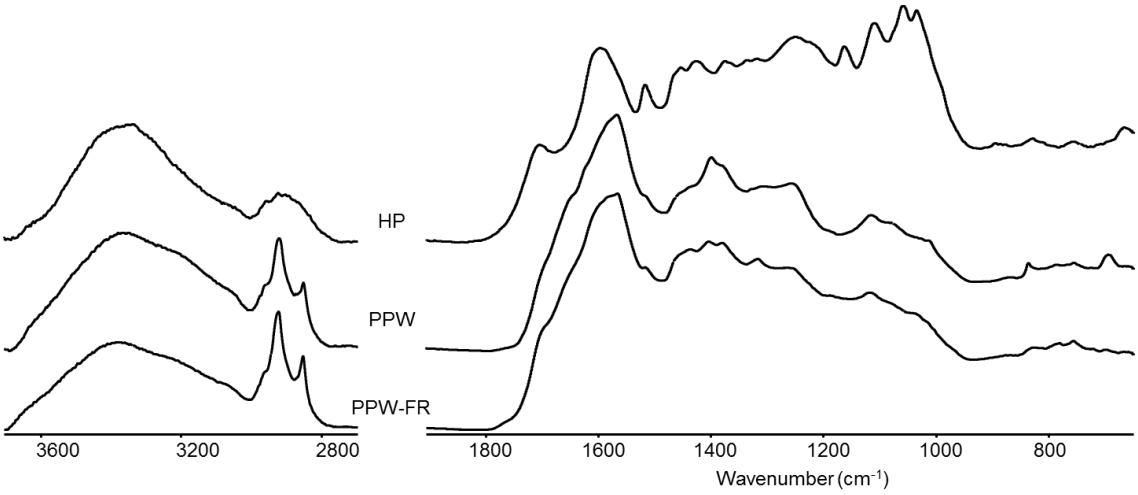

Figure 6.5 FTIR spectra of HP, PPW, and PPW-FR pyrolysis bio-char samples.

The calorific value, proximate analysis and BA measurement of bio-char samples from pyrolysis of HP, PPW, and PPW-FR were listed in Table 6.7. The calorific values for the three bio-char samples from pyrolysis of HP, PPW, and PPW-FR were respectively, 24.2, 20.3 and 23.5 MJ kg^{-1}. A high ash content of 25% was obtained in PPW bio-char, followed by 18% in PPW-FR bio-char and 5.6% in HP bio-char. The highest FC and lowest VM contents were obtained at 56 and 18% in PPW bio-char, and the lowest FC

and highest VM contents were each at 47% in HP bio-char, which were similar to proximate analyses of feedstock samples discussed earlier. The BA results of the bio-char samples from pyrolysis of HP, PPW, and PPW-FR were respectively, 0.8, 2.6 and 2.2%. Commercial activated carbon has a BA value of 21%. The PPW derived bio-char showed low BAs and this is likely attributed to a low surface area due to lower processing temperatures as compared to activated carbon. Similar results were also found by Mitchell et al. (2013) although mixed alkanes were used as adsorbents. Further work is required to improve the BA values of PPW based bio-char, such as carbonization or ozonolysis (Moreno-Pirajan and Giraldo, 2011).

Table 6.7 Calorific value, proximate analysis and BA measurement of HP, PPW and PPW-FR bio-char samples.

	HP	PPW	PPW-FR
Calorific value (MJ kg^{-1})	24.2	20.3	23.5
Ash (%)	5.6	25.4	17.6
Volatile matter (%)	47.6	18.3	34.1
Fixed carbon (%)	46.8	56.3	48.3
BA (%)	0.8	2.6	2.2

6.5 Conclusion

In this study, potato peel wastes were successfully converted to bio-oil and bio-char by pyrolysis. Results showed that the pyrolyzed PPW and PPW-FR generated lower yields of bio-oil and high yields of bio-char compared with HP. A significant amount of hydrocarbon compounds (alkanes and alkenes) in bio-oil derived from lipids and suberin of PPW and PPW-FR were found, which had similar aliphatic compounds with transport fuels. Furthermore, aromatic compounds were also produced and these can also be

upgraded to biofuels. The carbohydrate derived components in bio-oil water soluble fraction can be fermented into chemicals. The bio-chars from PPW and PPW-FR also exhibited better BA performances than HP, which exhibited potential use as an adsorbent. This preliminary study shows the potential of PPW as a feedstock for producing pyrolysis based chemicals and products.

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Chapter 7 Conclusion

7.1 Summary

Biological and thermochemical processes were successfully employed to convert potato peel waste (PPW) into value added bioproducts. Lactic acid was found as the dominant product of all anaerobic fermentations in both batch reactors and sequencing batch reactors (SBR) with undefined mixed cultures from different municipal wastewater treatment plants. The un-reacted PPW fermentation residue (PPW-FR) was also utilized for bio-oil and bio-char production with pyrolysis at 450°C.

The batch fermentation of PPW with undefined mixed cultures was studied under different pretreatment processes, enzymatic hydrolysis, temperature and solids loading conditions. The maximum yields of lactic acid, acetic acid, and ethanol were respectively, 0.22, 0.06, and 0.05 g g^{-1}, and the highest lactic acid concentration of 14.7 g L^{-1} was obtained from bioreactor with initial solid loading of 60 g L^{-1} at 35°C without additional enzymes. This batch fermentation exhibited the potential and possibility of producing lactic acid with undefined mixed cultures.

In order to further examine the suitability of lactic acid production from PPW using undefined mixed cultures, three types activated sludge samples from different municipal wastewater treatment plants across the country were used as inoculation starters to ferment PPW into predominantly lactic acid. Results showed that similar fermentation broth compositions were formed in the different fermenters. Genomics analysis of the bacterial populations by Illumina sequencing revealed that the microbial community

structures of three seed cultures were different but unique microbial community structures were formed with more than 96% of genus *Lactobacillus* in all three fermenters, as compared to <0.1% in seed cultures. The differences of *Lactobacillus* sp. among three fermented biomass samples were also observed with species-level taxonomic assignments which could be due to the differences in inocula.

A series of SBR reactors were conducted to investigate the influences of retention time and solids content to lactic acid production and the statistical results showed that decreasing retention time and solids content increased lactic acid production, and a good overall lactic acid yield and highest productivity (138.0 mg g^{-1} d^{-1}) were achieved in a 3.5 L upscale SBR reactor. A reasonable purity (91%) of aqueous lactic acid (50% w/w) was obtained through activated carbon adsorption, ion-exchange and vacuum condensation, and quantified and confirmed by HPLC and NMR.

The PPW-FR as promising alternative resource was conducted for further chemical characterization, and the results showed significant increase of lignin/suberin, C/H, and calorific value contents as compared with original PPW, which were further, verified by FTIR and NMR analyses. Thermal characterization by TGA and Py-GC/MS analyses indicated that PPW-FR required less energy for thermal conversion processes, such as pyrolysis, and more high value chemical compounds could be recovered. A lab scale pyrolysis experiment was conducted and the PPW was successfully converted to bio-oil and bio-char at 450°C. A significant amount of hydrocarbon compounds (alkanes and alkenes) in bio-oil derived from lipids and suberin of PPW-FR were found, which had similar aliphatic compounds with transport fuels. The bio-chars from PPW-FR also

exhibited better butane adsorption performances and exhibited potential use as an adsorbent. This preliminary study shows the potential of PPW-FR as a feedstock for producing pyrolysis based chemicals and products.

7.2 Further work

We have successfully demonstrated the integrated utilization of PPW into value added in laboratory. To further investigate its commercial applications, additional work to understand the mechanism of lactic acid production with undefined mixed cultures fermentation and the pilot-scale performance are necessary. The next phase of research should cover the following topics: (i) to investigate the real-time microbial community variation of fermentation process in order to understand the selection mechanism; (ii) to upscale reactor to 100 gallon and evaluate the lactic acid production in a longer term and develop integrated extraction and recovery methods; (iii) to assess different conversion processes to bioproducts such as anaerobic digestion and liquefaction to utilize the PPW-FR and compare the energy and economic advantages and disadvantages to pyrolysis; and (iv) to develop an integrated, realistic and economic feasible solution for PPW utilization.