EFFECT OF ETHANOL ORGANOSOLV PRETREATMENT ON SPENT COFFEE GROUNDS

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A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Chemical Engineering

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

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ABSTRACT

Lignocellulosic biomass is widely investigated as a sustainable feedstock to produce biofuels such as bioethanol. Spent coffee grounds (SCG) is discovered as a potential lignocellulosic biomass for bioethanol production due to its high hemicellulose and cellulose content. However, a prerequisite pretreatment step is necessary to remove the recalcitrance structure of biomass that hinders microbial and enzymatic attacks. In current study, SCG was subjected to aqueous ethanol-based organosolv pretreatment with sulfuric acid as catalyst. A parametric study emphasizing on four factors was conducted which included the solvent loading (10 – 17.5 % v/w), catalyst concentration (0 – 3 % v/v), reaction temperature (180 – 210 °C) and residence time (30 – 90 minutes). The purposes of this study are to examine the effects of various operating parameters in organosolv pretreatment and determine the most promising pretreatment condition. SEM, XRD, FTIR and TGA profiles of the pretreated SCG were analysed to study on the impacts brought by pretreatment process in terms of composition, morphological and structural changes, crystallinity, functional groups and thermal stability as compared with native SCG. The chemical composition of spent coffee grounds comprised 28.64 % alpha cellulose, 9.5 % beta cellulose, 36.17 % gamma cellulose and 25.69 % lignin. Organosolv pretreatment with solvent loading of 12.50 % v/w, 1 % v/v sulfuric acid concentration, at 190 °C for 60 minutes had optimum performance in terms of solid yield (75.9 %), alpha cellulose and beta cellulose (80.3 %) recovery, hemicellulose (66.5 %) removal and considerably delignification effect (76.3 %).

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α	alpha
β	beta
θ	theta
λ	wavelength
C	carbon
$CaCO_3$	calcium carbonate
Cl	chlorine
CS	coffee silverskin
Cu	copper
EOL	ethanol organosolv lignin
EU	European Union
FTIR	fourier transform infrared spectroscopy
HCl	hydrochloric acid
HMF	hydroxyl methyl furfural
HPLC	high performance liquid chromatography
H_2SO_4	sulfuric acid
LCB	lignocellulosic biomass
LSR	liquor to solid ratio
Mg	magnesium
NaOH	sodium hydroxide
NH_3	ammonia
O	oxygen
S	sulfur
SCG	spent coffee grounds
SEM	scanning electron microscopy
SHF	separate hydrolysis and fermentation
SSF	simultaneous saccharification and fermentation
TGA	thermogravimetric analysis
XRD	x-ray diffraction

CHAPTER 1

INTRODUCTION

1.1 Overview in Energy Consumption

Since the industrial revolution, energy and sustainable development has been a complex issue for mankind. Global energy consumption has been rising progressively over the years in line with the increment in world population as well as the development of the countries. With the significant grow in energy demand, its security and shortage issues have been considered as the utmost concern globally.

The four main energy end-use sectors can be classified as industrial, transportation, residential and commercial. According to International Energy Outlook 2018 announced by Energy information Administration in US, the total energy consumption in the four sectors from 1949 to February 2018 was recorded and shown in Figure 1.1. Based on Figure 1.1, the industrial sector had been the largest energy consumer since 1949 followed by transportation and residential. The commercial sector consumed the least amount of energy.

Undeniably, fossil fuels play a vital role in global energy production. Combustion of fossil fuels release huge amounts of energy, committing up to 80 % of the total energy supply, thus making it the largest source of energy currently (Zabed et al., 2016a). Since the formation of fossil fuels takes billions of years, most fossils fuels for example oil, natural gas and coal are treated as non-renewable resources which are resources that are unable to be replenished shortly once consumed. Hence, usage of this non-renewable resource is not sustainable in the long run.

There are few major concerns with the continuous usage of non-renewable resources such as fossil fuels. First of all, the supply of fossil fuels are finite. Due to the rapid expansion and development in industrialization globally, the supply is unable to support the immense demand of all kinds of fossil fuels, leading to rapid depletion of these fuels. Based on certain researches, the finite reserves of fossil fuels were estimated to be depleted within 65 years. In addition, burning of fossil fuels like oil and coal will incur negative impact to the environment. Fossil fuels combustion generates enormous amount of carbon dioxide and increasing in emission of greenhouse gases which results in climate change, rise of sea levels and global warming (Zabed et al., 2016a). Furthermore, huge fluctuation in fossil fuels prices

today causes economic recession, especially in certain developing countries, which leads to global and international conflict (Huang, Zhou and Lin, 2012).

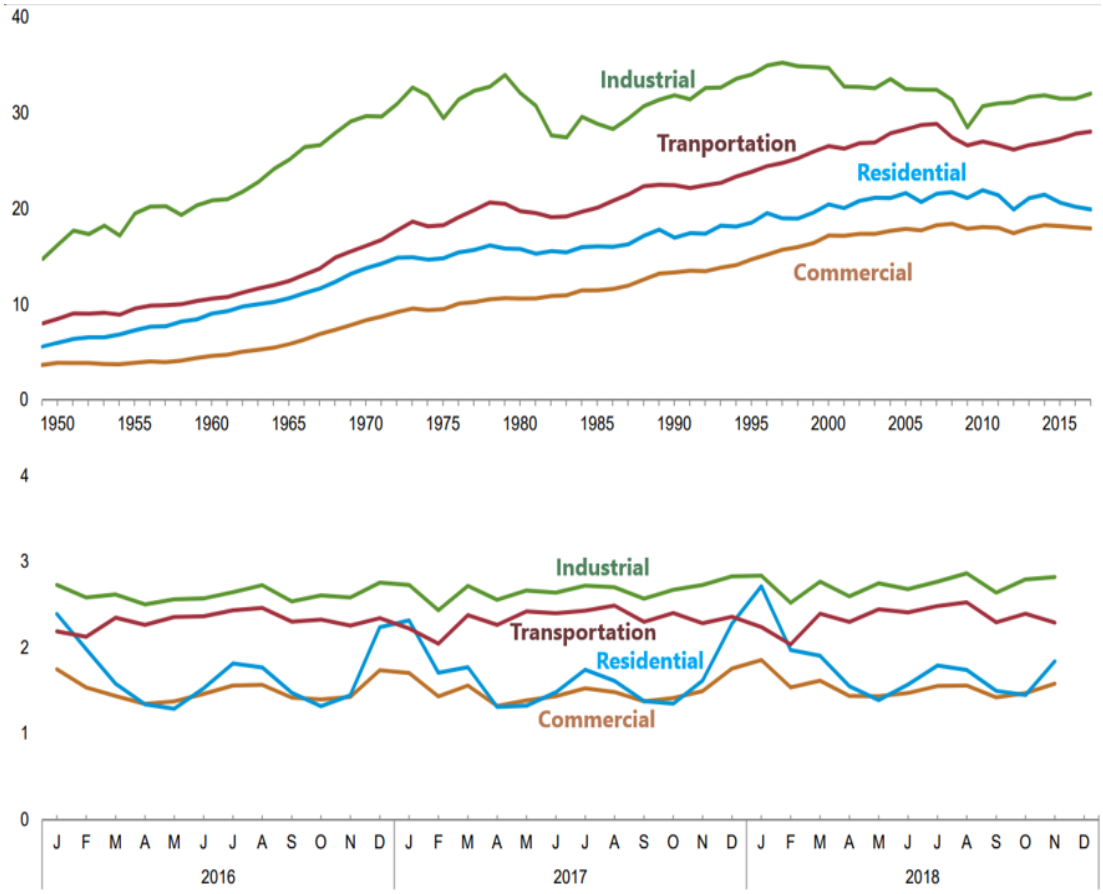

Figure 1.1: World Energy Consumption by End-used Sectors (U.S. Energy Information Administration, 2018)

As a consequence, the intention of discovering a sustainable, economical and renewable alternative energy sources with lesser greenhouse gases emission has become increasingly important in order to fulfil the demand of energy and reduce the environmental issue (Zabed et al., 2016a). Many renewable energy resources such as wind energy, nuclear energy, solar energy, bioethanol and biodiesel are still being explored to deal with the excessive usage of fossil fuels. Among several of renewable energy resources, biofuel is one of the alternative energy resources that acts as a potential candidate to substitute the utilization of fossil fuels as energy source.

1.2 Introduction of Bioethanol

The demand for biofuel has increased dramatically in recent times as it is sought as a new key solution to replace commercial petroleum-based fossil fuel and to counter its depletion problem. Biofuel refers to any hydrocarbon fuels generated in a short period of time from organic matter. It has almost similar working principle compares to conventional fossil fuels. However, it is defined as a renewable energy resource as it uses biomass for combustion. It consists of three primary types of biofuels: bioethanol, biodiesel and biogas (Kumari and Singh, 2018).

Recently, biomass to bioethanol production has drawn much attention as an eco-friendly alternative for fossil fuels. It contributes greatly in reducing the usage of fossil-based energy sources as well as greenhouse effects such as global warming that have become the main crisis nowadays. It had been classified as a clean alternative fuel source that can be renewed since the combustion of bioethanol does not emit additional carbon dioxide to the atmosphere (Zata Lini et al., 2017).

Ethanol has possessed a potential as a valuable substitute of gasoline in the transport fuel market. Bioethanol can be blended with fossil fuel like gasoline in engine to improve the combustion performance due to its higher-octane number, lower cetane number and higher heat of vaporization than gasoline. The amount of gasoline used in vehicles can be reduced up to 10 – 20 % if ethanol-blended gasoline is used (Park et al., 2010). Up to 10 v/v % ethanol is allowed to be blended with the gasoline without any car engines modification. On the other hand, ethanol blend up to 85 v/v % ethanol in mixtures with gasoline can be used in flexible fuel vehicles (FFV) and 100 v/v % ethanol is feasible to be applied on engines that are specifically designed (Morales et al., 2015).

Bioethanol has been evolving over time and can be classified into four groups which are the first generation, second generation, third generation and fourth generation biofuels based on the types of feedstock used in bioethanol production. Table 1.1 presents the descriptions of biofuel for four different generations.

Table 1.1: Types of Biofuel

Types of Biofuel	Descriptions	References
First Generation	Also known as conventional biofuels. Mostly generated from food or agriculture crops such as sugarcane, sweet potato, corn, palm oil etc. Derived from sugar, starch, animal or vegetable oil.	(Viesturs and Melece, 2014)
Second Generation	Often called "advanced biofuels". Produced from sustainable feedstock such as agricultural wastes, municipal solid wastes or food wastes that rich in lignocellulosic material such as rice straw and coconut husk. Non-edible feedstock.	(Naik et al., 2010)
Third Generation	Derived from microalgae and cyanobacteria feedstock. Feedstock has higher biomass productivity over plant-based biofuels.	(Viesturs and Melece, 2014)
Fourth Generation	Mostly produced from photo biological solar fuels and electro-fuels using solar energy by algae and cyanobacteria.	(Kumari and Singh, 2018)

Biomass that comprising of free fermentable sugars or complex carbohydrate that are fermentable after transforming into soluble sugars can be used as feedstock for bioethanol production (Zabed et al., 2016b). Starchy crops, sugar crops and lignocellulosic biomass (LCB) are the three main categories of the feedstock used in current bioethanol production. They can be differentiated by different extractions of the sugar solution from the feedstock before undergoing fermentation (Zabed et al., 2016a).

Feedstock that consists of high sugar content and rich in starch are mostly derived from edible sources such as corn and sugarcane. Ethanol that produced from edible feedstock is defined as first generation bioethanol. Edible sources are frequently used in bioethanol production as the process is comparatively simple. However,

production of first generation bioethanol appears to be unsustainable and has triggered the food insecurity issue which resulted in the "Food versus Fuel" dilemma. Additionally, it leads to increase in food prices globally and hence constitutes the biggest drawback of using first generation bioethanol (Naik et al., 2010).

Due to this reason, many researches have been carried out on developing commercial processes for second generation bioethanol production using lignocellulosic biomass, which are mainly from crop or food residues (Zabed et al., 2016b). Lignocellulosic bioethanol has been discovered to contribute lower impacts and proposed a positive energy balance in contrast with first generation bioethanol as well as gasoline. Table 1.2 shows the comparison between first and second generation bioethanol in terms of their advantages and drawbacks.

Table 1.2: Pros and Cons of First and Second Generation Bioethanol (Derman et al., 2018)

Types of Bioethanol	Advantages	Disadvantages
First Generation	Straightforward conversion process.	Results in "Food vs. Fuel" debate. Environmental degradation. Destruction of tropical forests.
Second Generation	Net greenhouse gases emission is lower. Energy and food security. Cheap and abundant feedstock. Environmentally friendly. Non-competitive with food.	Recalcitrant characteristics of the feedstock. Cost ineffective.

1.2.1 Lignocellulosic Feedstock

Recently, the utilization of lignocellulosic biomass (LCB) in bioethanol production had been steadily growing. Due to its high availability and cost effectiveness of the feedstock, LCB has been recognized as the most promising feedstock in terms of

economy as well as environmental friendliness (Sidiras and Salapa, 2015). The sources of LCB can be classified into four main groups which are hardwood, softwood, agricultural wastes and grasses. Lignocellulosic wastes from agriculture and food processing such as sugarcane bagasse and food wastes are the feedstock that are well-known for the production in second generation bioethanol (Hassan, Williams and Jaiswal, 2018). Figure 1.2 shows the variety sources of lignocellulosic biomass including examples.

Lignocellulosic biomass is primary composed of lignin, hemicellulose and cellulose. Generally, lignocellulosic biomass consist of 15 – 20 % of lignin, 25 – 30 % of hemicellulose and 40 – 50 % of cellulose (Tayyab, 2017). Cellulose is a branchless and crystalline homopolymer of glucose units whereas hemicellulose is a highly branched heteropolymer that consists of different monomers. Cellulose and hemicellulose are known as polysaccharides which form fermentable sugars through fermentation process. Unlike cellulose and hemicellulose, lignin is not a sugar unit. It is a complex aromatic polymer that deeply associated to hemicellulose and cellulose in the cell wall to form lignocellulosic complex (Kumari and Singh, 2018).

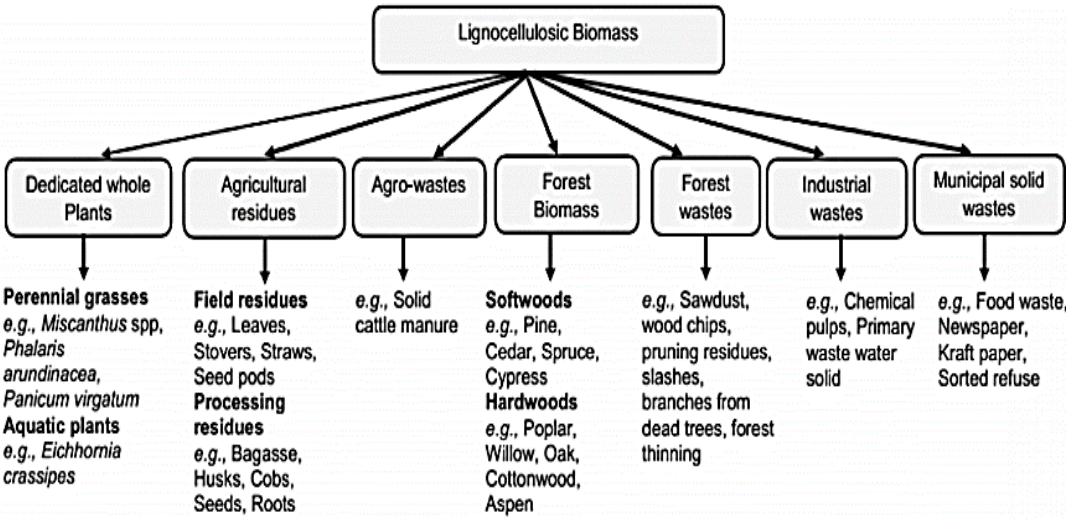

Figure 1.2: Sources of Lignocellulosic Biomass (Zabed et al., 2016b)

Lignocellulosic contents in biomass will differ with its sources as well as the physical properties of the biomass used. The variations in lignocellulosic contents have inspired more researches being devoted to investigate the lignin structure of different biomass. Based on the research, cereal residues like corn stover and rice straw will have higher lignocellulosic contents than grasses, fruit and vegetable residues.

Biomass with higher lignocellulose content are more preferable as feedstock for biorefineries as higher conversion of the biomass to usable fuels can be obtained (Hassan, Williams and Jaiswal, 2018).

1.3 Pretreatment of Lignocellulosic Biomass

Due to the complex hierarchical structure of lignocellulosic biomass along with the presence of lignin, it acts as a barrier in bioethanol production from LCB. The pretreatment step helps to degrade lignin by altering the lignocellulose structure, reinforce in cellulose recovery and ease of enzymatic hydrolysis which in turns provide higher glucose yield. Through the pretreatment process, the surface area and porosity of the biomass will be increased as well (Ebrahimi et al., 2017). Figure 1.3 illustrates the impact of pretreatment of lignocellulosic biomass.

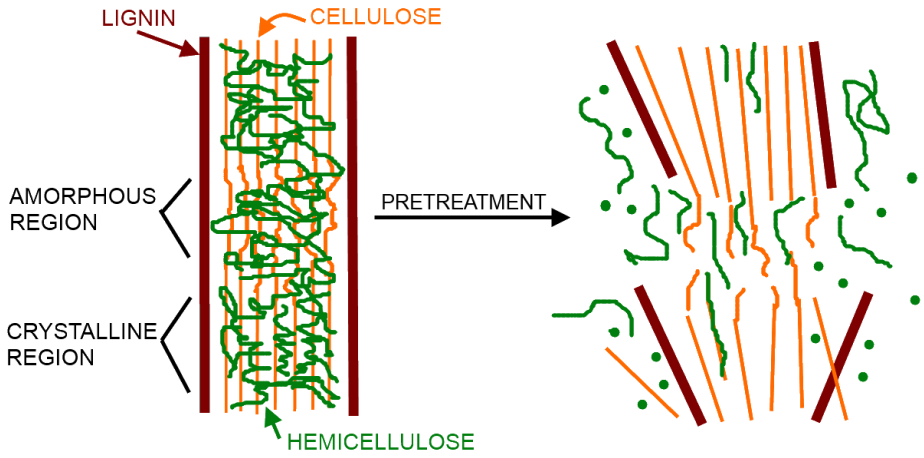

Figure 1.3: Impact of Pretreatment of Lignocellulosic Biomass (Zheng et al., 2014)

Pretreatment methods can be characterized into four main types which are biological, chemical, physical and physicochemical pretreatments. Each method of pretreatment affects differently on the lignocellulosic structures and none of the methods can be classified as standard due to each method possessing their own benefits and drawbacks (Seidl and Goulart, 2016). A suitable pretreatment method can be selected based on several criteria. First of all, the size reduction of biomass particles should be prevented. Secondly, the selected method must be able to preserve the hemicellulose fraction of the feedstock. Thirdly, the degradation in products and energy demands for the process must be minimized. Lastly, the selected method should apply a cost effective pretreatment catalyst or catalyst recycle system and regenerate

high value of lignin co-product (Kumar and Sharma, 2017). Figure 1.4 displays an overview of various pretreatment processes.

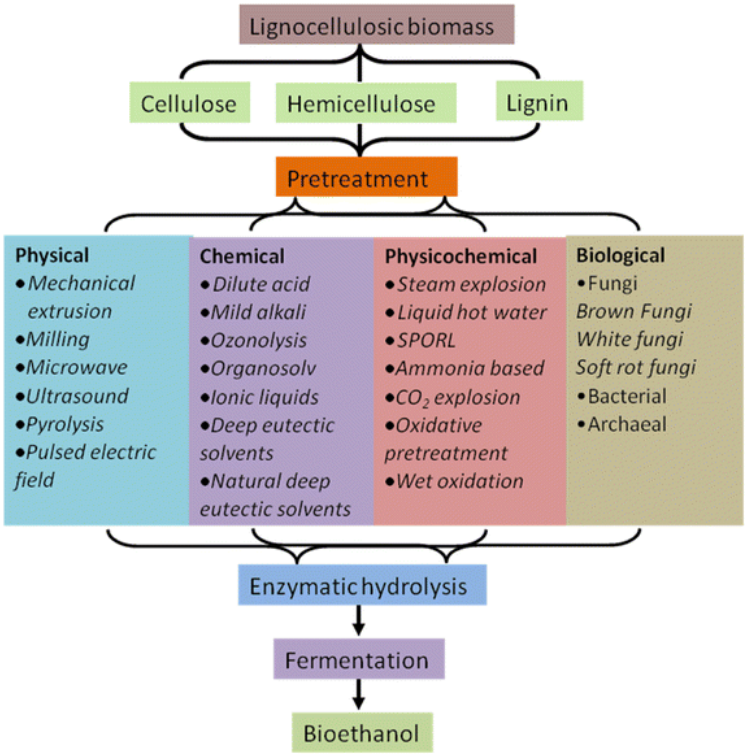

Figure 1.4: Overview of Various Pretreatment Processes (Kumar and Sharma, 2017)

1.3.1 Organosolv Pretreatment

Among the pretreatment methods, organosolv pretreatment under chemical methods is one of the most promising delignification methods that is widely applied. Organosolv pretreatment process is conducted by the addition of aqueous organic solvents to the biomass under particular pressure and temperature. Examples of common aqueous organic solvents are methanol, ethanol, glycerol, ethylene glycol and acetic acid. Among these organic solvents, ethanol organosolv pretreatment has been well-studied due to its volatility promotes extensively recycle, acts as a renewable solvent and more cost effective (Wildschut et al., 2013).

Organosolv pretreatment process can be conducted with or without the presence of catalyst which can be either organic or inorganic acid (eg. HCl and H_2SO_4), bases (eg. NaOH, NH_3 and $CaCO_3$) or salt catalyst (Widjaja et al., 2016). Strong mineral acids such as sulfuric acid is frequently used as reagent for this process because of their great efficiency and high reactivity. On the other hand, strong base

such as sodium hydroxide is the primary choice for most of the industries as it possessed a high dissolubility (Park et al., 2010).

The goals of this pretreatment are to increase the cellulose, and effectively diminish the hemicellulose or lignin content to degrade the complex crystalline structure of lignocellulosic biomass. The lignin degradation is more effective under condition of high solvent concentration. Small parts of hemicellulose will be dissolved and therefore increase the cellulose content (Widjaja et al., 2016). The pros and cons of organosolv pretreatment method are shown in Table 1.3.

Table 1.3: Pros and Cons of Organosolv Pretreatment (Widjaja et al., 2016; Zabed et al., 2016b)

Advantages	Disadvantages
Capable in producing high quality and relatively pure lignin as by-product.	Formation of inhibitors might occur at high temperature in presence of acidic catalyst.
Low energy consumption.	High energy recovery consumption.
High pretreated yield.	
Relatively low in cellulose and sugar degradation.	Large amount of solvent is required.
Easy recovery of solvents by distillation.	
Low Environmental impact.	

1.4 Problem Statement

The utilization of the bioethanol from lignocellulosic biomass is dramatically growing globally due to the depletion issues on non-renewable resources such as fossil fuel and rapid increment in energy consumption.

However, the production of bioethanol from lignocellulosic biomass is a relatively complicated conversion process due to the highly recalcitrant nature of the lignocellulosic network that hinders the accessibility of biomacromolecules for downstream processes (Salapa, Topakas and Sidiras, 2018). The major drawbacks of using lignocellulosic biomass in bioethanol production are the requirement of energy consumption in the pretreatment process, complicated overall conversion steps, extreme difference in the nature and composition between each biomass and generation of inhibitors.

The spent coffee grounds (SCG) is chosen as the potential lignocellulosic biomass for bioethanol production. Coffee is considered as one of the most favoured beverages globally. However, majority of the non-edible coffee residues will usually end up in disposal to a landfill after brewing, resulting in environmental and ecological problems. Therefore, the feasibility of using spent coffee waste as feedstock for bioethanol production was analysed in this study in order to recycle municipal waste and greatly utilized it as renewable source of energy.

As the pretreatment process is a very costly step compared to the other processes in bioethanol production, a stringent optimization on the pretreatment conditions plays a crucial role in bioethanol production to improve the cost effectiveness of these steps (Goh et al., 2011). There are several parameters that will significantly affect the output results of organosolv pretreatment and the interaction effects between these parameters will increase the complexity for optimizing the pretreatment process. Several common parameters for the process are operating temperature, residence time, solvent concentration, solvent loading, presence of catalyst, type of catalyst used and catalyst concentration.

As a result, a parametric study was performed to analyse on the influence of parameters on organosolv pretreatment process and to obtain the most promising conditions for organosolv pretreatment process.

1.5 Scope of the Study

This project report will be predominantly emphasized on the ethanol-based organosolv pretreatment of spent coffee grounds (SCG). A parametric study will be performed to gain insight in the effects of pretreatment parameters such as the ethanol solvent loading, sulfuric acid concentration, reaction temperature and reaction duration on the SCG. The most optimum pretreatment condition of the ethanol pretreatment on spent coffee grounds will be determined in this study and the effectiveness of process will be evaluated based on the solid yield, delignification, cellulose recovery and hemicellulose removal after the pretreatment process. Additionally, characterisation equipment including Fourier Transform Infra-Red Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Thermal gravimetric analysis (TGA) and X-ray Diffractometer (XRD) will be employed to give fundamental insight into the impacts that contributed by organosolv pretreatment process on SCG.

1.6 Aims and Objectives

This research project focuses on investigate the pretreatment of biomass waste from spent coffee grounds for bioethanol production. The aims and objectives of this current study include:

(i) To analyse the effects of ethanol solvent in organosolv pretreatment using spent coffee grounds.

(ii) To identify the optimum conditions of pretreatment parameters (Solvent loading, catalyst concentration, temperature and time).

(iii) To characterize the chemical and physical properties of the spent coffee grounds and its products after pretreatment.

1.7 Contribution of the Study

This study provides a fundamental insight in the effects of pretreatment parameters such as the ethanol solvent loading, sulfuric acid concentration, reaction temperature and reaction duration on the SCG. The optimum pretreatment condition could enhanced the cost effectiveness in bioethanol production and hence increased the demand for alternative renewable transportation fuel. Therefore, the dependency of fossil fuel able to reduce and slower down its depletion rate.

1.8 Outline of the Report

In this report, a comprehensive literature review on organosolv pretreatment was extensively discussed in Chapter 2. Chapter 3 elaborated and expounded on the methodology of the experiments conducted for pretreatment. Subsequently, the pretreatment results for each parameter study were investigated and discussed in Chapter 4. Lastly, the achievements and fulfilments of this were concluded and recommendations were made to improve the study in the future work.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Spent Coffee Grounds

Coffee is a famous beverage consumed around the world especially in the EU. The market demand for coffee has grown rapidly in recent years and it has evolved as the most traded goods other than petroleum. According to the data collected, the average annual coffee consumption in United States was estimated as 1.48 million tons per year (Liu et al., 2017). However, there were around 9.4 million tons of average annual global coffee production as stated by the International Coffee Organization (Ravindran et al., 2018).

Spent coffee grounds (SCG) is the unwanted solid residue after coffee brewing where the coffee powder was treated with hot water or steam for the instant coffee preparation. The figure of SCG is shown in Figure 2.1 below. More than 50 % of the fruit mass of residue was generated in the coffee production. It usually treated as industrial waste and ended up being disposed by landfill or composting as it has no commercial value. However, this disposal method is not recommended as it leads to environmental and ecological impacts such as increase in greenhouse gases in atmosphere and disrupting the soil ecosystem. According to Liu et al. (2017), the coffee production process possessed a momentous waste generation rate as approximately 0.91 g of SCG could be obtained from 1 g of coffee produced. With the increment in global coffee production annually, a large amount of SCG will be produced and may cause a severe issue on the treatment as well as disposal of SCG. Hence, a proper waste management plan that conforms to existing national regulations is required to handle this huge amount of SCG generated.

There are several ways to utilize the spent coffee grounds such as composting and gardening. In addition, research has been performed to investigate the feasibility of using spent coffee grounds as the source of biomass in bioethanol production in order to reduce the waste generation from coffee production.

Figure 2.1: Spent Coffee Grounds (Amoretti, n.d.)

2.1.1 Spent Coffee Grounds as Lignocellulosic Feedstock

Due to the rarity and depletion of fossil fuels, it is essential to discover renewable energy sources to fulfil the high energy requirements and overcome the fossil energy problems. As a result, bioethanol produced from biomass is being sought as one of the alternative energies to overcome this crisis and thus lignocellulosic biomass has been identified as a very promising feedstock in the future. Recently, bioethanol production by utilizing spent coffee grounds (SCG) as a sustainable lignocellulosic feedstock for waste reduction has seen an increased interest.

SCG is known as lignocellulosic material made up of polysaccharide polymers and phenolic polymers. It contains a huge amounts of organic compounds like lignin, cellulose, hemicellulose, fatty acids, protein, lipids and other polysaccharides (Ravindran et al., 2018). Unlike other lignocellulosic feedstocks, SCG contains more hemicellulose content in contrasted to cellulose content in its polysaccharide fraction. Due to the heterogeneous nature of hemicellulose, other than glucose, SCG is also abundant with monosaccharides for instance mannose, arabinose and galactose. Hence, it is applicable for a value-added product such as crude enzyme, bioethanol, monomeric sugars, sorbent materials and activated carbon. Furthermore, the large amounts of phenolic compounds in SCG with antioxidant activity also enables it to be a viable feedstock for natural antioxidants recovery (Ravindran et al., 2018).

2.2 Chemical Composition and Structure of Lignocellulosic Feedstock

Lignocellulosic biomass is mainly composed of lignin, hemicellulose, cellulose, protein and ash. Lignin, hemicellulose and cellulose are known as the three basic constituents of lignocellulosic biomass. Generally, most of the lignocellulosic materials consist 40 – 50 % cellulose, 25 – 30 % hemicellulose, 15 – 20 % lignin and remaining are the others composition (Tayyab, 2017). Table 2.1 shows the cellulose, hemicellulose and lignin contents in several examples of lignocellulosic biomass.

Table 2.1: The Cellulose, Hemicellulose and Lignin Contents in Several Lignocellulosic Biomass (Bajpai, 2016)

Type of lignocellulosic biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods	40 – 55	24 – 40	18 – 25
Softwoods	45 – 50	25 – 35	25 – 35
Corn Cobs	45	35	15
Wheat Straw	30	50	15
Switch Grass	45	31.4	12
Grasses	25 – 40	35 – 50	10 – 30

Cellulose is the crucial component which providing structural support in the cell wall of a plant. It is a linear, homopolymer of repeating units of glucose called β-D-glucopyranose moieties that connected together by $\beta - 1, 4$ glycosidic bonds. The cellulose chains, which have around 20 – 300 units, are packed into microfibrils and the long-chain of linear cellulose polymers with around 10,000 glucose units are grouped together to form cellulose fibers. As shown in Figure 2.2, the presence of van der Walls forces and hydrogen bonds in between the long chain of cellulose polymers packed the cellulose into microfibrils and arranged them in a parallel and orderly (crystalline) structure (Bajpai, 2016).

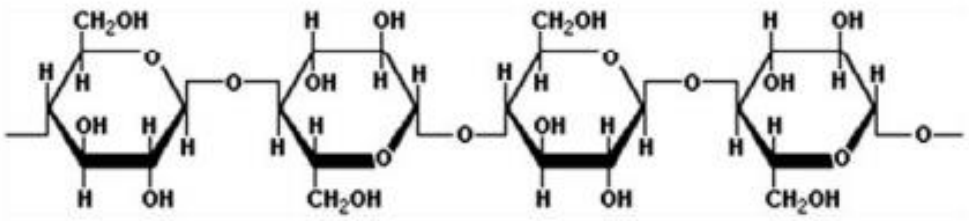

Figure 2.2: Cellulose Structure (Bajpai, 2016)

Hemicellulose is a short, linear and severely branched heteropolymer that consists of several monomers. It considered as the second most numerous polymers in lignocellulose after cellulose. Hexoses and pentoses are the main monomers found in hemicellulose. The examples of hexoses include α-D–galactose, β-D–glucose and β-D–mannose while for pentoses are α-L–arabinose and β-D–xylose respectively. Besides, α-D–galacturonic, α-D–glucuronic and α-D-4-O-methylgalacturonic acid are examples of sugar acids (also known as uronic acid) which appeared in hemicellulose as well. Glucomannans and xylans are the two well-known hemicelluloses. The extraction of xylan is much easier in an acid or alkali environment while for glucomannan, a highly alkali environment is required for extraction to be done (Bajpai, 2016). For hardwoods, agricultural residues and forest, municipal and industrial wastes, hemicellulose are mostly rich in xylans. For softwood, glucomannans will be the major hemicelluloses. In contrast to cellulose, hemicellulose has smaller chain size with around 50–300 units of sugar monomers and its highly branched nature in the main chain molecules introduced an amorphous structure and rendered it to exhibit poor resistance to chemicals (Zabed et al., 2016b). It acts as a 'coat' for cellulose-fibrils and therefore at least half of hemicellulose present in the biomass should be eliminated in order to enhance the cellulose digestibility (Bajpai, 2016). Figure 2.3 shows the hemicellulose structure.

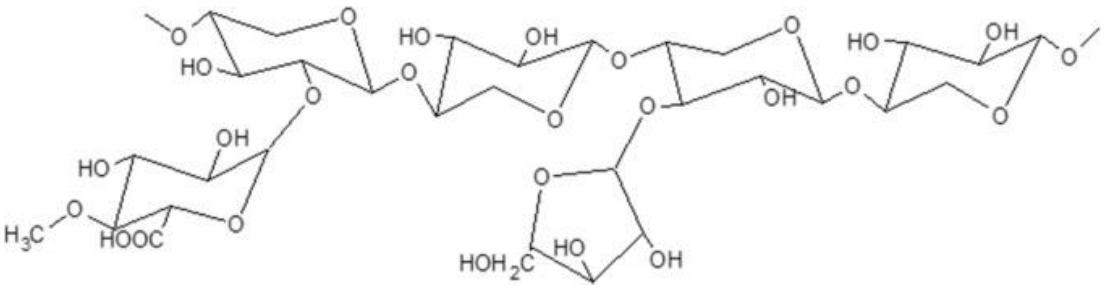

Figure 2.3: Structure of Hemicellulose (Kumari and Singh, 2018)

Lignin is known as a mononuclear aromatic polymer which is greatly branched and found in the cell walls, especially woody biomass. It is normally severely bounded with cellulose fibers to form complex lignocellulosic structure. The monomers of lignin are composed of three phenyl propionic compounds of phenyl alcohols which are the alcohols ρ-coniferyl, sinapyl.and coumaryl. It possesses hydrophobic nature to reduce water permeability to xylem vessels and it is tightly associated with cellulose and hemicellulose polymers. Lignin plays a role in providing cohesion and rigidness to the cell wall. It also protects polymers from microbial attack by forming a physico-chemical barrier (Kumari and Singh, 2018). Woody biomass mainly built up by lignin and cellulose polymers. For instance, softwood barks contains the highest lignin content followed by hardwood barks and lastly grasses and agricultural residues which are 30 – 60 %, 30 – 55 %, 10 – 30 % and 3 – 15 %, respectively (Zabed et al., 2016b). In Figure 2.4, it shows the structure of dominant lignin monomer in the biomass.

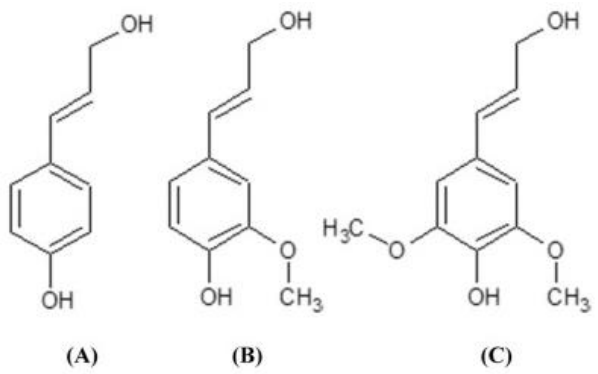

Figure 2.4: Structure of Dominant Lignin Monomer: (A) ρ-Coumaryl, (B) Coniferyl and (C) Sinapyl (Kumari and Singh, 2018)

2.2.1 Chemical Composition of Spent Coffee Grounds

As the spent coffee grounds (SCG) is recognized as lignocellulosic biomass, it is composed primarily of lignin and complex carbohydrates as well. Cellulose and hemicellulose are the two forms of polymerised sugars which constituted for the carbohydrate fraction of the SCG. As mentioned in Section 2.2, cellulose is a homopolymer of glucose that contains highly repeating units while hemicellulose is a greatly branched heteropolymer that composed mainly of galactose, mannose and arabinose. Unlike other lignocellulosic biomass, xylose is normally absent in spent

coffee grounds. SCG are rich in galactomannan and glucomannan due to the high hemicellulose content (Ravindran et al., 2018).

SCG consisted of a total lignin content around 23.15 ± 0.4 g per 100 g of SCG. According to this total lignin content, there were up to 17.50 ± 0.6 g per 100 g of SCG which contributed to insoluble lignin portion and the rest of 5.65 ± 0.4 g per 100 g of SCG were found to be soluble fraction. Besides carbohydrates and lignin, there was also a small percent of the total SCG weight committed to its lipid content which is around 13.4 %. However, this lipid content may be varied according to the type of coffee bean, the solvent applied and the number of reflux for lipid extraction and contributed up to the difference within the range of 10 to 20 % (Ravindran et al., 2018). The lipid content was estimated to fall approximately within the range of 12.29 – 14.88 % of total weight of SCG (Ahangari and Sargolzaei, 2013). The SCG also presented a high moisture content of around 56.7 % of the total weight of SCG in wet basis (Corrêa et al., 2014). The chemical composition (carbohydrates and lignin content) of the spent coffee grounds on a dry weight basis is shown in Table 2.2.

Table 2.2: Chemical Composition of Spent Coffee Grounds (% Dry Basis) (Hassan, Williams and Jaiswal, 2018)

Chemical Composition of SCG	Biomass on dry weight basis (% dry basis)
Cellulose	33.10
Hemicellulose	30.03
Lignin	24.52

2.3 Overview of the Bioethanol Production Processes

Lignocellulosic biomass has to undergo three major operations for bioethanol production which are biomass pretreatment, hydrolysis and reducing sugar fermentation process.

2.3.1 Pretreatment Process

The recalcitrant characteristics of lignocellulosic biomass (LCB) reduced the cellulose reactivity towards acid and enzymatic hydrolysis significantly. In order to enhance the enzyme accessibility, LCB is necessary to undergo a prerequisite pretreatment process prior to effective enzymatic hydrolysis and subsequent fermentation for bioethanol

production to remove the complex hierarchical structure of lignocellulosic biomass, increase the accessibility of the biomass for subsequent chemical or biological treatment and hence improve the rate and yield of reducing sugars. The complicated and recalcitrant structure of lignocellulosic biomass would be altered by lignin redistribution in the cell wall and decreased the degree of cellulose polymerization to gain fraction of amorphous cellulose, which could be easily converted into sugars by enzymatic attack (Sarkar et al., 2012). Greatest exposure of cellulose surface area for enzymes could be achieved for effective hydrolysis with extremely small consumption of energy and higher sugar recovery (Mohapatra et al., 2017). The overall efficiency of the pretreatment has to meet the equilibrium between substrate digestibility and inhibitory compounds formation for example furfural and hydroxy methyl furfural (Chen and Fu, 2016).

Pretreatment can be categorized into physical, physicochemical, chemical and biological methods or various combinations of these. Each method has their own distinct pros and cons.

2.3.1.1 Physical Pretreatment

In physical processing, methods include hammer milling, ball milling or other mechanical operations were used to breakdown the biomass feedstock into smaller particles. This method pretreated the lignocellulosic biomass by reducing the particle size and creating a larger surface area of cellulose to improve the accessibility to hydrolysable polymers within the lignocellulosic material (Kumari and Singh, 2018).

2.3.1.2 Physicochemical Pretreatment

Thermal pretreatment such as steam explosion utilizes heat energy to break down the hemicellulose and lignin in lignocellulosic biomass. The feedstock was usually heated between 150 °C – 180 °C during the pretreatment process. However, care is needed for this method as there is a risk of degradation of sugars under elevated temperature, around 250 °C. This is because the formation of phenolic compounds possess the ability to retard the fermentation process (Xiros, Topakas and Christakopoulos, 2013).

2.3.1.3 Chemical Pretreatment

Different chemicals may be employed in chemical pretreatment including acid, alkalis, organic solvent and other chemicals. Acid, alkaline and organosolv pretreatment are the most well-known chemical pretreatment methods. In acid pretreatment, the material was immersed in a dilute acid solution such as hydrochloric acid with concentration of around 0.5 – 1.5 % and heated to temperature within 140 °C – 200 °C under a certain period (normally few minutes to an hour) to hydrolyse hemicellulose and release fermentable sugars. Concentrated acid could also be used for pretreatment under lower temperature (40 °C – 100 °C) (Xiros, Topakas and Christakopoulos, 2013). In order to reduce inhibitors formation, selection of appropriate acid for pretreated lignocellulosic material is an essential step in the pretreatment process (Kumari and Singh, 2018).

Alkali pretreatment utilizes bases to increase internal surface area by causing swelling of fibrous cellulose and also destruction of bonds between lignin and carbohydrates. The effectiveness of this method is strongly depending on the amount of lignin presence in the feedstock. Biomass with low lignin content is more preferable (Amin et al., 2017). Several examples of base catalysts that frequently used in this pretreatment are potassium hydroxide, aqueous ammonia, sodium hydroxide and calcium hydroxide (lime).

On the other hand, organosolv pretreatment involves addition of aqueous organic solvent for example ethanol or acetone to the biomass in the presence or absence of catalyst under particular pressure and temperature for hemicellulose degradation and lignin removal. This pretreatment works better for biomass contains higher lignin content due to the high capability to break the internal links of hemicelluloses and lignin (Kumari and Singh, 2018).

2.3.1.4 Biological Pretreatment

Biological pretreatment dismantle the recalcitrant structures in the cell wall for hemicellulose and lignin degradation with the help of microorganism, microbes or enzymes as catalysts. It allowed cellulose to be more susceptible to enzyme and ease carbohydrate polymers to fermentable sugars conversion. Wood degrading fungi such as white rot, soft rot and brown rot fungi had been scouted as a potential source of enzyme for cell wall degradation for commercial plant (Amin et al., 2017). White-rot fungi possessed higher efficiency in selectively metabolize lignin and hemicellulose

which have low molecular weight while imparting only minor effect to cellulose. For soft and brown-rot fungi, they were usually useful in hemicellulose degradation but leaving lignin with little or no influence. Due to the long residence time required for biological pretreatment, it is not advisable for industrial purposes (Amin et al., 2017).

2.3.2 Hydrolysis

Hydrolysis is a process that required to maximize the conversion of glucan and xylan into glucose and xylose respectively, which are the fermentable sugars for the use in subsequent fermentation process (Derman et al., 2018). There are the major portions of cellulose and hemicellulose in lignocellulosic biomass. Acid hydrolysis that accomplished by using acid catalysts and enzymatic hydrolysis that using enzyme as catalysts are the two categories of hydrolysis.

For acid hydrolysis, it could be carried out by dilute acids hydrolysis (0.5 – 1.5 %) with high temperature and pressure in shorter reaction duration or by concentrated acids hydrolysis (70 – 90 %) with low temperature but longer reaction duration. Dilute acid treatment was more favourable due to its minimal contribution in environmental impact. However, sugars degradation and formation of inhibitors might be occurred at high temperature condition. Hence, the concentration of acid and temperature are commonly controlled within the range of 0.5 % – 1.5 % and 120 °C – 160 °C for dilute acid hydrolysis (Zabed et al., 2016b).

For enzymatic hydrolysis, it took place with the usage of enzyme under a mild condition of temperature and pressure to convert cellulose into fermentable sugars. It had been known as the most promising and effective processes that widely used due to the highly shape-specific active site on enzyme towards substrate (Zabed et al., 2016b). In contrast with acid hydrolysis, enzymatic hydrolysis operated at relatively lower temperature which requires lesser energy, produced higher yield in glucose, lower risk of inhibitors generation, and more environmental friendly (Chen and Fu, 2016). The efficiency of hydrolysis could be influenced by several factors including cellulose fibre crystallinity, lignin and hemicellulose content as well as the porosity of lignocellulosic biomass (Derman et al., 2018).

2.3.3 Fermentation

Fermentation process is a vital process in bioethanol production. Various microorganisms or yeasts were used to assist the fermentation process of sugars. Generally, the fermentation techniques for converting cellulose presented in lignocellulosic biomass to bioethanol could be classified into two processes: Separate Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF) (Derman et al., 2018).

In SHF process, the pretreated sample would perform enzymatic hydrolysis and fermentation separately in two sequential steps and done in separate units. The first unit was used to degrade the monomeric sugars in pretreated sample by using cellulases and xylanases as enzyme while the second unit was used to convert those sugar to ethanol (Mohapatra et al., 2017). These separate units in SHF process allowed both hydrolysis and fermentation processes to perform at different optimal operating conditions of pH and temperature, respectively. However, the cellulases activity would be strongly hindered and reduced dramatically after some period caused by the built-up of cellobiose and glucose which in turn minimized the yield of ethanol. Hence, this process was less likely to be applied in bioethanol production due to high energy requirement and higher chance of contamination in long process time.

SSF process is an excellent substitute for SHF process as it could improve the yield of ethanol by overcoming the end product inhibition issue faced by SHF process. The enzymatic hydrolysis and fermentation for SSF process would be conducted in a same reactor and thus it is more cost effective as lesser number of reactors is required. In the reactor, the glucose produced using cellulases would directly transform into ethanol by the fermenting microorganism. Hence, the inhibition of end product on cellulolytic activities could be minimized as glucose being removed from the medium continuously throughout the process (Xiros, Topakas and Christakopoulos, 2013). SSF process possessed several drawbacks as the enzymes for both hydrolysis and fermentation had different optimum temperature conditions (Sarkar et al., 2012). To tackle or overcome this problem, it was suggested to introduce a mixed culture of microorganisms or thermo-tolerant microbes and a hydrolysis step (Derman et al., 2018).

2.4 Overview of Organosolv Pretreatment

Several pretreatment processes had been developed to improve the enzyme accessibility. Recently, organosolv pretreatment with insignificant delignification damage, had seen growing concern in the transformation process from lignocellulosic biomass to hydrolysed cellulose and relatively pure lignin as secondary product for the bioethanol production. As mentioned in Section 1.3.1, there were numerous organic solvents that could be utilized in the pretreatment with or without catalysts. Acid catalysts were widely used to mitigate the high reaction temperature necessary for pretreatment, speed up the removal rate of lignin and facilitate hemicellulose decomposition. Without utilization of catalysts, the operating temperature for organosolv pretreatment was normally in range from room temperature up to 240 °C for sufficient delignification. Alcohols with high boiling point for instance glycerol and ethylene glycol had less requirements on operating temperature and pressure however required more energy for solvent recovery. Hence, low molecular weight alcohols with lower boiling point for example methanol and ethanol were more preferable as organic solvent for pretreatment due to high and easier solvent recovery as well as more cost-effective (Zhang, Pei and Wang, 2016).

There were two elemental fractionations occurring in this pretreatment. First of all, large amount of lignin and hemicellulose in biomass would be split into smaller fragments and dissolved in liquor after reacting with organic solvent at elevated temperature and pressure for some period (Wildschut et al., 2013). Cellulose, which was extremely liable to enzymatic hydrolysis, was hydrolysed and undergo fermentation to ethanol. Secondly, the dissolved lignin was separated from the lignin-rich liquids by dilution, drying and precipitation process to obtain solid lignin. The solvent would be recovered and recycled back to pretreatment to prevent inhibitory effect to enzymatic hydrolysis and fermentative microorganisms (Alvira et al., 2010). Figure 2.5 illustrates the organosolv pretreatment process of lignocellulosic biomass.

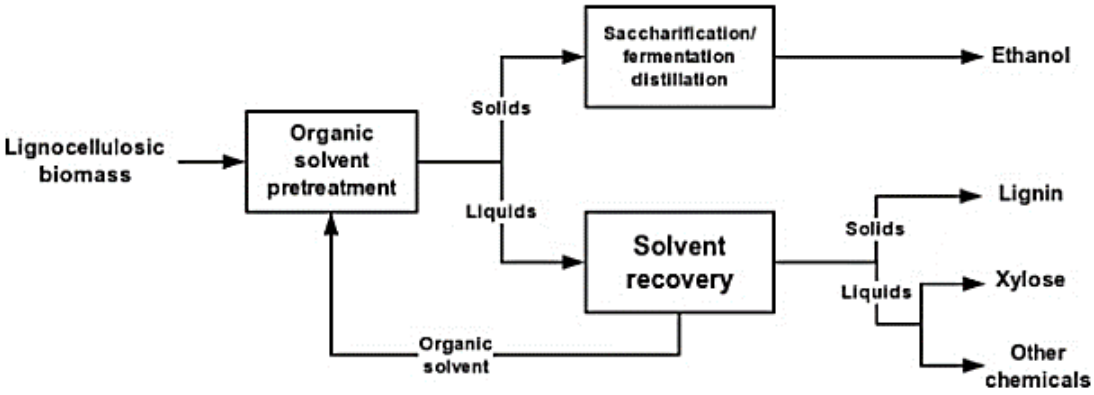

Figure 2.5: Flow Diagram of Organosolv Pretreatment of Lignocellulosic Biomass (Zhang, Pei and Wang, 2016)

2.4.1 Ethanol-based Organosolv Pretreatment

The current study focused on using ethanol as the organic solvent for pretreatment process. Organosolv pretreatment using ethanol as solvent had been studied extensively for many lignocellulosic biomass (Park et al., 2010; Wildschut et al., 2013; Jang et al., 2016). It was the primary choice as organic solvent in pretreatment process because it proposed several advantages including low cost solvent, non-toxic behaviour, highly miscible with water and relatively easy for solvent recovery by distillation. According to the Zhang, Pei and Wang (2016), the pretreatment temperature required for ethanol-based organosolv pretreatment was 190 °C and above, while for the operating time was more than 60 minutes.

2.5 Characterization Analysis of Pretreated Materials

After the organosolv pretreatment process, the pretreated spent coffee grounds (SCG) would suffer from physical and chemical changes. Some characterization techniques such as Scanning Electron Microscopy (SEM), X-ray Diffractometer (XRD), Fourier Transform Infra-Red Spectroscopy (FTIR) and Thermogravimetric analysis (TGA) were employed to give fundamental insight into the impacts that contributed by organosolv pretreatment process on SCG. Each technique has specific functions and is used to discriminate different characteristics.

2.5.1 Structural and Morphological Changes

The morphological and structural changes resulted on the surface of lignocellulose biomass due to pretreatment could be investigated through SEM analysis. Unlike other lignocellulosic substrates, the structure of SCG varied with regard to the particle size, shape and nature. Native SCG had numerous pores on the surface, non-fibrous and present in a sheet like structure (Ballesteros, Teixeira and Mussatto, 2014). The SEM micrograph of SCG with different magnification is shown in Figure 2.6.

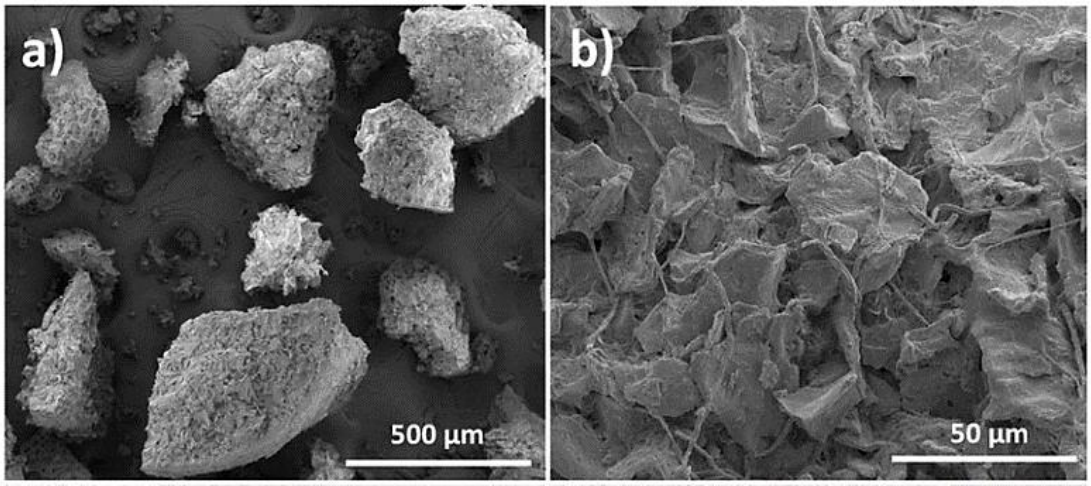

Figure 2.6: SEM Micrographs of Spent Coffee Grounds at Magnification of a) 200-Fold and b) 2000-Fold (Ballesteros, Teixeira and Mussatto, 2014)

During pretreatment process, an extensive structural disintegration of the cell of biomass would be occurred. Hemicellulose and lignin structures inside the biomass would be removed which otherwise would interfere the enzyme accessibility to cellulose. As a result, the SEM micrographs obtained for pretreated material showed a huge reduction in particle size as compared to the native material. As illustrated in Figure 2.7, the SEM micrograph of pretreated corncobs with 50 % v/v ethanol was highly porous compared to the untreated corncobs. The increment in pore size of the pretreated material led to higher surface area and eventually improved the efficiency of enzymatic hydrolysis (Ravindran et al., 2018).

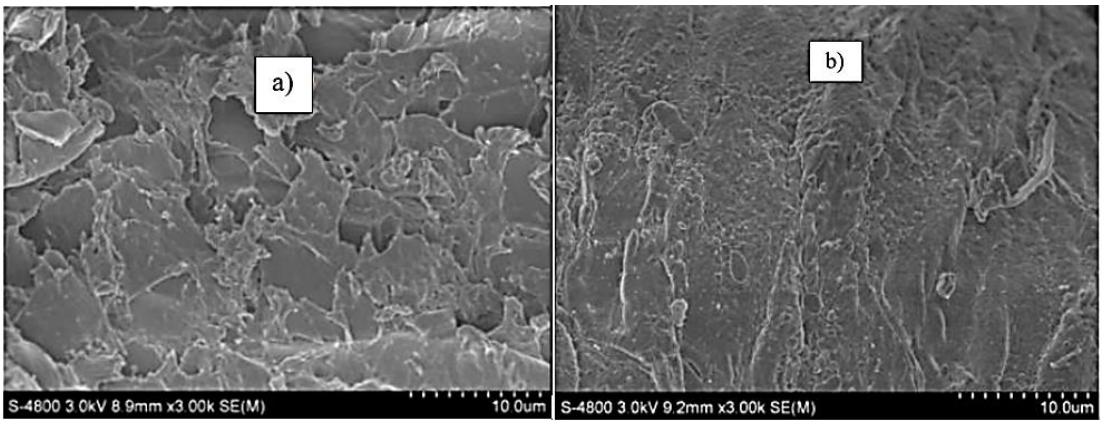

Figure 2.7: SEM Micrographs of Corncob Samples: (a) Untreated Corncobs (b) Pretreated Corncobs with 50 % v/v Ethanol (Varbanov et al., 2017)

2.5.2 Changes in Crystallinity

It is crucial to study on the crystallinity of both the native and pretreated material to evaluate the variation in composition after organosolv pretreatment process. The crystallinity of biomass could be analysed by conducting X-ray diffraction (XRD) by utilized a cellulose spectrum from the International Centre for Diffraction Data (ICDD) database as a reference (Ballesteros, Teixeira and Mussatto, 2014).

The presence of cellulose had greatly contributed in the crystallinity of lignocellulosic biomass as it has crystalline and amorphous regions in nature. Aggregation of cellulose in biomass occurred due to delignification of lignocellulose during pretreatment process which in turns caused an increment in crystallinity degree. Hence, the pretreated lignocellulosic biomass showed higher crystallinity than the untreated biomass. The crystalline cellulose hinders digestion by enzymatic hydrolysis although its amorphous counterpart able to convert into component sugars easily (Ravindran et al., 2018). Besides amorphous cellulose, amorphous structure in lignocellulosic biomass could be committed by hemicellulose and lignin as well which degraded easily and more susceptible to chemical attacks. The performance of the pretreatment could be featured by the rise in crystallinity degree which indicated the attrition of amorphous components (Ravindran et al., 2017). In a typical XRD profile, the presence of amorphous and crystalline structure of lignocellulosic biomass could be observed from the peaks located at 15° and 22° respectively as shown in Figure 2.8 due to the structure disorderliness resulted by pretreatment (Raghavi et al., 2016).

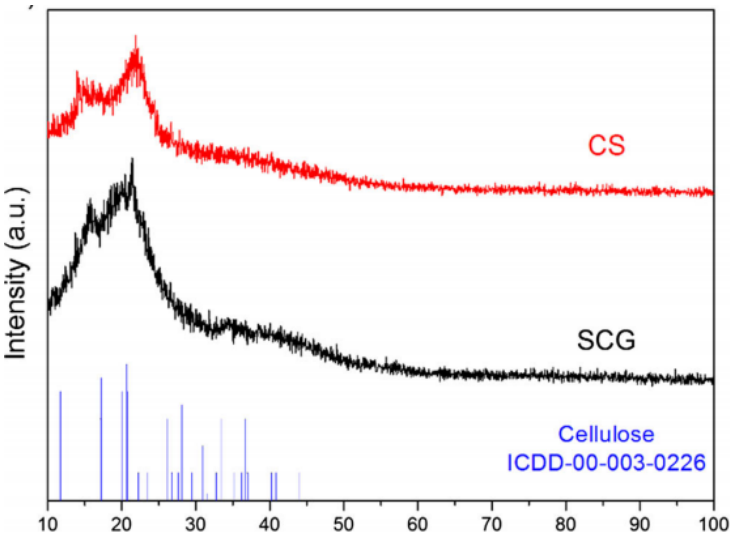

Figure 2.8: XRD Profile of Coffee Silverskin (CS) and Spent Coffee Wastes (SCG) (Ballesteros, Teixeira and Mussatto, 2014)

2.5.3 Changes in Functional Groups

The influences of pretreatment on functional groups of lignocellulosic biomasses could be analysed by FTIR. There are some typical spectrums can be observed after pretreatment. According to FTIR spectrums obtained by Ravindran et al. (2018) which studied on the organosolv pretreatment in SCG, the presence of peaks at 897 cm^{-1} reported the changes in β-glycosidic linkages between hemicellulose and cellulose. A drop in absorbance at this wavenumber for pretreated SCG could be explained by the intermolecular degradation in hemicellulose structure caused by pretreatment. Moreover, the existance of bonds between lignin and carbohydrate was observed by the peak located at 1035 cm^{-1} which was indicative of C–C and C–C–O bonds. Pretreated SCG had a lower intensity of band at that wavenumber showed the bond stretching effect due to pretreatment. The breakage of hydrogen bonds between hemicellulose and cellulose was distinguished as there was a decline in the peak at 1200 cm^{-1}. Any changes observed in the region of 1509, 1464 and 1422 cm^{-1} were signified by the removal of lignin or lignin degradation (Ravindran et al., 2017).

Furthermore, hemicellulose removal was determined from the decrease in absorption peaks at 1730 cm^{-1} and 1750 cm^{-1} that was indicative of the ester moisties in hemicellulose. The band widenning in range of 2800 – 3000 cm^{-1} represented the C–H stretching vibration in cellulose and breakage of hydrogen bonds. On the other hand, the broadening of absorption peak between 3000 and 3500 cm^{-1} was corresponded to the stretching vibration of O–H hydroxyl groups. Similar

observations were obtained in several studies conducted by Ballesteros et al. (2015), Ravindran et al. (2017) and Ballesteros, Teixeira and Mussatto (2014). Figure 2.9 shows the FTIR analysis of pretreated SCG performed by Ballesteros et al. (2015).

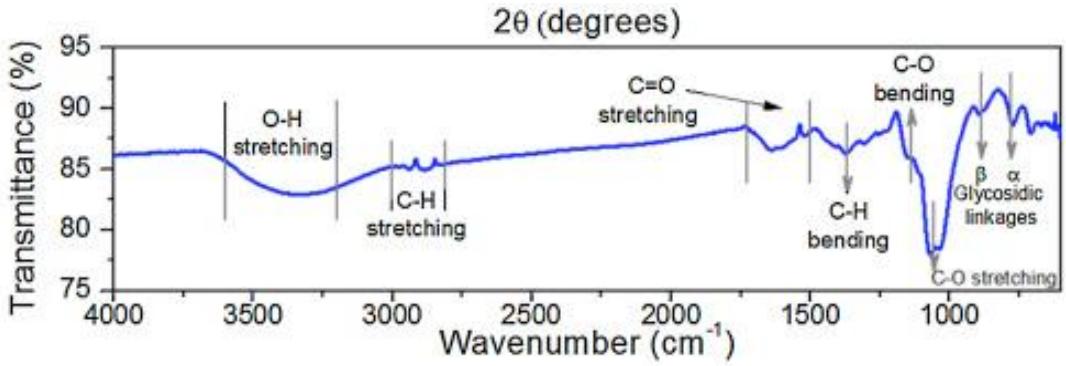

Figure 2.9: FTIR Analysis of Pretreated Spent Coffee Grounds (SCG) (Ballesteros et al., 2015)

2.5.4 Changes in Thermal Stability

Thermal gravimetric analysis (TGA) studied on the changes in mass of a material as a function of temperature or isothermally as a function of time under a controlled atmosphere of nitrogen, air, helium, other gas or in vacuum (Anderson Materials Evaluatio, Inc., n.d.). It could be used to examine the thermal stability of a material, moisture and solvent content as well as the material's composition. During the analysis, the sample was heated gradually in furnace and the mass changes was measured. A TGA thermal curve profile displayed the relationship of weight or weight precent against temperature or time and it revealed about the thermal transition in the sample (PhotoMetrics, Inc., n.d.).

The thermal stability and weight losses of raw and pretreated spent coffee grounds could be analysed through TGA. Generally, a TGA curve for spent coffee grounds consisted of three defined mass loss stages. Ballesteros, Teixeira and Mussatto (2014) had studied on the thermal stability of coffee silverskin (CS) and spent coffee grounds. According to the TGA curves achieved as shown in Figure 2.10, SCG and CS possessed identical trend with total three weight loss phases as the temperature increased to 600 °C. An insignificant reduction in weight of approximately 7.77 % at around 60.60 °C for SCG and 6.80 % at 61.58 °C for CS could be resulted by the dehydration of moisture content in the sample through evaporation. The most

prominent weight losses were occurred for both SCG (43.50 %) and CG (48.01 %) during second stage due to the occurrence of depolymerisation as well as break down of polysaccharides and oils in the sample. Lastly, the mass reduction of 33.08 % at 499.29 °C and 34.17 % at 457.24 °C for SCG and CS respectively could be corresponded to the decomposition of the samples at high temperature.

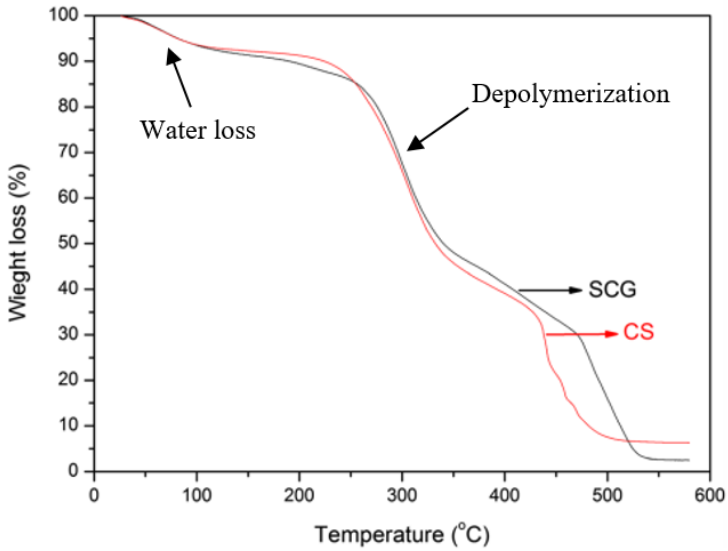

Figure 2.10: TGA Curves for Coffee Silverskin (CS) and Spent Coffee Grounds (SCG) (Ballesteros, Teixeira and Mussatto, 2014)

2.6 Parameters Affecting Organosolv Pretreatment

There are several parameters that affect the output results of organosolv pretreatment and the interaction effects between these parameters had been extensively studied (Huijgen, Reith and den Uil, 2010; Goh et al., 2011; Widjaja et al., 2016). Among the most important parameters are solvent loading, presence of catalyst and its concentration, operating temperature and reaction time. The interactions of these pretreatment parameters increased the complexity for optimizing the pretreatment process and numerous researches had attempted to investigate them (Huijgen, Reith and den Uil, 2010; Wildschut et al., 2013; Salapa, Topakas and Sidiras, 2018). A literature review was performed to gain insight into these parametric effects on the delignification, yield of lignin, enzymatic digestibility as well as xylan hydrolysis.

2.6.1 Effect of Organic Solvent Loading

Organic solvent loading is identified as one of the major factors in organosolv pretreatment. In this study, the organic solvent used was ethanol. It is crucial to assess the influence of the ethanol loading in the mixture for organosolv pretreatment.

Domínguez et al. (2014) had carried out organosolv pretreatment of Acacia dealbata wood, which is an invasive species, with liquor to solid ratio (LSR) of 10 g/g and 6 g/g at 230 °C for 60 and 90 minutes using 80 wt % glycerol as solvent to evaluate the effect of LSR on the pretreatment. The achieved solid yield and chemical compositions of the pretreated solid were tabulated in Table 2.3. The study determined that the higher the solvent loading, the greater the lignin reduction which could be supported by the lower lignin content (8.0 % and 8.3 %) obtained at LSR of 10 g/g for 60 and 90 minutes respectively as compared to LSR of 6 g/g (9.4 % and 10.6 %). This was due to dissolution of lignin at high solvent concentration during the process and hence greater delignification degree was achieved as well as enzyme hydrolysis of cellulose and hemicellulose. Besides, it could be seen that the pretreatment using low LSR had lower cellulose content in solids (82.1 % for 60 minutes and 81.2 % for 90 minutes) as compared to higher LSR which contained 85.0 % and 88.8 % of cellulose for reaction time of 60 and 90 minutes respectively. This indicated that cellulose preservation operated better at higher solvent loading.

However, xylan content in solid was reported to be lower at high LSR which were 4.90 % and 3.09 % for residence time of 60 and 90 minutes respectively. This showed that hemicellulose hydrolysis and enzymatic degradability of pretreated solid was lower in the presence of high solvent content. Hydrolysis process is known as a acid-catalysed process which weakens the linkages in the lignin-carbohydrate complex. At condition with high glycerol loading, the organosolv liqour became more alkaline and hence hydrolysis was less favoured. Hence, the hemicellulose hydrolysis was enhanced by lowering the glycerol loading.

Table 2.3: Operational Condition of Pretreatment and Results Obtained (Domínguez et al., 2014)

LSR (g/g)	Temperature (°C)	Time (minutes)	Solid Yield (%)	Cellulose (%)	Xylan (%)	Lignin (%)
6	230	60	50.6	82.1	6.61	9.4
6	230	90	47.9	81.2	5.54	10.6
10	230	60	50.3	85.0	4.90	8.0
10	230	90	47.2	88.8	3.09	8.3

In addition, the effect of solvent loading on delignification, cellulose recovery, hemicellulose removal and enzymatic hydrolysis yield were determined as well in the glycerol organosolv experiments of wheat straw performed by Sun and Chen (2007) with different glycerol loading from 10 g/g to 25 g/g at 240 °C for 4 hours. The result achieved was illustrated in Figure 2.11. According to the results, it was found that solvent loading of 10 g/g had highest pulp yield (74 %) but showed the lowest enzymatic hydrolysis yield of 48.7 % and 53.5 % after 24 and 48 hours respectively. This could be due to insufficient and uneven pretreatment of biomass at low glycerol level. A sharp increase in enzymatic hydrolysis yield was observed as the glycerol level increased up to solvent loading of 15 g/g, whereas caused decline in pulp yield as well as hemicellulose and lignin content. The drop in lignin contnet from 75 % to 52 % as the solvent loading increased from 10 to 15 g/g indicated that the delignification was enhanced as lignin was known to be more soluble in relatively high solvent concentration. The enzyamtic digestibility was improved as well due to the removal of lignin barrier.

However, further increased in solvent loading beyond 15 g/g showed no significant effect on puly yield but a small reduction on enzymatic hydrolysis yield were spotted after 24 and 48 hours. This revealed that the extensively high level of solvent content led to low delignification of wheat straw and increased in pulp yield which in turn lowering the occurrence of enzymatic hydrolysis of fiber fraction. As a result, glycerol loading of 15 g/g was selected as the optimum solvent loading which provided pulp yield of 51 %, 70 % lignin removal, 90 % hemicellulose removed and 95 % cellulose recovered. Under this solvent loading, 90 % and 92 % of enzymatic hydrolysis yields were obtained after one and two days respectively.

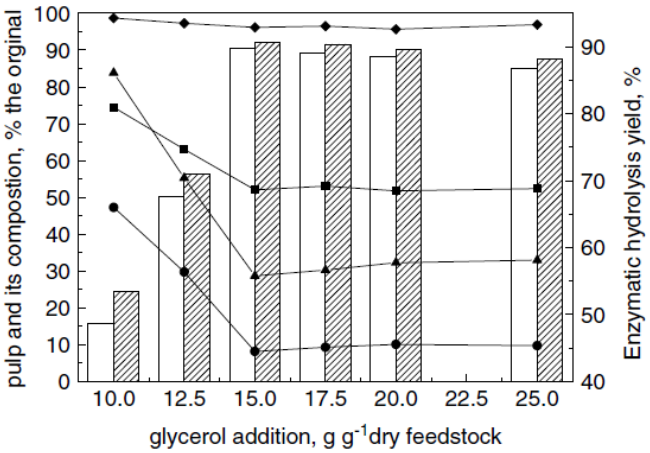

Figure 2.11: Effect of Varies Level of Glycerol Loading on Pretreatment of Wheat Straw at 240 °C for 4 hours. (Circle: Hemicellulose; Square: Pulp; Triangle: Lignin; Diamond: Cellulose; White Bar: 24 hours of Enzymatic Hydrolysis; Diagonal Hatched Bar: 48 hours of Enzymatic Hydrolysis) (Sun and Chen, 2007)

2.6.2 Effect of Catalyst Concentration

Catalyst concentration will bring an impact to the pretreatment process as well. In current study, sulfuric acid will be utilized as a catalyst in ethanol-based organosolv pretreatment. The presence of acid catalyst is believed to have the ability to enhance the fractionation process as well as the enzymatic hydrolysis of the cellulosic residues.

Salapa, Topakas and Sidiras (2018) had performed experiments of acetone organosolv pretreat barley straw with sulfuric acid as catalyst. The effect of sulfuric acid concentration was studied by carrying out several experiments with various operating conditions as shown in Table 2.4. From the results obtained, the delignification of pulp increased with an increment in acid concentration. The removal of lignin was largely influenced by the sulfuric acid concentration when the temperature and residence time were remained constant. The higher the pretreatment severity, the higher the lignin removal. In addition, effective xylan hydrolysis was promoted as well at higher acid concentration. At higher acid concentration (35 mol/m^3), the cellulose-to-glucose conversion by enzymatic hydrolysis was found to improve. As a consequence, the pulp yield was much lower when acid with higher concentration was used and the pulp was enriched in glucan.

Table 2.4: Pulp Yield Obtained for Each Run of Experiment (Salapa, Topakas and Sidiras, 2018)

Temperature (°C)	**Time (min)**	**Sulfuric Acid Concentration (mol/m^3)**	**Pulp Yield (%)**
140	20	10	77.0
140	20	35	48.5
160	40	10	70.9
160	40	35	40.6
180	20	10	59.9
180	20	35	35.7

Furthermore, the influence of acid concentration on the pretreatment performance could be studied as well based on the experiment conducted by Teramura et al. (2018). In the study, 25 % v/v butanol-based organosolv pretreatment process for sorghum bagasse was conducted. Five different concentrations of sulfuric acid (0.25, 0.5, 0.7, 1.0, and 2.0 %) were used to evaluate the impacts of acid concentration on solid fraction composition.

Based on the data collected, the yield of the solid fraction had a reversed relationship with the sulfuric acid concentration. In other words, the solid fraction yield dropped with an increment of acid concentration. The cellulose content in solid precipitate after pretreatment raised from 47.5 % to 73.4 % by increasing sulfuric acid concentration. This showed that high cellulose recovery (92 – 97.3 %) could be achieved in higher acidic condition. However, the hemicellulose and lignin (acid-soluble and acid-insoluble) content in solid residue dropped from 18.1 %, 22.1 % and 3.7 % to 3.4 %, 14.1 %, and 2.2 % respectively, when the concentration of acid raised. The hemicellulose and lignin recoveries declined dramatically by increasing acid concentration which were 61.6 – 7.2 % for hemicellulose and 74.0 – 29.0 % for total lignin. This proved that delignification degree increased at high acid concentration due to the degradation of the linkage between monomers of lignin, which is known as the β-O-4 structure. As the cellulose loading increased and content of lignin reduced in the solid residue, the enzymatic saccharification was favoured to obtain higher yield in glucose. Figure 2.12 displays the effect of sulfuric acid concentration on the solid fraction composition, solid fraction yield, cellulose and lignin recovery.

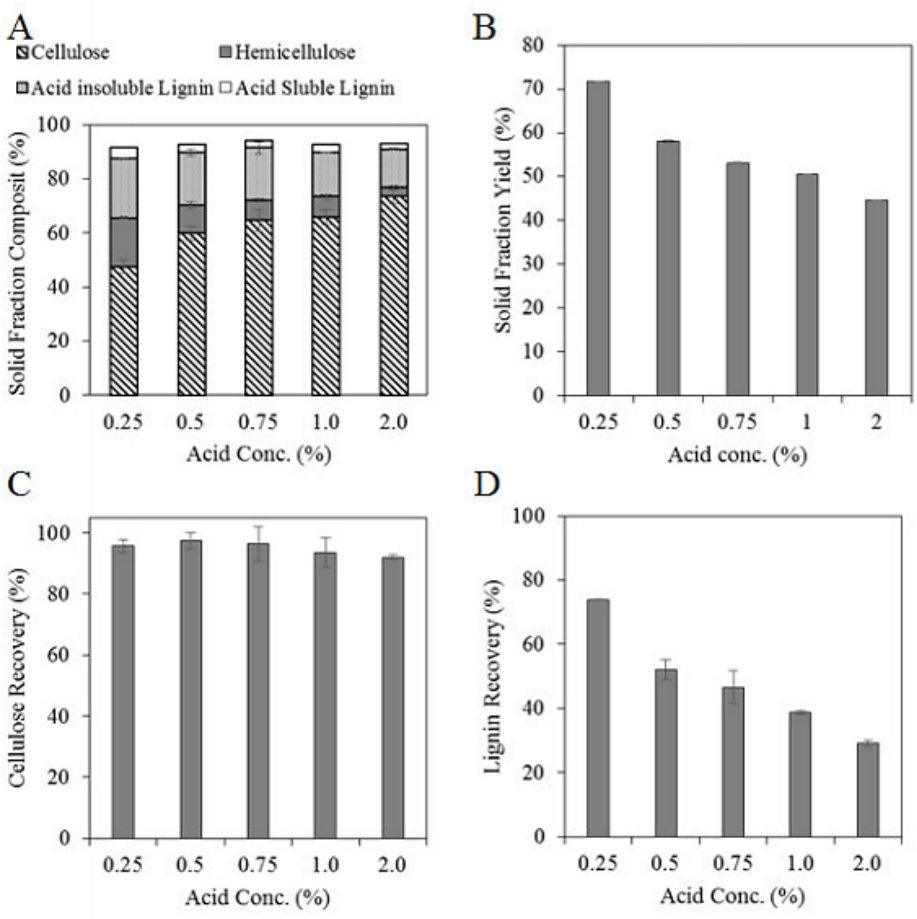

Figure 2.12: Effect of Sulfuric Acid Concentration on: A) Solid Fraction Composition, B) Solid Fraction Yield, C) Cellulose Recovery and D) Lignin Recovery (Teramura et al., 2018)

2.6.3 Effect of Reaction Temperature

The reaction temperature has a significant impact on degradation of lignocellulosic biomass. The influence of temperature had been studied by several researches and their results will be discussed in following section.

First of all, Wildschut et al. (2013) had studied the impact of temperature on the ethanol organosolv pretreatment of wheat straw in range between 160 – 210 °C for reaction time of 1 hour. The relationship between delignification, yield of pulp, enzymatic digestibility and xylan hydrolysis against temperature is illustrated in Figure 2.13. By referring to the experimental results as shown in Figure 2.13, the pulp yield decreased with increment in temperature. For instance, the pulp yield reported at 160 °C was 86.5 % but it was declinced to 53.5 % at 210 °C. This was due to the pretreatment severity at higher temperatures that enhanced the efficiency of

delignification and xylan hydrolysis. With increasing delignification effect, the yield of lignin solids collected by precipitation was increased from 0 % to 12.9 % when the temperature rised from 160 °C to 210 °C. As a result, the enzymatic digestivity of the wheat straw improved at higher temperature from 30.5 % (160 °C) to 56.1 % (210 °C). The experimental data obtained at different temperature is tabulated in Table 2.5.

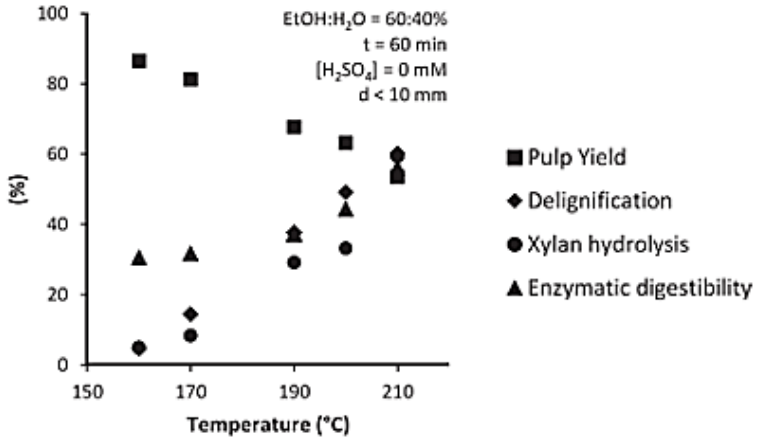

Figure 2.13: Organosolv Fractionation of Wheat Straw at Different Temperatures (Wildschut et al., 2013)

Table 2.5: Experimental Data Obtained at Different Temperature in 60 Minutes (Wildschut et al., 2013)

Temperature, °C	**Pulp Yield (%)**	**Delignification (%)**	**Xylan Hydrolysis (%)**	**Enzymatic Digestibility (%)**
160	86.5	4.7	5.0	30.5
170	81.2	14.4	8.4	31.7
190	67.7	37.7	29.2	37.2
200	63.2	49.1	33.2	44.4
210	53.5	60.1	59.3	56.1

In the experiments performed by Huijgen, Reith and den Uil (2010), the influence of reaction temperature showed similarities with the results obtained by Wildschut et al. (2013). Huijgen, Reith and den Uil (2010) performed studies on acetone-based organosolv pretreatment for wheat straw. From the results obtained, the delignification effect increased from 11 % to 79 % when the temperature elevated from

160 to 205 °C by using 50:50 % w/w acetone water and 60 minutes. Further increment in temperature to 220 °C resulted in slight increase in the pulp's lignin residual content to 8.3 % from 7.0 % at 205 °C as lignin condensation reactions occurred at high temperatures. As the delignification increased at elevated temperatures, the recovery of lignin from the organosolv liquor was enhanced as well. Enzyme digestibility of wheat straw improved at higher reaction temperature which reached 87 % at 205 °C but slightly dropped to 82 % at temperature of 220 °C as shown Figure 2.14. This might be due to the development of degradation products at high severity conditions.

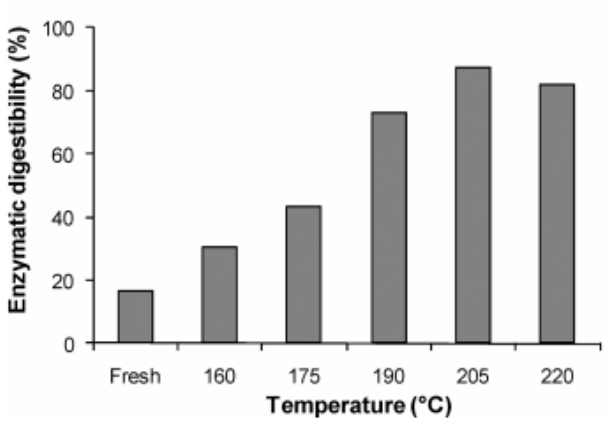

Figure 2.14: Influence of Enzymatic Digestibility on Temperature (Huijgen, Reith and den Uil, 2010)

2.6.4 Effect of Reaction Time

Reaction time is one of the factors that will influence the output result of pretreatment process. The influence of reaction time had been studied in many researches and discussed (Goh et al., 2011; Wildschut et al., 2013). According to literature, the delignification of lignocellulosic biomass would increase with an increment in reaction time.

Several experiments were performed by Wildschut et al. (2013) to assess the influence of reaction time on pretreatment process. The lignocellulosic biomass used for this ethanol organosolv pretreatment was wheat straw at temperature of 190 °C but at various residence time in range from 60 to 120 minutes. Based on the results obtained, the yield of wheat straw pulp declined gradually with an extension of the reaction duration. At long pretreatment duration, the deligninification degree increased due to the longer time available and led to higher yield in lignin solid. Therefore, the

enzyme digestivity was reported to be higher for pretreatment process under longer period. The xylan hydrolysis insignificantly affected by the duration of pretreatment and remained relatively constant in the variation of reaction time. The experiment results are tabulated and displayed in Table 2.6. A plot of delignification, yield of pulp, enzymatic digestibility and xylan hydrolysis against reaction time is shown in Figure 2.15.

Table 2.6: Experimental Data Obtained in Variety of Reaction Time at 190 °C (Wildschut et al., 2013)

Reaction Time, min	**Pulp Yield (%)**	**Delignification (%)**	**Xylan Hydrolysis (%)**	**Enzymatic Digestibility (%)**
60	67.7	37.7	29.2	37.2
90	65.6	46.5	28.5	39.5
120	64.0	48.8	29.7	42.0

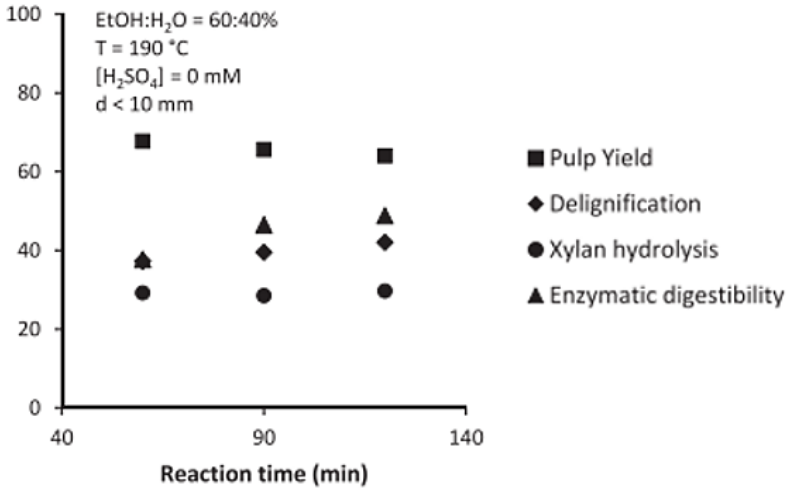

Figure 2.15: Organosolv Fractionation of Wheat Straw Against Reaction Time (Wildschut et al., 2013)

Besides that, Goh et al. (2011) had also investigated the effect of reaction time in 65 % ethanol organosolv pretreatment of empty palm fruit brunch (EPFB). The results obtained for the content of the filtrate and solid residue, the recovery of ethanol organosolv lignin (EOL) and the monomeric glucose yield were examined. As compared with the results obtained at reaction time of 45 minutes and 75 minutes, the

EOL recovery at 75 minutes was significantly higher than that for 45 minutes, which were 52 % and 48.7 % respectively. This was due to the prolonging reaction time that could increase the delignification degree of EPFB and hence higher amount of EOL could be obtained. The influence of reaction time was more significant under condition of high acid concentration as more lignin was able to be recovered in acidic medium. Furthermore, the glucose yield was comparatively higher at prolonged reaction duration. This could be due to sufficient time provided for dissolution of lignin and hemicellulose into aqueous ethanol solvent and promoted high digestibility of the pulps. The experiment results of pulp yield, glucose yield and EOL yield obtained in various pretreatment conditions are tabulated in Table 2.7.

Table 2.7: Experimental Data Obtained in Different Pretreatment Conditions (Goh et al., 2011)

Sulfuric Acid Concentration (%)	**Temperature (°C)**	**Time (min)**	**Pulp Yield (yield %)**	**Glucose Yield (yield %)**	**EOL (yield %)**
1.63	190	45	84.3	61.8	48.7
1.63	190	75	78.2	61.2	52.0

2.7 Overall Review on Parameter Affecting Organosolv Pretreatment

Based on the literature review on the Section 2.6, it can be seen that the complication in optimizing pretreatment process increased due to the interaction between the process parameters. Besides, the operating conditions for pretreatment must be controlled to avoid high severity condition especially in condition of very high temperature and high acid concentration that could favour the formation of degradation products, for instance, furfural and hydroxyl methyl furfural (HMF) from pentose sugars and hexose sugars respectively (Salapa et al., 2017).

CHAPTER 3

METHODOLOGY AND WORK PLAN

3.1 List of Materials and Equipment

All the materials, apparatus and equipment required in this study are listed in the following section.

3.1.1 Materials and Chemicals

There were various types of chemicals and raw material involved in current study which prepared prior the experiment. The spent coffee grounds was selected as the lignocellulosic biomass in current research to determine the effect of various parameters in organosolv pretreatment for bioethanol production. Spent coffee grounds was collected from the Starbucks and a complete list of required chemicals and raw materials for the organosolv pretreatment process are summarized in Table 3.1.

Table 3.1: List of Chemicals and Materials Required for the Experiments

Chemicals/Materials	Source	Brand and Purity	Usage
Spent Coffee Grounds	Local coffee shop	Starbucks	1. Lignocellulosic feedstock for organosolv pretreatment.
Ethanol	UTAR	Merck (95 %)	1. Organic solvent of pretreatment.
Sulfuric Acid	Merck Group, Malaysia	Merck (95 – 97 %)	1. Catalyst for organosolv pretreatment. 2. TAPPI method for lignin content determination. 3. TAPPI method for cellulose content determination.
Sodium Hydroxide	UTAR	Merck (99.99 %)	1. TAPPI method for cellulose content determination.
Ferrous Ammonium Sulfate	Merck Group, Malaysia	Merck (99.99 %)	1. TAPPI method for cellulose content determination.
Potassium Dichromate	UTAR	Merck (99.99 %)	1. TAPPI method for cellulose content determination.
Phenolphthalein	Merck Group, Malaysia	Merck (99.99 %)	1. Indicator solution for TAPPI method.
Distilled water	UTAR	100	1. For concentration dilution. 2. For washing process.

3.1.2 Apparatus and Equipment

There are few apparatus and equipment involved during the preparation, pretreatment and analysis process in current study. The specification and usage of each apparatus and equipment are listed in Table 3.2. Besides, the instruments used in the experiment are stated in Table 3.3.

Table 3.2: List of Apparatus and Equipment Used

Apparatus and Equipment	Specifications	Usage
Oven	Memmert	To dry the spent coffee grounds.
Sieving plate	ASTM no. 50 sieve	To remove coffee beans.
Hot plate	IKA RH Basic-2	For heating purpose.
Filter paper	90 mm diameter	To filter out solid precipitate.
Refrigerator	-	To store the filtrate from filtration.
Condenser	Flavorit 300 mm Graham Condenser	To reflux ethanol solution and sulfuric acid during organosolv pretreatment. To reflux sulfuric acid during lignin content determination.

Table 3.3: List of Instruments and Apparatus Used

Instrument and Apparatus	Specifications	Usage
Scanning Electron Microscope (SEM)	Hitachi SEM Model S– 3400N	To study on the topography, surface morphology and crystallographic of biomass.
Energy Dispersive X-ray Spectroscopy (EDX)	Ametek	To determine the elemental composition of biomass.

Table 3.3 (Continued)

Instrument and Apparatus	Specifications	Usage
X-ray Diffractometer (XRD)	Shidmazu XRD-6000	To characterize the crystallographic structure of the biomass.
Fourier Transform Infra-Red Spectroscopy (FTIR)	Nicholet IS10 FTIR	To determine the presence of cellulose and hemicellulose in biomass and the corresponding bonding.
Thermogravimetric Anlaysis (TGA)	NETZSCH Model STA 2500 Regulus	To gather carbon transformation temperature profile. To test the thermal stability of catalyst.

3.2 Research Methodology

The flow and research methodology of current study is shown in Figure 3.1.

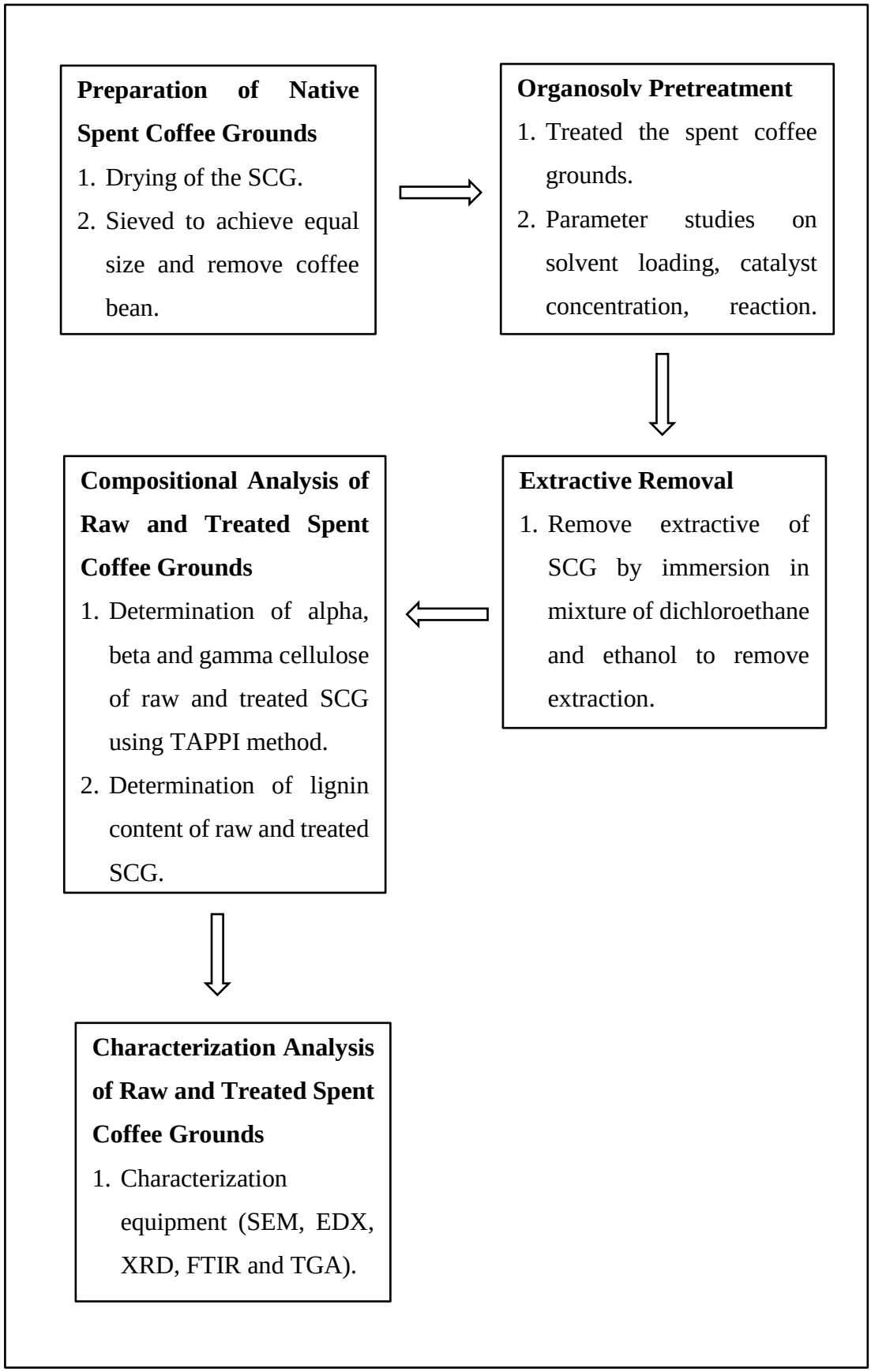

Figure 3.1: Overview of Research Methodology

3.3 Biomass preparation

The collected wet spent coffee grounds (SCG) was completely dried in an oven for 48 hours at 100 °C until constant weight was achieved. The SCG was sieved to remove coffee bean and kept under ambient-dry condition before use. Figure 3.2 illustrates the preparation of biomass.

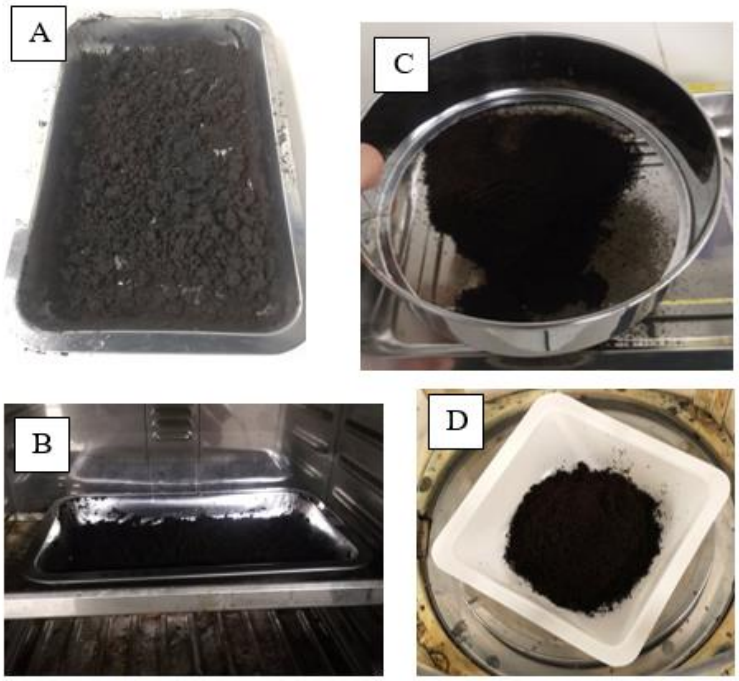

Figure 3.2: Overview of Biomass Preparation (A) Spent Coffee Grounds Collected from Starbucks (B) Drying of Spend Coffee Grounds in Oven (C) Sieve the Dried Spent Coffee Grounds (D) Dried Spent Coffee Grounds that Ready for Use

3.4 Organosolv Pretreatment Process

Organosolv pretreatment was carried out to treat spent coffee grounds using ethanol as solvent. Total four parameters were evaluated which included solvent loading, acid concentration, temperature and residence time in order to obtain the most promising operating condition. The parameter study was conducted via one-factor-at-a-time basis.

Figure 3.3 shown the experimental set up for organosolv pretreatment. The concentration of ethanol solvent was fixed at 60 % v/v for every experiment set due to higher yield of cellulose recovered (82 %) was reported in literature review (Pan et al., 2006). The round bottom flask was loaded with 10 g of SCG and 60 % v/v ethanol with varies solvent loading (solvent to solid ratio) from 10 % v/w to 17.5 % v/w (Jang et al., 2016). The suspension in vessel was stirred thoroughly throughout the process

by a magnetic stirrer with stirring rate of 200 rpm at the bottom of vessel. Sulfuric acid with concentration within 0 – 2.0 % was employed for the process as catalyst to speed up the performance (Sluiter et al., 2008). The pretreatment was performed under the reaction temperature from 180 to 210 °C and kept isothermally for a reaction time of 30 – 120 minutes which started to count from the moment when achieved desired temperature. Afterwards, the vessel was subsequently quenched into ice chamber to around 40 °C (Huijgen, Reith and den Uil, 2010).

After organosolv pretreatment, a filtration step was carried out by using vacuum filter and filter paper to separate the pretreated solid from organosolv liquor or filtrate as shown in Figure 3.4. The precipitated solid was washed by using 100 mL of aqueous ethanol with similar concentration as applied during pretreatment process which is 60 % v/v. Next, the solid residue was further rinsed with distilled water again for four times to achieve neutral pH. The mixture was stirred for 1 minute at 250 rpm at intervals of the washing process to provide sufficient washing (Figure 3.5). The pretreated solid residue was then oven-dried overnight at 100 °C and was stored in a desiccator for further analysis such as compositional analysis (cellulose, hemicellulose and lignin) and characterisation analysis (SEM, EDX, FT-IR and TGA).

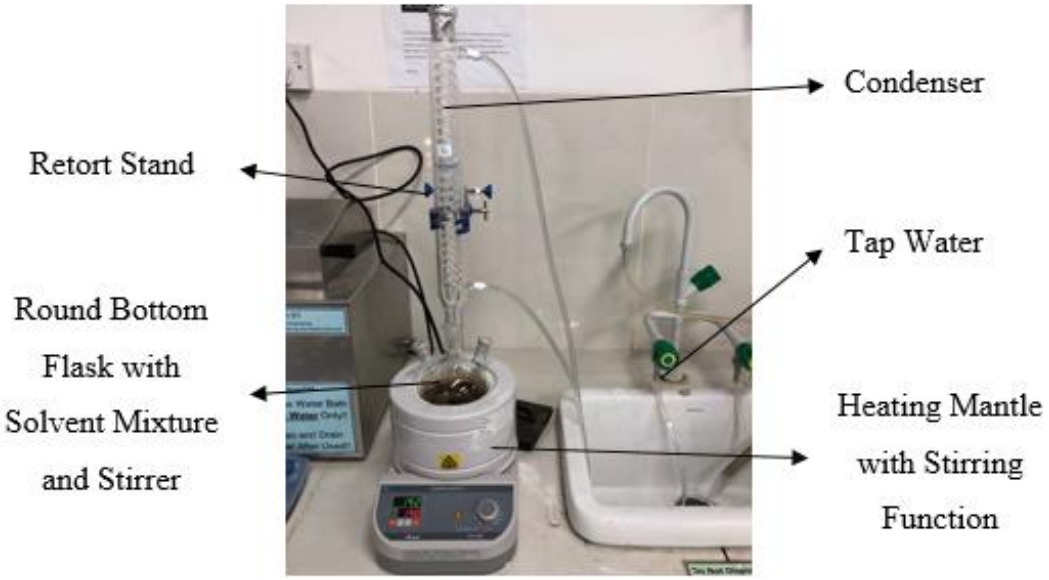

Figure 3.3: Experimental Set Up for Organosolv Pretreatment

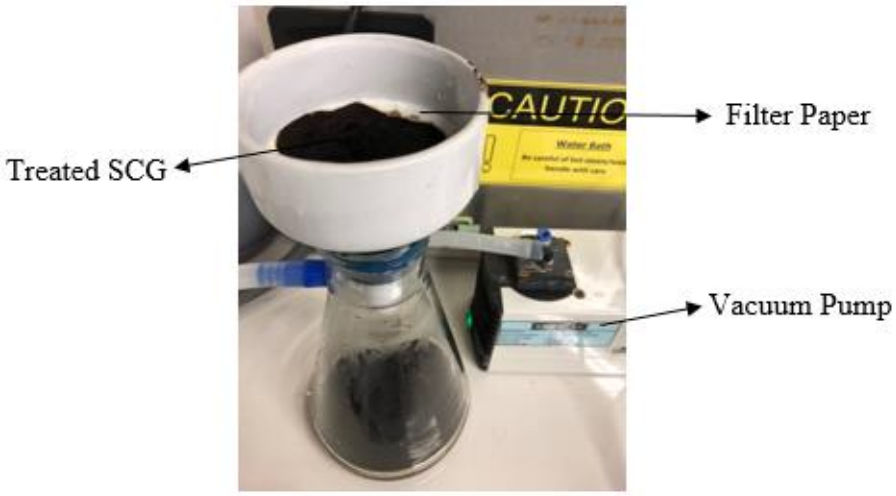

Figure 3.4: Filtration Step using Vacuum Pump

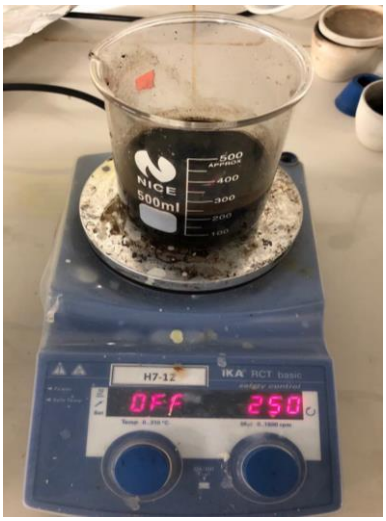

Figure 3.5: Stirring of Mixture at intervals of Washing Process at 250 rpm

3.4.1 Organosolv Pretreatment with Different Solvent Loading

In order to investigate the effect of operating parameters to ethanol-based organosolv pretreatment of spend coffee grounds, the study was begun with organosolv pretreatment with fixed mass of spent coffee grounds (10 g) and four different solvent loading (10.0, 12.5, 15.0, 17.5 % v/w). For example, for solvent loading of 10 % v/w, 10 ml of 60 % v/v diluted ethanol was added with respect to 1 g of biomass. The pretreatments were carried out at 190 °C for 60 minutes with 1 % v/v of concentration sulfuric acid as catalyst. The experiment sets with volume of 60 % v/v ethanol and concentrated sulfuric acid were tabulated in Table 3.4. The solvent loading with best pretreatment result was selected to proceed in Section 3.4.2.

Table 3.4: Experiment Sets with Variation in Solvent Loading

Experiment Set	Solvent Loading (% v/w)	Volume of 60 % v/v ethanol (ml)	Volume of Concentrated Sulfuric Acid (ml)
1	10.0	100	1.00
2	12.5	125	1.25
3	15.0	150	1.50
4	17.5	175	1.75

3.4.2 Organosolv Pretreatment with Variation in Sulfuric Acid Concentration

Concentrated sulfuric acid was chosen as the homogenous catalyst for organosolv pretreatment in this research. The effect of sulfuric acid concentration in range from 0 – 2 % v/v were studied. The experiments were carried out at 190 °C for 60 minutes with fixed mass of spent coffee grounds (10 g) and the best solvent loading that chosen based on the results in Section 3.4.1. The experiment sets are listed in Table 3.5. Experiment 1 with 0 % v/v acid concentration was served as a control to investigate the effect of the presence of homogenous catalyst.

Table 3.5: Experiment Sets with Different Acid Concentration

Experiment Set	Concentrated Sulfuric Acid Concentration (% v/v)
5 (Control)	0
6	1
7	2
8	3

3.4.3 Organosolv Pretreatment with Different Temperature

Similarly, the concentration of sulfuric acid with best pretreatment results in Section 3.4.2 was used as the pretreatment conditions for temperature study in range of (180 – 210 °C) for 60 minutes in this section. The experiment sets are shown in Table 3.6.

Table 3.6: Experiment Sets with Variation in Reaction Temperature

Experiment Set	Reaction Temperature (°C)
9	180
10	190
11	200
12	210

3.4.4 Organosolv Pretreatment with Variation in Residence Time

The best pretreatment results of reaction temperature was selected based on Section 3.4.3 and was proceed to the last parameter study with various residence time from 30 minutes to 120 minutes. The experiment sets are tabulated in Table 3.7. Table 3.8 summarised the experimental conditions for each experiment sets for organosolv pretreatment.

Table 3.7: Experiment Sets with Different Residence Time

Experiment Set	Residence Time (minutes)
13	30
14	60
15	90
16	120

Table 3.8: Experiment Sets for Organosolv Pretreatment at Variable Conditions

Experiment Set	Solvent Loading (% v/w)	Acid Concentration (% v/v)	Temperature (°C)	Residence Time (minutes)
Variation in Solvent Loading				
1	10.0	1	190	60
2	12.5	1	190	60
3	15.0	1	190	60
4	17.5	1	190	60

Table 3.8 (Continued)

Experiment Set	Solvent Loading (% v/w)	Acid Concentration (% v/v)	Temperature (°C)	Residence Time (minutes)
Variation in Concentration Sulfuric Acid Concentration				
5	A	0	190	60
6	A	1	190	60
7	A	2	190	60
8	A	3	190	60
Variation in Reaction Temperature				
9	A	B	180	60
10	A	B	190	60
11	A	B	200	60
12	A	B	210	60
Variation in Residence Time				
13	A	B	C	30
14	A	B	C	60
15	A	B	C	90
16	A	B	C	120

Notes: A – Optimum solvent loading for organosolv pretreatment chosen from set 1 – 4

B – Optimum acid concentration for organosolv pretreatment chosen from set 5 – 8

C – Optimum reaction temperature for organosolv pretreatment chosen from set 9 – 12

3.5 Extractive Removal

Generally, spent coffee grounds comprised of 10 – 15 wt % of oil content, depending on varieties of coffee (Somnuk, Eawlex and Prateepchaikul, 2017). Hence, oil extraction from dried and pretreated spent coffee grounds was performed before conducting compositional and characterization analysis of pretreated SCG to improve the accuracy of analysis as the results of analysis could be affected by presence of oil content in the sample. Solvent extraction method was applied to extract the lipid content in spent coffee grounds with solvent mixture of dichloroethane and ethanol in ratio of 2:1 v/v (Cequier-Sánchez et al., 2008). Besides, the SCG to solvent ratio of 1:10 w/v was used in this solvent extraction process. The pretreated SCG was extracted by immersion in the solvent mixture according to the required ratio for 24 hours in a

beaker. The beaker was wrapped with aluminium foil in order to prevent evaporation of solvent. Subsequently, the mixture was filtered and the residual solids were dried again in the oven until the constant weight of extracted SCG was obtained. The defatted and dried SCG was then used to proceed with compositional and characterization analysis. Figure 3.6 shows the pretreated SCG that placed in a cellulose thimble and soaked in solvent mixture based on the required ratio for 24 hours.

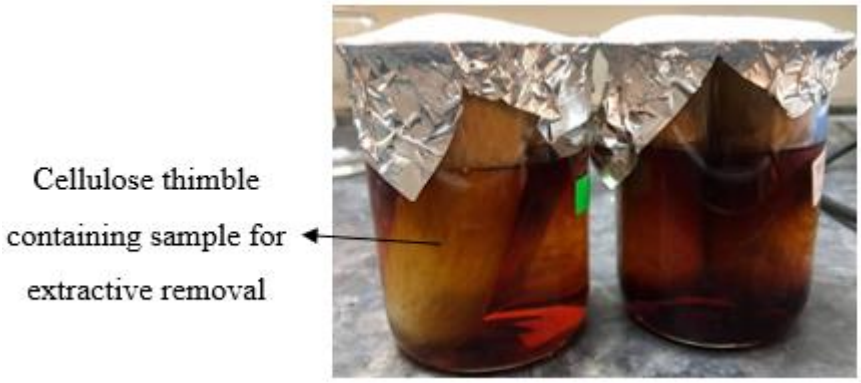

Figure 3.6: Soaking of Pretreated SCG in Solvent Mixture for 24 hours

3.6 Compositional Analysis

Compositional analysis was carried out to determine and evaluate the content of lignin, alpha cellulose, beta cellulose and hemicellulose in both raw and treated spent coffee grounds. Dried solid yield was determined as well for the treated spent coffee grounds after completed the organosolv pretreatment and removal of extraction.

3.6.1 Determination of Solid Yield

The treated spent coffee grounds was dried in oven at 100 °C after pretreatment process. The dried and treated SCG was weighted gravimetrically and the weight was recorded as shown in Figure 3.7. The solid yield in terms of percentage was obtained by following Equation 3.1:

$$Solid\ Yield\ (\%) = \frac{Mass\ of\ Dried\ and\ Treated\ Spent\ Coffee\ Grounds\ (g)}{Sample\ Mass\ (g) = 10g} \times 100\% \quad (3.1)$$

Figure 3.7: Dried and Treated Spent Coffee Grounds was Weighted Together with the Petri Dish

3.6.2 Determination of Alpha-, Beta- and Gamma-Cellulose Content

Alpha cellulose was an insoluble fraction of cellulose that resistant to and does not dissolved in 17.5 wt % of sodium hydroxide solution while beta cellulose and gamma cellulose were the soluble fraction (TAPPI, 1999). However, beta cellulose will be re-precipitated on acidification of the solution and leaving the gamma cellulose remained in the solution. Cellulose content of the spent coffee grounds was investigated by using TAPPI method T 203. The reagents required and the preparation procedure respectively were listed in the Table 3.9.

Table 3.9: Reagents Required and Their Preparation Procedure

Reagent	Preparation Procedure
17.5 wt% Sodium Hydroxide Solution	1. Weighted 17.5 g of sodium hydroxide pallets and added with 82.5 g of distilled water. 2. The solution was stirred thoroughly until all the solid dissolved.
0.5 N Potassium Dichromate Solution	1. Weighted 6.13 g of potassium dichromate and dissolved it in distilled water. 2. The solution was then diluted to 250 ml.
0.1 N, Ferrous Ammonium Sulfate Solution	1. 10.125 g of ferrous ammonium sulfate was dissolved in distilled water and added with 2.5 ml of concentrated sulfuric acid. 2. The solution was then diluted to 250 ml.

Table 3.9 (Continued)

Reagent	Preparation Procedure
3.0 N Sulfuric Acid	1. 83.5 ml of concentrated sulfuric acid was added to an excess of water and diluted to 1000 ml.

The following procedure was carried out to obtain the filtrate for determination of cellulose:

1. Approximate 1.5 g (± 0.1 g) of sample were placed into a 250 ml beaker and 75 ml of 17.5 wt % of sodium hydroxide solution was added.
2. The suspension was stirred by glass rod until it is completely dispersed and the glass rod was rinsed with additional 25 ml of 17.5 wt % of sodium hydroxide solution to remove the adhered sample.
3. The suspension was allowed to immerse and swell for a period of 30 minutes and the time started to measure right after the last drop of solution. The suspension was then covered with aluminium foil and placed at room temperature.
4. 100 ml of distilled water was added into the suspension after 30 minutes and the suspension was allowed to leave for another period of 30 minutes. The total extraction time was 60 ± 5 minutes.
5. After the 60 minutes period, the suspension was filtered. The first 10 to 20 ml of filtrate was discharged and about 100 ml of filtrate was collected in a clean and dry centrifuge tube.

3.6.2.1 Determination of Alpha Cellulose Content

The content of alpha cellulose in sample was analysed by following procedures:

1. 5.0 ml of filtrate and 10.0 ml of 0.5 N potassium dichromate solution were pipetted into a conical flask with working volume of 250 ml.
2. 30.0 ml of concentrated sulfuric acid was added cautiously into the flask while swirling the flask and allowed to leave for 15 minutes.
3. After 15 minutes, 50.0 ml of distilled water was added into the flask and allowed to cool to room temperature.
4. Six drops of phenolphthalein were added into the flask and the solution was titrated with 0.1 N of ferrous ammonium sulfate solution until colour changed from green to brown.

5. The procedure was repeated for blank solution where the filtrate was substituted by 12.5 ml of 17.5 wt % NaOH and 12.5 ml of distilled water.
6. The alpha cellulose content was calculated by following Equation 3.2:

$$Alpha\ Cellulose\ Composition\ (\%) = 100 - \frac{6.85(V_2 - V_1) \times N \times 20}{A \times W} \quad (3.2)$$

Where: V_1 = Titration of the filtrate, ml

V_2 = Blank titration, ml

N = Normality of the ferrous ammonium sulfate solution = 0.1 N

A = Volume of the filtrate used in oxidation, ml = 5.0 ml

W = Oven-dry weight of sample, g = 1.5 g

7. The alpha cellulose recovery rate for treated SCG was determined by using Equation 3.3:

$$Alpha\ Cellulose\ Recovery\ Rate\ (\%) = \frac{Extracted\ Solid\ Yield\ \ (g) \times Alpha\ Cellulose\ Composition\ of\ Treated\ SCG\ (\%) \times 100\%}{Extracted\ Sample\ Mass \times Alpha\ Cellulose\ Composition\ of\ Raw\ SCG\ (\%)} \quad (3.3)$$

3.6.2.2 Determination of Beta- and Gamma- Cellulose Content

The composition of beta- and gamma- cellulose was investigated by procedures as follow:

1. 50.0 ml of filtrate was pipetted into a 250 ml conical flask followed by 50.0 ml of 3 N of sulfuric acid. The flask was covered with aluminium foil and the mixture was mix thoroughly by inverting.
2. The conical flask was submerged in a water bath with temperature about 70 – 90 °C for five minutes to allow coagulation of beta cellulose.
3. The solution was kept in dry and clean centrifuge tube and the precipitate was allowed to settle overnight.
4. 10.0 ml of clear solution and 10.0 ml of 0.5 N potassium dichromate solution were pipetted into a conical flask with working volume of 250 ml.
5. 50.0 ml of concentrated sulfuric acid was added carefully into the flask and the solution was allowed to remain hot for 15 minutes.

6. 50.0 ml of distilled water was added into the flask after 15 minutes and allowed to cool to room temperature.
7. Six drops of phenolphthalein were added into the flask and the solution was titrated with 0.1 N of ferrous ammonium sulfate solution until colour changed from green to brown.
8. The procedure was repeated for blank solution by substituting solution with 12.5 ml of 17.5 wt % sodium hydroxide, 12.5 ml of distilled water and 25.0 ml of 3 N sulfuric acid.
9. The gamma cellulose composition was obtained by following Equation 3.4:

$$Gamma\ Cellulose\ Composition\ (\%) = \frac{6.85(V_2 - V_1) \times N \times 20}{5 \times W} \quad (3.4)$$

Where: V_1 = Titration of the filtrate, ml

V_2 = Blank titration, ml

N = Normality of the ferrous ammonium sulfate solution = 0.1 N

W = Oven-dry weight of sample, g = 1.5 g

10. The gamma cellulose removal rate for treated SCG was determined by using Equation 3.5:

$$Gamma\ Cellulose\ Removal\ Rate\ (\%) = 1 - \frac{Extracted\ Solid\ Yield\ \ (g) \times Gamma\ Cellulose\ Composition\ of\ Treated\ SCG\ (\%) \times 100\%}{Extracted\ Sample\ Mass \times Gamma\ Cellulose\ Composition\ of\ Raw\ SCG\ (\%)} \quad (3.5)$$

11. With known amount of alpha- and gamma cellulose content, the composition of beta cellulose was calculated by Equation 3.6:

Beta Cellulose Composition (%)

= 100 % – Total Alpha and Gamma Cellulose Composition (%) (3.6)

12. The beta cellulose recovery rate for treated SCG was calculated by using Equation 3.7.

$$Beta\ Cellulose\ Recovery\ Rate\ (\%) = \frac{Extracted\ Solid\ Yield\ \ (g) \times Beta\ Cellulose\ Composition\ of\ Treated\ SCG\ (\%) \times 100\%}{Extracted\ Sample\ Mass \times Beta\ Cellulose\ Composition\ of\ Raw\ SCG\ (\%)} \quad (3.7)$$

3.6.3 Determination of Lignin Content

Lignin is a highly branched mononuclear aromatic polymer which provided a rigid and amorphous structure. The content of lignin in biomass was determined by TAPPI method T222.

The composition of lignin was determined as following procedures:

1. Approximate 0.5 g (± 0.001 g) of the sample was weighted and placed into a 250 ml glass beaker.
2. A pre-hydrolysis of sample was performed by added 10 ml of 72 wt % of sulfuric acid into the beaker. The suspension was stirred gently by glass rod until it is completely dispersed.
3. Aluminium foil was used to cover the glass beaker and the suspension was allowed to stay overnight at room temperature.
4. On the next day, the sample was diluted to 3 wt % H_2SO_4 by addition of 325 ml of distilled water.
5. The suspension was then boiled under reflux at 200 °C for 4 hours.
6. The acidic lignin dispersion was filtered through vacuum filter and rinsed with distilled water until to achieve neutral pH.
7. The filtered solid was acid insoluble lignin which was dried at 100 °C to constant weight.
8. 3 ml of distillate was pipetted and diluted with 12 ml of distilled water.
9. A blank solution was prepared by addition of 2 ml of 3 wt % sulfuric acid to mix with 8 ml of distilled water.
10. The acid soluble fraction of lignin was obtained by measuring the absorbance of the diluted distillate and blank solution at 215 nm and 280 nm using Ultraviolet-visible spectroscopy (UV-Vis) which represented the wavelength of furan and HMF respectively.
11. Acid soluble lignin content was determined by using Equation 3.8.

$$Acid\ soluble\ lignin = \frac{[4.53 \times (A_{215} - A_{215,B})] - (A_{280} - A_{280,B})}{300} \quad (3.8)$$

Where: A_{215} = Absorbance of sample at 215 nm

$A_{215,B}$ = Absorbance of blank at 215 nm

A_{280} = Absorbance of sample at 280 nm

$A_{280,B}$ = Absorbance of blank at 280 nm

12. The total lignin content was obtained by the summation of acid insoluble and soluble lignin fraction.
13. The lignin composition of raw and treated SCG was obtained by following Equation 3.9.

$$Lignin\ composition\ (\%) = \frac{Total\ Lignin\ Content\ (g)}{Sample\ mass\ (g) = 0.5\ g} \times 100\% \qquad (3.9)$$

14. The lignin removal rate for treated SCG was calculated by using Equation 3.10.

$$Lignin\ Removal\ Rate\ (\%) = 1 - \frac{Extracted\ Solid\ Yield\ \ (g) \times Lignin\ Composition\ of\ Treated\ SCG\ (\%) \times 100\%}{Extracted\ Sample\ Mass \times Lignin\ Composition\ of\ Raw\ SCG\ (\%)} \qquad (3.10)$$

3.7 Characterisation Analysis

The chemical characterisation of the native and pretreated solid were analysed for comparison. There were several analysis methods that carried out by using different instrument for different purposes. The operating conditions and specifications of each instrument involved were further discussed in following section.

3.7.1 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is capable of performing a variety of signals on the surface of sample by using a focused beam of highly energetic electrons. In current study, SEM analysis with electron beam energy of 15 kV and magnification of 500X, 1000X and 2000X were performed by using Hitachi SEM Model S–3400N to examine the dried samples of both raw and pretreated SCG. The sample was coated in gold in a sputter coater before analysis (Ebrahimi et al., 2017). SEM images were used to determine the changes in structural morphology and fundamental physical properties of pretreated SCG.

3.7.2 Energy Dispersive X-ray Spectroscopy (EDX)

Energy dispersive x-ray (EDX) Ametek model was utilised coupled with SEM to identify the elemental contents and composition of native and pretreated SCG. The investigated elemental contents included carbon (C), oxygen (O), magnesium (Mg), sulphur (S) and chlorine (Cl).

3.7.3 X-ray Diffraction (XRD)

X-ray diffraction (XRD) is a technique that used to analyse powdered solid samples in terms of the crystallographic structure, crystallite size, phase identification and preferred orientation. Crystallinity of SCG could be studied by employing X-rays diffractometer with diffraction angles ranging between $5° < 2\theta < 60°$ at scan speed of 2° per minute. The radiation was emitted by Kα radiation source from Cu as target material ($\lambda = 0.154$ nm) at current of 30 mA and voltage of 40 kV (Ravindran et al., 2018). The effectiveness of enzymatic hydrolysis of pretreated SCG could be revealed as well through the XRD analysis.

3.7.4 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) analysis was conducted on native and pretreated SCG by using Nicholet IS10 FTIR to analyse the potential changes in structural as a result of the alteration in the functional groups after pretreatment process. The FTIR spectra within mid-IR range (4000 – 400 cm^{-1}) were recorded with thirty-two scans per spectrum and at 4 cm^{-1} of resolution in transmission mode. The effect of pretreatment on SCG were determined by comparing between the FTIR spectra of untreated and pretreated sample.

3.7.5 Thermogravimetric Analysis (TGA)

Thermal gravimetric analysis (TGA) was carried out to analyse the thermal stability and weight losses of raw and pretreated SCG when exposed to heating up to 1000 °C. In TGA analysis, 10 mg of sample was taken in an aluminium pan while using empty pan as a reference for comparison. The sample was heated from room temperature (25 °C) to 1000 °C at a rate of 10 °C per minute in constant nitrogen atmosphere. The physical and chemical changes based on thermal behaviour of sample were observed.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Native Spent Coffee Grounds

4.1.1 Compositional Analysis of Raw Spent Coffee Grounds

Compositional analysis was carried out to determine and evaluate the content of lignin, alpha cellulose, beta cellulose and hemicellulose in raw spent coffee grounds (SCG). The chemical composition of raw spent coffee grounds is summarized in Table 4.1.

Table 4.1: Chemical Composition of Raw Spent Coffee Grounds in Dry Weight Basis

Chemical Component	Percentage (wt % dry basis)
Alpha Cellulose	28.64
Beta Cellulose	9.50
Gamma Cellulose	36.17
Lignin	25.69

4.1.2 Surface Morphology of Raw Spent Coffee Grounds

Surface morphology of native SCG was determined using scanning electron microscope (SEM). Figure 4.1 (a), (b) and (c) show the images of raw SCG taken under magnification of 500, 1000 and 2000 respectively. According to Figure 4.1, the SEM image of SCG showed that SCG had a very porous, honeycomb like structure. Thick bundle of cellulose that attached with each other could be observed from Figure 4.1. Majority of the surfaces of SCG were considered smooth even though there were tiny dents and pores that were more prominently observed in Figure 4.1 (c) under 2000 magnification which could be due to mechanical process of size reduction during brewing process (Zein, Gyamera and Skoulou, 2017). Identical observation was also obtained by Safarik et al. (2012) as well where the SEM image of SCG showed a high porosity, honeycomb like structure which providing larger surface area for adsorption for chosen organic xenobiotic . Besides, there were also several dotted spots visualized on the surfaces which could possibly due to the presence of impurities. This could be supported by the result obtained from energy-dispersive X-ray (EDX) where all the composed elements in SCG were investigated. EDX results were shown in Table 4.2. It showed that SCG was mainly composed of carbon (55.68 wt %, 62.88 at %) and

oxygen (43.11 wt %, 36.55 at %) followed by impurities such as magnesium (0.53 wt %, 0.29 at %), sulphur (0.41 wt %, 0.17 at %) and chlorine (0.27 wt %, 0.10 at %).

Table 4.2: Atomic Composition of Raw Spent Coffee Grounds

Element	Weight Percent (%)	Atomic Percent (%)
Carbon	55.68	62.88
Oxygen	43.11	36.55
Magnesium	0.53	0.29
Sulfur	0.41	0.17
Chlorine	0.27	0.10

4.1.3 Fourier Transform Infrared (FTIR) Analysis

FTIR analysis was carried out to investigate and evaluate the chemical bonding of native SCG. The FTIR spectra of native SCG was shown in Figure 4.2 which involved wavenumber range from 500 to 4000 cm^{-1}. This FTIR spectra would act as a control to compare the difference in chemical bonding of SCG after pretreatment. The conspicuous absorbance peaks reported and its corresponded functional group are listed in Table 4.3.

4.1.4 X-ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis was conducted to assess the crystallinity of the raw SCG. The radiation was emitted at voltage of 40 kV and current of 30 mA using copper tube. The range of scattering angle of 2θ was measured from 5° to 60° at the scan speed of 2° per minute. The crystallinity of the biomass was greatly contributed by the presence of cellulose.

Figure 4.3 shows the XRD spectra of raw SCG which would serves as a control for the comparison with pretreated SCG. From the result obtained, the strongest three peaks were reported at 16.04°, 20.16° and 21.48°. Nearly similar observation was also observed by Ballesteros, Teixeira and Mussatto (2014) where the strongest peaks located at 15° and 22° respectively. The peaks that observed at around 38° and 45° were contributed by the presence of carbon in the sample (Unni et al., 2016; Barnthip et al., 2017).

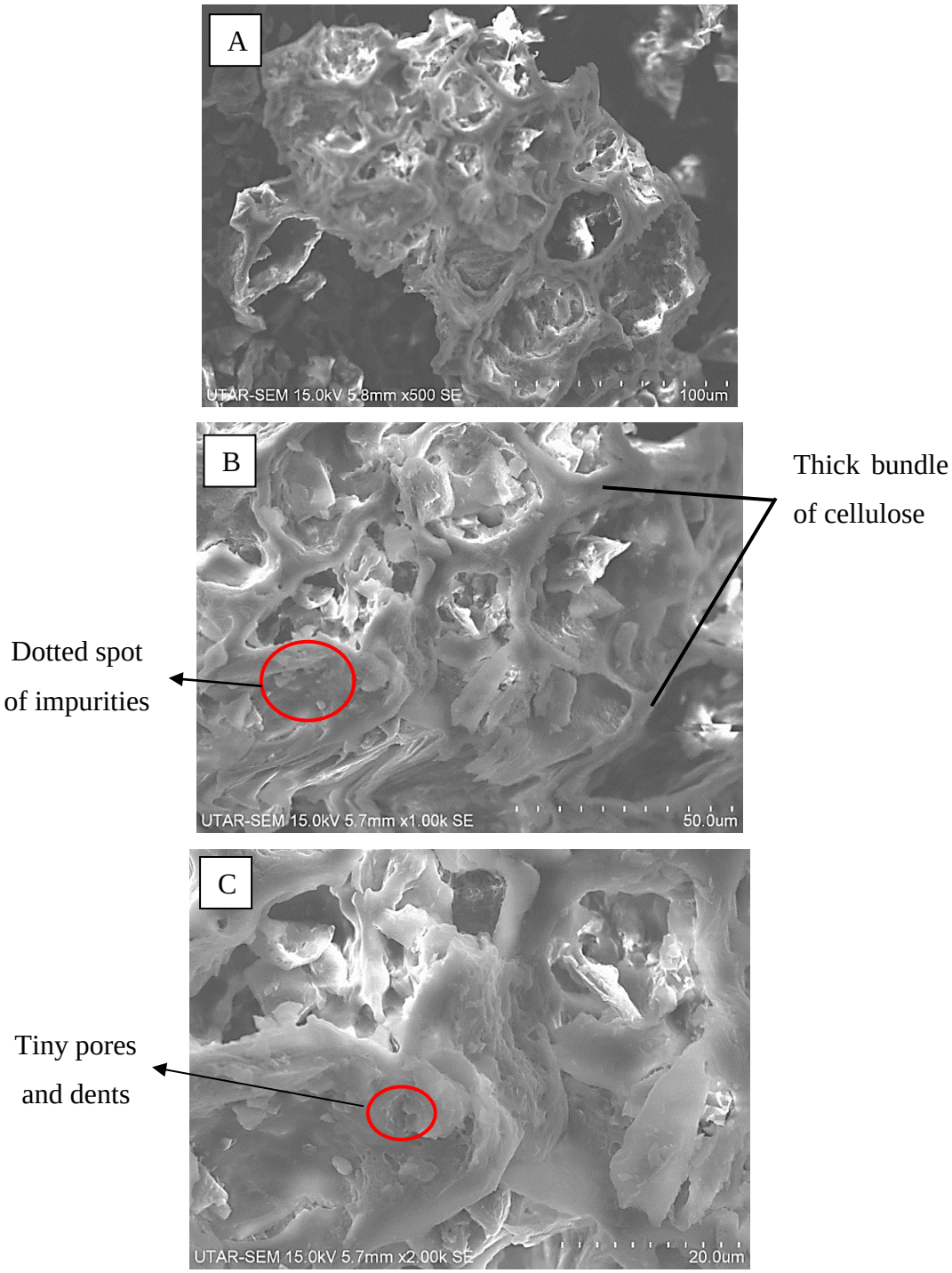

Figure 4.1: Surface Morphology of Raw Spent Coffee Grounds under Magnification of (A) 500X (B) 1000X and (C) 2000X

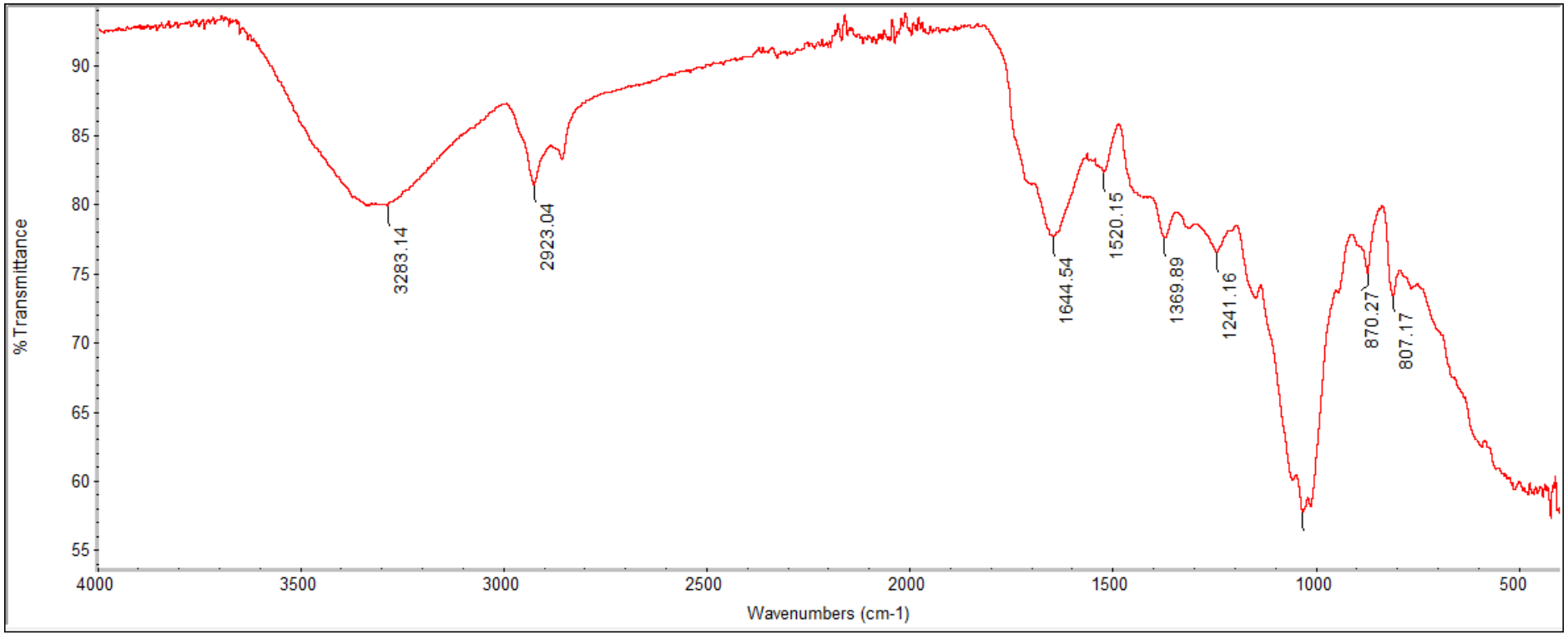

Figure 4.2: FTIR Spectra of Raw Spent Coffee Grounds

Table 4.3: FTIR Absorption Bands of Native SCG and Its Functional Group Respectively

Wavenumbers (cm^{-1})	**Functional Group**	**Polymer**	**Reference**
870	Glycosidic bonds	Hemicellulose	Xu et al. (2013)
			Ballesteros et al. (2015)
1035	C-O, C=C and C-C-O stretching	Cellulose, hemicellulose, lignin	Xu et al. (2013)
1146	C-O-C asymmetry stretching	Cellulose and hemicellulose	Xu et al. (2013)
1241	C-O stretching	Hemicellulose	Bekiaris et al. (2015)
1369	C-H bending	Cellulose, hemicellulose and lignin	Xu et al. (2013)
1520	Aromatic ring vibration	Lignin	Xu et al. (2013)
1644	C=O stretch	Lignin	Xu et al. (2013)
			Ballesteros et al. (2015)
2923	C-H stretching	Lignin	Xu et al. (2013)
			Ballesteros et al. (2015)
3283	O-H stretching	Lignin	Xu et al. (2013)
			Ballesteros et al. (2015)

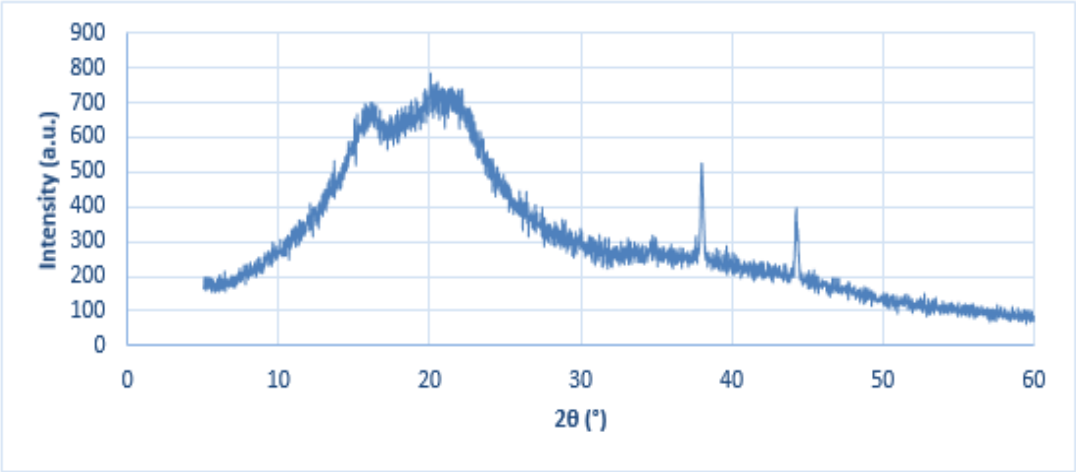

Figure 4.3: XRD Diffractograms of Raw Spent Coffee Grounds

4.1.5 Thermogravimetric Analysis (TGA)

Cellulose and hemicellulose are known as polysaccharides which form fermentable sugars through fermentation process. Thermogravimetric analysis was conducted to study on the weight loss of the polysaccharides extracted from SCG when exposed to heating from 30 °C to 1000 °C under nitrogen atmosphere.

The TGA curve with three defined mass loss stages was obtained and shown in Figure 4.4. First stage started at temperature around 62 °C to 100 °C where a slight weight loss of approximately 6 % was reported. This could be resulted by the dehydration of the sample where the adsorbed and structural water evaporated. For second stage, a significant transformation and weight loss were observed at temperature around 249 °C to 374 °C with a prominent drop in mass from 91 % to 45 %. The huge reduction in weight at this stage indicated the occurrence of depolymerisation as well as break down of polysaccharides and oils in the sample. After second stage, the mass began to drop gradually to reach approximately 13 % as the increment in temperature to 1000 °C. This thermal stage could be related to the polysaccharides decomposition into degradation products such as hydroxymethylfurfural (HMF) and furfural due to the high temperature condition (Ballesteros et al., 2015).

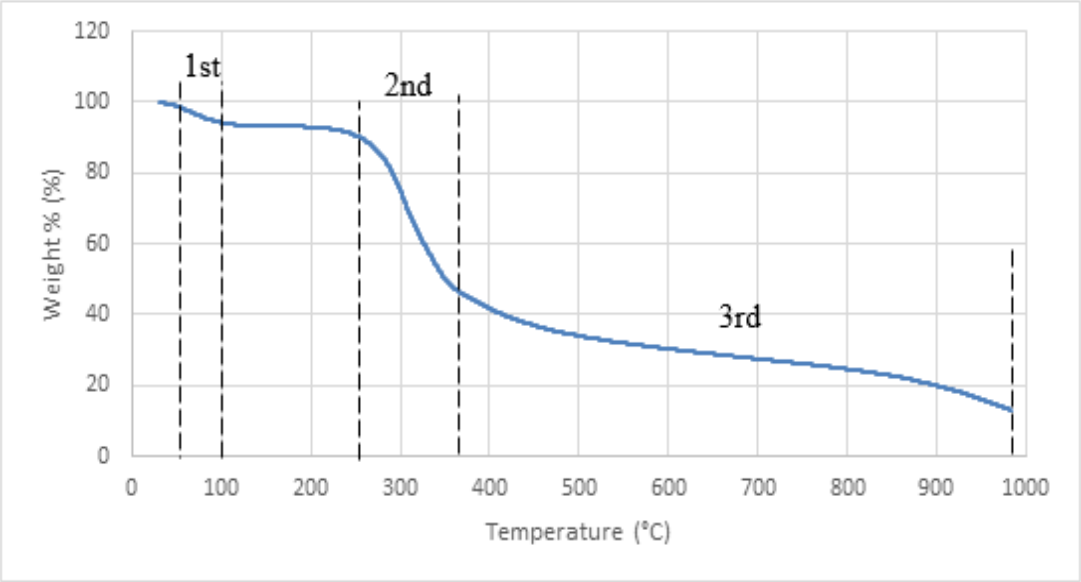

Figure 4.4: TGA Curve of Native Spent Coffee Grounds

4.2 Organosolv Pretreatment

Organosolv pretreatment is a process that used to treat lignocellulosic biomass using organic solvent. In this study, organosolv pretreatment was carried out to treat spent coffee grounds using ethanol as solvent. There were a total of four parameters being evaluated which included solvent loading, acid concentration, temperature and residence time in order to obtain the most promising operating condition. The pretreated spent coffee grounds were evaluated and examined by Scanning Electron Microscope (SEM), Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD) and Thermogravimetric Analysis (TGA).

4.2.1 Effect of Solvent Loading

Organosolv pretreatment was performed using fixed concentration of 60 % v/v diluted ethanol and 1 % v/v concentrated sulfuric acid as catalyst at 190 °C for 60 minutes with different solvent loading (10 % v/w, 12.5 % v/w, 15 % v/w and 17.5 % v/w). Figure 4.5 (a) and (b) shows the results of pretreatment when varying solvent loading.

According to Figure 4.5 (a), the solid yield with solvent loading of 10.0 % v/w was the lowest (69.40 %) while solvent loading of 12.5 % v/w showed the highest solid yield of 75.90 %. Further increment in solvent loading to 15.0 % v/w and 17.5 %v/w revealed moderately drop in solid yield which were 75.00 % and 74.31 % respectively. The high solid yield for solvent loading of 12.5 % could be explained by observing the

results as presented in Figure 4.5 (b) where the alpha and beta cellulose recovery rate (80.3 %) and gamma removal rate (66.5 %) were the highest. As the solvent loading increased, the hemicellulose hydrolysis and enzymatic degradability of pretreated SCG reduced because the activity of hydrolytic enzymes would be hindered by ethanol (Huijgen, Laan and Reith, 2008). Since the gamma-cellulose was mainly consisted of hemicellulose, the gamma cellulose removal rate dropped from 66.5 % to 57.2 % when solvent loading increased from 12.5 % to 17.5 %.

American Paper and Pulp Association (TAPPI) stated that alpha cellulose was an undegraded and unbranched polymer that had high resistance and remained insoluble in 17.5 wt % of sodium hydroxide solution while beta cellulose was the degraded cellulose that soluble in 17.5 wt % of sodium hydroxide solution. In terms of alpha and beta recovery rate, the rate increased from 71.8 % for solvent loading of 10.0 % v/w to the highest rate of 80.3 % for solvent loading of 12.5 % v/w then declined to 74.5 % and 70.1 % when further increasing the solvent loading to 15.0 % v/w and 17.5 % v/w. This could be due to the enzymatic degradability of pretreated SCG reduced with an increase in solvent loading.

Huijgen, Laan and Reith (2008) stated that the delignification effect increased as the lignin was more soluble in relatively high ethanol content. In this parameter study, the lignin removal improved at higher ethanol content where lignin removal rate of 74.5 % was reported at lowest solvent loading (10 % v/w) and gradually increased to 77.9 % at solvent loading of 15 % v/w. However, a drop in lignin removal rate (73.8 %) was observed at solvent loading of 17.5 % v/w due to the reprecipitation of lignin occurred at extensively high ethanol content (Bensah and Mensah, 2013). In addition, a higher ethanol content generated a high pH condition which would slow down or reduce the cleavage of bonds within the lignocellulose complex and lignin, causing reduction in delignification (Huijgen, Reith and den Uil, 2010).

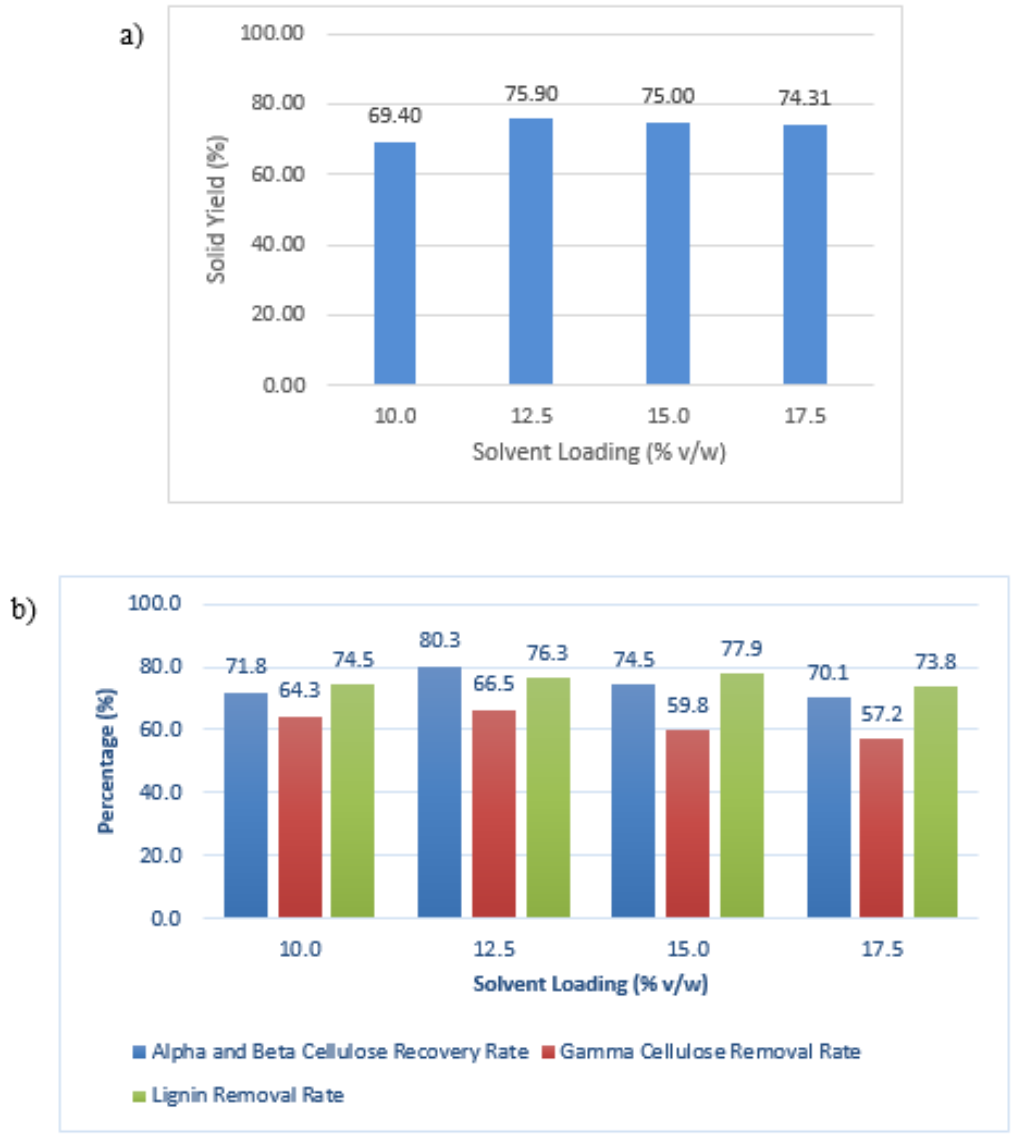

Figure 4.5: Pretreatment Performance at 190 °C for 60 Minutes Using 1 % v/v Acid But Varying Solvent Loading (a) Solid Yield after Organosolv Pretreatment (b) Alpha and Beta Cellulose Recovery Rate, Gamma Removal Rate and Lignin Removal Rate

Based on the pretreatment performance in compositional analysis, solvent loading of 12.5 % v/w showed the best result in overall performance while solvent loading of 17.5 % v/w was the worst. Hence, characterisation analysis was carried out for these two solid loadings to compare the difference in performance. Figure 4.6 illustrates the FTIR spectra of raw SCG and treated SCG under solvent loading of 12.5 % v/w and 17.5 % v/w. The prominent peaks of absorption were discussed. Transmittance at 3305, 2910 and 1640 cm^{-1} corresponded to O-H, C-H and C=O stretching of lignin compounds (Xu et al., 2013; Ballesteros et al., 2015). As compared

to the raw SCG, the treated SCG under solvent loading of 12.5 % v/w had lower transmittance at 3305, 2910 and 1640 cm^{-1} indicated the reduction of lignin compounds. In contrast with raw SCG, a high transmittance at 3305 and 1640 cm^{-1} were clearly observed for treated SCG under solvent loading of 17.5 % v/w which denoted high amount of lignin present which supported by the lower lignin removal rate as shown in Figure 4.5 (b) where the cleavage of bonds in lignin might reduce and lignin reprecipitated at extensively high ethanol content. The transmittance of solvent loading of 17.5 % v/w at 2910 cm^{-1} was significantly lesser compared to both raw SCG and treated SCG under solvent loading of 12.5 % v/w could be explained due to lesser composition of impurities such as magnesium (0.15 at %), sulfur (0.15 at %) and chlorine (0.07 at %) elements present in the solid. This statement was supported by the EDX result as shown in Table 4.4.

Besides, the transmittance at 1030 cm^{-1} represented C-O, C=C and C-C-O stretching of cellulose, hemicellulose and lignin compounds (Xu et al., 2013). The reduction at this peak for both treated SCG indicated the reduction of cellulose, hemicellulose and lignin component in the solid. The transmittance at this peak for treated SCG under solvent loading of 12.5 % v/w was higher than that for 17.5 % v/w which proven that solvent loading of 12.5 % v/w had higher total cellulose component due to the higher cellulose recovery.

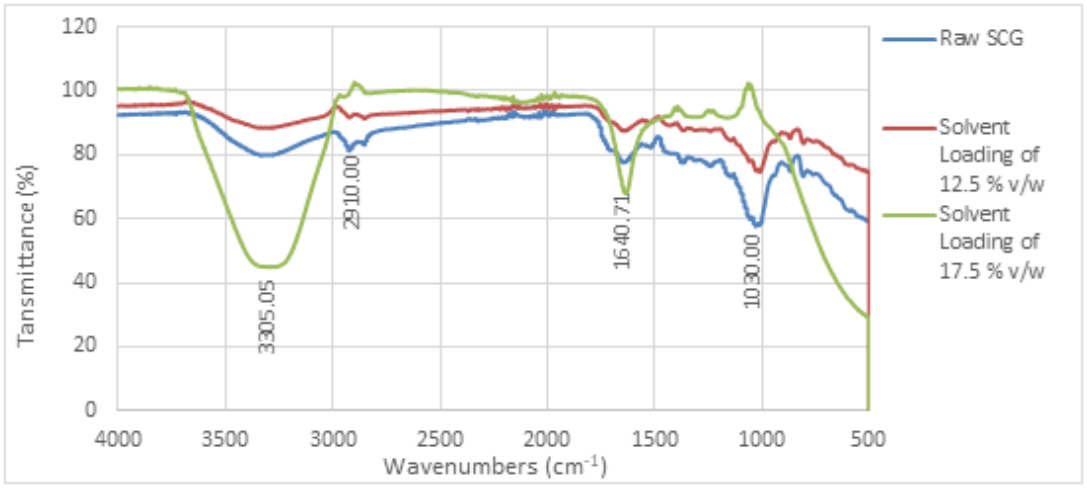

Figure 4.6: Comparison of FTIR Spectra between Raw and Treated Spent Coffee Grounds with Solvent Loading of 12.5 % v/w and 17.5 % v/w

Table 4.4: Atomic Composition of Treated Spent Coffee Grounds with Solvent Loading of 12.5 % v/w and 17.5 % v/w

Element	Weight Percent (%)	Atomic Percent (%)
Solvent Loading of 12.5 % v/w		
Carbon	59.99	66.85
Oxygen	39.17	32.76
Magnesium	0.32	0.18
Sulfur	0.40	0.17
Chlorine	0.12	0.05
Solvent Loading of 17.5 % v/w		
Carbon	62.91	69.54
Oxygen	36.25	30.08
Magnesium	0.28	0.15
Sulfur	0.36	0.15
Chlorine	0.20	0.07

Figure 4.7 (a) displays the SEM images of the raw SCG while (b) and (c) show the images of treated SCG with solvent loading of 12.5 % v/w and 17.5 % v/w respectively under 1000X magnification. As shown in Figure 4.7 (a), the surface of raw SCG appeared to be smoother than treated SCG even though it possessed tiny pores. Raw SCG had thick bundles of cellulose attached with each other in a close structure. In contrast to raw SCG, pretreated SCG depicted to be rougher, more porous and more spacious between the fibers which indicated a size reduction after pretreatment due to hemicellulose and lignin removal. However, the treated SCG in Figure 4.7 (b) with solvent loading of 12.5 % v/w had higher severity where the structure was in high porosity and the cellulose structure were disrupted as some degraded cellulose was peeled off from the fiber strand.

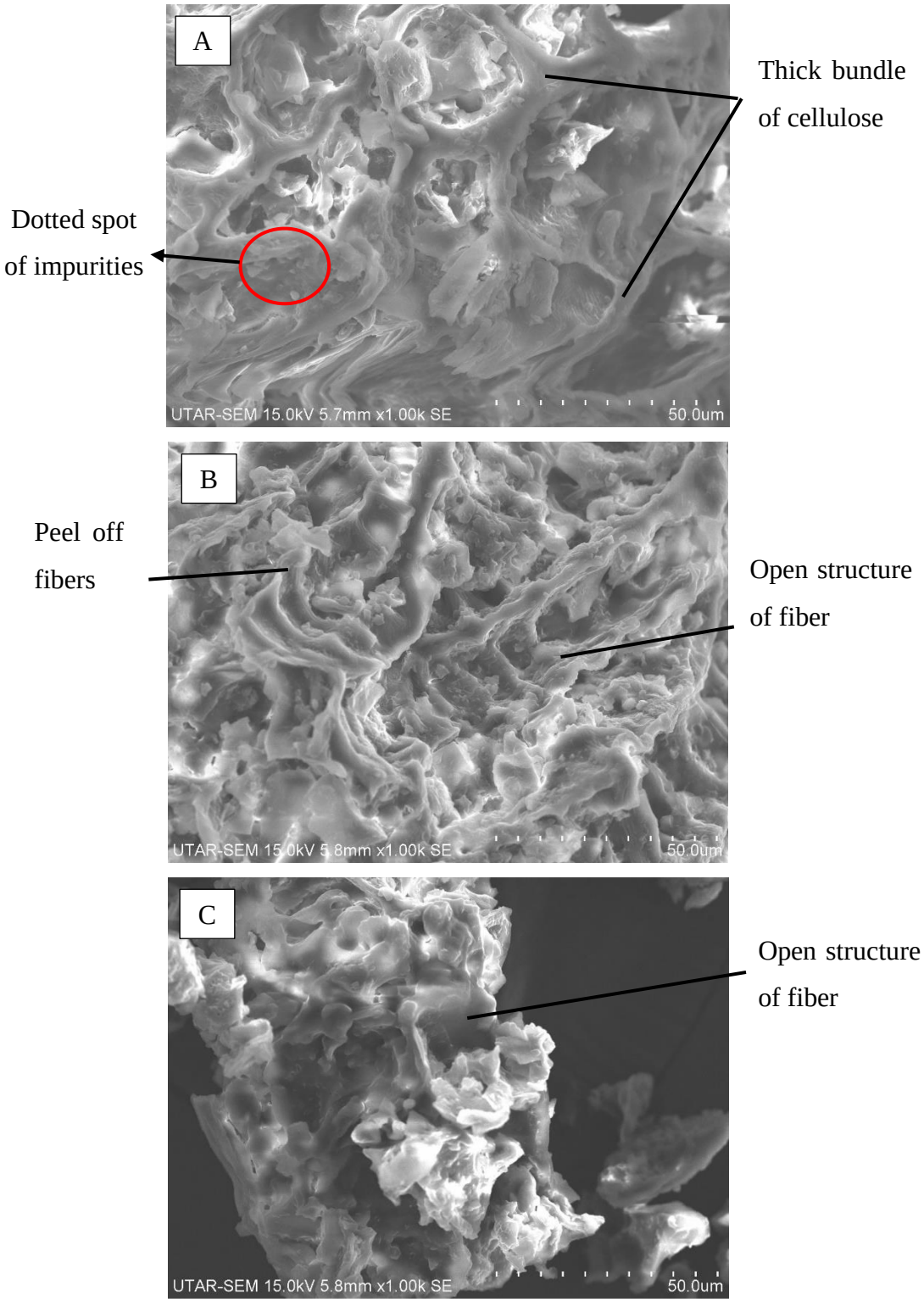

Figure 4.7: Surface Morphology of Raw and Treated Spent Coffee Grounds under Magnification of 1000 (A) Raw Spent Coffee Grounds (B) Treated Spent Coffee Grounds with Solvent Loading of 12.5 % v/w (C) Treated Spent Coffee Grounds with Solvent Loading of 17.5 % v/w

Apart from SEM and FTIR analysis, X-ray diffraction (XRD) analysis was utilised to determine the crystallinity of SCG before and after pretreatment. X-ray diffractogram of raw SCG and pretreated SCG under solvent loading of 12.5 % v/w from 5° to 60° are presented in Figure 4.8. Generally, the presence of amorphous and crystalline structure of lignocellulosic biomass could be observed from the peaks located at 15° and 22° (Raghavi et al., 2016). As illustrated in Figure 4.8, treated SCG showed higher crystallinity than the native SCG which attributed to the effectiveness of the pretreatment. Aggregation of cellulose in biomass occurred due to delignification of biomass during pretreatment resulted to an increment in crystallinity degree for treated SCG. Analogous observations were reported by Raghavi et al. (2016) for the pretreatment of sugarcane trash.

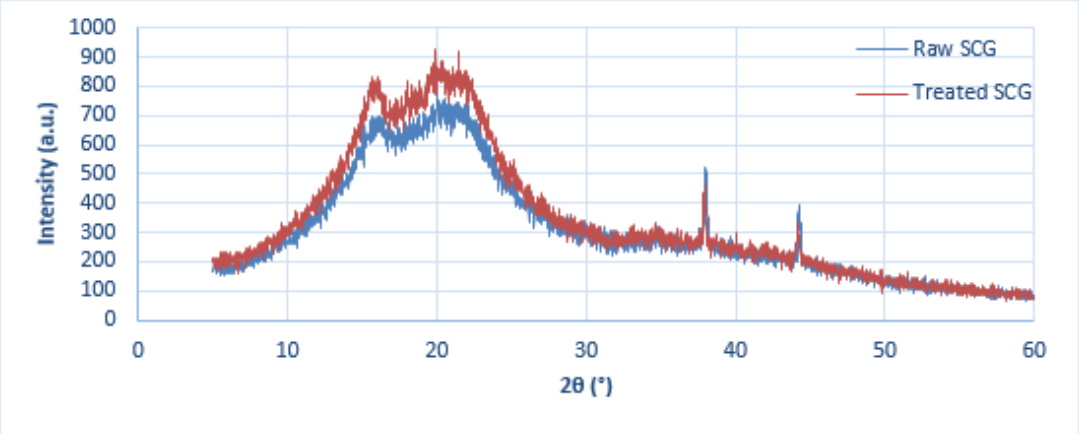

Figure 4.8: X-ray Diffractogram of Native and Pretreated Spend Coffee Grounds

Besides, thermogravimetric analysis was conducted to compare the thermal stability of the raw and pretreated SCG under solvent loading of 12.5 % v/w. The TGA curves obtained are illustrated in Figure 4.9. According to the result, raw and pretreated SCG revealed an identical trend of curve with three defined weight loss phases. The curve for both raw and treated SCG were highly overlapped with each other in the first and second phase of weight loss. The first weight reduction occurred at approximately 62 °C (99 %) and became stable at 93 % when reached temperature of 124 °C. This insignificant loss in weight was most likely due to the removal of volatile materials or moisture in the sample through evaporation. At the second phase, the result showed a sharp decline in weight from 91 % to 45 % between temperature ranges of 250 °C to 375 °C as a result of the depolymerisation and decomposition of polysaccharides and

possibly oils that present in SCG. Finally, the mass loss observed in the third phase showed the occurrence of thermal decomposition in sample into degradation products due to the high severity condition. By referring to Figure 4.9, the TGA curve of treated SCG showed a more prominent reduction in weight (37 %) as compared to the raw SCG (30 %) which indicated a lower thermal stability for treated SCG due to removal hemicellulose and lignin components after pretreatment. Hence, the cellulose was more vulnerable and subjected to degradation due to the loss of protection. The analogous TGA curve was reported by Ballesteros, Teixeira and Mussatto (2014) where the thermal stability of spent coffee grounds and coffee silverskin were studied. The study reported that coffee silverskin had lower thermal stability than SCG in the last thermal stage as coffee silverskin started to decompose at temperature above 457.24 °C and had 34.17 % weight loss while the decomposition of spent coffee grounds began at 499.29 °C with 33.08 % weight loss.

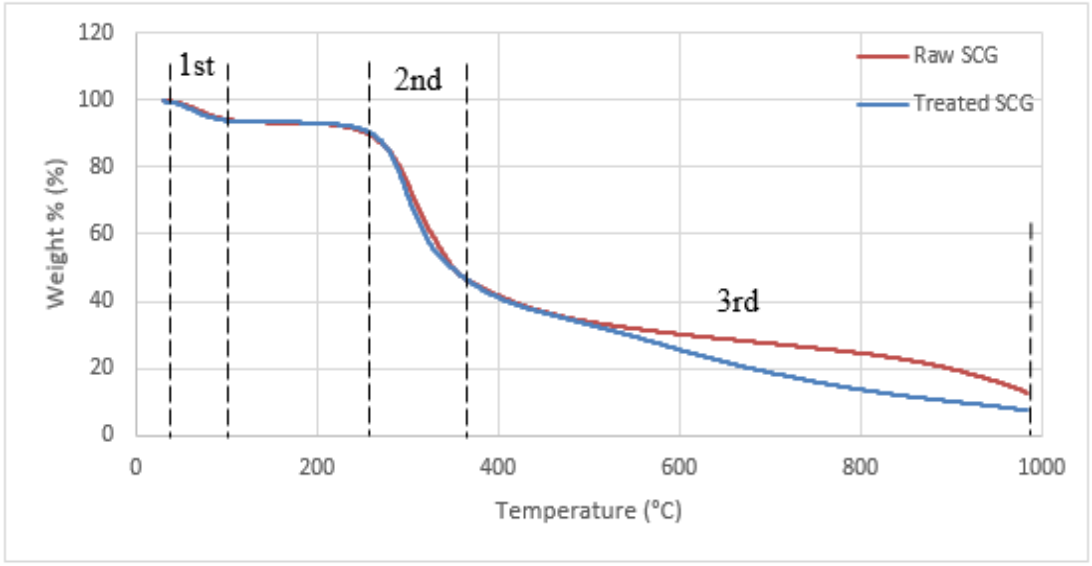

Figure 4.9: Comparison of TGA Curve between Raw and Treated Spent Coffee Grounds with Solvent Loading of 12.5 % v/w

After completion of both compositional and characteristic analysis, the optimum performance was obtained for solvent loading with 12.5 % v/w due to relatively high cellulose recovery (80.3 %) and lignin removal (76.3 %). Hence, this solvent loading was used to proceed to subsequent parameter studies.

4.2.2 Effect of Acid Concentration

Concentration sulfuric acid was used as the catalyst for the ethanol-based organosolv pretreatment in this study. The pretreatment was taken place at 190 °C for 60 minutes with solvent loading of 12.5 % v/w but various sulfuric acid concentration (0, 1, 2 and 3 % v/v). The pretreatment results obtained are shown in Figure 4.10 (a) and (b). Noted that the experiment set with 0 % v/v acid was performed as a control to determine the effect of the pretreatment process with and without the presence of catalyst.

Figure 4.10 (a) shows the solid yield obtained of each experiment with various acid concentration. According to the results obtained, the solid yield dropped moderately from 82.07 % to 67.05 % with an increment in acid concentration from 0 % v/v to 3 % v/v as the delignification of SCG was encouraged at high concentration of acid due to the high pretreatment severity. This statement was proven by the increment in removal rate of lignin dramatically from 63.4 % (without catalyst) to 76.3 % (with 1 % v/v acid) and continue to increase to achieve 78.9 % (with 3% v/v acid) based on Figure 4.10 (b).

In addition, the gamma cellulose removal rate was enhanced from 55.7 % for 0 % v/v acid to 68.6 % with 3 % v/v acid as catalyst. This could be explained because the hemicellulose hydrolysis and enzymatic degradability of pretreated SCG were promoted at high acid concentration (Salapa, Topakas and Sidiras, 2018). For the alpha and beta cellulose recovery, it showed the highest value (80.3 %) when 1 % v/v of acid was used and declined with further increment in acid concentration. It could be concluded that the structure of cellulose was not only disrupted and degraded by the strong corrosive power of acid but cellulose content in the biomass were broke down as well.

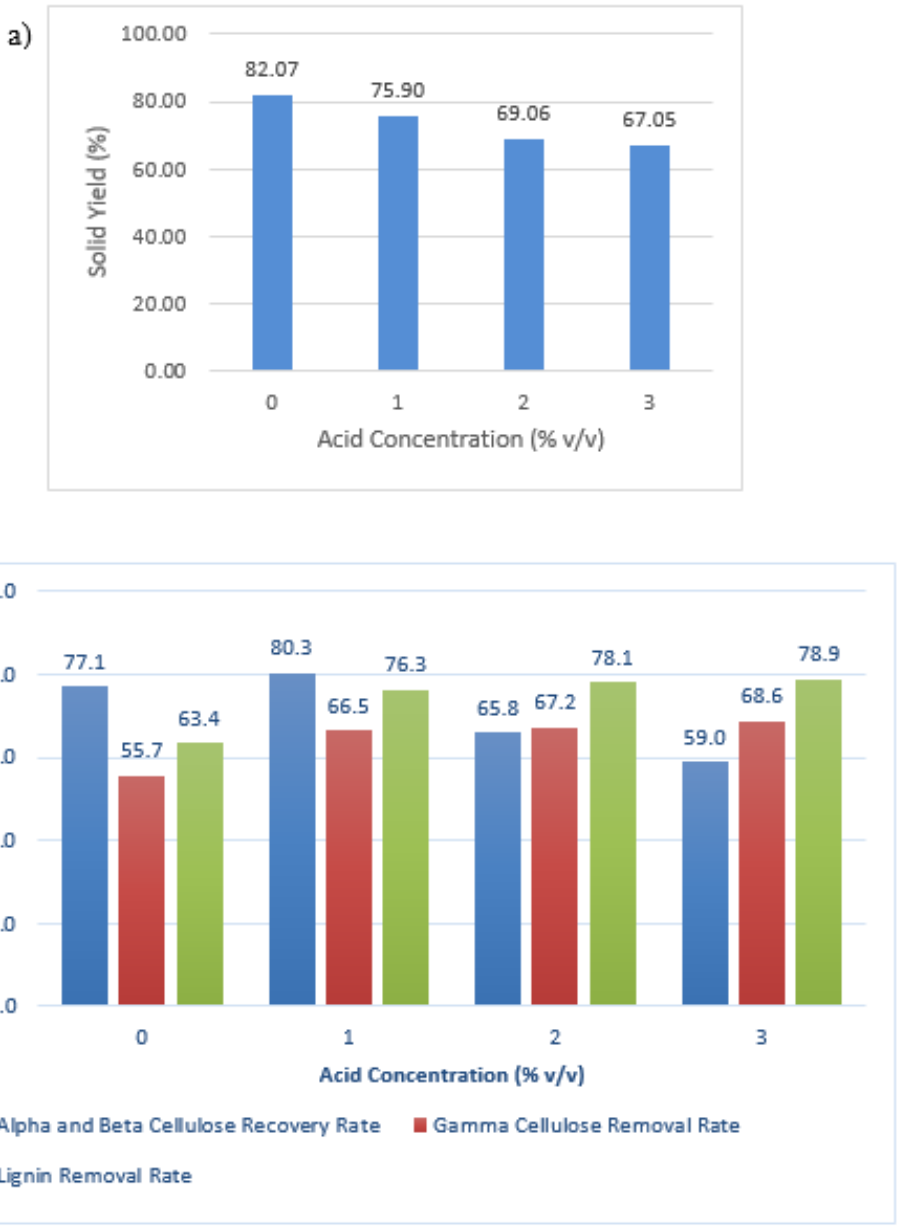

Figure 4.10: Pretreatment Performance at 190 °C for 60 Minutes under Solvent Loading of 12.5 % v/w But Varying Sulfuric Acid Concentration (a) Solid Yield after Organosolv Pretreatment (b) Alpha and Beta Cellulose Recovery Rate, Gamma Removal Rate and Lignin Removal Rate

Figure 4.11 illustrates the FTIR spectra between raw SCG and treated SCG with acid concentrations of 0, 1 and 3 % v/v. There were four conspicuous peaks reported where 3300, 2915 and 1640 cm^{-1} symbolized the O-H, C-H and C=O stretching of lignin compounds respectively while 1030 cm^{-1} represented the C-H, O-H and C=O stretching of cellulose, hemicellulose and lignin compounds (Xu et al., 2013; Ballesteros et al., 2015). According to the results obtained, there was

insignificant difference or overlapped in FTIR spectra between raw SCG and the treated SCG without catalyst. This had proven the low effectiveness of the organosolv pretreatment performance in the absence of catalyst. Treated SCG with 1 % v/v acid had the highest transmittance in overall spectra implied that it was the optimum acid concentration for the pretreatment. However, the transmittance for the treated SCG with 3 % v/v acid had slightly lower in transmittance as compared to that with 1 % v/v. This result was opposed to the pretreatment results obtained in Figure 4.10 (b) where the deligninfication effect and the hemicellulose removal were expected to be higher when acid concentration increased (Salapa, Topakas and Sidiras, 2018). This could be due to the higher composition of impurities in the solid than that for 1 % v/v acid. According to EDX results of both acid concentrations that tabulated in Table 4.5, 3 % v/v had higher magnesium, sulfur and chlorine element (0.21 % Mg, 0.18 % S and 0.09 % Cl) than 1 % v/v acid (0.18 % Mg, 0.17 % S and 0.05 % Cl).

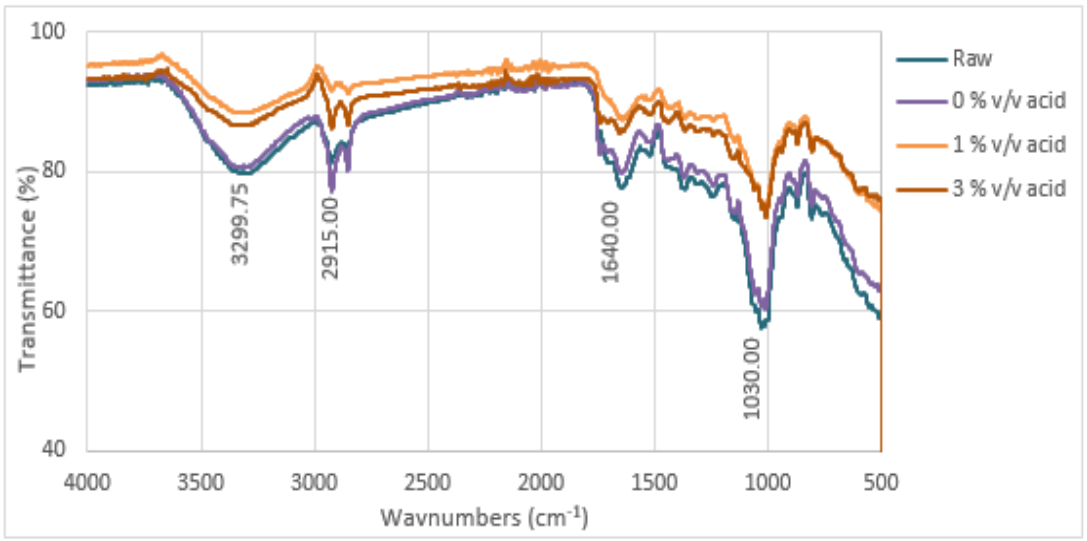

Figure 4.11: Comparison of FTIR Spectra between Raw and Treated Spent Coffee Grounds with Acid Concentration of 0, 1 and 3 % v/v

Table 4.5: Atomic Composition of Treated Spent Coffee Grounds with Acid Concentration of 1 % v/v and 3 % v/v

Element	1 % v/v acid		3 % v/v acid	
	Wt (%)	At (%)	Wt (%)	At (%)
Carbon	59.99	66.85	62.98	65.19
Oxygen	39.17	32.76	36.39	34.33
Magnesium	0.32	0.18	0.37	0.21
Sulfur	0.40	0.17	0.61	0.18
Chlorine	0.12	0.05	0.06	0.09

The pretreatment performance between acid concentration of 1 % v/v and 3 % v/v were further compared through SEM analysis. The surface morphology for both treated SCG are displayed in Figure 4.12. The analysis of treated SCG using acid concentration of 1 % v/v revealed rougher surface as shown in Figure 4.12 (a). This indicated that the cellulose structure was disrupted as some degraded cellulose was peeled off from the fiber strand and caused uneven surface. Besides, there were some cracking lines which could be observed from the surface morphology of both treated SCGs. Basically, the treated SCG would possess greater porosity and higher pore volume by subjecting to higher concentration of acid (Teramura et al., 2018). This was proven as the treated SCG in Figure 4.12 (b) had more spacious structure which indicated a size reduction after pretreatment due to high hemicellulose and lignin removal as shown in Figure 4.10 (b).

Based on the compositional analysis, FTIR analysis and SEM analysis, pretreatment with 1 % v/v acid concentration was selected as the optimum for this parameter due to the highest alpha and beta cellulose recovery and satisfied gamma cellulose and lignin removal. Furthermore, pretreatment with 1 % v/v acid concentration had the best performance in FTIR analysis. On the other hand, even though the surface morphology of pretreatment with 3 % v/v acid concentration showed better result due to the high severity condition, the performance for pretreatment with 1 % v/v acid concentration was still considered optimum.

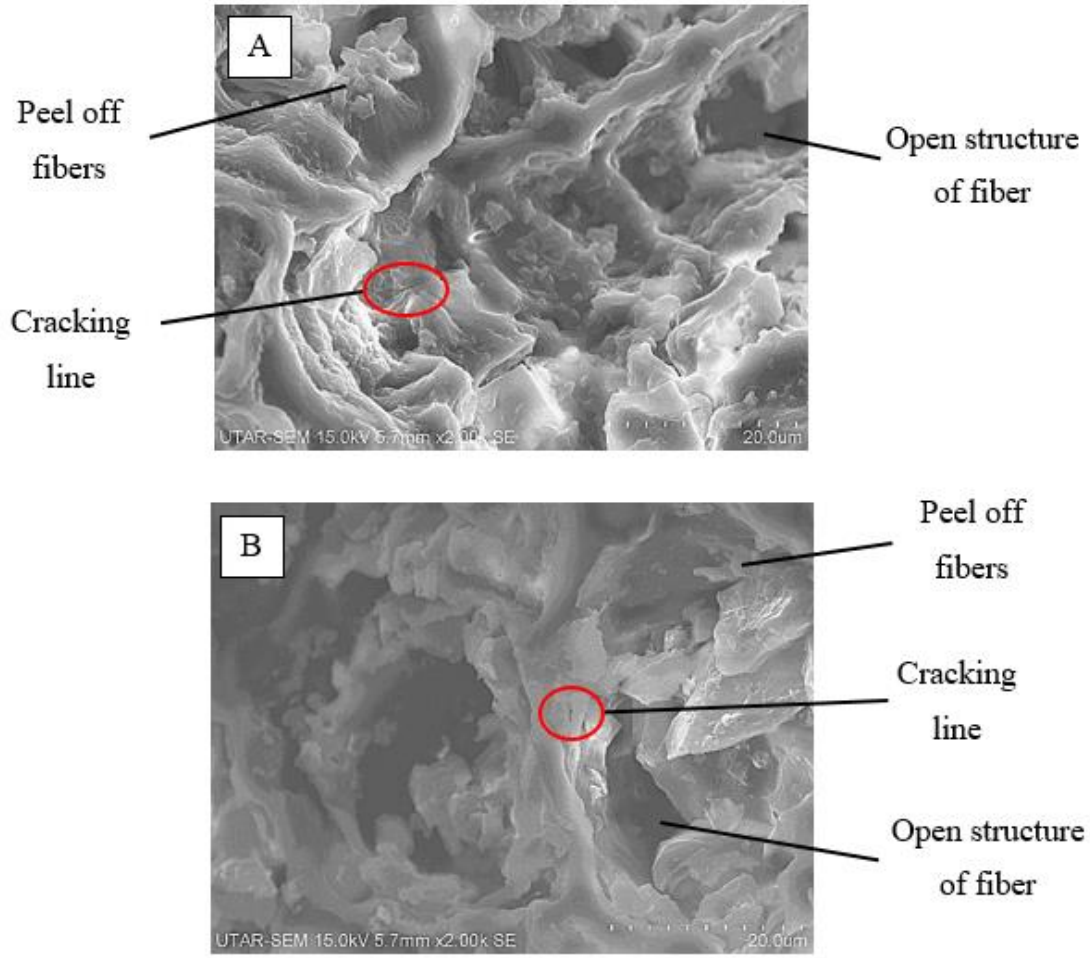

Figure 4.12: Surface Morphology of Treated Spent Coffee Grounds under 2000X Magnification (A) Acid Concentration of 1 % v/v (B) Acid Concentration of 3 % v/v

Hence, the treated SCG with 1 % v/v acid concentration was used to compare the crystallinity of treated SCG with and without catalyst by carried out XRD analysis in order to evaluate the impact of acid concentration on SCG. Comparison of XRD profile between pretreated SCG without catalyst and with 1 % v/v acid as catalyst was depicted in Figure 4.13. The performance of the pretreatment could be featured by the rise in crystallinity degree at 15° and 22° which indicated the attrition of amorphous components. As shown in Figure 4.13, the treated SCG with 1 % v/v acid concentration had higher crystallinity compared to the treated SCG without catalyst which revealed high removal of amorphous components such as lignin and hemicellulose which in turn increased the crystallinity index (Raghavi et al., 2016).

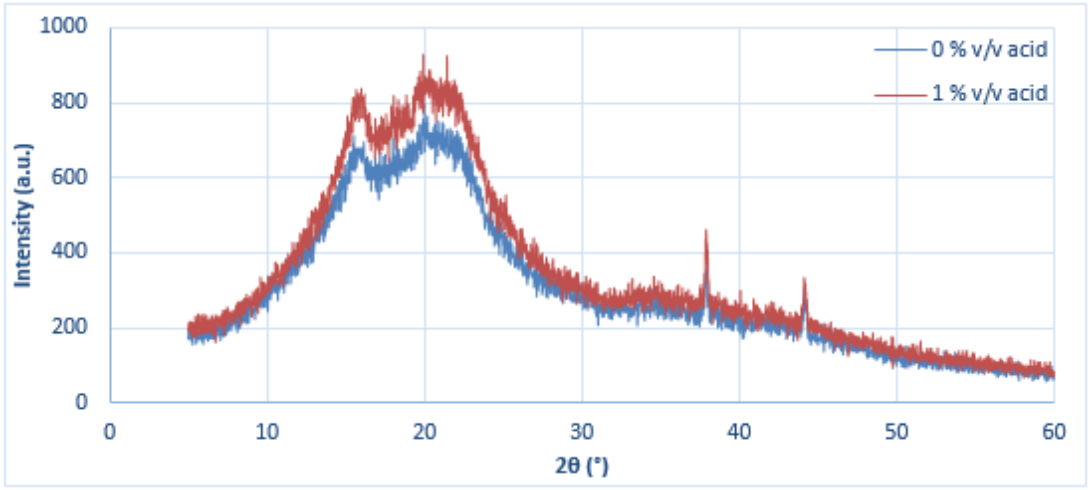

Figure 4.13: X-ray Diffractogram of Pretreated Spend Coffee Grounds with 1 % v/v of Acid and Without Catalyst

Furthermore, the thermal stability of treated SCG with and without catalyst was examined by carried out thermogravimetric analysis. Figure 4.14 shows the weight loss curves for treated SCG without catalyst and with 1 % v/v acid as catalyst. The TGA curves revealed that the treated SCG for both conditions had similar trend with three defined weight loss stages. The minor weight loss observed in first stage was attributed to desorption of water in the sample though evaporation (Filho et al., 2007). Treated SCG with 1 % v/v acid in this stage had lower mass loss (6 %) than that without catalyst (8 %). This indicated that treated SCG with 1 % v/v acid had less absorbed water in polysaccharide structure. During second stage, the greatest weight losses occurred which related to the decomposition of polysaccharides structure in samples, providing 46 % and 48 % weight losses for treated SCG with 0 % v/v acid and 1 % v/v acid respectively. The last weight loss stage occurred at temperature above 400 °C where the weight of both treated SCG were gradually decreased with increment of time. This could be explained by the presence of thermal decomposition event in the sample into degradation products due to high severity condition. In this stage, treated SCG without catalyst showed higher thermal stability by providing lesser weight loss (28 %) than that for treated SCG with 1 % v/v acid (33 % weight loss) as temperature raised from 400 °C to 1000 °C. The lower thermal stability indicated that polysaccharides were more vulnerable and subject to degradation at high temperature due to lower hemicellulose and lignin components present in the sample which was a desired outcome for pretreatment.

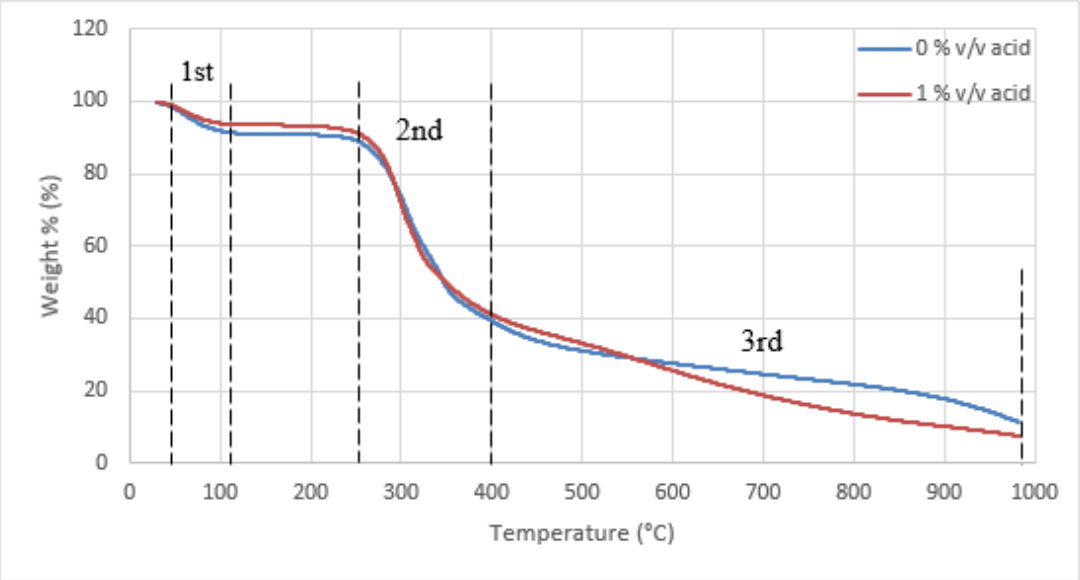

Figure 4.14: Comparison of TGA Curve between Pretreated Spend Coffee Grounds with 1 % v/v of Acid and Without Catalyst

Hence, at the end of this parameter study, acid concentration of 1 % v/v was concluded as the optimum acid concentration for organosolv pretreatment after analysed all the results obtained from compositional and characteristic analysis.

4.2.3 Effect of Reaction Temperature

In section 4.2.2, pretreatment condition with 1 % v/v sulfuric acid as catalyst had the best performance and hence was applied to proceed for the parameter study on reaction temperature in this section. The pretreatment were performed for 60 minutes at temperature of 180 °C, 190 °C, 200 °C and 210 °C with 12.5 % v/w solvent loading and 1 % v/v sulfuric acid as catalyst. The data obtained are illustrated in Figure 4.15.

The study reported a decline in solid yield from 78.06 % to 69.50 % when the reaction temperature increased from 180 °C to 210 °C due to the enhanced delignification effect at high temperature. Contrary trends were observed for gamma cellulose and lignin removal rate which increased moderately with elevation of temperature from 180 °C to 200 °C. However, a further increase to temperature of 210 °C caused a minor reduction for both gamma cellulose removal (from 67.1 % to 64.4 %) and lignin removal (from 78.1 % to 76.3 %) rate. This was possibly due to the occurrence of lignin condensation reactions and formation of degradation products such as furfural at high temperature (Huijgen, Reith and den Uil, 2010).

For the alpha and beta cellulose recovery rate, a maximum recovery rate of 80.3 % had been obtained at temperature of 190 °C. A further elevation of temperature slightly decreased the cellulose recovery rate as shown in Figure 4.15 (b). As the enzymatic digestibility of the cellulose improved substantially with the pretreatment severity, more cellulose was disrupted, degraded as well as broken in structure resulting slight reduction in cellulose recovery at high severity condition.

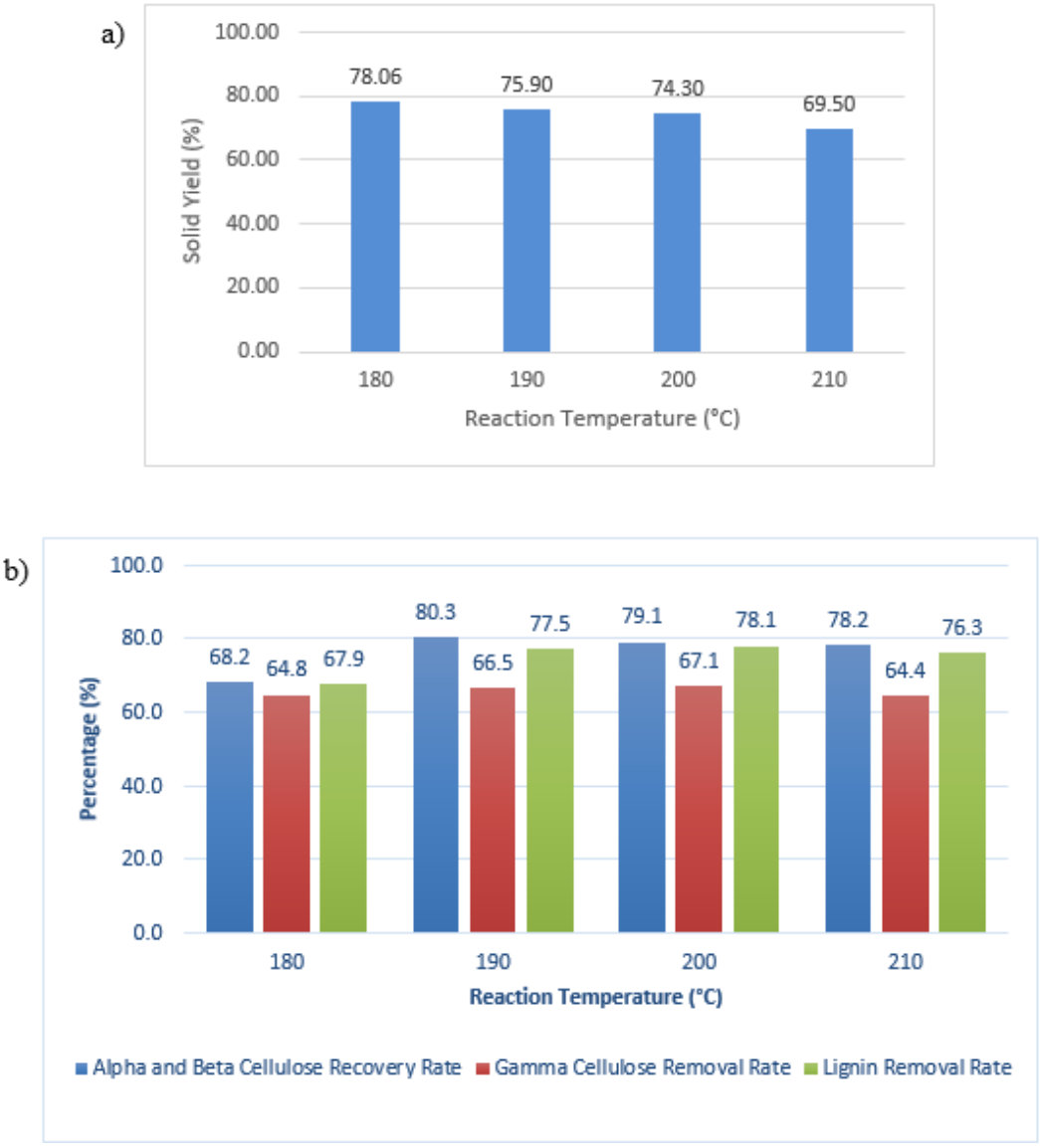

Figure 4.15: Pretreatment Performance under Solvent Loading of 12.5 % v/w with 1 % v/v acid for 60 Minutes But at Varying Reaction Temperature (a) Solid Yield after Organosolv Pretreatment (b) Alpha and Beta Cellulose Recovery Rate, Gamma Removal Rate and Lignin Removal Rate

The pretreatment performance at 180 °C was relatively poor as compared to others which indicated insufficient pretreatment temperature. For pretreatment temperature within 190 °C to 210 °C, pretreatment performance at 190 °C showed the best result. However, pretreatment performance at 210 °C was less desired due to low effectiveness. The performance of these two pretreatment temperature was further supported by FTIR spectra as depicted in Figure 4.16.

Figure 4.16 shows the comparison of FTIR spectra of treated SCG at temperature of 190 °C and 210 °C. Five conspicuous absorption peaks were observed where 3320 cm^{-1}, 2928 cm^{-1} and 1640 cm^{-1} represented the O-H, C-H and C=O stretching of lignin compounds respectively, 1034 cm^{-1} symbolized the presence of C-O, C=C and C-C-O stretching of cellulose, hemicellulose and lignin compounds and lastly, 1745 cm^{-1} represented the ketone or aldehyde C=O stretching of hemicellulose (Xu et al., 2013; Ballesteros, Teixeira and Mussatto, 2014). The FTIR spectra showed analogous result as shown in Figure 4.15 (b) where the peaks at 3320 cm^{-1} and 1640 cm^{-1} were lower for treated SCG at 190 °C than that at 210 °C which supported the high lignin removal rate at 190 °C. However, a contrary observation reported at 2928 cm^{-1} which supposed to observe a smaller peak could be explained due to the presence of high carbon element as well as impurities in the solid. The comparison between the atomic composition of treated SCG at 190 °C and 210 °C are shown in Table 4.6. At wavelength of 1034 cm^{-1}, the transmittance of treated SCG at 210 °C was higher than that for 190 °C. In other words, treated SCG at 210 °C had lesser total cellulose components. This statement was supported by the high alpha and beta cellulose recovery rate of 80.3 % at 190 °C as shown in Figure 4.15(b). Besides, there was an outstanding peak at 1745 cm^{-1} for treated SCG at 190 °C which could be due to the higher composition of carbon (66.85 %) in solid SCG treated at 190 °C than at 210 °C (66.67 %).

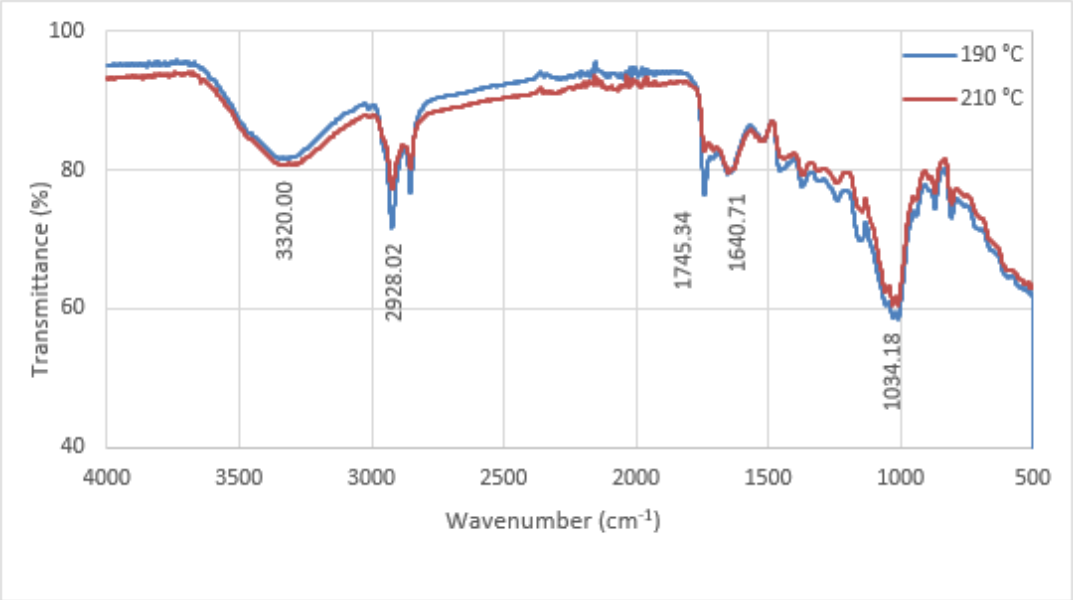

Figure 4.16: Comparison of FTIR Spectra of Treated Spent Coffee Grounds between Temperature of 190 °C and 210 °C

Table 4.6: Atomic Composition of Treated Spent Coffee Grounds at Temperature of 190 °C and 210 °C

Element	**190 °C**		**210 °C**	
	Wt (%)	**At (%)**	**Wt (%)**	**At (%)**
Carbon	59.99	66.85	59.77	66.67
Oxygen	39.17	32.76	39.31	32.93
Magnesium	0.32	0.18	0.23	0.12
Sulfur	0.40	0.17	0.48	0.20
Chlorine	0.12	0.05	0.21	0.08

Surface morphology of treated SCG at 190 °C and 210 °C were examined from SEM analysis and the SEM images are shown in Figure 4.17. The SEM images for both conditions revealed an empty space between fiber bundles, indicated the absence of lignin compounds. Furthermore, traces of hemicellulose and lignin could be observed throughout the surface of fiber bundles for both pretreatment conditions. Presence of cracking lines, tiny pores and dents could be marked on the surface as well. However, the treated SCG at 190 °C appeared to be more porous and higher severity

than the treated SCG at 210 °C. Hence, the optimum temperature for pretreatment was selected to be at 190 °C.

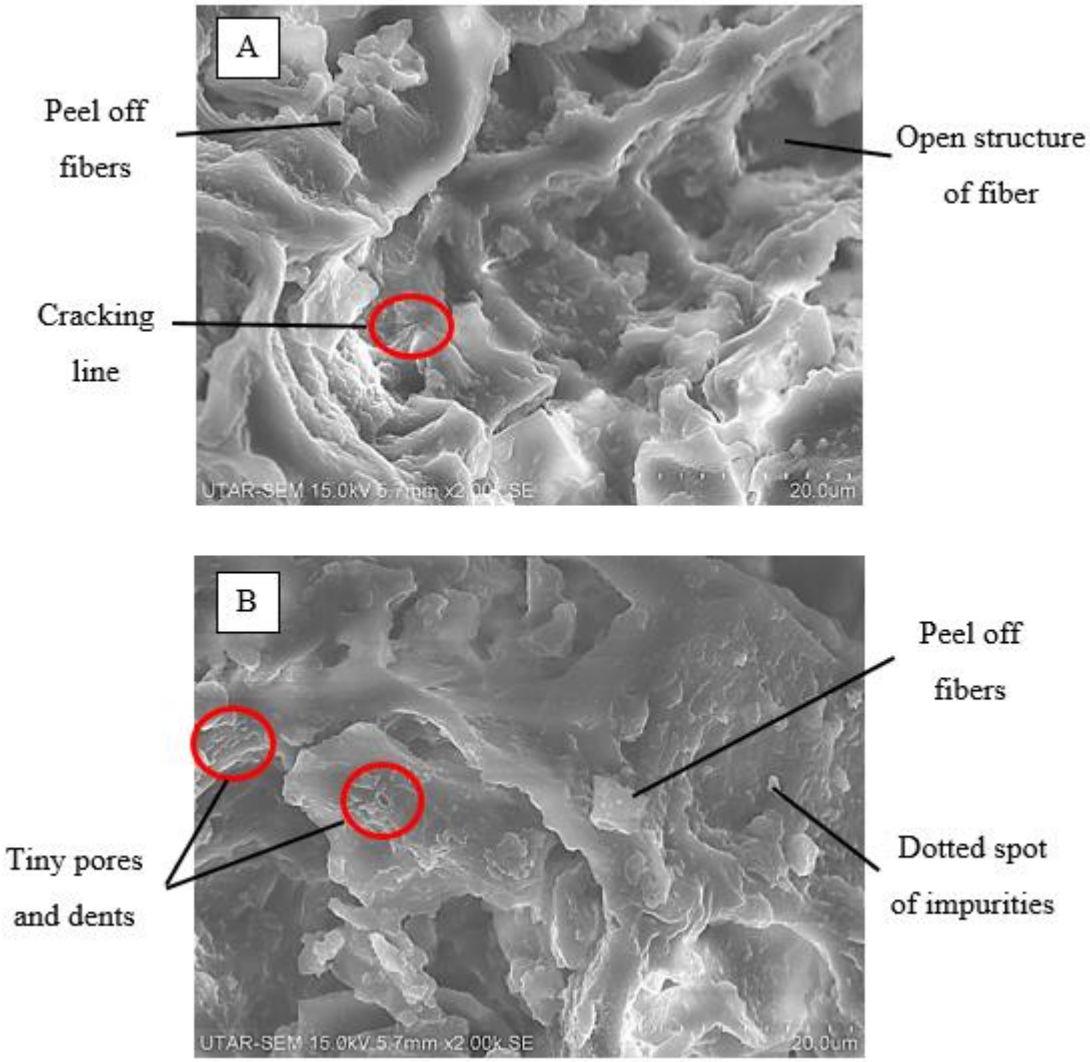

Figure 4.17: Surface Morphology of Treated Spent Coffee Grounds under 2000X Magnification at (A) 190 °C (B) 210 °C

Figure 4.18 illustrates the XRD profile of treated SCG at temperature of 190 °C and 210 °C. From the result obtained, the XRD profile for both temperatures were highly overlapped with each other. However, the peaks located at 15° and 22° for treated SCG at 190 °C was obviously higher than the treated SCG at 210 °C, revealing an increment in crystallinity. This again coincided with result obtained earlier where treated SCG had higher hemicellulose and lignin removal rate at 190 °C at 66.5 % and 77.5 % respectively.

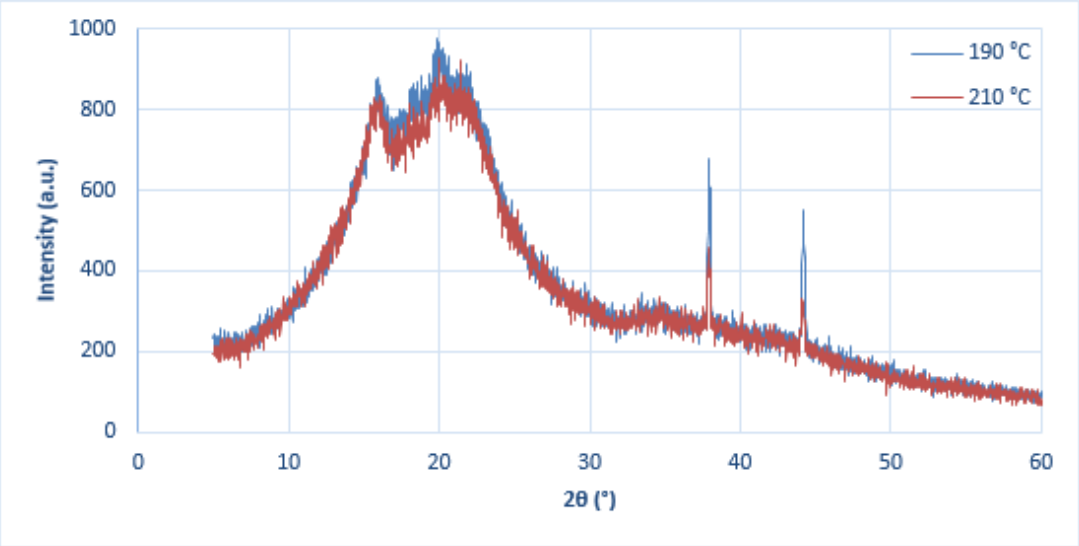

Figure 4.18: X-ray Diffractogram of Treated Spend Coffee Grounds at Temperature of 190 °C and 210 °C

At the end of the parameter study on reaction temperature in this section, temperature of 190 °C was chosen as the optimum temperature for organosolv pretreatment due to the relatively high cellulose recovery (80.3 %), sufficiently high hemicellulose removal (66.5 %) and better deligninfication effect (77.5 %). This selection was supported by the results of characterisation and compositional analysis. Hence, the subsequent parameter study would be operated at this optimum temperature.

4.2.4 Effect of Residence Time

A parameter study on residence time was performed based on the optimum parameters obtained from section 4.2.3 (solvent loading of 12.5 % v/w, 1 % acid concentration and 190 °C). Different residence time (30, 60, 90 and 120 minutes) were conducted and the result are illustrated in Figure 4.19.

By referring to Figure 4.19 (a), it could be noticed that the solid yield declined gradually from 82.24 % to 70.56 % as the residence time increased from 30 to 120 minutes. It was expected that the solid yield reduced as the residence time increased because prolonged residence time enhanced the lignin removal as well as hemicellulose removal. This statement was supported by the reported results as shown in Figure 4.19 (b) where the gamma cellulose and lignin removal rate increased from 61.8 % to 70.1 % and 55.2 % to 80.1 %, respectively with an extension of residence time. A contrary trend was achieved for alpha and beta cellulose recovery rate where

the cellulose recovery rate dropped from 81.7 % to 78.2 % as the residence time increased from 30 to 120 minutes. This insinuated that prolong residence time had given sufficient time to disrupt, degrade or even break down the cellulose structure to a high extent, causing higher difficulty in recovery back into its original structure and hence resulted in a decline of cellulose recovery rate. The pretreatment performance was further supported by FTIR spectra as depicted in Figure 4.20.

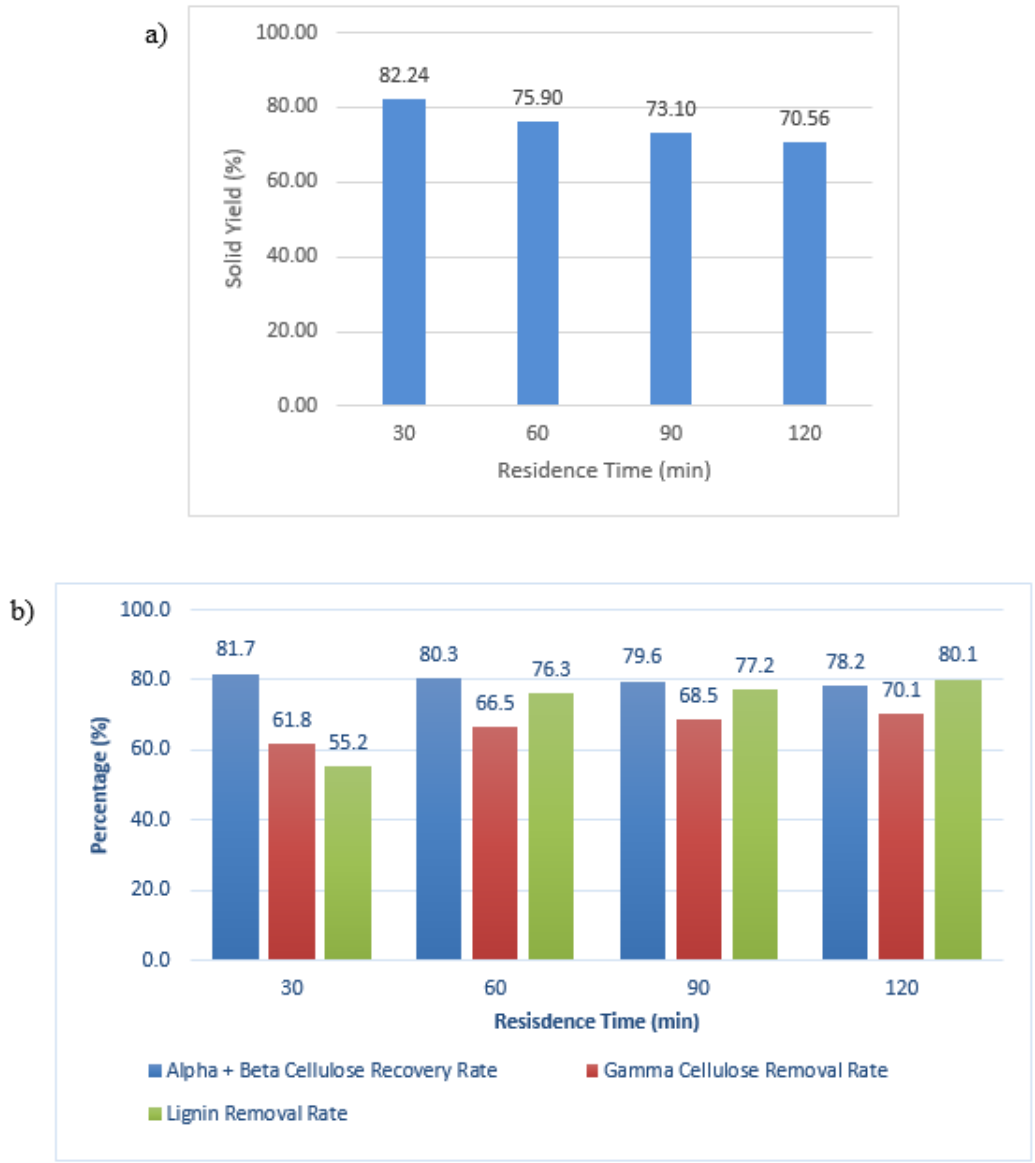

Figure 4.19: Pretreatment Performance at 190 °C under Solvent Loading of 12.5 % v/w with 1 % v/v acid But Varying Residence Time (a) Solid Yield after Organosolv Pretreatment (b) Alpha and Beta Cellulose Recovery Rate, Gamma Removal Rate and Lignin Removal Rate

Figure 4.20 shows the comparison of FTIR spectra for treated SCG at 30, 60 and 120 minutes to determine the effect of the residence time on the pretreatment process. The peaks located at 3307, 2924 and 1663 cm^{-1} symbolized the presence of O-H, C-H and C=O stretching of lignin compounds respectively while absorption peak at 1037 cm^{-1} represented the presence of C-O, C=C and C-C-O stretching of cellulose, hemicellulose and lignin compounds. By referring to Figure 4.20, the absorption peaks reduced with an extension of the reaction duration. The FTIR spectra for treated SCG at 120 minutes had highest transmittance at 3307, 2924 and 1663 cm^{-1} as compared to 30 and 60 minutes, showing the high effectiveness in delignification where highest lignin removal rate (80.1 %) were obtained. The reduction of absorption peak at wavelength of 1037 cm^{-1} for treated SCG at 120 minutes revealed that the amount of total cellulose, hemicellulose and lignin components were lesser in the solid. This statement was proven by the low alpha and beta cellulose recovery (78.2 %) and high hemicellulose and lignin removal (70.1 % and 80.1 %) at 120 minutes.

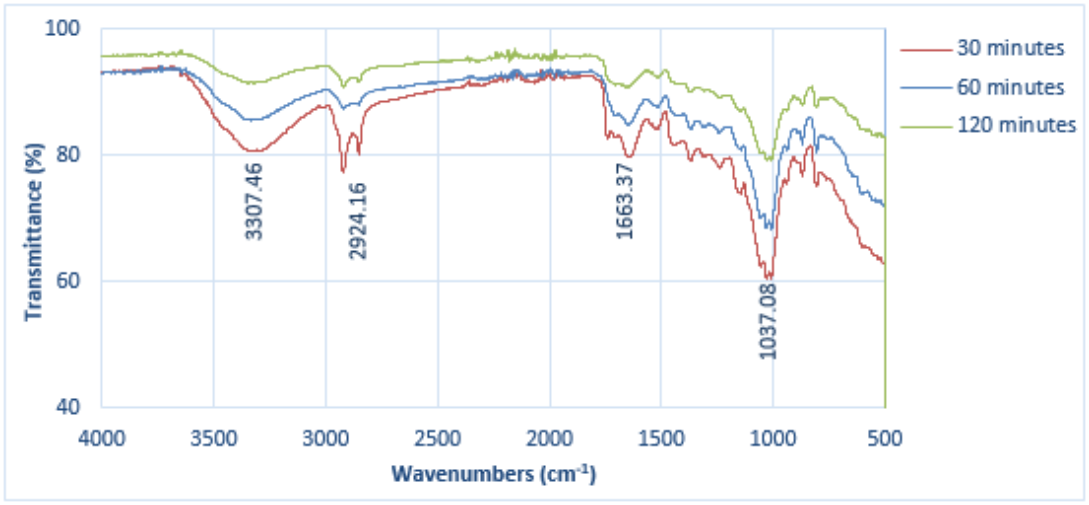

Figure 4.20: Comparison of FTIR Spectra for Treated Spent Coffee Grounds at Residence Time of 30, 60 and 120 minutes

Figure 4.21 illustrates the surface morphology of the treated SCG under residence time of 60 and 120 minutes that examined from SEM analysis. Traces of hemicellulose and lignin compounds were observed on the surface of fiber bundles for both pretreatment at 60 and 120 minutes which resulted in rough and uneven surface. The presence of cracking lines and empty spaces between fiber bundles could be spotted on the surface for both residence time as well which indicated the absence of

lignin compounds. However, pretreatment at 60 minutes showed higher severity and porosity as well as more rupture of fiber bundle compared to pretreatmet at 120 minutes which attributed to the high effectiveness in pretreatment process.

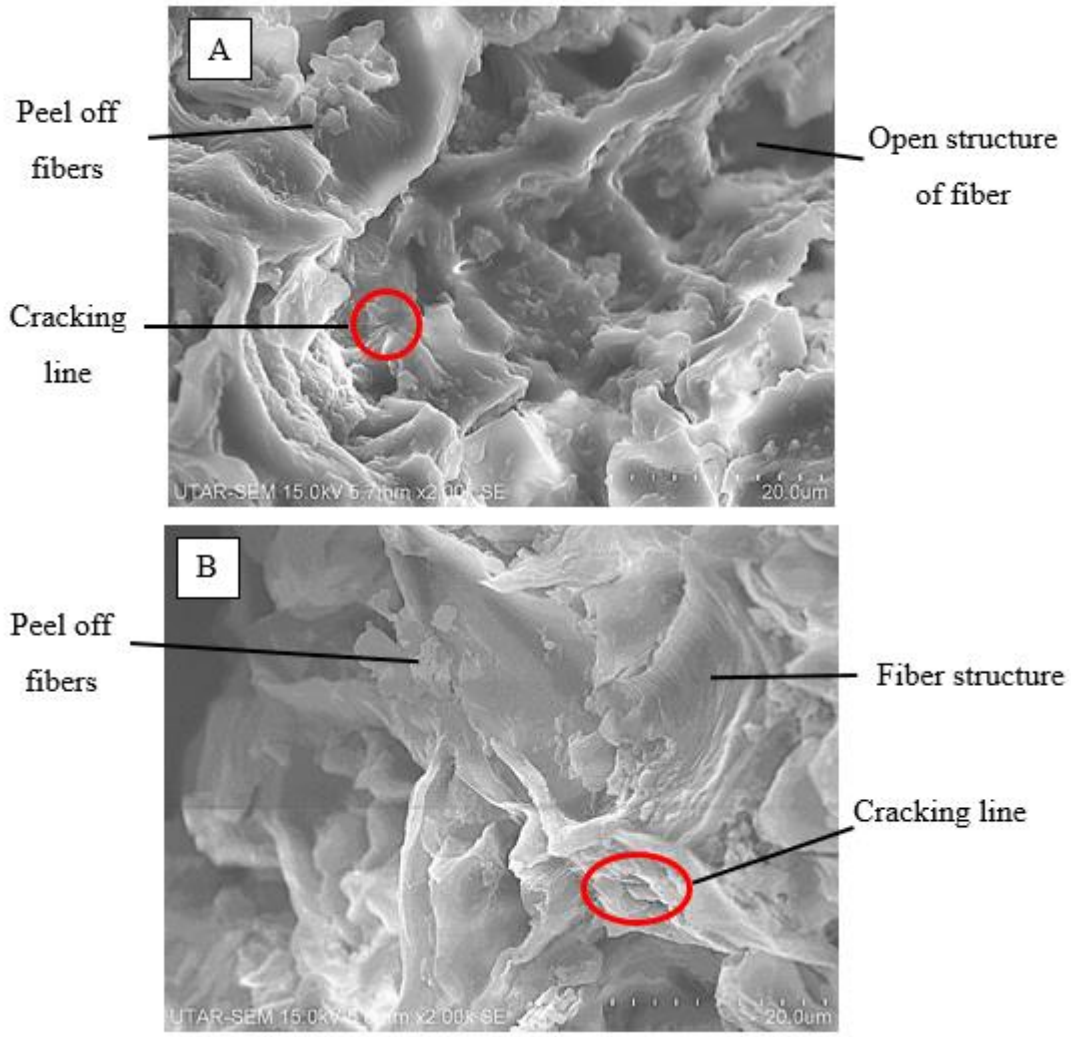

Figure 4.21: Surface Morphology of Treated Spent Coffee Grounds under 2000X Magnification at (A) 60 minutes (B) 120 minutes

Apart from FTIR and SEM analysis, XRD analysis was conducted as well to compare the pretreatment performance between residence time of 60 and 120 minutes. Figure 4.22 shows the XRD profiles obtained for two different pretreatment durations. The XRD profiles for both reaction durations were greatly overlapped with each other. Since the presence of amorphous and crystalline structure were generally located at 15° and 22°, the intensity for both treated SCG at these two degrees were compared. As it could be seen in Figure 4.22, the treated SCG at 60 minutes showed slightly higher

peaks with intensity of 720 a.u. at 15° and 828 a.u. at 22° than 120 minutes with intensity of 650 a.u. and 734 a.u. at 15° and 22° respectively. Hence, the treated SCG had higher crystallinity when the pretreatment was carried out for 60 minutes duration. This could be explained by the comparably higher cellulose recovery (80.3 %) for treated SCG at 60 minutes where the crystallinity of lignocellulosic biomass was significantly contributed by the presence of cellulose due to its crystalline and amorphous regions in nature (Ravindran et al., 2018).

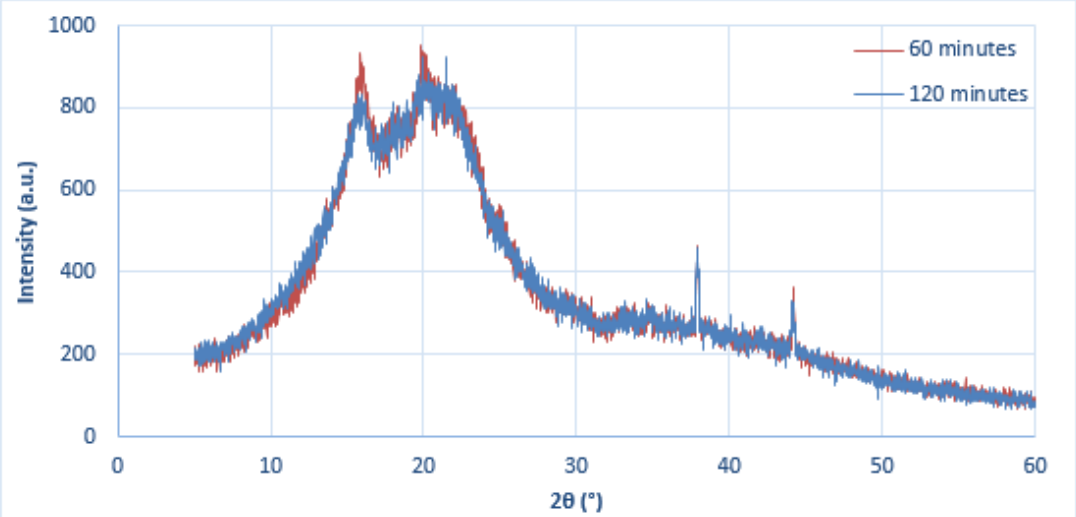

Figure 4.22: X-ray Diffractogram of Treated Spend Coffee Grounds at Residence Time of 60 and 120 Minutes

By comparing all the results obtained from compositional and characteristic analysis, residence time of 60 minutes was determined as the most optimum reaction duration which retained 80.3 % of alpha and beta cellulose, and removed 66.5 % of gamma cellulose and 76.3 % of lignin.

4.3 Comparison of Organic Solvent Used in Organosolv Pretreatment

Similar pretreatment process was carried out and studied by other final year student using 80 wt % glycerol as organic solvent. According to the study, the optimum glycerol-based pretreatment condition was carried out at 180 °C for 60 minutes with solvent loading of 12.5 % v/w and 2 % v/v of sulfuric acid as catalyst. The solid yield, alpha and beta cellulose recovery rate, gamma removal rate and lignin removal rate obtained from pretreatment process using ethanol and glycerol were analysed and

compared to determine to the effectiveness of organosolv pretreatment process on spent coffee grounds. The results of pretreatment performance for both solvents shown in Figure 4.23. It should be noted that the preliminary optimum ethanol-based pretreatment parameter obtained from this study was conducted at 190 °C for 60 minutes with solvent loading of 12.5 % v/w and 1 % v/v of sulfuric acid as catalyst.

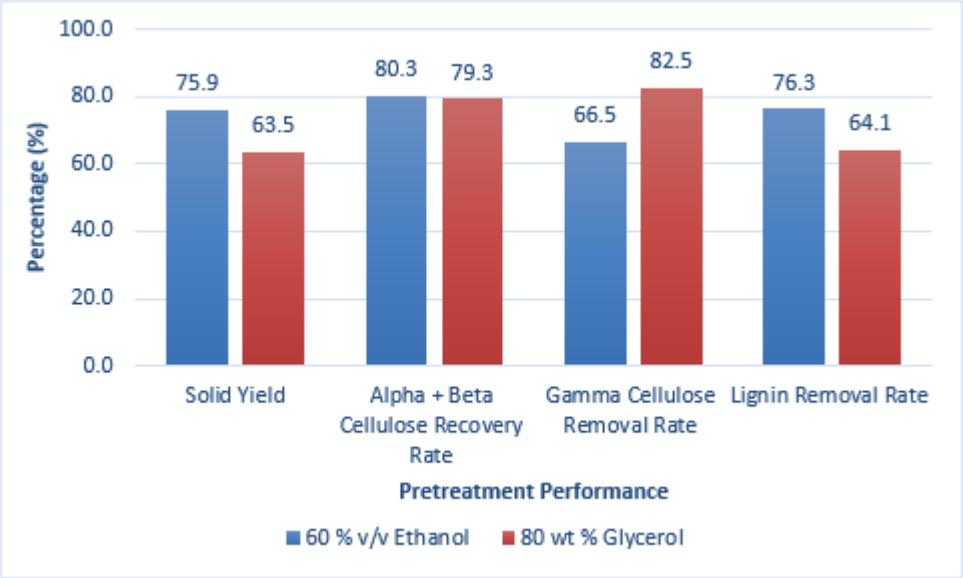

Figure 4.23: Comparison of Pretreatment Results with Ethanol and Glycerol

By referring to Figure 4.23, the ethanol-based pretreatment showed better performance in terms of solid yield, cellulose recovery rate and lignin removal rate, which were 75.9 %, 80.3 % and 76.3 % respectively, as compared to glycerol-based pretreatment. However, a contrary trend was observed for gamma cellulose removal rate where glycerol-based pretreatment possessed an intensely high percentage in gamma cellulose removal (82.5 %). This indicated that hemicellulose hydrolysis of pretreated SCG was more effective when using glycerol as solvent for pretreatment. The performance of organosolv pretreatment process depended on the ability to remove hemicellulose and lignin as much as possible and at the same time retain the cellulose, thus improving the rate of enzymatic hydrolysis and cellulose yield. Hence, in an overall view of the performance, ethanol-based organosolv was more promising which provided higher solid yield, cellulose recovery rate and lignin removal rate even though a lower removal in hemicellulose (66.5 %) was reported.

Nevertheless, Sun and Chen (2007) disclosed that glycerol-based pretreatment performed with 15 g/g dry wheat straw at 240 °C for 4 hour and washed at 80 °C retained 95 % of cellulose and removed more than 70 % of lignin. The output of the enzymatic hydrolysis of the pretreated wheat straw was 90 % and 92 % of hypothetically achievable sugar after 24 and 48 hours respectively. The result reported was comparable with that of the ethanol-based pretreatment of hybrid poplar that yielded 88 % solids fraction with cellulose recovery of 82 % and 74 % of lignin recovered under the pretreatment condition of 180 °C for 1 hour with 60 % v/v ethanol and 1.25 % v/v sulfuric acid as catalyst (Pan et al., 2006; Sun and Chen, 2007). An adverse result obtained in this study with literature reviews could be due to the low severity in glycerol-based pretreatment of SCG which provided insufficient pretreatment condition. Table 4.7 shows the comparison between pretreatment outcomes obtained from parameter studies conducted and literature studies.

Table 4.7: Comparison between Pretreatment Results Obtained from Parameter Studies Conducted and Literature Studies

Pretreatment	Optimum Conditions	Results	Reference
100 % v/v Glycerol	240 °C for 4 hours with glycerol addition of 15 g/g dry wheat straw.	81 % pulp yield. 95 % cellulose recovery. 90 % hemicellulose removal. More than 70 % lignin removal.	(Sun and Chen, 2007)
80 wt % glycerol + 2 % v/v of sulfuric acid	180 °C for 60 minutes with glycerol loading of 12.5 % v/w.	63.5 % solid yield. 79.3 % cellulose recovery. 82.5 % hemicellulose removal. 64.1 % lignin removal.	From parameter performance obtained by other final year student.
60 % v/v Ethanol + 1.25 % v/v sulfuric acid	180 °C for 1 hour.	88 % pulp yield. 85 % cellulose recovery. 72 % hemicellulose removal. 74 % lignin removal.	(Pan et al., 2006)
60 % v/v Ethanol + 1.25 % v/v sulfuric acid	190 °C for 60 minutes with ethanol loading of 12.5 % v/w.	75.9 % solid yield. 80.3 % cellulose recovery. 66.5 % hemicellulose removal. 76.3 % lignin removal.	From parameter performance obtained in this study.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, the chemical composition of the native spent coffee grounds was examined which comprised of 28.64 % alpha cellulose, 9.5 % beta cellulose, 36.17 % gamma cellulose and 25.69 % lignin. The ethanol-based organosolv pretreatment of spent coffee grounds (SCG) were performed under several pretreatment conditions to investigate their effects on SCG. The parameter study on solvent loading, acid concentration, temperature and residence time were carried out and the relationship of each parameter in organosolv pretreatment was successfully studied.

There are several important findings can be concluded based on the results obtained from the parameter studies conducted. Firstly, the hemicellulose hydrolysis and enzymatic degradability of pretreated SCG reduced at higher ethanol content which hindered the hydrolytic activity of enzyme and resulting in lower hemicellulose removal. The high ethanol content also promoted the reprecipitation of lignin which reduced the delignification effect. Secondly, the delignification and hemicellulose removal were encouraged in the presence of high acid concentration but the cellulose recovery was diminished. Thirdly, lignin and hemicellulose removal could be improved with increment of temperature but formation of degradation products would be enhanced at extremely high temperature (above 210 °C) which caused a drop in both lignin and hemicellulose removals. Cellulose recovery exhibited similar trend as well where the cellulose recovery reduced at high severity conditions. Lastly, prolonged residence time in pretreatment increased the ability of lignin and hemicellulose removal. However, it also resulted in a decline in cellulose recovery due to sufficient period provided for the occurrence of cellulose degradation.

Hence, ethanol-based organosolv pretreatment at 190 °C for 60 minutes under solvent loading of 12.5 % v/w using 60 % v/v ethanol as solvent and in the presence of 1 % v/v sulfuric acid given the most optimum result in terms of performance in terms of solid yield (75.9 %), alpha and beta cellulose recovery (80.3 %), gamma cellulose removal (66.5 %) and considerably delignification effect (76.3 %). These results obtained by ethanol-based organosolv pretreatment was more promising as compared to that using glycerol as solvent with pretreatment condition at 180 °C for

60 minutes with solvent loading of 12.5 % v/w and 2 % v/v of sulfuric acid as catalyst, which provided 79.3 % cellulose recover, 82.5 % hemicellulose removal and 64.1 % delignification effect.

The performance of treated SCG at this optimum condition were supported by the results obtained from characterization analysis. FTIR spectra revealed that hemicellulose and lignin were removed during organosolv pretreatment. This was accompanied by an increment in crystallinity of residual cellulose as observed from the XRD profile. The surface morphology of treated SCG under optimum condition showed high severity, more porous and rupture of fiber bundle with the presence of cracking lines indicated absence of lignin compounds which attributed to the high effectiveness in pretreatment process. Furthermore, the thermal stability of treated SCG would reduce at temperature above 400 °C where the decomposition of SCG began.

In the nutshell, all objectives were fulfilled for this study. The relationship of each parameters and their respective effects on organosolv pretreatment were successfully studied. The optimum pretreatment condition of organosolv pretreatment was determined. The physical and chemical properties of the SCG before and after pretreatment were investigated and discussed as well.

5.2 Limitations and Recommendations

Experiment was completed accordingly to the scope of study. However, some aberration occurred in the experiment were overlooked due to time constrained. The variation of results could be resulted by human errors or the limitation of equipment and experimental methods. There were several suggestions being proposed in order to enhance the accuracy and consistency as well as the reliability of the result by overcoming the limitations of this research study. Proposed recommendations on the study are listed below.

(i) The chemical compositions such as ash or lipid extractive that presence in spent coffee grounds should be determined as well.

(ii) Investigate the feasibility of spent coffee grounds as promising biomass for pretreatment by conduct chemical composition analysis on other biomass and make comparison.

(iii) Perform additional parameter study on pretreatment parameters such as type of solvent used, solvent concentration and types of homogenous catalyst to investigate the effect of these parameters on pretreatment performance.

(iv) Increase the number of experiment sets for each parameter to increase the accuracy of the optimum conditions.

(v) The equipment used should be consistent for every set of experiment to ensure the consistency of the experiment environment.

(vi) Compositional and characterisation analysis should be carried out as soon as the samples were produced to minimize or avoid the contamination of samples.

(vii) Conduct High Performance Liquid Chromatography (HPLC) to determine the concentration of compounds such as furans, glucose and phenolic compounds in the solution mixture which affect the accuracy of UV-Vis spectrophotometry.

(viii) Carry out acid hydrolysis and fermentation processes to have a better grasp in understand the effect of organosolv pretreatment on bioethanol production.

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