FERULIC ACID PRODUCTION FROM BANANA STEM WASTE: KINETIC STUDY

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ABSTRACT

For an agriculture-intensive country like Malaysia, the abundantly-available organic wastes such as banana stem provide an opportunity for the conversion into highly-sought compounds such as ferulic acid. Ferulic acid offered variety of physiological benefits used in cosmetics, food preservation and bio-production of vanillin. In this research, the soil mixed culture was mixed with banana stem wastes in an incubator shaker followed by sample collection at every 6-hours interval for the next 60 hours. Next, HPLC (high performance liquid chromatography) analysis was performed to determine the amount of ferulic acid presence. The purpose of this study was to determine the kinetic constants (Kx, Km and Vmax) from the modified biomass and Michaelis –Menten equation by using the Runge-Kutta 4^{th} method with the aids of Microsoft Excel Solver. The kinetic constants were reported to be Kx = 0.005/ hour, Km = 0.044 mmol/ L and Vmax = 3.66 x10^{-6} μmol/ min. The yield for ferulic acid production was 14.54 mg/g. Furthermore, the R^2 for biomass and substrate concentration were 0.86 and 0.89 respectively. In this research, the kinetic constants and the yield for ferulic acid production were taking precedent compared to other researches due to the banana stem waste used was high in nutrient content and easy to access by soil mixed cultures. Hence, the microbes grow well and large amount of enzyme feruloyl esterase can deliberated promptly to cleave the ester linkage joined between the cross-link of polysaccharides ends with more ferulic acid formation.

ABSTRAK

Bagi sebuah negara pertanian intensif seperti Malaysia, terdapat pelbagai sisa organik yang sedia ada seperti batang pisang memberi peluang untuk penukaran ke dalam sebatian yang amat diminati seperti asid ferulic. Asid ferulic menawarkan berbagai-bagai manfaat fisiologi dan digunakan dalam kosmetik, pemeliharaan makanan dan bio pengeluaran untuk vanillin. Dalam kajian ini, hidupan dalam tanah campuran telah bercampur dengan sisa batang pisang dalam shaker incubator. Kemudian, ia diikuti dengan koleksi sampel setiap 6 jam berselang untuk tempoh sepanjang 60 jam. Seterusnya, HPLC (kromatografi cecair prestasi tinggi) analisis telah dijalankan untuk menentukan jumlah asid ferulic. Tujuan kajian ini adalah untuk menentukan pemalar kinetik (Kx, Km dan Vmax) daripada persamaan biojisim dan Michaelis-Menten yang diubah suaikan dengan menggunakan kaedah Runge Kutta keempat dalam Microsoft Excel Solver. Pemalar kinetik dilaporkan adalah Kx = 0.005 / jam, Km = 0,044 mmol / L dan Vmax = 3.66 x10 $^{-6}$ μmol / min. Hasil untuk pengeluaran asid ferulic adalah 14.54 mg / g. Tambahan pula, R^2 bagi kepekatan biojisim and substrat adalah 0.86 dan 0.89 masing-masing. Dalam kajian ini, pemalar kinetik dan hasil untuk pengeluaran asid ferulic mengambil duluan berbanding dengan kajian lain kerana sisa batang pisang yang digunakan mengandungi kandungan nutrient yang tinggi dan mudah untuk diakseskan oleh hidupan dalam tanah campuran. Oleh itu, mikrob membesar dengan baik dan sejumlah besar enzim feruloyl esterase boleh dilepaskan dengan segera untuk memecahkan jalinan ester yang menyertai antara salib-link pada polisakarida dan berakhir dengan pembentukan asid ferulic yang banyak.

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LIST OF ABBREVIATIONS

μ	cell growth rate
μmax	maximum cell growth rate
A	frequency factor
CLSM	co focal laser scanning microscopy
$\frac{dS}{dt}$	rate of substrate utilization
$\frac{dP}{dt}$	rate of product formation
$\frac{dX}{dt}$	rate of biomass utilization
Ea	activation energy
HPLC	High Performance Liquid Chromatography
Km	Michaelis constant
K	specific reaction rate
Ks	substrate constant
Kx	biomass constant
LCC	lignin–carbohydrate complexes
LM	light microcopy
MOA	Ministry of Agricultural
ODE	ordinary differential equation
PDE	partial differential equation
R	gas constant (8.314 J/mol. K)
[S]	substrate concentration
SEM	scanning electron microscopy
T	absolute temperature, K
[X]	biomass concentration
Vmax	maximum rate of reaction
Yp/s	yield of product on substrate

1 INTRODUCTION

1.1 Motivation, problem statement and brief review

Previously, when logging activities or after a harvest operation from paddy or wheat farm was carried out, the residues were burnt or used as solid foods for livestock (Gil et al., 2011). This will deteriorate the surrounding environment and bring a serious impact to the health of human beings. Therefore, utilization of lignocellulosic wastes such as banana stem waste has gained much attention in the face of growing environment awareness among the mass populations.

It is generally known that lignocellulosic wastes are abundantly-available, possess high intrinsic economic-value and deemed as environmental-friendly. Hence, lignocellulosic waste is gradually replacing the use of food crops as raw material. It brings huge benefits to mankind, animals and all living creatures that had a demand on food to survive. Apart from that Prakash and Hara (2010) emphasized that the nature of lignocellulosic waste makes it able to adapt well to chemical processing to yield end- products with affixation value.

Generally, ferulic acid (3-methoxy-4-hydroxycinnamic acid) is an organic compound that is rich in hydroxycanamic acid. Graf (1992) revealed that ferulic acid offers many physiological benefits. It can be used in cosmetics, food preservation (can inhibit fatty acid peroxidation), bio-production of vanillin and also healthy supplement for suppressing cancerous cells for anti-cancer activities.

Significantly, mixed culture is a group of colony microorganisms that present in a specific, localized location (Sharif, 2009). Rhizophere is a common name given to the microbe that grows and lives around the soil particles or near the root of a plant. Soil mixed culture plays the role as decomposer in the organic compound. It can be employed in the degradation of lignin (delignification) and polysaccharide (cellulose degradation).

Apart from that, Jelena et al. (2012) have mentioned that kinetic model assist in the process of controlling the fermentation process, increasing the productivity and quality of the product, reducing the operating costs and eliminate disturbances. Therefore, the Michaelis –Menten equation is used to obtain kinetic parameters of ferulic acid production from banana stem waste using soil mixed culture.

In a tropical country like Malaysia, bananas are produced in large quantities. In 2011, the total planted area of banana in Malaysia was estimated to be 33,704.2 hectares (MOA, 2006).Upon harvest, the remaining banana stems were left to degrade. In this research, the unwanted banana stem waste will be used back as the raw material for the production of ferulic acid. The use of soil mixed culture as microbe for the production of ferulic acid also makes the fermentation process becomes more economic since the material cost plays a conclusive role in the industries which employ fermentation processes. On the other hand, due to the use of soil mixed culture as microbe and the kinetic study for ferulic acid production using banana stem waste is not in further investigation, all of the above contribute to the inspiration of conducting this research to find out the suitable kinetic constants used for scaling up.

1.2 Objectives

To study on the kinetic of ferulic acid production from banana stem waste using soil mixed culture.

1.3 Scopes of study

In the acclimatization step, the banana stem wastes and soil sludge contained the soil mixed culture were collected then put into the reactor under ambient temperature. Soil mixed culture was relying on the uptake of banana stem waste as substrate to survive and grow. In the biological processes, feruloyl esterase was produced to break the ester bond between the hemicelluloses and lignin of banana stem waste. Subsequently, ferulic acid was released as the end product. When conducted the experiment, the entire 22 samples were prepared in the conical flask and arranged into the incubator shaker. In every 6 hours, the sample was taken

out from the incubator shaker and the supernatant was poured into the universal bottle for HPLC analysis. The remaining precipitate was taken out together with the centrifuge tube and microwaved at 60 ^{o}C for drying in order to obtain the dry weight of biomass. This procedure was repeated continuously for two and half days (60 hours) with the time interval of 6 hours. Followed by HPLC (high performance liquid chromatography) analysis to determine the amount of ferulic acid presented in the sample. The main focus of this research was to study the kinetic for ferulic acid production from the banana stem waste using soil mixed culture. The modified Michaelis –Menten constant and biomass equation was used to study on the variety of kinetics parameters such as biomass utilization, ferulic acid production. Finally, the kinetic constant (Kx, Km and V_{max}) was determined by using Runge-Kutta 4th method solved by Microsoft Excel Solver.

1.4 Organization of this thesis

The structure of the thesis was outlined as follow:

Chapter 2 provided the descriptions on the overview to ferulic acid and its applications in food, pharmaceutical and cosmetics industry. Furthermore, the lignocellulosic wastes generally used for the production of ferulic acid were written. This chapter also briefly discussed on the advantageous of using soil mixed culture instead of others microbes. Instead of some other equations like Monod Equation, the ultimate selection on the Michaelis-Menten Equation as the kinetic model was included with clear justification.

Chapter 3 represented the methodology of the research. This chapter gave a review on the experiment executed to determine the biomass and product concentration. The useful data obtained experimentally was used for the kinetic study for the production of ferulic acid on banana stem waste by using soil mixed culture in this research.

Chapter 4 devoted to the results on the experimental data and discussion based on the results as well as the comparison with other researchers.

Chapter 5 drawn together a summary of the thesis and outlines the future work which might be derived from the model developed in this work in the recommendation part.

2 LITERATURE REVIEW

2.1 Ferulic acid

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) was a major hydroxycinnamic acid with phenolic compound that commonly found in plant cell wall. It was covalently linked with lignin by ether bond (MacAdam & Grabber, 2002). Ferulic acid played an important role in the structure of cell wall as it linked with others polysaccharide chains via dimerisation reaction, In 1966s, ferulic acid was undergo isolation from a commercial resin and chemically synthesis in 1985s. In 1970s; Japanese researchers discovered that ferulic acid has ubiquitous antioxidant properties. Ferulic acid was attributed to the presence of phenolic nucleus and the extended side chains that enable it to from a stabilizing resonance of free radical. When small amount of free radical produces by body, it helped in human metabolic activities. However, it was lead to the formation of cancerous cell, accelerated ageing and weakens the immune system when numerous of free radical produced. Therefore, ferulic acid with the properties to stabilize the body's free radical added into the healthy supplement to multiply the human health benefits.

In an addition, the artificial of chemically synthesized flavour was not allowed to produce the natural flavour under current US legislation. According to Food and Drug Administration (FDA), the products were obtained from animal, plant, microbial and enzyme was considered as natural. Ferulic acid can used as the precursor for bio-production of vanillin (synthetic flavouring agent used in place of natural vanilla extract) which was widely apply in food, pharmaceutical and cosmetics industry.

Feruloyl esterase classified as the subclass of carboxylic ester hydrolases which was able to hydrolyze the ester linkage found between the hydroxycinnamic acids and sugars. Due to the biotechnological importance of feruloyl esterease, these enzymes have been studied from a huge number of microbial sources (fungal and bacterial sources). Furthermore, Anvar & Mazza (2008) revealed that ferulic acid can released from lignin complexes in herbaceous

crops via the enzyme- feruloyl esterase in a biological process. There were several types of lignocellulosic wastes adopted for the production of ferulic acid by using different bacteria strains as shown in Table 2.1. Cited the example: *Staphylococcus aereus* was isolated from soil and cultured in a medium broth contained beef extract and peptone. Then, the wheat bran obtained from soft wheat (*T. aestivum*) was used as the substrate and mixed with acclimatized microbes. In the aftermath, the enzyme feruloyl esterase was released for the production of ferulic acid through the biological degradation. (Prakash and Hara, 2010). Moreover, sugar beet pulp was another popular lignocellulosic waste chosen for the production of ferulic acid by *Streptomyces tendae* after incubation of 5 days at 160 rpm. (Fereirra et al., 2007). Despite, corn- cobs fermented by *Escherichia coli* also used for the same production. In this research, the soil mixed culture was used as microbes instead of the isolated pure culture. Soil mixed culture rely on the banana stem waste to grow. During the time, feruloyl esterases were released to break the ether bond of lignin; consequently the desired end product, ferulic acid was acquired. The advantages of using soil mixed culture were easily obtainable and free unlike other microbes that need to be purchased at a high cost with additional charges emerged from the needs to cultivate it. Enzymatic reaction was employed for the production of so-called 'natural vanilla aroma' from ferulic acid also avoided the additional spending on unnecessary clean-up of chemical wastes from industrial processes.

Phenolic acids, both benzoic acid/ cinnamic acid derivatives (Figure 2.1) had the ability to donate electrons but also of their stable radical intermediates to inhibit the oxidation of fatty acids and oils in various food ingredients. Therefore, ferulic acid was used in food preservative. (Kuenzig, 1984) Apart from that, the strong link between inflammation and oxidative stress making ferulic acid enhanced itself with significant properties against inflammation diseases. Ferulic acid also bought zillion of benefits to chemist, biotechnologist and dermatologist .This can well explained in 'Journal of Investigate Dermatology' which emphasized that ferulic acid have the ability to double the skin photo-protection and hence can protected human's skin from sun damage that can led to skin cancer if severe. Generally, vitamin C was well known due to its properties to brighten the skin tone and effectiveness against wrinkles and fine line. However, it was unstable and oxidized in fast pace when exposed to air. Oxidized vitamin C can increased the formation of free radical. With the additional of ferulic acid into the vitamin C visible increased the stability and effectiveness of

vitamin C. For further information for the chemical structure and synthesis of ferulic acid and related compounds in plants was shown in Figure 2.2.

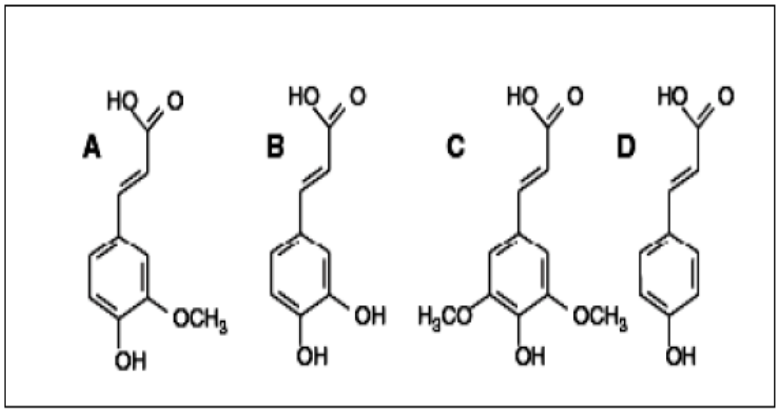

Figure 2.1 Structure of phenolic acids

A. Ferulic acid

B. Caffeic acid

C. Sinapic acid

D. *p*-Coumarin acid

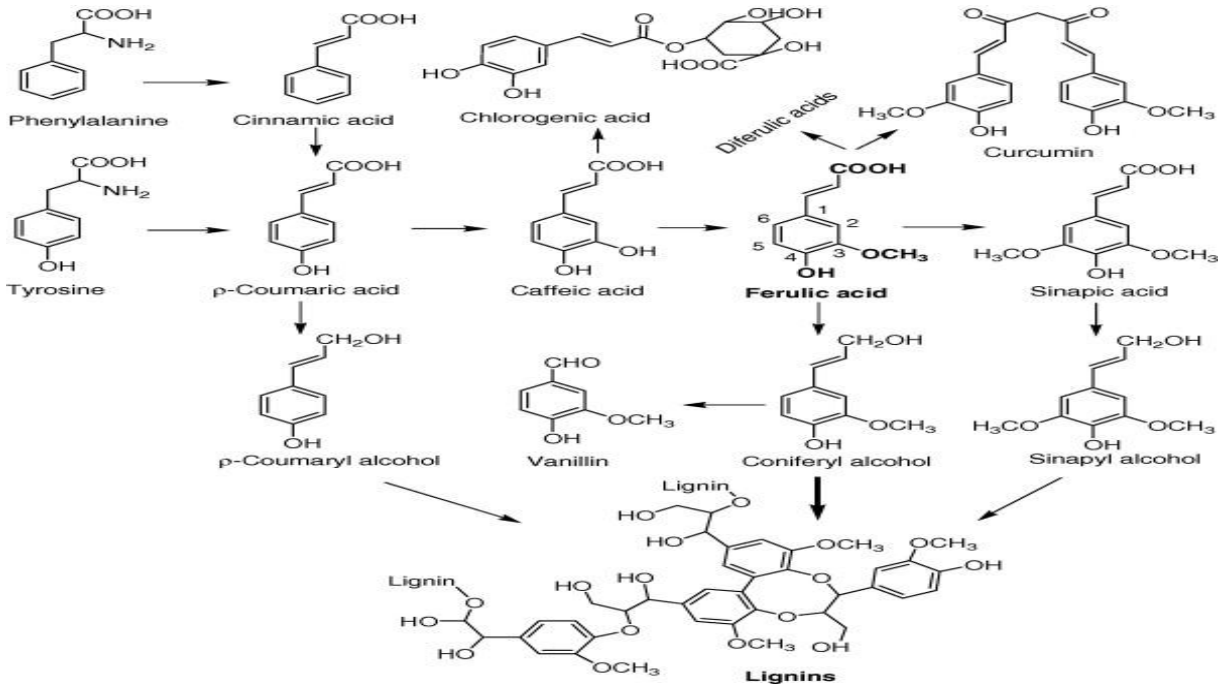

Figure 2.2: Chemical structure and synthesis of ferulic acid and related compounds in plants.

Table 2.1: Various source for the ferulic acid production (Miller and Van, 1989).

Microbial sources	Fungi: *Aspergillus sp.* *Aureobasidium sp.* *Sporotrichum sp.* *Penicilium sp.* *Staphylococcus sp* Yeast : *Saccharomyces sp.* *Candida sp.* *Aureobasidium pullulans* Bacteria: *Streptomyces sp.* *Bacillus sp.* *Pseudomonas sp.*
Plant sources	Barley, Finger millet, wheat bran, sugar beet pulp
Animal sources	Mamalian liver, Brain, serum, dental composites

2.2 Lignocellulosic waste

In milling, brewing, and various food industries, the yearly piled up of agro waste materials generated has led to the consideration of extracting high value residues to counteract the expenses of treating and disposing of the remaining residues. Biological degradation, for both economic and environment purposes had become a prevalent alternative for treatment of agricultural wastes. Lignocellulosic wastes were environmentally sound, renewable and costless. The nature of agricultural waste was making it suitable for chemical processing to

acquire ended-product with high affixation value. Lignocellulosic biomass was acquired from variety of sources such as agricultural, kitchen and forest waste.

Lignocelluloses plant structures have variety of specific chemicals named as extractives such as resins, phenolics and minerals (calcium, magnesium, potassium, and others) that can turned into ash when burned (Mussatto and Teixeira, 1998). It was normally resistant to physical, chemical, and biological attack. However, cellulose and hemicelluloses component in lignocellulosic waste can be broken down via hydrolysis to produce fermentable, simple sugars. There are three major component in lignocellulosic waste *viz* lignin , cellulose and hemicelluloses. In Table 2.2 showed the chemical composition of some typical cellulose-containing materials. The majority of biomass weight contributes by cellulose with 40% to 60% cellulose, followed by 20% to 40% hemicellulose, and 10% to 25% lignin depends on different type of wood.

Table 2.2: Chemical composition of some typical cellulose-containing materials. (Hons, 1996)

Source	Composition (%)			
	Cellulose	Hemicellulose	Lignin	Extract
Wheat straw	30	50	15	5
Bagasse	40	30	20	10
Softwood	40-44	25-29	25- 31	1-5
Hardwood	43-47	25-35	16-24	2-6
Flax (retted)	71.2	20.6	2.2	6
Jute	71.5	13.6	13.1	1.8
Henequen	77.6	4-8	13.1	3.6
Ramie	76.2	16.7	0.7	6.4
Cotton	95	2	0.9	0.4

2.2.1 Lignin

Lignin was a very complex molecule derived from wood. It was covalently linked with hemicelluloses and crosslink with cellulose. In nature, lignin was deemed as the third most abundant natural polymer after cellulose and hemicelluloses. Argyropoulos and Menachem (1998) mentioned that there was approximately 300 billion metric tonnes of lignin in the planet with an annual biosynthetic production rate of 20 billion metric tonnes. Lignin provided support though strengthening of wood in tree, internal transport of water and nutrients, form barrier against microbial attack. There were three phenyl propionic alcohols exist as monomers of lignin: *p* -coumaryl(1), coniferyl alcohol (2) and sinapyl alcohol (3) (Figure 2.3).

Lignin was linked with carbohydrate though covalent bonds at C-4 in the aromatic ring and α-carbon to form lignin–carbohydrate complexes (LCC). Baucher et al. (1998) portrayed that in soft wood plants, hydroxycynnamic acids (*p*-coumaric and ferulic acid) normally associated to lignin and hemicelluloses though ester and ether bonds as bridges between them to form lignin–carbohydrate complexes. The biomass's enzymatic digestibility which resulted in the production of biological products was mainly relying on lignin content (Adler, 1977). Hence, extraction of lignin from soft wood plants was utmost importance since the bio-products such as vanillin and ferulic acid which largely depend on the utilization of extracted lignin.

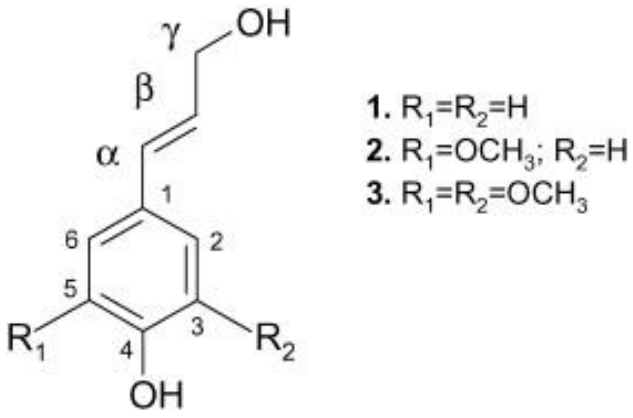

Figure 2.3: Lignin monolignols.

2.2.2 Cellulose

Cellulose was known as the most abundant organic compound in Earth. The elementary bedding of cellulose was the exiting of wood as the topmost source from lignocelluloses in forests. Other cellulose-containing materials include agriculture residues, water plants, grasses, and other plant substances. Serge and Daniel (2010) emphasized that cellulose composed of D-glucose unite linked by β-1, 4 glycoside bonds. Cellulose was making up from very large polymer molecule composed of many hundreds or thousands of glucose molecules (polysaccharide). Moreover, it was un-branched polymer with long chains of sugar molecules. The molecular linkages in cellulose were rigid, able to withstand chemical attack and high in stability. The strands of cellulose formed extended ribbon. Both intra- and intermolecular hydrogen bonding can found in cellulose. Inter-chain H-bonding allowed multi-chain interaction responsible for the formation of sheet-like nature of the native polymer as shown in Figure 2.4. Apart from that, the percentage of alpha cellulose in various lignocellulosic samples was shown in Table 2.4.

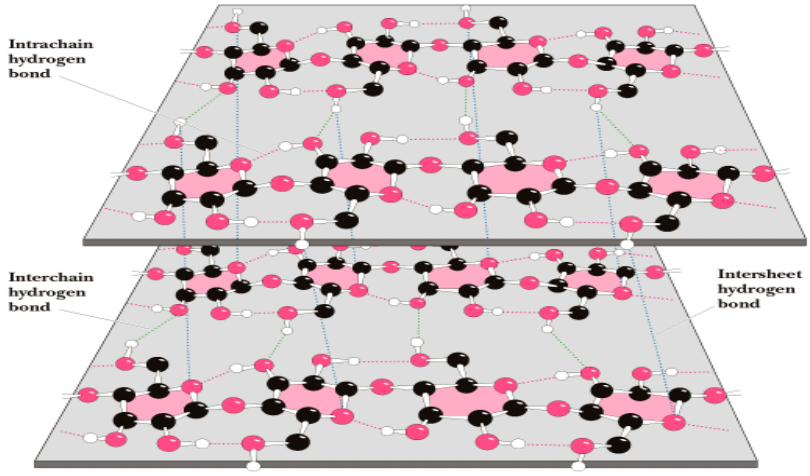

Figure 2.4: The structure of cellulose.

Table 2.3: The percentage of alpha cellulose in various lignocellulosic samples. (Alfred, 2012)

Lignocellulosic Sample	Composition of Alpha cellulose (%)
Walnut green skin	21.5
Groundnut shell	65.2
Maize husk	62.96
Rice husk	43.33
Sorghum stalk	47.36
Banana stem	63.9
Pineapple leave	73.4
Leaf waste	50.1

2.2.3 Hemicellulose

Hemicellulose was comprised of short and branched chains of sugars. The backbone of the chains of hemicellulose generally make up of sugar repeat units named as homopolymer or a heteropolymer which was the mixture of different sugars. It mainly comprised of 5 different sugar such as five-carbon sugars (D-xylose and L-arabinose) and six-carbon sugars (D-galactose, D-glucose and D-mannose). The structure for constituent of hemicellulose as shown in Figure 2.5. The hemicellulose content in softwood had more six-carbon sugars compared to hardwood. The significant difference between cellulose and hemicelluloses was its sugar composition. Hemicellulose had shorter and branched chains and able to change the shape easily (amorphous) which make its structure easier to hydrolyze. It is easily hydrolyze by dilute acid or base, but nature provides an arsenal of hemicellulase enzymes for its hydrolysis. These enzymes were commercially important because they opened the structure of wood for easier bleaching.

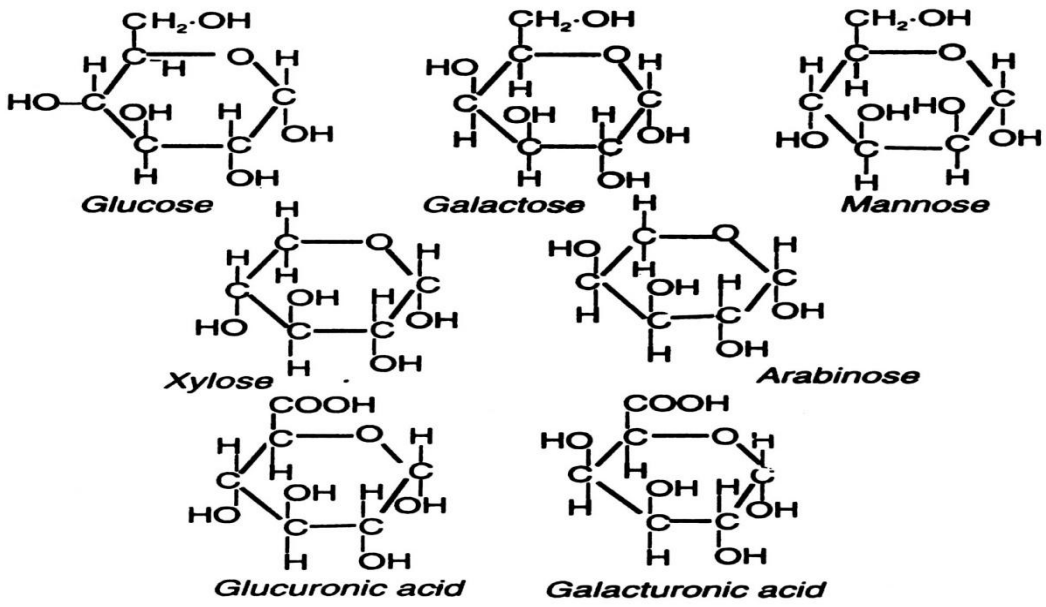

Figure 2.5: Structure for constituents of hemicelluloses.

2.2.4 Potential agricultural wastes for ferulic acid production

In recent decades, populaces have placed a high awareness on forest conservation and preservation as well as the sensible use of forestry and lignocelullosic wastes. This trend was highly urged by the predicament of raising consumption for wood fiber based products relative to the shrinking non-renewable resources. The application of lignocellulosic wastes had many advantages: cheap, recyclable and environmental sound. The common agricultural wastes for the production of ferulic acid were corncob and wheat bran. According to Paola (2008) indicated that in corn industry, corncob was the essential side- product produced. There were roughly 18 kg of corn cobs produced for every 100 kg of corn grain. By comparison, in flour industry for every 100 kg of wheat approximately 22 kg of wheat bran were produced (Chun & Zhen, 2010). In this research, banana stem waste was used as the substrate for the production of cost-effective product: ferulic acid. Banana stem was ample natural resources in subtropical and tropical region. It was used for the production of profitable product as manure and solid feedstock for domestics (Ultra et al., 2005). After harvested of banana, large amount of banana stem abandoned in banana plantation for natural degradation to become organic waste and it caused the environmental pollution. The exploitation of the waste of banana stem waste was dramatically beneficial to the

environment and ushered in additional income to the farmers (Li et al., 2010). Apart from that, banana stem waste had a high organic content (83%); with 15–20% (w/w) lignin and cellulose which gave it a sheath-like texture (Kalia & Sonakya, 2000). The analysis of chemical composition and anatomical structure of banana stem was carried out by using Light Microcopy (LM), Scanning Electron Microscopy (SEM) and Co focal Laser Scanning Microscopy (CLSM). The chemical analysis indicated that banana stem had high holocellulose and low lignin content if compared to some other non-wood fiber resources as shown in Table 2.4. In tropical country like Malaysia, statistics revealed that 279 000 metric tons of banana produced annually. This amount was massive compared to cereal production (wheat and corn) with only 118 000 metric tons per annum. Once again, it showed the untapped potential of banana stems as raw material for ferulic acid production.

Table 2.4: The chemical composition of banana stem waste and some other raw material.

Percentages (%)	Cellulose	Holocellulose	Kiason lignin	Acid soluble lignin	Ash content	Extracts	Pectin
Banana stem (Li et al., 2010)	39.12	72.71	8.88	1.90	8.20	3.05	0.27
Straw (Liu et al., 2003)	36.20	63.1	11.30	4.25	12.87	7.45	-
Pine (Cai and Tac, 2007)	45	71-83	24.57-29.85	0.37	0.27-0.28	1.11-3.51	-

2.3 Soil microbes

Microbes were very tiny organisms that observed under the microscope. It was divided into seven groups which were bacteria, archaea, fungi, prototist, microscopic animals, microscopic plants and viruses. There were ubiquitous on Earth. Some microbes thrived in extremely high temperature (thermophilic bacteria) while some can lived in freezing cold environment (mesophilic bacteria). Commonly, there were two types of soil bacteria namely: aerobic and anaerobic bacteria. Aerobic bacteria need oxygen to survive and it was found in a well drained area .On the contrary, anaerobic bacteria can lived without oxygen supply and mostly found in wet, poorly drained soils such as swamp and morass areas. Soil also abound with zillion of living organisms that can served as decomposers or degradation agents for lignin. Moravec (2012) emphasized that one teaspoon of soil may composed up to several million of soil mixed culture. In an addition, there are 10-20 million bacteria, 100,000 fungi, 50,000 algae and 30,000 protozoa in per gram of fertile soil (Patel, 1999). There are 1000 species or organisms with population densities in the order of 10^6 m^{-2} for nematodes, 10^5 m^{-2} for micro arthropods and 10^4 m^{-2} for other invertebrate groups found in a square metre of an agricultural soil, and that one gram of soil may consist over a thousand fungal hyphae and up to a million or more individual bacterial colonies (Altieri, 1999). The variability of microbial populations in each gram of soil was shown in Table 2.5.

In an addition, the role play by beneficial soil bacteria was to put the nitrogen in the soil into liquid form so that it can feed to plants. The need for usage of manure declined when the nitrogen-fixing bacteria was sufficient in the soil. Moreover, the soil bacteria were broken down the organic matter and resolved the pesticide residues in the soil. Some soil bacteria were able to suppress the soil pathogens that could cause disease in plant and reduce the usage of fungicides. There were dozens of benefit bring by root fungi such as *Mycorrhizae* which can work along with the bacteria to suppress miscellaneous of plant pathogens. Beneficial fungi helped the plants to grow healthy by producing several of natural growth hormones.

Table 2.5: The variability of microbial populations in each gram of soil (Metting, 1993)

Organisms	Per g of soil
Bacteria	10^{8-} 10^{9}
Actinomycetes	10^{7}-108
Fungi	10^{5}-10^{6}
Microalgae	10^{3}-10^{6}
Protozoa	10^{9}-10^{10}
Nematodes	10^{1}-10^{2}

2.4 Kinetic study

In a fermentation processes, it was tough to understand and control the process as it involved various of components such as effect of substrates, products inhibition, and the interaction of microbial observation. Since observation was rarely aids in optimizing the process and solved the encounter real problem in industry regarding the profitability and productivity. Therefore, a successful evaluation of the kinetic constant offered a better understanding into the bioreactor operation as well as the microorganism's degradation capacities. Kinetic data obtained in small scale of reactors was used as the mass transfer data for scale up purpose. In other words, kinetic study assisted in scaling up. In lieu of this, kinetic study was carried out to represent the complex processes by simple mathematical or kinetic models. A good mathematical modelling focuses on important aspects of a particular process to yield useful results. To model a fermentation process, the bioreactor performance such as flow pattern, mixing and mass transfer of the process should be taken into consideration. Moreover, microbial kinetics which involved cell growth rate, population model, yield of product was several crucial considerations.

In 1913, Michaelis and Menten was developed a mathematical model of the kinetics of simple enzyme-catalyzed reaction named as Michaelis-Menten kinetics. This model was

based on batch reactors with constant liquid volume in which the initial substrate, so was known. At high substrate concentration, all the sites were occupied by substrates and the enzyme was saturated. Saturated kinetics was obtained from a simple reaction scheme that involved a reversible step for enzyme-substrate complex formation as shown in Equation 2.1.

$$E+S \underset{K_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (2.1)$$

The rate of product formation:

$$V = \frac{dP}{dt} = K_2\,[ES] \qquad (2.2)$$

Where V, the rate of product formation or substrate consumption.

The rate of variation of the ES complex:

$$\frac{d[ES]}{dt} = k_1\,[E][S] - k_{-1}\,[ES] - k_2\,[ES] \qquad (2.3)$$

The conservation of enzyme yield:

$$[E] = [E_0] - [ES] \qquad (2.4)$$

The quasi-steady state assumption was substituted into Equation 2.3 forms the Equation 2.5

$$[ES] = \frac{K^1[E][S]}{K^{-1}+K^2} \qquad (2.5)$$

Next, substituted the enzyme conservation Equation 2.4 into Equation 2.5 yield

$$[ES] = \frac{K^1([E_0]-[ES])[S]}{K_{-1}+K_2} \qquad (2.6)$$

Solving Equation 2.6 for ES

$$[ES]= \frac{[E_O][S]}{\frac{K_{-1}+K_2}{K_1}+[S]} \quad (2.7)$$

After that, Equation 2.7 was substituted into Equation 2.2 to get Equation 2.8

$$V = \frac{dP}{dt} = \frac{K^2[E_O][S]}{\frac{K_{-1}+K_2}{K_1}+[S]} \quad (2.8)$$

$$V= \frac{dP}{dt} = \frac{V\,max[S]}{Km+[S]} \quad (2.9)$$

Where $\frac{dP}{dt}$ the rate of product formation, V_{max} was the maximum forward velocity, Km was the Michaelis-Menten Constant and [S] represented the substrate concentration.

Moreover, Michaelis–Menten theory was proved to be a powerful approach to analyze enzyme kinetics. This equation defined the relationship between initial velocities and substrate concentrations. Initial velocities were usually determined by linear regression of initial data points of the progress curves at different substrate concentrations as shown in Figure 2.6. However, the trend for concentration of product and substrate versus time shown in Figure 2.7. In Michaelis-Menten equation, Km and Vmax were the two constants that need to determine. Fogler (2010) mentioned that Vmax changed with the amount of enzymes. Indirectly; it implied that any increased in the substrate concentration will not alter the Vmax. The constant Km represented the ability of substrate to bind to an enzyme. Therefore, low Km value translated into higher affinity and resulted in higher velocity at any substrate concentration. In this research, an assumption make where the biomass dry weight contained the amount of substrate plus inoculum in equity ratio. Hence, an equation (2.10) was modified from the elemental Michaelis- Menten Equation, (2.9) to study on the kinetic constant: Km and Vmax.