OPTIMIZATION OF XYLOSE PRODUCTION FROM RICE STRAW USING RESPONSE SURFACE METHODOLOGY (RSM)

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ABSTRACT

Rice straw is a source of xylose which can be used as a raw material for production of many high value products. Xylose is a pentose sugar formed from xylan, one of the substrate in hemicelluloses. The aim of this study is to optimize xylose production from rice straw by manipulating the agitation time, pH and substrate concentration using Response Surface Methodology (RSM) based on central composite design (CCD). Acid and alkaline hydrolysis method was used for pretreatment of rice straw to remove lignin compound. After pretreatment, hemicellulose was used to produce xylose using enzymatic hydrolysis method. The enzyme used for enzymatic hydrolysis was *Thermomyces Lanuginosus* xylanase to degraded xylose from hemicelluloses. Enzymatic hydrolysis of rice straw was performed at 45°C and 250 rpm using agitation time (2-12 hours), pH (2-10) and substrate concentration (0.1-1.1 mg/ml). The best conditions for agitation time, pH and substrate concentration were approximately 4 hours, 6 and 0.9 mg/ml respectively. These values were used for optimization process using RSM. The optimized conditions from RSM were 4.14 hours of agitation time, pH 6.14 and 0.89 mg/ml of substrate concentration which produce 29.517 mg/ml of xylose. Before optimization, the xylose concentration was 24.694 mg/ml which the production of xylose was increased by 19.53%. As conclusion, the combination between agitation time, pH and substrate concentration can increase the production of xylose from rice straw.

ABSTRAK

Jerami padi merupakan sumber xilosa yang boleh digunakan sebagai bahan mentah untuk penghasilan pelbagai produk yang bernilai tinggi. Xilosa ialah gula pentosa yang terbentuk daripada xilan, salah satu substrat di dalam hemiselulosa. Kajian ini bertujuan untuk mengoptimumkan pengeluaran xilosa daripada jerami padi dengan memanipulasikan waktu pengadukan, pH dan kepekatan substrat menggunakan Kaedah Tindak Balas Permukaan(RSM) berdasarkan reka bentuk komposit pusat (CCD). Kaedah hidrolisis asid dan alkali digunakan sebagai pra-rawatan jerami padi bagi membuang sebatian lignin. Setelah dirawat, hemiselulosa digunakan untuk menghasilkan xilosa menggunakan kaedah hidrolisis enzim. Enzim yang digunakan untuk hidrolisis enzim adalah *Thermomyces Lanuginosus* xilanase supaya xilosa terdegradasi daripada hemiselulosa. Hidrolisis enzim daripada jerami padi dilakukan pada 45°C dan 250 rpm dengan waktu pengadukan (2-12 jam), pH (2-10) serta kepekatan substrat (0.1-1.1 mg / ml). Keadaan terbaik untuk masa pengadukan, pH dan kepekatan substrat maing-masing adalah sekitar 4 jam, 6 dan 0.9 mg / ml. Nilai-nilai ini digunakan untuk proses pengoptimuman menggunakan RSM. Keadaan optimum daripada RSM adalah 4.14 jam waktu pengadukan, pH 6.14 dan 0.89 mg / ml konsentrasi substrat yang mana telah menghasilkan 29.517 mg / ml xilosa. Sebelum pengoptimuman, konsentrasi xilosa adalah 24.694 mg / ml yang mana penghasilan xilosa telah meningkatkan sebanyak 19.53%. Kesimpulannya, gabungan antara waktu pengadukan, pH dan kepekatan substrat dapat meningkatkan pengeluaran xilosa daripada jerami padi.

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LIST OF SYMBOLS/ABBREVIATIONS

ANOVA - Analysis of variance
CCD - Central composite design
g - Gram
g/L - Gram per litre
hr - Hour
L - Litre
M - Molar
mg - Miligram
min - Minutes
ml - Mililitre
mM - Milimolar
OD - Optical density
OFAT - One factor at time method
RSM - Response surface methodology
rpm - Round per minute
T - Temperature
U - Unit (enzyme activity)
°C - Degree Celsius
% - Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Rice is the world's second largest cereal crop after wheat but can produce high amount of crop residues, about 330 million tones. According to the Food and Agriculture Organization of the United Nation (FAO) statistics, world annual rice production in 2007 was about 650 million tons. Every kilogram of grain harvested is accompanied by production about 1-1.5 kg of the straw. Ninety percent of the world production comes from developing countries of East and Southeast Asia where straw is the main feed for ruminants. Rice straw is low in digestibility and protein. Particularly, in India and other countries of southeast Asia where virtually all arable land is in crop production and animals are dependent upon straw, garbage, by-products and other wastes. Ninety percent of cereals go for human consumption, so that sufficient availability of supplements for animal feeding is a problem. Plant breeding has been devoted to maximize the grain yield with less interest in straw. This has resulted in short varieties in which the proportions of straw and leaf blade are reduced (Detroy *et al.*, 1980).

Rice straw predominantly contains cellulose (32- 47%), hemicellulose (19–27%) and lignin (5–24%). The pentoses are dominant in hemicellulose, in which xylose is the most important sugar followed by arabinose and the hexoses. The carbohydrate content of rice straw typically involves glucose (41–43.4%), xylose (14.8–20.2%), arabinose (2.7–4.5%), mannose (1.8%) and galactose (0.4%).

In rice straw, the ash components of K, Ca, Mg, Mn, Fe, Al, Cu, Zn, Si, and P exist in ionic state (Qiu, 2001). These elements are indispensable to the metabolism of crop, some of which act as enzyme activator and regulate the enzyme activity (e.g. K+, Mg2+) and some of which participate in enzymatic reaction as an component (e.g. Fe3+, Cu2+, Zn2+) (Yang *et al.*, 2005).

The straw has traditionally been removed from the field by the practice of open-field burning. This practice clears the field for new plantings and cleans the soil of disease-causing agents. Recently, the impact of open-field burning of rice straw on air quality has led to legislation which will in the future strictly control this practice. In the search for viable alternatives, the California rice growers are considering straw as a source of liquid fuels and energy (Yu *et al.*, 1997).

In California, the Rice Burning Reduction Act has motivated growers and government agencies to investigate economically viable uses for rice straw. Rice straw, as is true of all straw-type fibrous plant material, is composed primarily of cellulose, hemicellulose, and lignin. The realization that agro-based lignocellulosic fibers constitute a resource, rather than a waste product necessitating disposal, has heightened the interest in using agro-based fibers in a variety of composite materials, including fiber and particle board (Inglesby *et al.*, 2005)

Hemicellulose is generally heteropolymeric and composed of more divers' saccharide monomers and compositional arrangement, including numerous side shains on an otherwise linear backbone. The array of monosaccharide constituents found in terrestrial hemicelluloses includes pentoses and the example is D-xylose and L-arabinose. It is like hexoses (e.g. D-glucose, D-galactose, and D-mannose).

Hardwood and softwood hemicelluloses often include an additional variety of uronic acids including 4-methyl-D-glucuronic, D-glucuronic and D galacturonic acids. It depends on the tree species. The monomeric sugars and uronic acids present in hemicelluloses are polymerize (generally 150-200 sugar residues/molecular backbone) into array of glycan including glucans, xylans, mannans, galactans and glucuronides, with molecular masses that are generally lower than those of the extended cellulose chains.

Xylose is a recucing sugar that degrades from hemicelluloses. Xylose or wood sugar, is an aldopentose a monosaccharide containing five carbon atoms and including an aldehyde functional group. It has chemical formula $C_5H_{10}O_5$. Xylose is found in the embryos of most edible plants that free carbonyl group, it is a reducing sugar.

Xylose is also the first saccharide added to the serine or threonine in the proteoglycan type O-glycosylation and so it is the first saccharide in biosynthetic pathways of mostanionic polysaccharides such as heparan sulfate and chondroitin sulfate (Boons *et al.*, 2006)

The pretreatment method most often used is steam explosion, heating the lignocellulosic material by steam injection to a temperature of 190-230°C (Saddler *et al.,* 1993). Another method, which has been used in this study, is wet oxidation. In the wet oxidation process, the material is treated with oxygen at alkaline conditions at 150-185°C. Wet oxidation produces fewer toxic compounds from the carbohydrate part of the lignocellulosic material than steam explosion. Effective pretreatments must improve enzymatic hydrolysis and prevent formation of by-products that might inhibit subsequent hydrolysis and fermentation steps.

Recent researches for enzymatic hydrolysis are usually carried out under mild conditions such as using low pressure and long retention time in connection to the hydrolysis of hemicelluloses were studied about the hydrolysis of rice straw using 5–10% H_2SO_4 at 80–100 °C. They reported the best sugar yield at 100°C with 10% H_2SO_4 for 240 min.

Yin *et al.*, (1982) has studied about the hydrolysis of hemicelluloses fraction of rice straw with 2% H_2SO_4 at 110–120°C, where they succeeded to hydrolyze more than 70% of pentose. Next research carried out hydrolysis of rice straw was carried out by (Karimi *et al.,* 2004) with different acids with varying concentrations (0.5–1% H_2SO_4, 2–3% HCl and 0.5–1% H_3PO_4) and they found that after 3 h retention time, rice straw pentosans converted to a solution of monosaccharides, suitable for fermentation.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques widely used to determine the effects of several variables and to optimize different biotechnological processes. Empirical models and statistical analysis are extremely important to elucidate basic mechanisms in complex situations, thus providing better process control and understanding. The main objective of RSM is to determine the optimum operational conditions for the system or to determine a region that satisfies the operating specifications (Roberto *et al.,* 2000).

1.2 Problem Statement

Rice production is expected to increase significantly in the near future in order to feed the rising human population. Today, paddy rice culture produces 660 million tons of rice, along with 800 million dry tons of agricultural residues, mainly rice straw. This biomass is managed predominantly through rice straw burning and soil incorporation strategies.

Rice straw burning leads to significant air pollution and has been banned in some regions, whereas stubble and straw incorporation into wet soil during land preparation is associated with enhanced methane emissions (Escribá *et al.*, 2009). Rice straw burning produce Carbon dioxide (CO_2). Carbon dioxide (CO_2) is one of the major pollutants in the atmosphere. The concentrations of CO_2 in the air around 1860 before the effects of industrialization were felt, is assumed to have been about 290 parts per million (ppm). In the hundred years and more since then, the concentration has increased by about 30 to 35 ppm that is by 10 percent (Vadiveloo, 2005).

Usually, rice straw was used for animal feeding. However, rice straw supply is exceeded and makes it not productive because many rice straw not use complete for feeding. Many ideas have been developed to use straw especially for pulp and paper, construction materials, soil incorporation, compost and fuel. However, because of other competitive resources, the prospect of immediate use of straw for these purposes is dim. Vehicle emissions' share in black cloud pollution is 32 per cent, followed by open burning 30 per cent because farmers have no alternative to burn the straw to get the land ready for the winter cultivation cycle (Hägerdal *et al.,* 2009)

1.3 Objective

The purpose of this research is to optimize the production of xylose from rice straw using response surface methodology (RSM).

1.4 Research scope

In order to achieve the objective of the research study, several scope of study has been identified such as to study the effect of agitation time, pH and substrate concentration on xylose production from rice straw. Besides that, to optimize the production of xylose from rice straw using Response Surface Methodology (RSM).

1.5 Rationale & Significance

In using of rice straw as raw material can consider as a low cost because rice straw was abundant and inexpensive in Malaysia. Otherwise, the composition of the xylose is plenty in rice straw. Production of xylose from rice straw can reduce pollution in environment. The food industry, medical industry and also chemical industry can get more profit because of the inexpensive of the rice straw.

It also benefits to use waste to useful product that can reduce cost of production. Besides that, it benefits to utilize waste from agriculture because salvage from agriculture has a big potential in producing many product such as xylose, cellulose and glucose.

The production of xylose has a potential in a future because from the xylose, many valuable product can be produce such as a bio-ethanol which is the fuel that has same function with the crude oil like petrol but the bio-ethanol has a lower cost and lower price that it.

Xylitol is one of the products from xylose that has higher demand because it widely used in the pharmaceutical industry. Xylitol has a big potential in preventing dental caries otitis and in children. This organic compound, with the general formula $C_5H_{12}O_5$, has a sweetening power similar to that of sucrose, being recommended to obese and diabetic patients, due to its insulin-independent metabolism. It is also used as a food additive to improve color and texture of products in which it is incorporated, without causing any undesirable change during stocking (Roberto *et al.,* 2001).

CHAPTER 2

LITERATURE REVIEW

2.1 Raw Material

Rice straw is attractive as a fuel because it is renewable and consider to be carbon dioxide neutral but has not yet been commercially. The nature of rice straw is limited by the great bulk of material, slow degradation in the field, harboring of rice diseases and high mineral content. However, the straw must be disposed of in order to make way for the next crop. Rice straw is an agricultural residue containing xylose, which represents up to 90% of the total sugar present in the hemicellulosic fraction of this residue and can be converted to different products (Mosier *et al.,* 2008).

One of these products, xylitol, is widely used in food and pharmaceutical industries (Zeid *et al*, 2008). Rice straw predominantly contains cellulose (32–47%), hemicellulose (19–27%) and lignin (5–24%). The pentoses are dominant in hemicellulose, in which xylose is the most important sugar followed by arabinose and the hexoses. The carbohydrate content of rice straw typically involves glucose (41–43.4%), xylose (14.8–20.2%), arabinose (2.7–4.5%), mannose (1.8%) and galactose (0.4%) (Karimi *et al.*, 2006).

According to the research conversion of rice straw to sugars by dilute-acid hydrolysis from Guo *et al.*, (2009), the main sugars from rice straw contain glucose (36.6%) and xylose (16.1%). The lignin content accounts for 14.9% of dried materials. However, the amount of acid-soluble lignin is only 1.9% of rice straw. The ash content was apparently high due to plenty of silica in this raw material. Table 2.1 presented the constituent of original rice straw in this study.

Table 2.1: Constituent component of original rice straw

Component	% (w/w) of dry rice straw
Glucose	36.6
Xylose	16.1
Lignin	14.9
Ash	14.5

The other research from Chang *et al.,* (2009) was founded the conversion of rice straw by pretreatment in freezing under atmospheric pressure. Table 2.2 summarizes the composition of the raw material used in this study.

Table 2.2: Composition of rice straw

Composition	% (w/w) of dry rice straw
Glucose	41.06
Hemicellulosic sugars	13.88
Xylose	12.38
Arabinose	1.02
Galactose	0.48
Mannose	0.00
Lignin	25.33
Moisture	9.26
Ash	10.46

Cellulose was the primary component, forming 41.06% of the sample. Hemicellulosic sugars accounted for 13.88% of raw material and xylose was the other major constituent (12.38%). Arabinose and galactose accounted for only a small portion of the biomass, while mannose was not detected. Cellulose (as glucose) and lignin content (41.06% and 25.33%, respectively) were in the range reported for other agricultural residues.

2.2 Hemicellulose

The hemicelluloses fraction of agricultural and hardwood ligno-cellulosic materials contains xylose as the major sugar component. These cheap and abundant natural resources, upon hydrolysis with acid, yield xylose-rich hydrolysates that can be recovered in good yields The term hemicelluloses refer to a group of homo- and heteropolymers consisting largely of anhydro-D-xylopyranose, mannopyranose, glucopyranose and galactopyranose main chains with a number of substituent. Hemicelluloses are generally found in association with cellulose in the secondary walls of plants, but they are also present in the primary walls (Sjoman *et al.*, 2008).

Figure 2.1 shows the structure of molecules cellulose, hemicelluloses and pectin. From the figure, cellulose enzyme is responsible to degrade molecules of cellulose. At the hemicelluloses structure, xylanase enzyme is an enzyme that can react with the molecules. Same as pectin molecules, pectinase is a specific enzyme to degrade it to the simple sugar (Lee *et al.*, 1982).

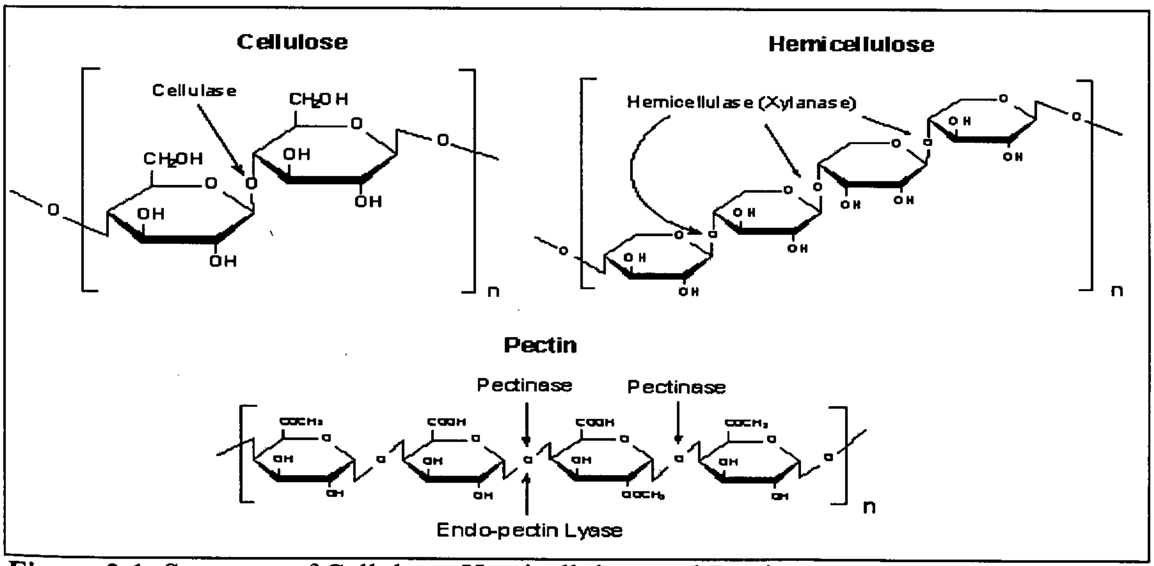

Figure 2.1: Structure of Cellulose, Hemicellulose and Pectin.

The conversion of hemicelluloses in to simple sugar could be presented from Lavarack *et al.*, (2002) that was using the acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose and glucose. Figure 2.2 shows a typical plot of the aqueous xylose and arabinose concentration versus time.

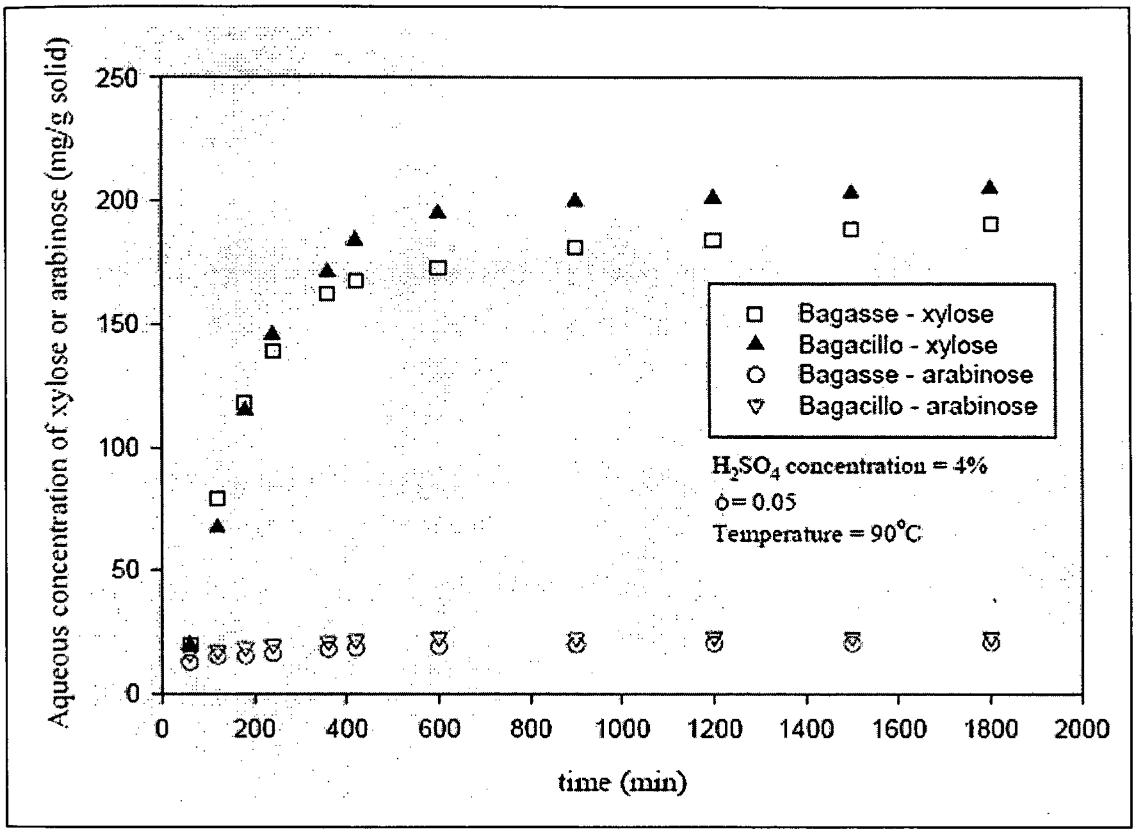

Figure 2.2: Hydrolysis of bagasse hemicellulose to produce xylose and arabinose

It can be seen that the concentration of xylose increases relatively rapidly over the first 300–600 min and follows a trajectory that would be expected for a first order reaction. This period has been interpreted as representing the hydrolysis of the easy to hydrolyze fraction of the xylan from hemicelluloses.

2.3 Xylose Production

Xylose is a sugar with five carbon atoms in each molecule, found in the cell walls of many plants. As it cannot be broken down in humans, it does not increase blood sugar levels, so is used in sugar-free foods for people with diabetes or a need to control their weight (Khawari *et al.*, 1996). A pentose sugar, referred to in the early literature as L-xylose. It is present in many woody materials. The polysaccharide xylan, which is closely associated with cellulose, consists practically entirely of D-xylose. Corncobs, cottonseed hulls, pecan shells and straw contain considerable amounts of this sugar.

Rahman *et al.*, (2005) proved production of xylose from oil palm empty fruit bunch fiber using sulfuric acid. Analysis of acid hydrolysate showed that highest release of xylose from hemicellulose was 31.1 (g/l) when acid concentration was 2% which was 91.2% of potential concentration of xylose. On the other hand with 4 and 6% sulfuric acid, maximum xylose released were 30.7 and 30.27 (g/l), respectively. This is shown in Figure 2.3.

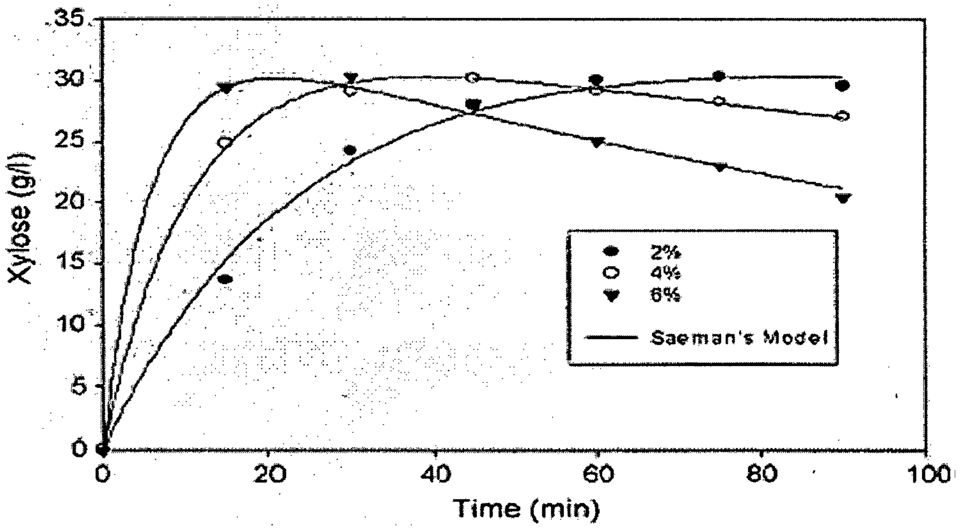

Figure 2.3: Effect of sulfuric acid concentration and reaction time on xylose production.