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

NUR'ATIQAH BINTI NORHALIM

UNIVERSITI MALAYSIA PAHANG

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Nama Penyelia
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NUR'ATIQAH BINTI NORHALIM

A thesis submitted in fulfillment of

the requirements for the award of the degree of

Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering

Universiti Malaysia Pahang

DECEMBER 2010

I declare that this thesis entitled "*Optimization of Xylose Production from Rice Straw using Response Surface Methodology (RSM)*" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Name : Nur'Atiqah Binti Norhalim

Date : 3 December 2010

Special Dedication to:

My mother, Puan Kamariah Jaafar,
My father, Encik Norhalim Sirome,
My family members,
My fellow lecturers,
My friends and
My fellow colleagues.

For all your concern, support and faith in me.

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ABSTRACT

The rice straw is an abundant biomass produced every year in Malaysia and also well known as agricultural residues in renewable resource. The biomass residues often burned at open field and caused in environmental problem. The rice straw contains about 25% hemicelluloses, the next most abundant polysaccharides in nature. The hemicelluloses will degrade into its reducing sugar which is xylose in enzymatic hydrolysis process by using xylanase. This research is study on optimization of xylose production from rice straw by using Response Surface Methodology (RSM) based on Central Composite Design (CCD). The parameters used to obtain xylose production were temperature, agitation rate and xylanase concentration during enzymatic hydrolysis. The rice straw was treated by alkaline hydrolysis and acid hydrolysis continuously. Then, the conventional method was used to determine the best ranges of parameters. The best ranges were used in RSM for optimization process. The optimum production of xylose was achieved at temperature of 55°C, agitation rate of 155 rpm and 2.06 mg/ml of xylanase concentration. The xylose production obtained was 0.2716 mg/ml after optimization process. Before optimization, the xylose concentration was 0.2051 mg/ml. As a conclusion, the production of xylose has been successfully optimized from rice straw using RSM with increment of 32.43%.

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LIST OF SYMBOLS/ABBREVIATIONS

ANOVA	-	Analysis of variance
CCD	-	Central composite design
DNS	-	Dinitrosacyclic
DOE	-	Design of Experiment
g	-	Gram
g/l	-	Gram per litre
hr	-	Hour
L	-	Litre
M	-	Molar
mg	-	Miligram
mg/ml		Miligram per mililitre
min	-	Minutes
ml	-	Mililitre
nm	-	Nanometer
OD	-	Optical density
OFAT	-	One factor at time method
RSM	-	Response surface methodology
rpm	-	Round per minute
Uv-Vis	-	Ultraviolet Visible Spectroscopy
w/v	-	Weight per volume
µg	-	Microgram
µg/ml	-	Microgram per mililitre
˚C	-	Degree Celsius
%	-	Percentage

CHAPTER 1

INTRODUCTION

1.1 Research Background

Rice straw is a waste after the grain or seed and chaff have been removed. This straw is an agriculture residue well known in Malaysia. Even though, rice straw will be reuse throughout a few method such as feed stocks for animal, biofuel and make papers. In major practices, the rice straw which remains unused and burned at rice field will cause environmental problem, such as air pollution and fire disaster. The rice straw consist carbohydrate composition of 35% cellulose, 25% hemicelluloses and 12% lignin whereby throughout hydrolysis process would produce xylose (Saha *et al.,* 2003).

Xylose known as the simple sugar which represent up to 90% of the total sugar present in the hemicelluloses fraction of this residue (Azza *et al.,* 2008). The xylose present in this fraction may utilize in bioconversion process for development of fermentation process besides attempt to reduce the adverse impact on the environment (Brownell and Nakas, 1991). The production of xylose was the main sugar obtained from hemicellulose, but other by-products such as glucose, acetic acid, and furfural were also produced in low amounts during the hydrolysis process (Silva *et al.,* 1998).

Therefore, the hydrolysis processes is required to be able to break the polysaccharides bonds into simple sugar (monosaccharide). Hence, the lignin component acts as a physical barrier and must be removed to make the carbohydrates available for further transformation processes (Kadam *et al*, 2000). In the hydrolysis process it is understood that initially the lignin protective layer around the hemicelluloses fiber is softened under elevated temperature and pressure which allows the acid to penetrate the layer and hydrolyze the amorphous xylan to form xylose (Rahman *et al.*, 2006).

Usually, the chemical hydrolysis pretreatment methods used for lignin removal from feedstock are dilute acid, alkaline, organic solvent, ammonia, sulfur dioxide, carbon dioxide or other chemicals to make the biomass more digestible by the enzymes (Patel, 2007). The pretreatment of lignocellulosic biomass is crucial before enzymatic hydrolysis. According to Kovacs *et al.* (2009) who claimed that the complete hydrolysis of cellulose and hemicelluloses requires various enzymes including xylanase to act on side chains in hydrolysis process. In addition, Harada *et al*. (2008) stated the rice bran was incubated with xylanases enzyme that responsible to degrade the hemicelluloses from rice bran into xylose.

The optimization of xylose production using Response surface methodology (RSM) as utilizes to optimize the conditions. Moreover, it have been used to model the responses of sensory attribute and chemical analysis to generate predictive equations which correlate the consumer response with the variables studied in food product design and the process (Schutz, 1983; Gacula, 1993; Hu, 1999). These predictive equations (models) can be used to optimize the formulation or the process and to estimate the expected consumer response to combination of factors not directly tested (Moskowitz, 1994). Therefore, Lee *et al.* (2006) were optimized the enzymatic clarification process of banana juice using RSM by indicating the optimum conditions at incubation temperature of 43.2%, incubation time of 80 min and 0.084% enzyme concentration for clarified the banana juice.

1.2 Problem Statement

In major practices, rice straw is burned. Consequences from these activities might cause pollution such as air pollution and also global warming. Although burning of rice residues were cost-effective and the predominant method of disposal in areas under combined harvesting in the Indo-Gangetic Plains (IGP) (Samra *et al.,* 2003) of South Asia. However, disposal of crop residues by burning is often criticized for accelerating losses of Soil Organic Matter (SOM) and nutrients, increasing carbon emissions, causing intense air pollution and reducing soil microbial activity (Biederbeck *et al.,* 1980). Estimated emissions of greenhouse gases caused by burning of rice straw are thus substantial. Besides contributing to the greenhouse effect, the large-scale burning of rice straw results in serious health hazards as is evident from the reported increase in respiratory and eye problems among the local population (Grace *et al.,* 2004).

1.3 Objective

This research is aim to optimize the production of xylose from rice straw by using Response Surface Method (RSM).

1.4 Research Scopes

There are research scopes that have been identified for this study to achieve the objective:

i. To study the effect of temperature on xylose production
ii. To study the effect of agitation rate on xylose production
iii. To study the effect of enzyme concentration on xylose production
iv. To optimize the temperature, agitation rate and enzyme concentration on xylose production using Response Surface Methodology (RSM)

1.5 Rationale & Significance

The production of xylose from rice straw will reduce the environmental problem than burn the rice straw. The rice straw contain xylose up to 90% of the total sugar present in the hemicellulosic fraction and can produces different of product by converted it. These products are widely used in food, pharmaceutical industries and also agro-industrial process. In Austria, Danisco Sweeteners factory were invested by the global food ingredients producer to secure and increase the production of xylose, a monosaccharide and the raw material for xylitol production.

Xylose production will be used for further fermentation to produce alcohols such as xylitol and ethanol. Thus, its known as renewable resources and as an alternative potential of fuel. The reason is because demands on ethanol of biofuel nowadays and as a result, xylose highly potential to produce it by converted xylose and glucose into ethanol. There are approve that conversion of cellulose and hemicelluloses which mainly produce glucose respectively will further converse into ethanol (Saha *et al.,* 2003).

The production of reducing sugars studied on the effects of temperature, agitation rate and enzyme concentration during enzymatic hydrolysis. The optimization of production recovered using Response Surface Methodology (RSM). It is also known as the best techniques to solve a problem in an optimization of bioprocesses. Therefore, RSM is used to generate a lot of samples for consumer evaluation and solve the problem in a short period of time (Rudolph, 2000).

CHAPTER 2

LITERATURE REVIEW

2.1 Rice Straw

Rice straw is considered to account for the largest portion of available biomass feedstock in the world about 7.31 x 10^{14} of dry rice straw per year and Asia is responsible for 90% of the annual global production (Kim and Dale, 2004). Rice straw is attractive as a fuel because it is renewable and consider to be carbon dioxide neutral (Atchison, 1996) 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 (Daniel *et al*., 2002). Rice straw has been utilized in much bioconversion process.

Therefore, rice straw have been investigated its potential as the sole feedstock to produce biogas because contains a high percentage of polysaccharides and lignin (He *et al.,* 2008). In recent years, many attentions have been focused on the biotechnological process for production of several useful feedstock and food products from agro-forest residues and agriculture residues such as rice straw (Azza *et al.,* 2008). However, lignocellulose such as rice straw is difficult to hydrolyze using only enzyme due to its recalcitrant and heterogenous structure, which primarily consists of cellulose, hemicelluloses and lignin (Chandra *et al*., 2007).

2.2 Hemicellulose

Hemicellulose is plant cell wall polysaccharide and third most abundant polymer in nature. In rice bran, hemicellulose is a heteropolysaccharide consisting of xylan as a backbone and several sugars such as arabinose, galactose and glucose as side chain (Harada *et al*., 2005) next to xylose as the major simple sugar found in hemicelluloses. However, among biomass components consist of hemicelluloses which mainly composed of xylan that provide an important source such as xylose and xylo-oligosachharides potentially utilize in chemical, food and pharmaceutical industries (Fook *et al*., 1999).

An extensive research has been undertaken to convert hemicelluloses derived carbohydrates particularly xylose into useful products (Rao *et al.,* 2006). The degradation of hemicellulose through hydrolysis to yields its simple sugar. The hydrolysis of hemicelluloses recovered crystalline xylose (Curreli *et al.,* 2002). Therefore, several technologies were proposed for fractionation or extraction of hemicelluloses from feedstock such as using conventional techniques based on chemical treatment (Shevchenko *et al.,* 2000).

Hemicellulose also is considered to be amorphous components (Gharpuwy *et al*., 1983; Jeoh *et al.,* 2007). An enzymatic hydrolysis is an efficient method that hydrolyzes the formless xylan to form xylose at optimum temperature and agitation rate in incubator by application of xylanase. Specifically, hemicellulose has three basic forms: 1.4-β-D-xylans, 1,3- and 1,4-β-D-galtictuns. and 1,4-β-D-mannans. The complete hydrolysis of complex composition and structure of hemicelluloses into monosaccharides requires the combined action of several enzymes. These enzymes include β-D-xylanases, β-D- galactanases, β-D-mannanases, as well as glycosidases β-D-xylosidase, *x*- and β-D-galactosidase and β-D-mannosidase. Esterases also participate by hydrolysis of acetylated carbohydrates (Saha *et al.,* 2003).

2.3 Production of Xylose

Xylose is a monomer found in hemicellulose after hydrolysis process. The xylose can be used as substrate for production of a wide variety of compounds by chemical and biochemical processes (Silva *et al.,* 1995). Thus, Rahman *et al*. (2006) had been recovered the production of xylose from oil palm empty fruit bunch fiber using sulphuric acid. The xylose was completely converted from polymer xylan of empty fruit bunch fiber without formation of any decomposition products.

Xylose obtained as the major sugar component in the hemicelluloses fraction of agricultural. Upon hydrolysis with acid, the xylose can be recovered in these cheap and abundant natural resources (Nigam and Singh, 1995). Rice straw hydrolysate increased the xylose and total sugars without any toxic compounds (Azza *et al*., 2008). On the other hand, optimization on the recovery of xylose from sugarcane baggase had been done by Boussarsar *et al.* (2009) using hydrothermal treatment.

The dilute acid pretreatment modification using a mixture containing aqueous solution of a dilute acid and a metal salt catalyst such as $FeSO_4$ for hydrolyzing cellulose and hemicelluloses in lignocellulosic biomass could obtain higher overall fermentable sugar yields (Nguyen and Tucker, 2002). The xylose monomer and xylotriose degradation significantly increased by adding inorganic salts in water at 180°C. This inorganic salt enhanced the recovery of xylose monomer and degradation of xylotriose.

2.4 Pretreatment Process

Pretreatment of the rice straw has proved to increase both its physical and chemical properties thereby, minimizing the costs of transport, handling and storage. These applications concerned with improving combustion efficiency and reducing pollution emission. Acid hydrolysis has been investigated as a possible process for

treating lignocellulosic materials such as wood chips, rice straw (Almeida, 1991), sugar beet pulp (Chamy *et al.,* 1994) and wheat straw (Fanta *et al.,* 1984).

Rice straw can be hydrolyzed using dilute acid to obtain a mixture of sugars with xylose as the major component. However, in the hydrolyzate some by-products generated in the hydrolysis, such as acetic acid, furfural, phenolic compounds or lignin degradation products, can be present. These are potential inhibitors of a microbiological utilization of this hydrolyzate (Dominguez *et al.,* 1996). Treatment with dilute sulphuric acid at moderate temperatures in the first stage of acid hydrolysis has proven to be an efficient means of producing xylose from hemicellulose (Roberto *et al.,* 1994; Silva 1996).

In the other case, the pretreatment used to remove lignin which known as forms a three-dimensional network inside the cell wall beside associate with other cell wall polysaccharide. They are sparingly insoluble in water and partially in organic solvent because of hydrogen bonds between polysaccharides and linkage of lignin to polysaccharides. Thus, the pretreatment have been proven to be one of simple and effective methods to improve biodegradability and biogas production of lignocelluloses materials (He *et al.,* 2008). The biodegradable of lignocelluloses materials may convert into reducing sugar.

There is chemical pretreatment such as alkaline hydrolysis that decreased the lignin content while enhances the enzymatic saccharification. The chemical processes are based on cellulose hydrolysis of acids or cellulose solvents such as alkaline hydrogen peroxide (Mosier *et al*., 2005). The releasing of sugars from lignocelluloses biomass was facilitated by pretreatment process.

The lignocelluloses pretreatment used other chemical such as ionic liquids to dissolve cellulose in biomass. Ionic liquids can be reused after treatment and easily applied to enhance the enzymatic hydrolysis and efficiently recover fermentable sugars such as glucose and xylose from lignocellulosic biomass source (Li *et al.,* 2006). Therefore, Nguyen *et al.* (2010) had studied the pretreatment method using ammonia and ionic liquid for the recovery of bio-digestible cellulose from lignocellulosic by-product which effect on the enzymatic glucose conversion.

Lignocellulosic biomass cannot be saccharified by enzyme in a high yield without a pretreatment procedure because the lignin in the cell wall is a barried to enzyme action (Sewalt *et al.,* 1997). Rice straw was selected as a substrate for saccharification in research had done by Jeya *et al.* (2009). The lignin components were decreased of 37% in pretreated rice straw with 2% of sodium hydroxyide.

2.5 Enzymatic Hydrolysis

The hemicellulosic fraction can then be enzymatically hydrolyzed to xylose. Enzymatic hydrolysis of biomass hemicellulose does not produce toxic products. The hydrolysis of hemicellulose is accelerated at elevated temperatures owing to relatively high activation energy in the solid-liquid phase reaction. At high temperatures part of the xylose released from hemicellulose can be degraded rapidly and cellulose in the amorphous region can yield glucose (Banerjee, 1989). The enzymes that involve in degradation of hemicelluloses listed in Table 2.1 (Selinger *et al*., 1996).

Table 2.1: Enzyme involve in the hydrolysis of complex hemicelluloses

Enzyme	**Mode of action**
Endo-xylanase	Hydrolyzes mainly interior β-1,4-xylose linkages of the xylan backbone
Exo-xylanase	Hydrolyzes β-1,4-xylose linkages releasing xylobiose
β-Xylosidase	Releases xylose from xylobiose and short chain xylooligosaccharides
α-Arabinofuranosidase	Hydrolyzes terminal nonreducing α-arabinofuranose from arabinoxylans
α-Glucuronidase	Releases glucuronic acid from glucuronoxylans
Acetylxylan esterase	Hydrolyzes acetylester bonds in acetyl xylans
Ferulic acid esterase	Hydrolyzes feruloylester bonds in xylans
γ-Coumaric acid esterase	Hydrolyzes γ-coumaryl ester bonds in xylans

Endo-xylanases are much more common than β-xylosidases, but the latter are necessary in order to produce xylose. Activity was optimum at pH 3.3 and 52 °C. β-Glucuronidase acts in synergism with xylanases and β-xylosidases to hydrolyze glucuronoxylan. The yield of xylose greatly increases in the presence of this enzyme (Puls *et al.,* 1980). Thus, Wang and Zhang (2006) produced xylose from corncobs by xylanase through hydrolysis under concentrated ultrafiltration with polyamide (PA) capillary fibres. Most important is endoxylanase cleaves β-1,4-xylose backbone in hydrolysis (Sharma *et al*., 2010).

Therefore, it is necessary to develop enzymes and microorganism that are resistant to such inhibitory substances or to employ additional step to remove the inhibitor. The pretreatment and enzymatic hydrolysis steps to achieve fermentable sugar are currently known to have much more room for reducing processing cost than other processes (Lynd *et al.,* 2008). The enzymatic hydrolysis utilized on recovery of reducing sugar. The crystalline structure of cellulose has been

hydrolyzed by cellulase during enzymatic hydrolysis (Jeoh *et al*., 2007). The loading of cellulase increased the production of glucose (Bak *et al*., 2009).

Furthermore, the enzyme enhanced conversions of pretreated substrate. Thus, Kovacs *et al.* (2009) had been studied on enzymatic hydrolysis of steam-pretreated lignocellulosic materials with *Trichoderma atroviride* enzymes produced in-house. In the research claimed the supplementation of *Trichoderma atroviride* with xylanase enzyme resulted in an increase of 40% in the xylose level and an improvement of 21% in the glucose concentration.

2.6 Factors Affecting the Production

2.6.1 Effects of Temperature

The degradation of hemicelluloses can be generally be achieved by either high temperature and short residence time (270°C, 1 minutes) or lower temperature and longer residence time (190° C, 10 minutes) (Saha, 2003). It had been reported by Morjanoff and Gray (1987) that enzymatic saccharification of 100g sugarcane bagasse after steam explosion with 1% H_2SO_4 at 220°C for 30 seconds yielded 65.1 sugars. As a result, it been approved that the temperature involved in sugar production from sugar bagasse.

According to Karunanithy *et al.* (2009) who constructed the influence of high shear bioreactor parameters on carbohydrate release from different biomasses. It had been studied on effects of the barrel temperature and screw speed of high shear bioreactor from 50 to 200°C and 50 to 200 rpm, respectively. The highest glucose and combined sugar conversion of 22.76 and 68.33% respectively were recorded at 50°C and 150 rpm for corn stover.

The temperature also affect on degradation and biomass production. The effect of the temperature was studied by Wang *et al.* (2005) in response surface analysis to evaluate the influence of pH, temperature and substrate concentration on the acidogenesis of sucrose-rich waste water. The optimum conditions resulted in the research were at pH 5.6, 33.5°C of temperature and sucrose concentration 24.2 g/l. It also defined that the parameters studied was affect on the production of volatile fatty acid (VFA) and H_2 from sucrose-rich wastewater.

The effect of various operating variables such as pretreatment temperature on the degree of lignin removal and the enzymatic digestibility were investigated by Ko *et al*. (2009). The application of varying temperatures (50 - 160°C) during alkaline pretreatment was recovered in production process of ethanol from rice straw. In addition, rice straw which was pretreated using ammonia aqueous solution at moderate temperature to enable production of the maximum amount of fermentable sugars from enzymatic hydrolysis.

2.6.2 Effects of Agitation Rate

Agitation is important for adequate mixing, mass transfer and heat transfer. It assists for mass transfer between the different phases present in the culture (Kongkiattikajorn *et al*., 2007). The experimental were carried out in order to study the effect of agitation speed on cell and ethanol production of the mixed culture at rotatory shaker of 0, 50, 100, 150 and 200 rpm for 18 hours. The agitation increased the assimilation of sugars.

Furthermore, the agitation rate play an important role in understanding the production pattern, Consequently, Teramoto *et al*. (2007) had done pretreatment of wood chips for enzymatic hydrolysis, whereas the mixture were incubated at 45°C in a rotary shaker set at 250 rpm and sampled periodically. The conversion of cellulose to glucose was recovered during enzymatic hydrolysis.

The homogeneous chemical and physical conditions could be maintained by using agitation during enzymatic hydrolysis. Silva et al. (2010) has been studied on the fermentation of cellulosic hydrolysates obtained by enzymatic saccharification of sugarcane bagasse pretreated by hydrothermal processing. In the enzymatic hydrolysis process, the substrate mixed with cellulase and β-glucosidase was incubated in a rotary shaker at 100 rpm, 45°C for 72 hours. After that, the production of glucose, cellobiose and xylose concentration in the hydrolysates were quantitatively determined by HPLC.

2.6.3 Effect of Enzyme Concentration

The endo-xylanase, β-xylosidase, and α-L-arabinofuranosidase used for hemicelluloses bioconversion which reported by Saha (2003). Thus, there require several different enzymes with different specificities for complete hydrolysis of enzyme. Accordingly, the xylanase used in hydrolysis of xylan to xylose (Wang and Zhang, 2006). The variation of xylan conversion was carried out at 50°C in bath with 1.5 µg/ml of enzyme concentration. The hydrolysis with higher enzyme concentration produced more amounts of xylose.

The studied on enzyme concentration is used on xylose production in order to determine an optimum concentration of xylose. Beside, the study on effect of enzyme concentration will measured the enzyme activity. Hence, Ko *et al.* (2009) had been recovered that the xylanase supplementation in the hydrolysis of pretreated rice straw affect on degradation of xylan polymers. The higher glucose yields were achieved when xylanase amount were used to enhance the enzymatic hydrolysis.

2.7 Optimization using Response Surface Methodology (RSM)

The design of experiment using response surface methodology (RSM) is to obtain an optimum condition of production. RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions, successfully been used in the optimization of bioprocesses (Hao *et al*., 2006). In order to optimize the xylose production by hydrolyzing sugarcane bagasse, RSM was used to maximize the temperature and sulphuric acid concentration of the samples (Paiva *et al*., 2002). Based on the principle of design experiment (DOE), the methodology encompasses the use of various types such as experimental designs, generation of polynomial equations and mapping of the response over the experimental domain to determine the optimum product (Box and Draper, 1987).

Statistical experimental design was used to optimize hydrolysis parameters such as pH, temperature, and concentrations of substrates and enzymes to achieve the highest saccharification yield. Enzyme concentration was identified as the limiting factor for saccharification of rice straw (Jeya *et. al.*, 2009). On the other hand, Silva and Roberto (2001) have been used RSM to optimize the production of xylitol by *Candida guilliermondii* FTI 20037 based on effect of initial xylose concentration and inoculums level. The optimum xylose concentration and inoculums level were found to be 82 g/l and 3 g/l respectively with xylitol concentration of 52 g/l.

Response Surface Methodology may evaluate multiple parameters and its interaction meanwhile reducing the number of experimental trials. Thus, the process condition such as pressure, temperature, camel hump fat ratio, water content and incubation time were optimized at five different levels using RSM had been done by Shekarchizadeh *et al.* (2009). A second order polynomial response surface equation was developed indicating the effect of variables on cocoa butter analog yield.

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, there are three parameters were investigated in order to obtain xylose production. The parameters involved were temperature, agitation rate and enzyme concentration during enzymatic hydrolysis. The conventional method was conducted to study the effect of these parameters on production of xylose. Then, the xylose production was optimized by using Response Surface Methodology (RSM). Figure 3.1 show an overall process on xylose production from rice straw using RSM.

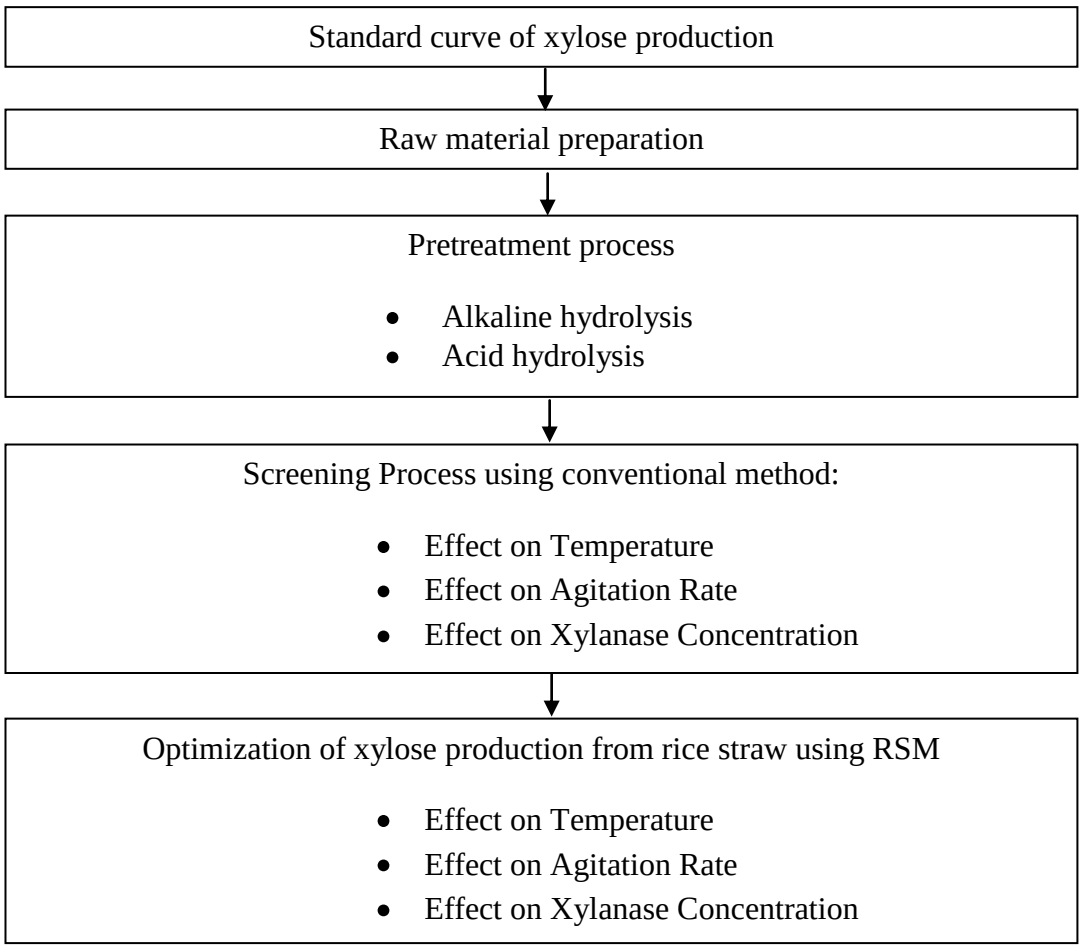

Figure 3.1: Overall process for production of xylose

3.2 Materials, Chemicals and Equipments

All materials, chemicals and equipments were provided by Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang. The materials used in this research were Erlenmeyer flask, volumetric flask, schott bottle, spatula, dropper, beaker, funnel, vial, test tubes, pipette and tips. The chemicals used are sodium hydroxide, citric acid, sulphuric acid, D-xylose, Dinitrosalicyclic (DNS) reagent and xylanase from *Thermomyces Lanuginosus* . The equipments that used were analytic balance, water bath, incubater shaker and Uv-Vis Spectrophotometer Hitachi U-1800.

3.3 Standard Curve of Xylose Production

The standard curve was calibrated by using Miller (1959) method as guideline for xylose concentration determination. The xylose with different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0, mg/ml) was prepared in sodium citrate buffer solution with pH 5.3. Then, 2 ml of each concentration was added with 2 ml of DNS reagent. The Optical Density (OD) of the samples were observed using Uv-Vis Spectrophotometer at 540 nm. Then, the standard curve of xylose production was obtained by plotting a graph of OD reading versus xylose concentration.

3.4 Preparation of Raw Material

. A bunch of rice straw from small farms in Teluk Intan, Perak. This rice straw was dry under sunrise at open space. After several hours, the dry rice straw was ground in a blender to become powder.

3.5 Pretreatment Process

3.5.1 Alkaline Hydrolysis

Firstly, 8.0 g of dry rice straw was placed into 2L beaker to carry out alkaline hydrolysis to remove lignin. The raw material was then soaked in sodium hydroxide of 1% (w/v) NaOH solution at 90°C for 2 hours in water bath. After two hours, the insoluble solid is separated from its aqueous solution by filtration. Meanwhile for the separation process, the hot water was poured in the insoluble water to drain out the alkaline solution from pretreatment process. The solid was dry in oven for 24 hours at 60°C before use in acid hydrolysis process. The pretreated rice straw was ensured dry its constant weight.

3.5.2 Acid hydrolysis

After a day in oven, the dry weight of rice straw was took out and placed in 2L beaker to continue the pretreatment process by acid hydrolysis. The dry rice straw was soaked in dilute sulfuric acid of 1% (w/v) H_2SO_4 solution at room temperature for 90 min. This pretreatment process also removed the lignin from rice straw. At the end of the dilution acid process, the substrate was separated from its aqueous solution by filtration and the dilution acid was drain out using water. Lastly, the substrate or insoluble solid was again dry in oven for 5 hours at 80°C. The pretreated rice straw was ensured dry its constant weight.

3.6 Screening Process for Temperature, Agitation Rate and Enzyme Concentration on Xylose Production Using Conventional Method

In the enzymatic hydrolysis, the xylanase was used to convert hemicellulose into xylose. The experiment used one factor at time (OFAT) method by varying one parameter meanwhile the other two parameters remains constant. This method would be investigated the best ranges of parameters in this study. The best ranges would be used in optimization process using RSM.

3.6.1 Effect of Temperature on Xylose Production

Firstly, 0.5 g of dry rice straw after pretreatment is placed into 250 mL Erlenmeyer flask which contained 25 ml of sodium citrate buffer (pH 5.3). Then, 0.25 mL of xylanase with concentration 1.5 mg/ml was added in the buffer solution. The experiment is carried out by incubate the samples in rotary shaker at different temperature of 15, 25, 35, 45, 55 and 65°C. The constant agitation rate of 150 rpm was used for 4 hours. Lastly, the samples were analyzed using Dinitrosalicyclic Colorimetric (DNS) method to obtain xylose concentration. The graph of xylose concentration versus temperature was plotted.

3.6.2 Effect of Agitation Rate on Xylose Production

The experiment was continued to obtain the effect of agitation rate on production of xylose. As previous experiment, 0.5 g of dry rice straw after pretreatment was placed into 250 mL Erlenmeyer flask that containing 25 ml of sodium citrate buffer (pH 5.3). Then, 0.25 mL of xylanase enzyme with concentration of 1.5 mg/ml was added into the sample. The experiment was carried out by incubate the sample in rotary shaker at different agitation rate of 60, 90, 120, 150, 180, 210 and 240 rpm. The maximum temperature from previous experiment was used as constant value in this process for 4 hours. Lastly, the samples were analyzed using Dinitrosalicyclic Colorimetric (DNS) method to obtain xylose concentration. The graph of xylose concentration versus agitation rate was plotted.

3.6.3 Effect of Xylanase Concentration on Xylose Production

The experiment was continued to obtain the effect of xylanase concentration on production of xylose. Firstly, 0.5 g of dry rice straw after pretreatment was placed into 250 mL Erlenmeyer flask that containing 25 ml of sodium citrate buffer (pH 5.3). The experiment was carried out by added 0.25 mL of xylanase enzyme with different concentrations, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml into different Erlenmeyer flask. Then, the samples were incubated in rotary shaker for 4 hours using maximum value of temperature and agitation rate that were obtained from previous experiments. Lastly, the samples were analyzed using Dinitrosalicyclic Colorimetric (DNS) method to obtain xylose concentration. The graph of xylose concentration versus xylanase concentration was plotted.

3.7 Optimization of Xylose Production using Response Surface Methodology (RSM)

Response Surface Methodology (RSM) was used to obtain the optimum temperature, agitation rate and xylanase concentration that produce higher production of xylose concentration. The best ranges were determined in conventional method to be use in RSM for optimization process. The optimized condition was arranged using RSM based on Central Composite Design (CCD). the experimental range and level of the independent variables for optimization in RSM was shown in Table 3.1.

Table 3.1: The experimental design of low and high levels of variables using Response Surface Methodology

Variables	Unit	Range and levels	
		Low level	High level
Temperature	℃	50	60
Agitation Rate	rpm	120	180
Xylanase Concentration	mg/ml	1.5	2.5

3.8 Dinitrosalicyclic Colorimetric Method (DNS Assay)

The determination of xylose concentration was analyzed by Dinitrosalicyclic (DNS) method. The DNS reagent was prepared by adding 0.5 g of sodium sulphite, 10 g of 3,5-dinitrosalicyclic acid, 10 g of sodium hydroxide, 182 g of potassium sodium tartarate and 2 g of phenol before dissolved the mixture with one liter of distilled water. The DNS reagent was kept in dark place.

After enzymatic process, the sample was analyzed using this method. About 2 ml of the sample was added to 2 ml of DNS reagent. The mixture containing DNS reagent was carried out with boil in water bath at 90˚C for 10 minutes. After that, the sample was cooled down to room temperature in a few minutes. The sample was determined the optical density (OD) by using Uv-Vis spectrophotometer at 540 nm of wavelength.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Enzymatic Hydrolysis of Xylose Production

In this chapter, the effect on temperature, agitation rate and xylanase concentration were studied on xylose production during enzymatic hydrolysis. The enzymatic hydrolysis process was produced reducing sugar, xylose from rice straw as the substrate by adding xylanase enzyme to increase the production. The conventional method was used to determine the maximum value of each the effect studied. Then, the low level and high level of parameter were picked before and after the maximum value respectively. The range levels were used in Response Surface Methodology to carry out an optimization on xylose production.

4.2 Effect of Temperature on Xylose Production

The enzymatic hydrolysis with different temperature of 15, 25, 35, 45, 55, and 65°C were tested to obtain the maximum production of xylose concentration using constant value of both of agitation rate and xylanase concentration. During this experiment, the enzymatic hydrolysis was carried at 150 rpm of incubator shaker and 1.5 mg/ml of xylanase concentration for 4 hours of incubated process. The result for effect of temperature on xylose concentration was illustrated as show in Figure 4.1.

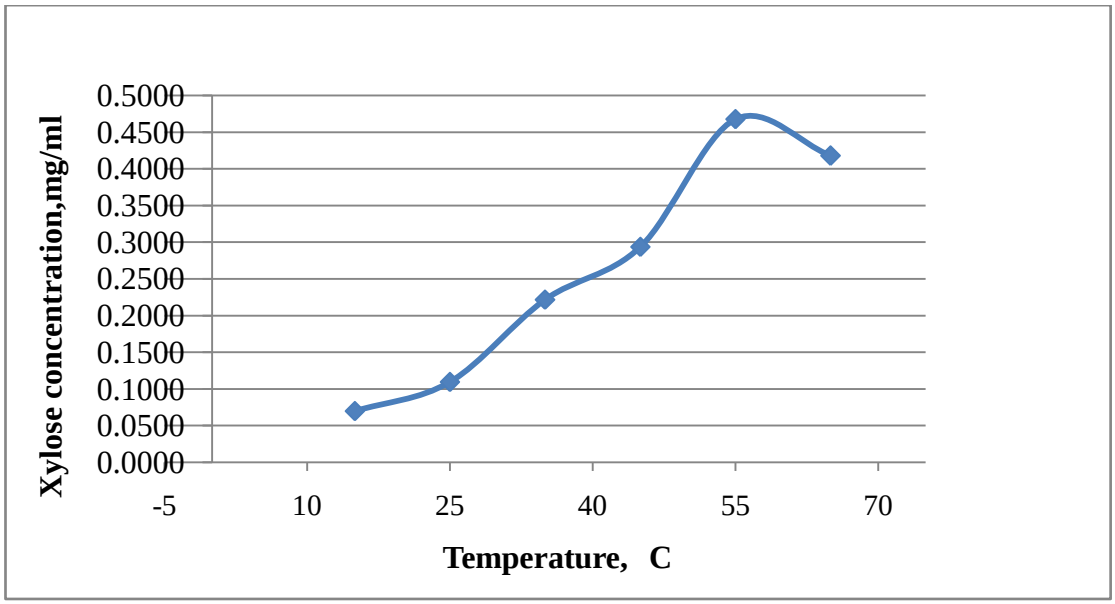

Figure 4.1: Effect of temperature on production of xylose concentration.

Based on the Figure 4.1, the highest xylose concentration produced at temperature of 55°C is 0.4678 mg/ml. At first, the production of xylose increase as temperature increase from 15 – 55°C but then it is decreased at temperature of 65°C. Thus, the best range of temperature selected is 45 – 65°C.

This result supported by other research on cloning, expression, characterization and high cell-density production of recombinant endo-1,4-β-xylanase from *Aspergillus niger* in *Pichia pastoris*. It was demonstrated that the xylanase activities increased from temperature of 45 – 55°C and the activities decreased after temperature of 55°C (Ruanglek *et al.*, 2007). It also stated recombinant activities of xylanase obtained at the optimal temperature of 55°C. The enzyme activity may denature due to high temperature usage. The temperature increases the rate of enzymatic reactions, hence the rate of clarification, as long as the temperature is below denaturation temperature for the enzyme (Lee *et al*., 2006).

4.3 Effect of Agitation Rate on Xylose Production

The maximum value of temperature obtained from the effect of temperature on xylose production was used as constant value to study on effect of agitation rate. The enzymatic hydrolysis with different agitation rate value of 60, 90, 120, 150, 180 and 210 rpm were tested to obtain the maximum production of xylose concentration. During this experiment, the enzymatic hydrolysis was carried out at temperature of 55°C of incubator shaker and 1.5mg/ml of xylanase concentration for 4 hours of incubated process. The result for effect of agitation rate on xylose production was illustrated as show in Figure 4.2.

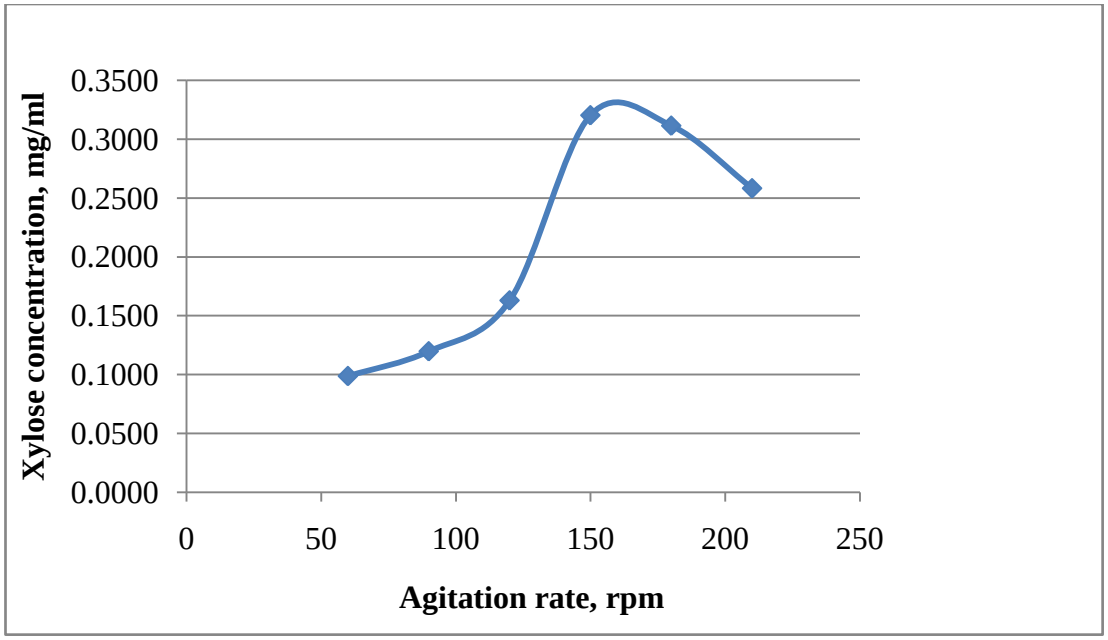

Figure 4.2: Effect of agitation rate on production of xylose concentration.

Based on the Figure 4.2, the production of xylose increase as agitation rate applied increase from 60 – 150 rpm. Then, the xylose production decrease after agitation rate applied more than 150 rpm. The study on effect of agitation rate was obtained the highest xylose production at agitation rate of 150 rpm is 0.3204 mg/ml.

This result supported in other research which conducted by Rajagopalan and Robert (1990) toward effect of agitation on ligninase activity and ligninase production by *Phanerochaete chrysosporium*. The research reported that the production increase as agitation of culture increase. Agitation rate applied for adequate mixing, mass transfer and heat transfer but also maintains homogeneous chemical and physical conditions. However, an agitation rate can creates shear forces, which affect microorganisms in several ways, causing morphological changes, variation in their growth and product formation and also damaging the cell structure (Mittal, 1992).

4.4 Effect of Xylanase Concentration on Xylose Production

The experiment is continued by using the maximum value of temperature and agitation rate in the both result of effect of temperature and agitation rate to study the effect of xylanase concentration. The enzymatic hydrolysis with different xylanase concentration of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml were tested to obtain the maximum production of xylose concentration. During this experiment, the enzymatic hydrolysis was carried out at temperature of 55°C and 150 rpm for 4 hours of incubated process. The result for effect of xylanase concentration on xylose production was illustrated as show in Figure 4.3.

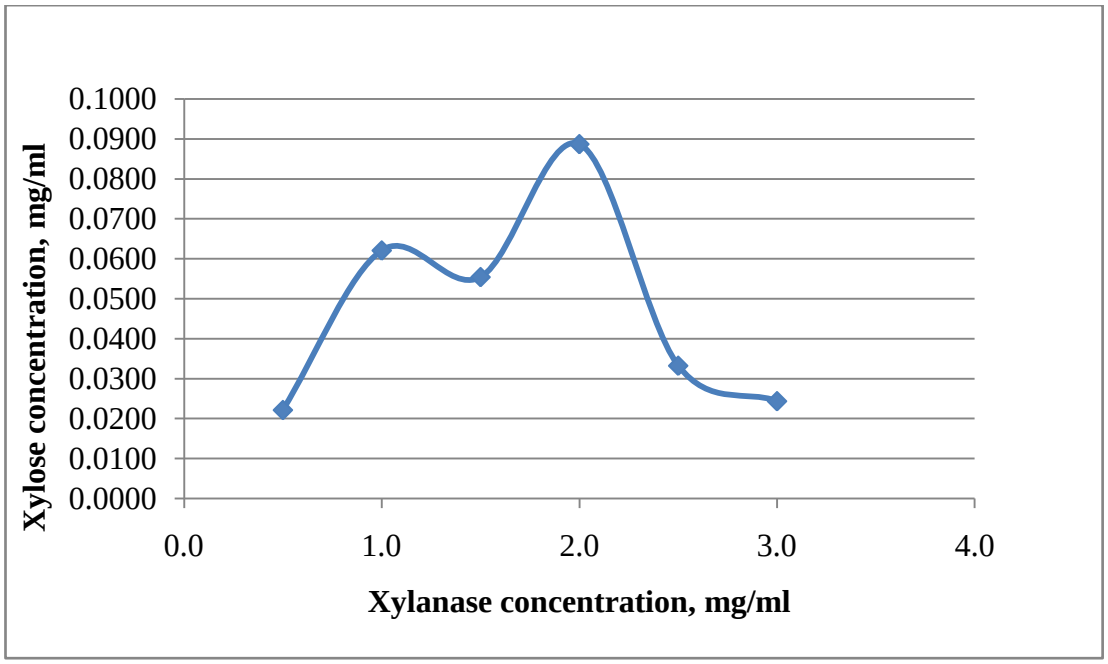

Figure 4.3: Effect of xylanase concentration on production of xylose concentration.

Based on the Figure 4.3, an increase used the xylanase concentration of 0.5 mg/ml and 1.0 mg/ml in enzymatic hydrolysis led to increase the production of xylose concentration but then it is decreased slightly when used 1.5 mg/ml of xylanase concentration. Then, the xylose production increased at 2.0 mg/ml of xylanase concentration before decreased constantly whenever used 2.5 mg/ml and 3.0 mg/ml of xylanase concentration. The highest xylose concentration produced is 0.0887 mg/ml when used 2.0 mg/ml of xylanase concentration. The result supported in the effects of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein (Haslaniza *et al.*, 2010). The research reported that the production of nitrogen content increased as enzyme concentration used increased.

The addition of xylanase to cellulase enzymes enhanced both the glucan and the xylan conversions of a high-xylan-containing pretreated substrate, due to better accessibility of cellulose after xylan degradation (Berlin *et al.*, 2007). Upon increasing the enzyme amount further, the reaction rate did not increase significantly which may be due to the lack of substrate to access the active site of enzyme, and/or

difficulty in maintaining uniform suspension of the biocatalysts at higher enzyme concentration (Wei *et al*., 2003). Therefore, enzyme concentration was identified as the limiting factor for saccharification of rice straw (Jeya *et al*., 2009)

4.5 Optimization of Xylose Production by using RSM

Response Surface Methology (RSM) used in Design Expert version 6.0.8 to optimize the xylose production by analysis the data. This research used the low level value and high level value by using Central Composite Design (CCD) to identify the optimum values of xylose production as demonstrate in Table 4.1.

Table 4.1: The low level and high level for three parameters experimented.

Name	Unit	Low level	High level
Temperature	°C	50	60
Agitation rate	rpm	120	180
Xylanase concentration	mg/ml	1.5	2.5

Based on Table 4.1, the low level and high level selected in between the maximum value from each of three parameters studied. The experimental data is constructed by using RSM analysis equation from analysis of variance (ANOVA) as in Equation 4.1.

$$xylose\ concentration = 0.29 + 0.012A + 0.011B + 3.606 \times 10^{-3}C - 0.077A^2 - 0.032B^2 - 0.013C^2 + 8.037 \times 10^{-3}AB + 8.375 \times 10^{-4}AC - 3.063 \times 10^{-3}BC \quad \textbf{(Equation 4.1)}$$

The temperature is indicated by code A while code B presented for agitation rate and code C showed for xylanase enzyme concentration in Equation 4.1. Then, the parameters in CCD were designed in a total of 17 experimental trials including three central points arranged as in Table 4.2.

Table 4.2: Experimental data arranged by Response Surface Methodology (RSM)

Standard order	**Factors**			**Actual Value**	**Predict Value**
	Temperature (°C)	Agitation rate (rpm)	Xylanase Concentration (mg/ml)	Xylose Concentration (mg/ml)	Xylose Concentration (mg/ml)
1	50.00	120.00	1.50	0.1463	0.150
2	60.00	120.00	1.50	0.1452	0.150
3	50.00	180.00	1.50	0.1541	0.160
4	60.00	180.00	1.50	0.2084	0.200
5	50.00	120.00	2.50	0.1519	0.160
6	60.00	120.00	2.50	0.1774	0.170
7	50.00	180.00	2.50	0.1707	0.160
8	60.00	180.00	2.50	0.2051	0.200
9	46.59	150.00	2.00	0.0610	0.055
10	63.41	150.00	2.00	0.0887	0.095
11	55.00	99.55	2.00	0.1896	0.180
12	55.00	200.45	2.00	0.2106	0.220
13	55.00	150.00	1.16	0.2561	0.250
14	55.00	150.00	2.84	0.2550	0.260
15	55.00	150.00	2.00	0.2794	0.290
16	55.00	150.00	2.00	0.2982	0.290
17	55.00	150.00	2.00	0.2982	0.290

Based on the Table 4.2, the highest production of xylose indicated in standard order 16 and 17 which produced 0.2982 mg/ml. The parameter conditions for those standard orders were temperature of 55°C, 150 rpm for agitation rate and used 2.0 mg/ml of xylanase concentration. The lowest production of xylose indicated in

standard 9 is 0.061 mg/ml were at temperature of 46.59°C, agitation rate of 150 rpm and use 2.0 mg/ml of xylanase concentration.

The result of xylose concentration in the form of analysis of variance (ANOVA) for response surface quadratic model as showed in Table 4.3.

Table 4.3: The analysis of variance (ANOVA) for response surface quadratic model in produced of xylose concentration.

Source	**Sum of Squares (E^{-3})**	**DF**	**Mean Square (E^{-3})**	**F value**	**Prob > F**	
Model	74.0000	9	8.202	57.02	< 0.0001	*Significant*
A	1.8670	1	1.867	12.98	0.0087	*Significant*
B	1.7100	1	1.710	11.89	0.0107	*Significant*
C	0.1776	1	0.1776	1.23	0.3032	*Not significant*
A^2	66.0000	1	66.0000	461.51	<0.0001	*Significant*
B^2	12.0000	1	12.0000	82.57	<0.0001	*Significant*
C^2	1.8630	1	1.8630	12.95	0.0088	*Significant*
AB	0.5168	1	0.5168	3.59	0.0999	*Significant*
AC	0.0056	1	0.0056	0.039	0.8491	*Not significant*
BC	0.0750	1	0.0750	0.52	0.4936	*Not significant*
Residual	1.0070	7	0.1439			
Lack of Fit	0.7714	5	0.1543	1.31	0.4864	*Not significant*
Pure Error	0.2356	2	0.1178			
Cor Total	75.0000	16				

Table 4.3 show the *P*-values for model obtained were small compare to a desired significance level, 0.05. This model terms are indicated as significant. The response indicates that lack of fit for the model was insignificant. In this case the value of A, B, A^2, B^2 and C^2 were significant model terms. Meanwhile the value of C, AC, and BC were not significant as the values indicated greater than 0.1 in the

model terms. The model may improve by using model reduction if there are many insignificant model terms and this not counting those required to support hierarchy.

Then, the regression analysis for the production of xylose concentration showed in Table 4.4. The model indicated precisely by the determination of coefficient (R^2) and correlation coefficient (R).

Table 4.4: Regression analysis for response surface quadratic model

Model Terms	Values
R^2	0.9865
Adj R^2	0.9692
Pred R^2	0.9089
Adeq precision	25.733

Based on Table 4.4, the coefficient of determination R^2 that was found to be close to 1 (0.9865) also advocates a high correlation between observed and predicted values. The Pred *R*-squared of 0.9089 is in reasonable agreement with the Adj *R*-squared of 0.9692. Adequate precision measures the signal to noise ratio and a ratio value greater than 4 is desirable. The model ratio of 25.733 indicates an adequate signal and approve to be used in navigate the design space.

The production value also had been observed from the respective response surface contour plots. The estimation of xylose production and selectivity over independent variables of temperature, agitation rate and xylanase concentration in terms of response surfaces are shown in Figure 4.4 – 4.5.

The effect of reaction temperature and reaction agitation rate on xylose production when xylanase concentration was selected at 2.0 mg/ml as the centre point is shown in Figure 4.4.

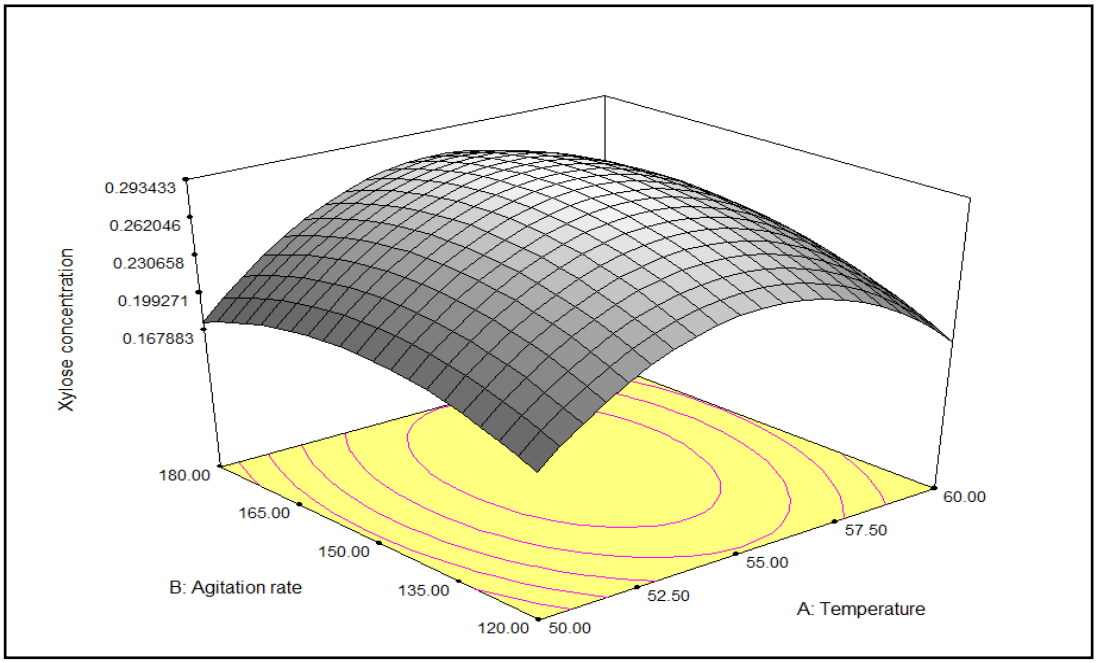

Figure 4.4: Response surface plot of the combination effect of temperature and agitation rate on production of xylose concentration

Figure 4.4 showed the effect of temperature and agitation rate on xylose production when xylanase concentration was maintained at 2.0 mg/ml. The figure presented an elliptic characteristic along the reaction agitation rate and xylanase concentration axis. This indicated that the temperature is the most important among the three independent variables on xylose production.

The increasing of temperature affect on increasing of xylose production. The optimum temperature indicated from response surface was at 55°C which produced 0.2982 mg/ml of xylose concentration. The similar figure pattern was supported by Chou *et al.* (2010) in the research of evaluation on effects of temperature, NaOH concentration and time on solubilization of palm oil mill effluent (POME) using Response Surface Methodology (RSM). The research indicated the addition of NaOH significantly improved COD solubilization when its concentration was below than optimal value.

The effect of reaction agitation rate and xylanase enzyme concentration when temperature was selected at 55°C as the centre point is shown in Figure 4.5.

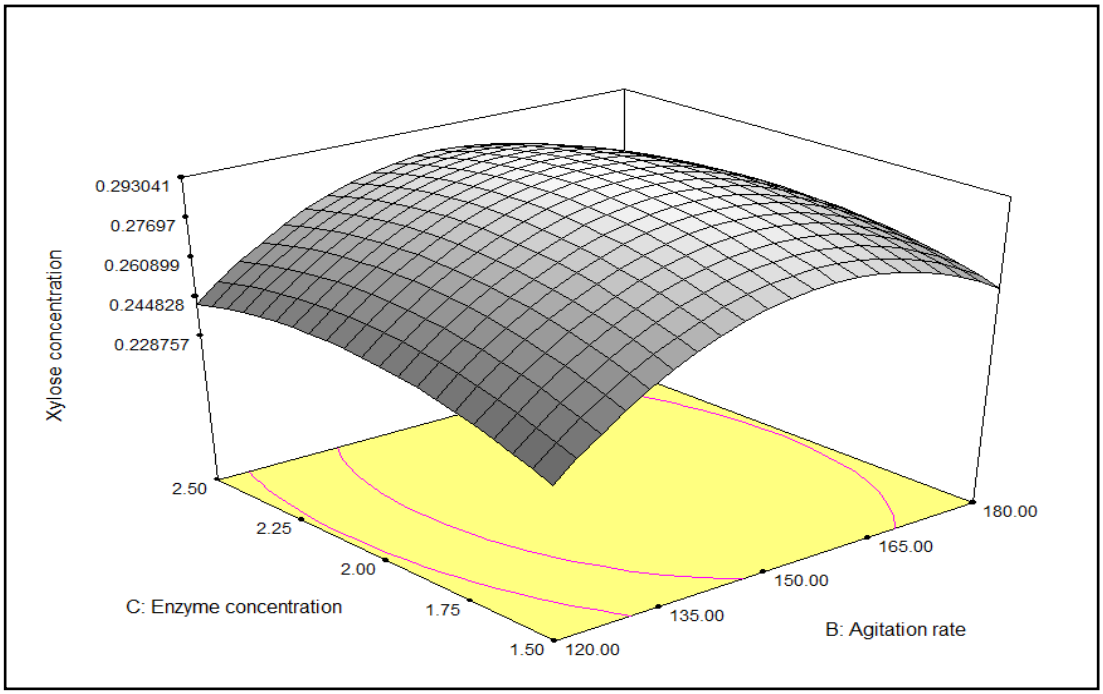

Figure 4.5: Response surface plot of the combination effect of temperature and agitation rate on production of xylose concentration

Based on the Figure 4.5, the production of xylose increase as the agitation rate applied increase. The optimum agitation rate indicated as 150 rpm which produced the xylose concentration of 0.2919 mg/ml. The high agitation rate may affect the shear force also as mentioned in the research of effect of pH, agitation and aeration on hyaluronic acid production by *Streptococcus Zooepidemicus* (Micheal *et al.*, 1994). However, there is limitation that might cause lower biomass concentration if applied higher shear force (Illias and Hoq., 1998).

The effect of xylanase enzyme concentration for production of xylose could be observed in Figure 4.5. The production of xylose increase as the xylanase concentration increased. The optimum xylanase concentration obtained as 2.0 mg/ml that produced xylose concentration of 0.2919 mg/ml. According to Ohgren *et al.* (2007) of the effect of hemicelluloses and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. The research stated that the xylanase supplementation increased the glucose and xylose yields for all pretreatments.

4.6 Optimization of Temperature, Agitation Rate and Enzyme Concentration using Response Surface Methodology (RSM) on Xylose Production

The result of an optimization using Response Surface Methodology (RSM) showed the optimum condition for maximum production of xylose. In order to validate the prediction, another experimental was carried out at temperature of 55°C, agitation rate of 155 rpm and 2.0 mg/ml of xylanase concentration. The predicted value of optimize xylose production is 0.2982 mg/ml. However, the actual production of xylose through experimental is 0.2716 mg/ml which close to the predicted value. This also indicated that RSM is valid to optimize the production of xylose. The optimization on xylose production using Response Surface Methadology (RSM) is show in Table 4.5.

Table 4.5: The optimization on production of xylose concentration using Response Surface Methadology (RSM)

Parameter	**Before Optimization**		**After Optimization**		
	Value	**Xylose Concentration (mg /ml)**	**Value**	**Xylose Concentration (mg/ml)**	
				Actual Value	**Predicted Value**
Temperature (°C)	60	0.2051	55	0.2716	0.2982
Agitation Rate (rpm)	180		155		
Xylanase Concentration (mg/ml)	2.5		2.0		

Table 4.5 show that the production of xylose concentration before and after optimized using RSM is 0.2051 mg/ml and 0.2716 mg/ml respectively. The production condition before the optimization process was at temperature of 60°C, 180 rpm of agitation rate and 2.5 mg/ml of xylanase concentration for 4 hours. After optimization using RSM, the xylanase concentration used is 2.0 mg/ml less than before optimization. However, the production of xylose concentration after optimization higher than the production obtained before the oprimization. Throughout the optimization may conclude that the usage of xylanase may reduce while produced high production of xylose concentration. The cost consumption for enzyme may reduce and beneficial supply the production of xylose or simple sugar to consumer of the other sector.

After optimization, less consumption of temperature instead produced higher concentration than previously. The optimized of xylose usage the agitation rate lower than before optimization. Thus, the energy of energy consumption may reduce as well as produced high production of xylose. As conclusion, the cost of xylose production may reduce as well as used less amount of xylanase and less consumption of energy in the process.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The optimization of xylose production from rice straw was successfully achieved by using Response Surface Methodology (RSM). The conventional method used gave the best range of temperature from 50 – 60 °C while the best range of agitation rate was within 120 – 180 rpm and 1.5 – 2.5 mg/ml of xylanase concentration.

The optimum conditions was observed by using Response Surface Methodology (RSM) for highest production of xylose concentration were at temperature of 55 °C, with 150 rpm and 2.0 mg/ml of xylanase concentration. The predicted value of xylose concentration is 0.2982 mg/ml however the actual value of xylose concentration obtained is 0.2716 mg/ml. Before optimization the xylose concentration yield is 0.2051 mg/ml lower than yield of xylose concentration after optimization. Thus, the production of xylose has been successfully optimized from rice straw using RSM with increment of 32.43%.

5.2 Recommendation

An optimization on production using Response Surface Methodology (RSM) may conduct on different independent variables such as substrate dose, agitation time and pH (Li *et al.,* 2006). Besides, study on different pretreatment process by thermal pretreatment using microwave or conventional heating to enhance enzymatic digestibility on production of simple sugar (Wen and Hu, 2008).

The hemicelluloses were degraded during enzymatic hydrolysis where β – xylosidase are necessary in order to produce xylose other than Endo-xylanase (Puls *et al*., 1987). The simple sugar production from another substrate such as corncob, corn stover, sugarcane bagasse, and empty fruit bunch may study on its high concentration of production. It is also recommend that to obtain the concentration of another sugar or product such as glucose, arabinose and ethanol (Wang *et al.*, 2010).

Besides, the purification and extraction of xylose could be use in further study on xylose production. The solution obtained had been through several steps before adding 50% v/v of ethanol to be allowed precipitation of xylose as a white crystalline paste (Curreli *et al.,* 2002). The weight of crystalline may measure to indicate as the production in next research.

In next research is suggested to use kinetic modeling of xylose concentration. The kinetic model was modified due to the difficulty in finding a strict mechanism for hydrolysis reactions (Aguilar *et al*., 2002). Kinetic models were developed to explain the variation with time of the main products generated. The determination of kinetic parameters for xylose formation and degradation had been done by (Yat *et al.,* 2008). Then, the kinetic parameters obtained can be used in reactor models of various configurations to identify optimum conditions for biomass conversion to fermentable sugars in the future.

There also recommend to analysis the xylose production using High Performance Liquid Chromatograph (HPLC). The column use is SUPELCOSIL LC-NH_2 and RI detector. Aqueous acetonitrile of 75% used as mobile phase with flow rate of 1.5 ml/min at 50°C (Rahman *et al*., 2007).

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APPENDIX A

MATERIALS AND METHOD

Appendix A1

Preparation of Xylanase Concentration in Sodium Citrate Buffer

Mix in the following proportions to get the required concentration of xylanase

Xylanase Concentration (mg/ml)	Mass of xylose (mg)	Volume of buffer solution (ml)
0.5	50	100
1.0	100	100
1.5	150	100
2.0	200	100
2.5	250	100
3.0	300	100

APPENDIX B

RESULT AND DISCUSSION

Appendix B1

Experiment Data for Standard Curve of Xylose

Table B-1: Standard Curve of Xylose Concentration

Concentration (mg/ml)	Optical Density (OD)
0.0	0.000
0.2	0.148
0.4	0.358
0.6	0.646
0.8	0.710
1.0	1.095

Appendix B2

Screening Process the Effect of Parameters on Xylose Production (Conventional Method)

Table B-2: Effect of Temperature on Xylose Production

Temperature (°C)	Xylose Concentration (mg/ml)
15	0.0698
25	0.1098
35	0.2217
45	0.2938
55	0.4678
65	0.4180

Table B-3: Effect of Agitation Rate on Xylose Production

Agitation rate (rpm)	Xylose Concentration (mg/ml)
60	0.0987
90	0.1197
120	0.1630
150	0.3204
180	0.3115
210	0.2583

Table B-4: Effect of Xylanase Concentration on Xylose Production

Enzyme concentration (mg/ml)	Xylose Concentration (mg/ml)
0.5	0.0222
1.0	0.0621
1.5	0.0554
2.0	0.0887
2.5	0.0333
3.0	0.0244

Appendix B3

Enzymatic hydrolysis using RSM suggested parameters

Table B-5: Experimental data based on RSM suggested parameters

Std	Factor 1 Temperature (°C)	Factor 2 Agitation rate (rpm)	Factor 3 Enzyme concentration (mg/ml)	Absorbance (OD)	Xylose concentration (mg/ml)
1	50.00	120.00	1.50	0.132	0.1463
2	60.00	120.00	1.50	0.131	0.1452
3	50.00	180.00	1.50	0.139	0.1541
4	60.00	180.00	1.50	0.188	0.2084
5	50.00	120.00	2.50	0.137	0.1519
6	60.00	120.00	2.50	0.211	0.2339
7	50.00	180.00	2.50	0.154	0.1707
8	60.00	180.00	2.50	0.185	0.2051
9	46.59	150.00	2.00	0.106	0.1175
10	63.41	150.00	2.00	0.080	0.0887
11	55.00	99.55	2.00	0.171	0.1896
12	55.00	200.45	2.00	0.190	0.2106
13	55.00	150.00	1.16	0.231	0.2561
14	55.00	150.00	2.84	0.230	0.2550
15	55.00	150.00	2.00	0.252	0.2794
16	55.00	150.00	2.00	0.269	0.2982
17	55.00	150.00	2.00	0.269	0.2982

Appendix B4

Response Surface Methodology (RSM) Analysis

Predicted Vs. Actual Analysis

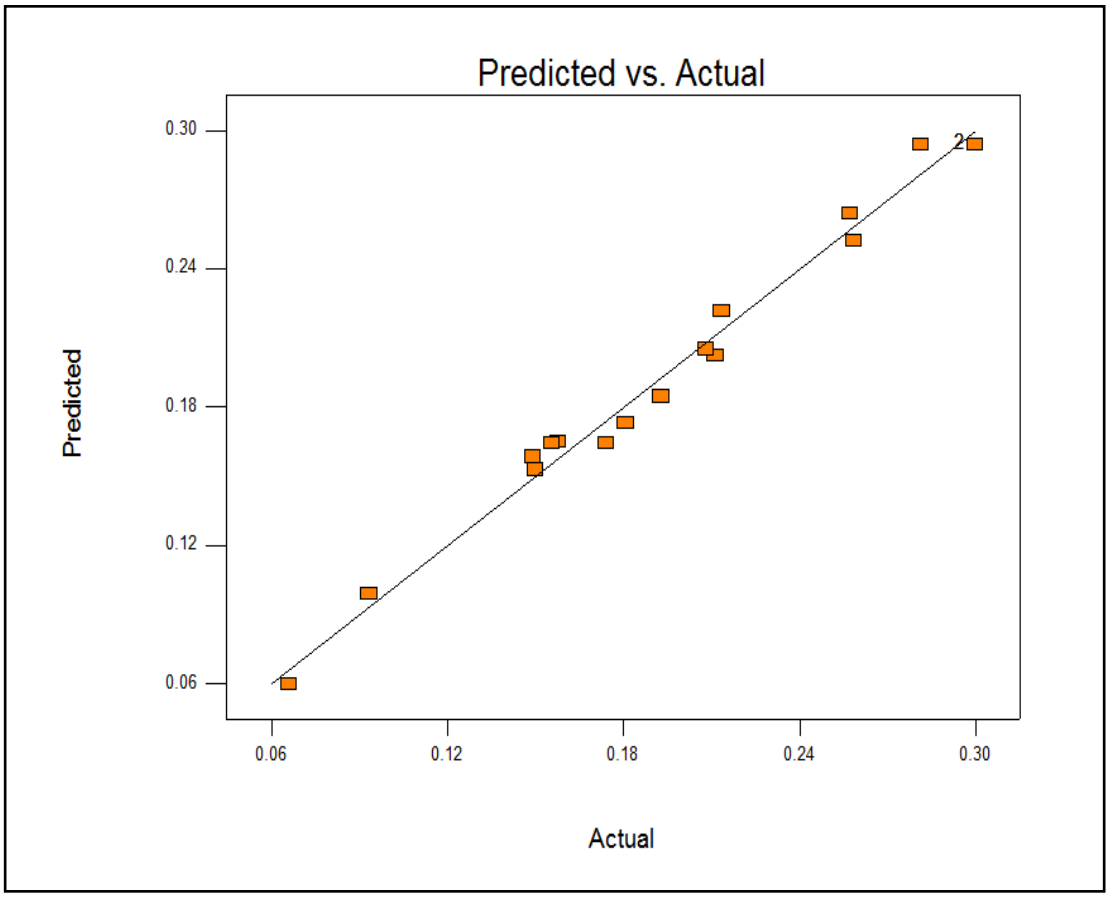

Figure B-1: Graph of Predicted Vs. Actual Analysis

Outlier T Analysis

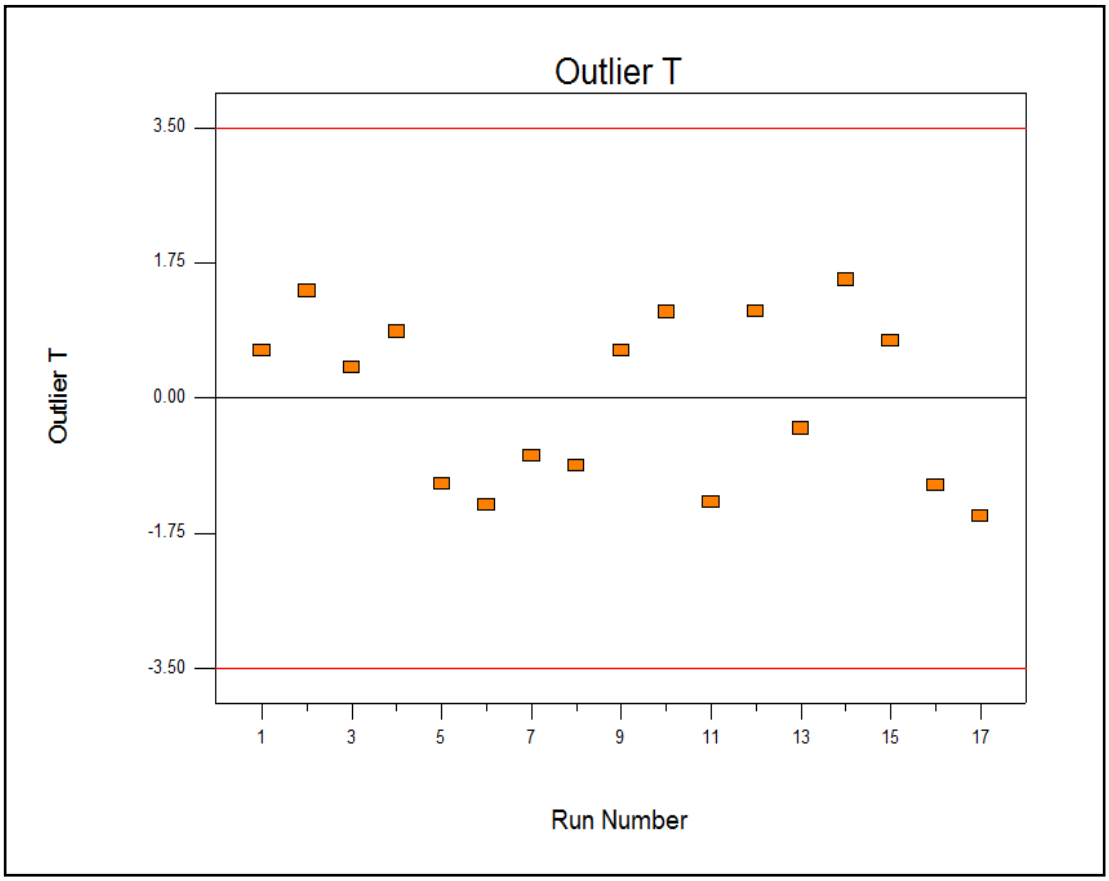

Figure B-2: Graph of Outlier T Analysis

Residuals Vs. Temperature

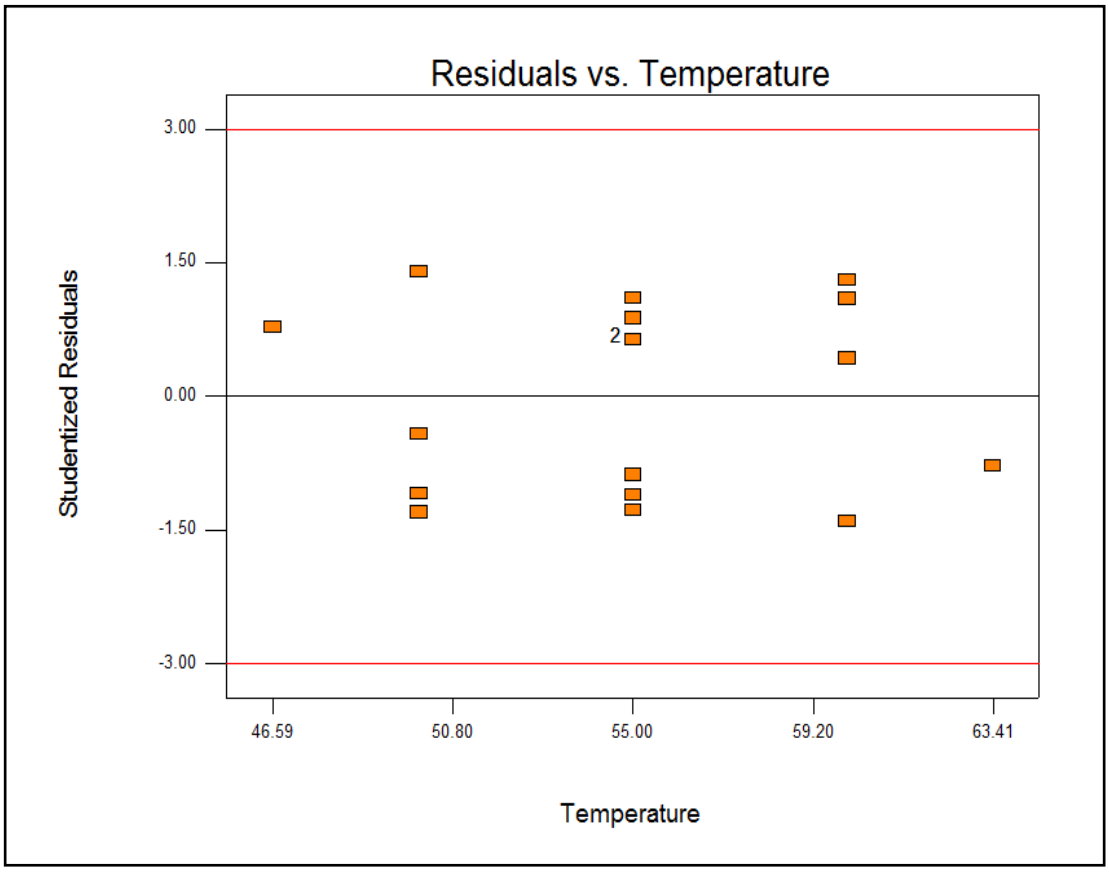

Figure B-3: Graph of Residuals Vs. Temperature

Residuals Vs. Agitation Rate

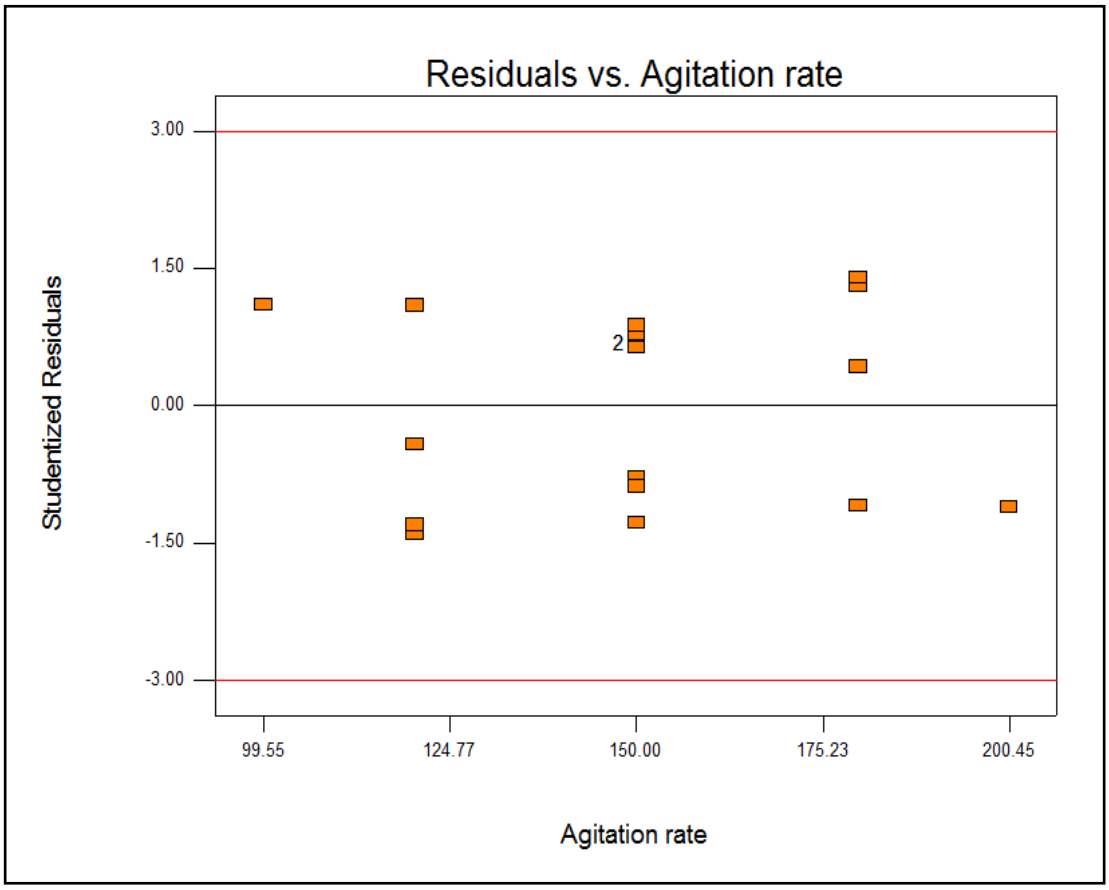

Figure B-4: Graph of Residuals Vs. Agitation Rate

Residuals Vs. Enzyme Concentration

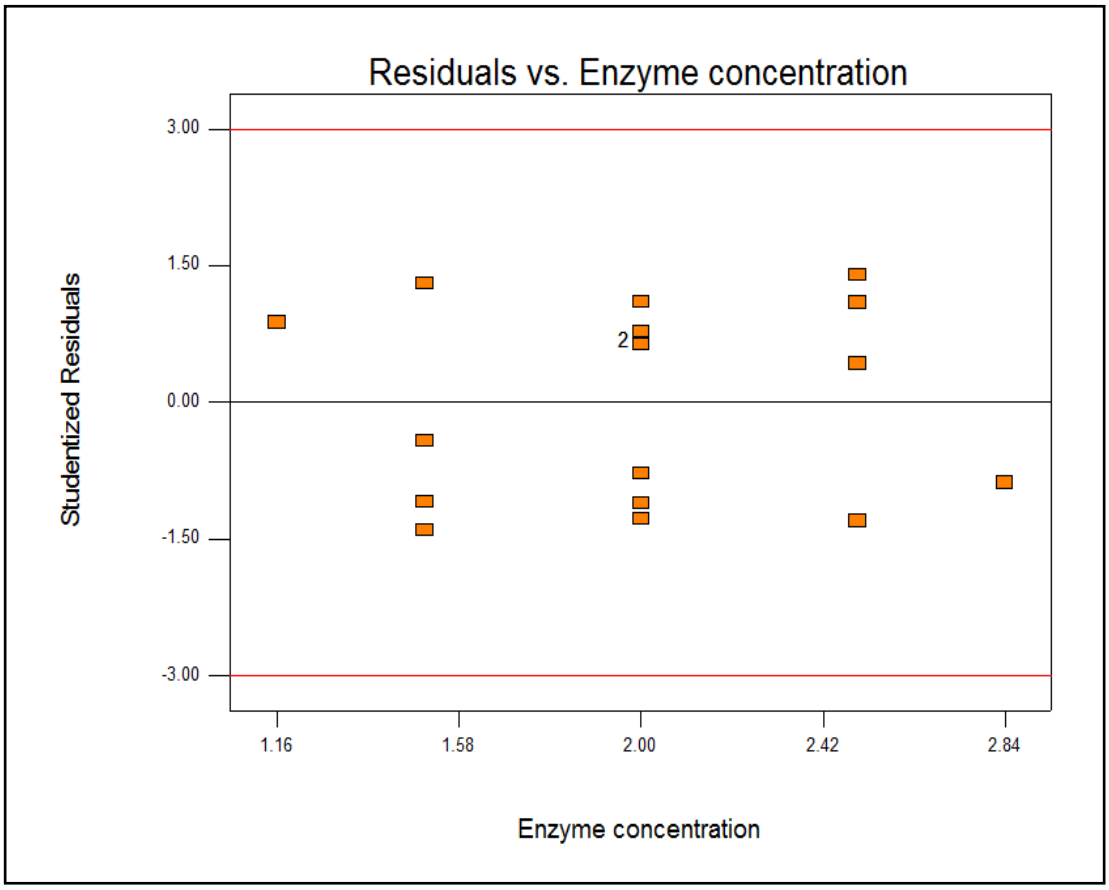

Figure B-5: Graph of Residuals Vs. Enzyme Concentration

Box-Cox Analysis

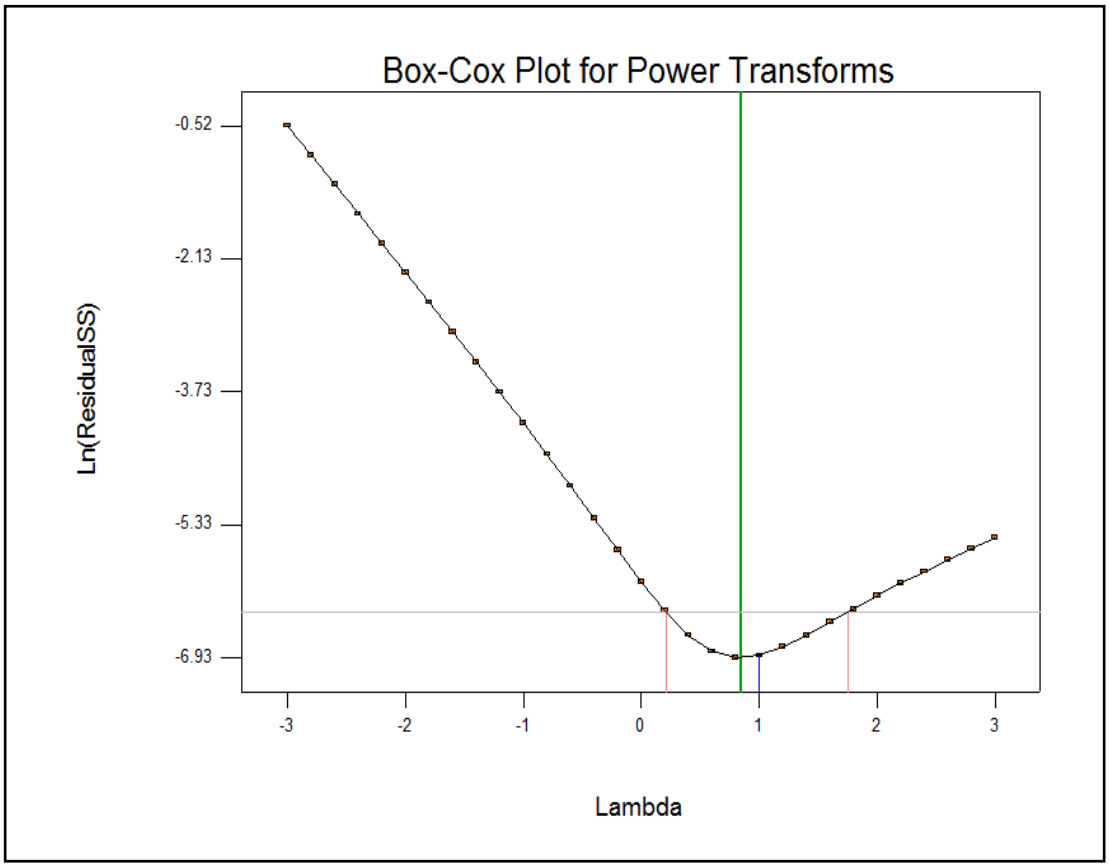

Figure B-6: Graph of Box-Cox Analysis