OPTIMIZATION OF XYLOSE PRODUCTION FROM SUGARCANE BAGASSE USING RESPONSE SURFACE METHODOLOGY (RSM)

PRAKASH GUNAH

UNIVERSITI MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

JUDUL : **OPTIMIZATION OF XYLOSE PRODUCTION FROM SUGARCANE BAGASSE USING RESPONSE SURFACE METHODOLOGY (RSM)**

SESI PENGAJIAN: **2010/2011**

Saya **PRAKASH GUNAH**
(HURUF BESAR)

mengaku membenarkan tesis Projek Sarjana Muda (PSM) ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut:

1. Hakmilik kertas projek adalah di bawah nama penulis melainkan penulisan sebagai projek bersama dan dibiayai oleh UMP, hakmiliknya adalah kepunyaan UMP.
2. Naskah salinan di dalam bentuk kertas atau mikro hanya boleh dibuat dengan kebenaran bertulis daripada penulis.
3. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian mereka.
4. Kertas projek hanya boleh diterbitkan dengan kebenaran penulis. Bayaran royalti adalah mengikut kadar yang dipersetujui kelak.
5. *Saya membenarkan/tidak membenarkan Perpustakaan membuat salinan kertas projek ini sebagai bahan pertukaran di antara institusi pengajian tinggi.
6. **Sila tandakan (✓)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
✓	TIDAK TERHAD	

Disahkan oleh

_____ (TANDATANGAN PENULIS)

Alamat Tetap: No.131 Tmn Bukit Zamrud,
71300 Seremban,
Negeri Sembilan Darul Khusus.

Tarikh: 30 November 2010

_____ (TANDATANGAN PENYELIA)

ROHAIDA CHE MAN
Nama Penyelia

Tarikh: 30 November 2010

CATATAN:
* Potong yang tidak berkenaan.
** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT atau TERHAD.
◆ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penylidikan, atau disertai bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (PSM).

SUPERVISOR'S DECLARATION

"I hereby declare that I have read this thesis and in
my opinion this thesis is sufficient in terms of scope and
quality for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)"

Signature : .....
Supervisor : Rohaida Che Man
Date : .....

OPTIMIZATION OF XYLOSE PRODUCTION FROM SUGARCANE BAGASSE USING RESPONSE SURFACE METHODOLOGY (RSM)

PRAKASH GUNAH

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

November 2011

I declare that this thesis entitled "Optimization of Xylose Production from Sugarcane Bagasse Using Response Surface Methodology" 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."

Signature : ……

Name : PRAKASH GUNAH

Date : 30 November 2010

To my beloved mother and father

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my sincere thanks and appreciation to my thesis supervisor, Pn Rohaida Che Man for encouragement, guidance, critics and insightful comment. This publication of this study would not be possible without her encouragements and advices. As for all the lessons, guidance and unparalleled knowledge shared will not be forgotten.

I am very thankful to Universiti Malaysia Pahang (UMP) for providing good facilities in the campus. To all the staff in Faculty of Chemical & Natural Resources Engineering, a very big thanks you to all.

My sincere appreciation also extends to all my fellow colleagues and others who have provided assistance and guidance in preparing this thesis. Their views and tips are useful indeed. Thank you for the time sacrificed to accompany me. And last but not least, I am grateful to all my family members especially to my mother and father for their continues support for me to successfully accomplish my project.

ABSTRACT

Hemicellulose is defined as several polysaccharides that are more complex than a sugar and less complex than cellulose, found in plant cell walls. Xylose is a pentose sugar formed by the hydrolysis of xylan, the substrate found in hemicellulose. Sugarcane bagasse widespread with sugar sources contains about 27% of hemicellulose. After extracting the juice content in sugarcane, the large portion of excess bagasse is burn as waste material causing air pollution. Acid hydrolysis method was used for the pretreatment of sugarcane bagasse to extract hemicellulose compound. The main objective of this research is to optimize the production of xylose from sugarcane bagasse. To achieve the objective, this research was conducted in identified scopes. The scopes of this research includes the study of the effect of agitation time, pH and substrate concentration on xylose production using sugarcane bagasse and to optimize the production of xylose using Response Surface Methodology (RSM). Response Surface Methodology (RSM) is a method in Design Expert software which uses mathematical technique to optimize production based on different parameters. After the pretreatment, hemicellulose was obtained and used to produce xylose using enzymatic hydrolysis method. At the end of this research, it was found that the optimized xylose production from sugarcane bagasse was obtained at agitation time of 5.36 hour, pH 8.73 and substrate concentration of 70.6mg/ml which produce 19.64 mg/ml of xylose. Before optimization, the xylose production was only 13.22 mg/ml and the production of xylose was increased by 49% after optimization.

ABSTRAK

Hemisellulosa didefinasikan sebagai polisakarida yang lebih kompleks daripada gula kurang kompleks berbanding sellulosa. Xilosa adalah gula pentosa yang terhasil daripada hidrolisis xylan, substrat yang terdapat dalam hemisellulosa. Hampas tebu mengandungi kandungan hemisellulosa sebanyak 27%. Biasanya, selepas mengekstrak kandungan air gula dari tebu, hampas tebu yang banyak dibakar sebagai sisa mentah yang menyebabkan pencemaran udara. Objektif utama kajian ini adalah untuk mengoptimumkan penghasilan gula xylosa daripada hampas tebu. Bagi mencapai objektif ini, kajian ini dijalankan berdasarkan skop yang dikenalpasti. Skop kajian ini termasuk kajian masa, pH dan kepekatan substrat terhadap pengnghasilkan xylosa daripada hampas tebu dan untuk mengoptimumkan penghasilkan xylosa menggunakan Kaedah Permukaan Tindakbalas (RSM). RSM adalah kaedah didalm perisian Design Expert yang menggunakan teknik pengiraan matematik bagi mengoptimumkan penghasilan berdasarkan kepada parameter yang berlainan. Selepas dirawat, hemisellulosa digunakan untuk menghasilkan xilosa dengan menggunakan kaedah hidrolisis enzim. Pada penghujung kajian, didapati bahawa penghasilan xilosa daripada hampas tebu yang dioptimumkan dicapai pada masa 5.36 jam, nilai pH 8 and kepekatan substrat 70.6mg/ml yang menghasilkan xilosa sebanyak 19.64 mg/ml. Sebelum pengoptimuman, penghasilan xilosa adalah hanya 13.22 mg/ml dan penghasilan telah meningkat sebanyak 49% selepas pengoptimuman.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Lignocellulosic biomass is one of the most available and renewable resources which represent a promising low cost raw material for the production of biofuel, bioenergy and added value biomolecules. Hemicellulosic materials such as agricultural residues (sugarcane bagasse) offer this possibility (Boussarsar, 2009). Lignocellulosic feedstock is considered as an attractive raw material not only for the liquid transportation fuel but also for the production of chemicals and materials, i.e. the development of carbohydrate-based because of its availability in large quantities. Corn stover, wheat straw, sugar begasse, rice straw, rice hull, corn cob, oat hull, corn fiber, woodchip and cotton stalk have attracted the most interest of research (Gray *et a*l., 2006). The major chemical components of lignocellulosic biomass lignocellulosic biomass are cellulose, hemicellulose and lignin. Cellulose is a linear polymer of anhydroglucopyranose units linked by ether bonds. Hemicellulose as cellulose, are polymers constituted of sugar units. They differ from cellulose by being smaller and branched polymers usually containing more than one sugar type; they are also amorphous polysaccharides. Lignin is a complex, crosslinked, three-dimensional polymer formed with phenylpropane units (Marion *et al*., 2010).

Nowadays about 50% of generated sugarcane bagasse is used to generate heat and power to run the sugar mills and ethanol plants. The remaining portion is usually stockpiled. However, because the heating value of carbohydrates is approximately half of that lignin, it would be beneficial to develop a more economical use of carbohydrates. One such possibility would be to extract the hemicelluloses before cellulose to convert them to higher value-added products such as prebiotic xylooligosaccharides or polymers and composites for chemical and pharmaceutical applications (Brienzo *et al*., 2009). Agricultural residues, such as sugarcane bagasse contain about 25% of hemicellulose (Roberto *et al*., 2003). The increasing interest in biotechnological processes employing lignocellulosic residues is quite justifiable because these materials are cheap, renewable and widespread sugar sources. Hemicellulosic hydrolysates made from such residues have been frequently utilized instudies for developing a technically and economically viable bioprocess (Roberto *et al*., 2003).

Among biomass components, hemicelluloses which are mainly composed of xylans, provide an important source of interesting molecules such as xylose and xylo-oligosaccharides which have potential applications in different areas, notably in chemical, food and pharmaceutical industries (Boussarsar, *et al* 2009). The quantity and quality of hemicelluloses in the crops are quite variable and depend on the considered species. They are usually defined as polymers that are solubilized from plant cell walls by alkali. There are biopolymers of a limited number of sugars, mainly the xylose, mannose, glucose, galactose, arabinose, acid glucuronic. Xylose is always the sugar monomer present in the largest amount in hemicellulose (Derriche *et al*., 2007). A hemicellulose can be any of several heteropolymers (matrix polysaccharides), present in almost all plant cell walls along with cellulose. Hemicellulose has a random, amorphous structure with little strength. Therefore it is easily hydrolyzed by dilute acid or base (Rahman *et al*., 2006).

Xylose is the second most abundant sugar in lignocellulosic materials after glucose and its efficient conversion is one of the prerequisites for lignocellulosic industrialization. Xylans, a major wood cell wall hemicellulose with a β-1,4-linked xylopyranosyl backbone, comprise up to 35% of hardwood and 14% of softwood (Ishihara *et al*., 2002) Xylose, is 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. With its free carbonyl group, it is a reducing sugar. This xylose can be used as a substrate to produce a wide variety of compounds or fuels by chemical or biotechnological processes.

Reduction of xylose by catalytic hydrogenation produces the sugar substitute xylitol. Xylitol is a polyol whose properties have attracted the attention of at least three types of industries: food (for its sweetening power and insulin-independent metabolism), odontological (for its anticariogenicity, tooth rehardening and remineralization properties) and pharmaceutical (for its capability of preventing otitis and its possibility of being used as a sweetener or excipient in syrups, tonics and vitamin formulations) (Roberto *et al*., 2003).

The cellulose and hemicellulose content of sugarcane bagasse can be hydrolyzed chemically or enzymatically to obtain reducing sugar such as xylose. Dilute-sulfuric acid hydrolysis is a chemical hydrolysis for either the pretreatment before enzymatic hydrolysis or the conversion of lignocellulose to the corresponding sugars (Taherzadeh *et al*., 1997). In dilute-acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulose fraction. If higher temperature or longer retention times are applied, the formed monosaccharides will be further hydrolyzed to other compounds. It is therefore suggested that the hydrolysis process be carried out in at least two stages, the first stage at relatively milder conditions during which the hemicellulose fraction is hydrolyzed and a second stage can be carried out by enzymatic hydrolysis or dilute

acid hydrolysis at higher temperatures during which the cellulose is hydrolyzed (Karimi *et al*., 2006).

Roberto *et al*., (2003) stated the optimization of xylose production from oil palm empty fruit. The objective of the study was to determine the effects of H_2SO_4 concentration and reaction time on the production of sugars (xylose, glucose and arabinose) and on the reaction byproducts (furfural, hydroxymethylfurfural (HMF) and acetic acid). Dilute sulfuric acid was used as a catalyst for the hydrolysis of rice straw at 121 °C hydrolysis reactor. Rationale for conducting this study was determined based on a central composite statistical design. Response surface methodology (RSM) was adopted to optimize the hydrolysis conditions aiming to attain high xylose selectivity. The optimum H_2SO_4 concentration of 1% and reaction time of 27 min was found. Under these conditions, 77% of xylose yield and 5.0 g g^{-1} of selectivity were attained.

1.2 Problem Statement

The amount of organic waste obtained from the agriculture industry is abundant in Malaysia but the utilization is still limited. Sugarcane (*Saccarhum officinarum*) bagasse is a residue produced in large quantities by sugar industries. In general, 1 tonne of sugarcane bagasse generates 280 kg of bagasse, the fibrous by-product remaining after sugar extraction from sugarcane. Since bagasse is a by-product of the sugarcane industry, the quantity of production in each country is in line with the quantity of sugarcane produced. However, the utilization of sugarcane bagasse is still limited and is mainly used as a fuel to power the sugar mill. When burned in quantity, it produces sufficient heat energy to supply all the needs of a typical sugar mill, with energy to spare. This resulting CO_2 emission to the atmosphere which causes environmental pollution. (Sun *et al*., 2004).

After extracting the juice content in sugarcane, the large portion of excess bagasse is burn as waste material. Field burning is the major practice for removing sugarcane bagasse, which increases the air pollution and consequently affects the public health. But Sugarcane bagasse mainly contains cellulose (32–47%), hemicellulose (19–27%) and lignin (5–24%) which can utilize for various commercial purposes, especially the demand of xylose from hemicellulose increases in many industries (Brienzo *et al*., 2009). Therefore this research is aimed to optimize the xylose production using sugarcane bagasse.

1.3 Objectives

The main objective of this research is to optimize the production of xylose from sugarcane bagasse using Response Surface Methodology (RSM).

1.4 Scopes of Research

The scopes of study on the production of xylose are:

i. To study the effect of agitation time on xylose production from sugarcane bagasse
ii. To study the effect of pH on xylose production from sugarcane bagasse.
iii. To study the effect of agitation time on xylose production from sugarcane bagasse.
iv. To optimize the production of xylose from sugarcane bagasse using Response Surface Methodology (RSM).

1.5 Rationale and Significance

Malaysia is one of the major country which produces sugarcane every year. In 2009 the total amount of sugarcane production is 755,770 metric tons which consist of 13,880 hectar. From year 2007 to 2012, there will be about 18 percent in excess of the area needed for domestic sugar self-sufficiency, to an aggregate hectare that will supply feedstock for both sugar starting from 2008 as needed, without affecting sugar self-sufficiency (Charles *et al*., 2003). The large amount of sugarcane bagasse should be utilized in beneficial way rather than burning it. By doing this, environmental pollution can be minimized.

Response Surface Methodology (RSM) will provide the optimal condition to increase the xylose production from sugarcane bagasse. By regulating different parameters that affect the production of xylose, the optimization of the xylose from sugarcane bagasse can be obtained effectively. The advantage of RSM is it is fast, can investigate the interaction between parameters as well as increase the production rate of xylose, thus fulfill the commercial demand (Psomas *et al*., 2007).

CHAPTER 2

LITERATURE REVIEW

2.1 Xylose

Xylose can be use as substrate to produce a wide variety of compounds or fuel by chemical or biotechnological processes. With sustained hydrolysis xylose may be degraded to such decomposition products as furfural, hydroxymethylfurfural and furan resins (Lavarack *et a*l., 2002). Xylose can also be hydrolyzed from xylan-rich materials like rice husk, corn stalk, wheat straw and flax straw. Xylose is hydrogenated at high pressure to xylitol by using a nickel catalyst. (Sjöman *et al*., 2008). In health perspective, xylitol can be a better alternative for people with diabetes because it does not raise insulin level in human body. Xylitol is a sugar alcohol having sweetness equal to sucrose but it does not cause dental carries and thus it is used as a sweetener by the confectionary industry. Xylose is also a versatile sugar compound and has many applications such as sugar source for non-nutritive agent in pharmaceutical industry, additive in colour photography and brightener in zinc electroplating (Murthy *et al*., 2005). Potential sources for xylose are birch and other hardwoods that have a xylan rich hemicellulose structure. In chemical wood pulping processes hemicelluloses are hydrolyzed and xylose is found in rejected spent liquor (Alen *et al*., 2000).

2.2 Hemicellulose

Hemicelluloses are known as valuable in pulp additives, natural barrier for packaging films and as components of skin substitutes in case of damage of superficial epidermal layers. As hemicelluloses are relatively tightly bound in the plant cell wall network to lignin and cellulose, it is difficult to separate them without significant modification of their structure. Different treatments have been applied to hemicellulose extraction. Acid hydrolysis and hydrothermal methods are usually much preferable (Brienzo *et al*., 2009). In addition, wood-based hemicellulose hydrolyzates contain lignosulfonates and inorganic ions (Sjöman *et al*., 2008). There are several xylose purification operation from hemicellulose hydrolyzate, acid hydrolysis, enzymatic hydrolysis to produce xylose which could partially improve purity and yield in commercial xylose production. In several plants the majority of hemicelluloses is xylan which can be hydrolyzed into xylose. Particularly the hemicellulose of hardwood is rich in xylan. Consequently it is possible to obtain xylan and xylose as by-products from cellulose industry using hardwood. (Gunda *et al.,* 1970)

2.3 Hemicellulose Conversion to Simple Sugar

Different treatments have been applied to hemicellulose extraction and heat treatment is often combined with addition of chemicals such as alkali, acid or hydrogen peroxide. In order to obtain fast enzymatic hydrolysis of biomass with a high sugar yield (for both hexoses and pentoses), the two main protective coats around cellulose, hemicellulose and lignin need to be removed or altered without degrading the hemicellulose sugars. Hemicellulose forms a physical barrier around the cellulose (Öhgren *et al.,* 2006). Acid hydrolysis is an effective agent for both delignification and solubilization of hemicelluloses. In these conditions carbohydrates are less damaged and delignification is more efficient. However, the use of acid treatment of bagasse

requires prior removal heavy metals with chelating agents. The metals catalyze decomposition of the peroxide anion in the alkaline medium leading to the formation of hydroxyl radicals which cause hemicellulose depolymerization and diminish its recovery. However, chelation not only removes heavy metals, but also alkali earth metals which act as natural stabilizers of the peroxide during the treatment . This is the reason why magnesium ions are added later in a surplus to the chelating agents to prevent hemicellulose degradation (Brienzo *et al*., 2009).

According to Neureiter *et al*. (2002), acid concentration is the most important parameter affecting sugar content, while for the formation of sugar degradation products, temperature has the highest impact. The main problem encountered in this process is the generation of a large number of degradation products that can strongly affect the microbial metabolism. To overcome this problem, it is necessary to select satisfactory reaction conditions to keep the degradation products at low levels because their type and levels depend on the severity of the hydrolysis reaction.

2.4 Enzymatic Hydrolysis

Enzymatic hydrolysis is a process in digestion in which macromolecules are split from food by the enzymatic addition of water. Enzymatic hydrolysis can not only economize energy on account of the relatively mild reaction conditions, but also avoid using toxic and corrosive chemicals (Xu *et al*., 2007). Enzymatic hydrolysis of biomass hemicellulose does not produce toxic products. Alternatively, xylose can be produced by enzymatic hydrolysis xylan. Generally, in lignocellulosic biomass, xylan exists in xylan–lignin complex and becomes resistant to hydrolysis (Zhu *et al*., 2006). Therefore xylose production is carried out in two stages: alkaline extraction of xylan from lignocellulosic biomass followed by enzymatic hydrolysis (Akpinar *et al*., 2009).The enzyme hydrolysis is

catalyzed by xylanase enzyme for xylose production. The greatest potential for sugar production from biomass also lies in enzymatic hydrolysis of cellulose and hemicellulose using cellulase and hemicellulase enzymes. Although the structure of hemicellulose is more complex than cellulose and requires several different specificities for complete hydrolysis, the polysaccharide does not form tightly packed crystalline structures like cellulose does and thus is more accessible to enzymatic hydrolysis. In hemicellulose, enzymatic hydrolysis requires mild conditions and long periods of time (Saha *et al*., 2004).

From the research done by Rahman *et al*. (2006) it was revealed that under controlled treatment conditions, acid hydrolysis of lignocellulosic biomass mainly produced xylose from xylan with cellulosic and lignin fractions remaining unaltered. The solid residue can further be utilized for production of ethanol or in pulp processing for making high grade paper. In the hydrolysis process it is understood that initially the lignin protective layer around the hemicellulose fiber is softened under elevated temperature and pressure which allows the acid to penetrate the layer and hydrolyze the amorphous xylan to form xylose. On the other hand the condition is not severe enough to hydrolyze the crystalline structure of cellulose which remains as insoluble solid. Although xylose was the main sugar obtained from hemicellulose, other byproducts such as glucose, acetic acid, furfural, etc. were also produced in low amount during the hydrolysis process.

2.5 Factors Affecting the Hemicelluloses Degradation

2.5.1 Effect of Agitation Time

Time is one of the most important factor in enzymatic hydrolysis process. Generally, production rate of reducing sugar increases as time increases. But after a certain time period the production rate will start to decrease. It is speculated that a reduction in the reaction rate may be due to the limitation of the enzyme activity by formation of reaction products at high degrees of hydrolysis as time increases.

Roberto *et al*. (2006) investigated the conversion of rice straw into reducing sugar (xylose) in a semi-pilot reactor Hydrolysis of rice straw by dilute sulfuric acid at different time were investigated. The hydrolysis method was carried out in a 350-L reactor with pressure (35 bar) and acid concentration (1.25%) at different time (0 – 50 min). The results show the ability of first stage hydrolysis to depolymerize xylan to xylose with a maximum yield of 77% at 180min.

According to Xu *et al*. (2007) the pretreated rice straw was hydrolyzed using cellulase in stoppered Erlenmeyer flasks to determine the optimal agitation time. The hydrolysis was performed in 0.1 M citrate buffer (pH 4.8) at 150 rpm at 50 °C under shaking. To determine the effectiveness of time on enzymatic hydrolysis, enzyme to substrate ratio was maintained at 30 FPU/g of substrate. The experiment was conducted at hydrolysis time (6 – 48 h) on enzymatic hydrolysis of soybean straw. The highest yield of xylose obtained at 36hr with the recovery of 51.22%.

2.5.2 Effect of pH

pH has strong effect on hemicellulose degradation to produce reducing sugar (xylose). Generally, the yield of xylose produced is higher at pH range of 3-6. According to Zabihi *et al* (2010), the hydrolysis rate for the production of xylose from wheat straw is higher at pH 4.8 with temperature (50 ℃) and agitation speed (240 rpm).

Chen *et al*. (2010) investigated the optimum pH level for xylose production. The enzymatic saccharification of hemicellulose in dried residue after lime treatment or bio-degradation was performed by shaking gently (120 rpm) at 50 ℃ in 250 mL Erlenmeyer flask containing buffer H3PO4. The substrate content for reaction was 10.0% (w/v) and Tween-80 as surfactant (1.0%, v/v) was used. Before enzyme loading, slurry was acclimated by incubating at 50 ℃ on a rotatory shaker (ZHWY-211B, Tocan Scientific, Shanghai, China) at 120 rpm for 30 min. At end of the research it is found that production of xylose is higher at pH 5.3 with the production rate of 29.87mg/ml.

Martinez *et al*. (2003) also investigated the influence of pH on continuous production of xylose from sugarcane bagasse hemicellulosic hydrolysate by *C. guilliermondii*. Experiments were carried out in a reactor with 1.25 l of treated hydrolysate, at 30 ℃ and 300 rpm using different pH level (4.0 – 7.0). It is found that the yield of xylose concentration from sugarcane bagasse hemicellulosic hydrolysate at pH level of 7.

2.5.3 Effect of Substrate Concentration

The substrate concentration is another variable that influenced the xylose yield and xylose conversion during the enzymatic hydrolysis. After reaching the optimal condition, the yield of reducing sugar starts to fall**.** Such effect can be attributed to end-product inhibition caused by high concentration of substrate, and mass transfer limitations within the reaction mixture due to the high viscosity of the slurry. Other factors that can contribute to the low degree of carbohydrate conversion at high substrate concentration, mainly when low enzyme loadings are employed, include the decrease in the reactivity of cellulosic material in the course of hydrolysis.

Xu *et al*. (2007), conducted studies on enzymatic hydrolysis of pretreated soybean straw. The effect of substrate concentration (2–20% w/v) on xylose production together with the effects of other parameters was investigated. The hydrolysis rate increased up to substrate concentration of 5%. Maximum hydrolysis rate of 43.73% was achieved at substrate concentration of 5%. Further increase in the substrate concentration decelerated the rate of hydrolysis.

2.6 Response Surface Methodology

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful developing, improving and optimization processes. The most extensive applications of RSM are in the particular situations where several input variables potentially influence some performance measure or quality characteristic of the process. Central composite design is a type of response surface methodology. It is a general linear model in which attention is focused on characteristics of the fit response function, in particular, where optimum response value is occur. The yield data were analyzed for model fit using the RSM software (Design Expert) (Corredor *et* al., 2006).

Response surface methodology (RSM) uses quantitative data from appropriate experiments to determine and simultaneously solve multivariate equations. It is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and analyzing optimum conditions of factors for desirable responses. It has been successfully utilized to optimize compositions of fermentation medium, conditions of enzymatic hydrolysis, synthesis parameters for polymers and parameters for food processes (Li *et al*., 2006).

A successive response surface method is an iterative method which consists of a scheme to assure the convergence of an optimization process. The scheme determines the location and size of each successive region of interest in the design space, builds a response surface in this region, conducts a design optimization and will check the tolerances on the response and design variables for termination. This RSM method has been widely used to evaluate and understand the interaction between different physiological and nutritional parameters (Hounjg *et al.,* 1989)

Psomas *et al*. (2007) stated that the response surface methodology (RSM) was applied to optimize the cultural conditions for xanthan gum production by *Xanthomonas campestris*, to maximize cell and xanthan production in batch experiments using a synthetic broth (Luria-Bertani plus glucose, LBG). The interactive effects of three independent variables (agitation rate (100–600 rpm), temperature (25–35 °C), time of cultivation (24–72 h) on xanthan gum and were studied. A second ordered polynomial model was fitted and optimum conditions were estimated. At end of the research the optimal xanthan gum production was found at 600 rpm 30 °C at 72 h and biomass at 600 rpm, 25 °C at 72 h.

Cruz *et al*. (2010) conducted a research aimed to optimize probiotic yogurt containing glucose using Response Surface Methodology (RSM) and to determine the levels of glucose and glucose oxidase that minimize the concentration of dissolved oxygen and maximize the *Bifidobacterium longum* count by the desirability function. RSM mathematical models adequately described the process, with adjusted determination coefficients of 83% for the oxygen and 94% for the *B. longum*. The desirability function indicated that 62.32 ppm of glucose oxidase and 4.35 ppm of glucose was the best combination of these components for optimization of probiotic yogurt processing.

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, several methods were taken to investigate the effect of different parameters for the production of xylose from sugarcane bagasse. First, acid hydrolysis method used for pretreatment to delignified the sugarcane bagasse. Next, the pretreated sugarcane bagasse undergoes enzymatic hydrolysis method to obtain xylose. Then based on results obtained from one factor at a time method (OFAT), optimization for the xylose production was carried by using Response Surface Methodology (RSM). Experiment was conducted according the data that obtained from Response Surface Methodology. Figure 3.1 shows the overall flow of xylose production from sugarcane bagasse.

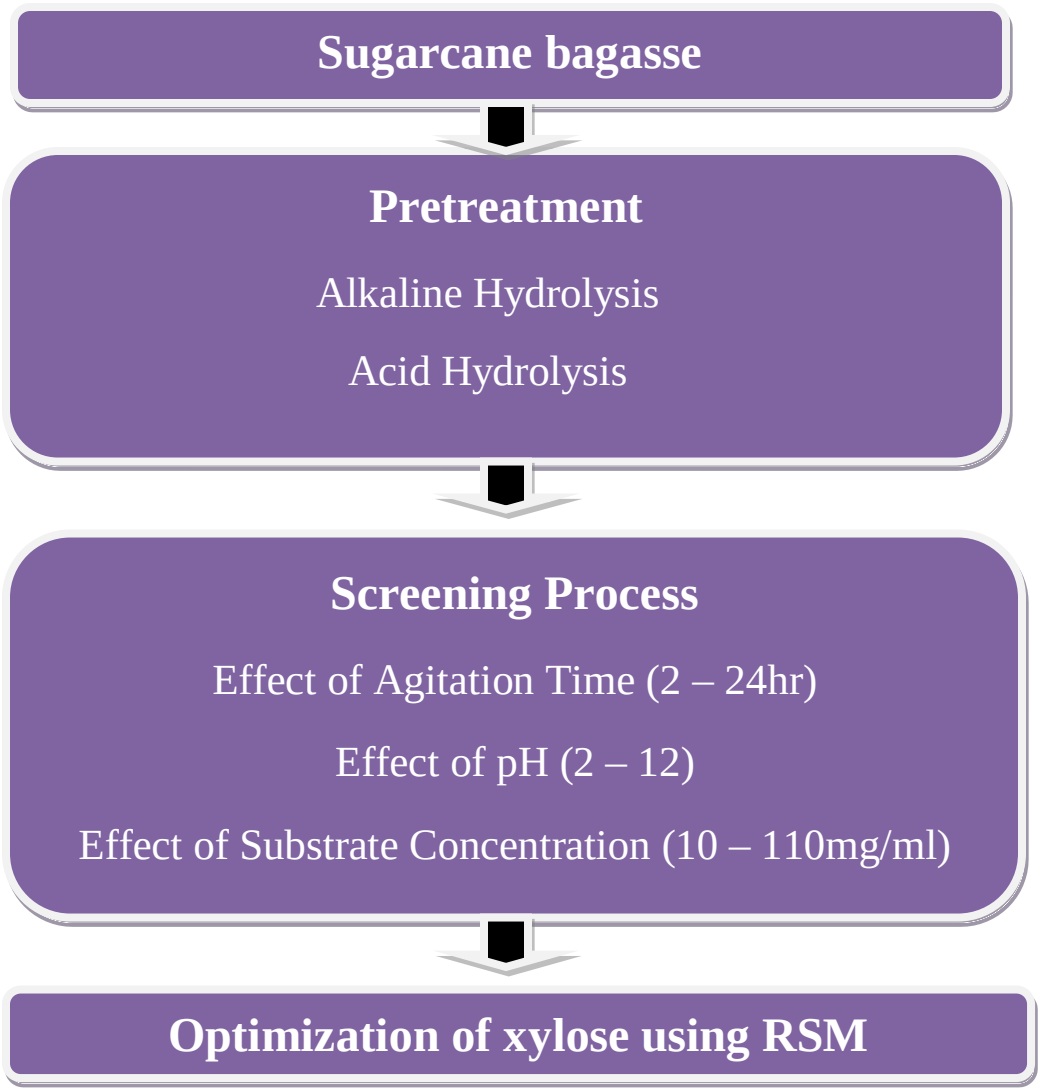


Figure 3.1: Overall flow of xylose production

3.2 Materials

The materials used in this study are sugarcane bagasse, enzyme xylanase, xylose, dinitrosalicyclic acid, sodium citrate, acetic acid, sodium phosphate, Tris, absolute ethanol, hydrochloric acid, sodium hydroxide, magnesium sulphate, hydrogen peroxide, ethylenediamine tetraacetic acid (EDTA) and sulphuric acid

3.3 Preparation of Raw Material

Sugarcane bagasse obtained from sugarcane juice making shops in Kuantan. The samples were sun dried and milled to attain particles with ≈1 cm long and 1 mm thick.

3.4 Pretreatment Process Using Acid and Alkaline Hydrolysis

The pretreatment was initiated by placing the weighed dry sugarcane bagasse in to a 125 ml Erlenmayer flask. Solution containing 1% of sodium hydroxide (NaOH) was added to sugarcane bagasse. The sugarcane bagasse was placed in water bath at 100℃ for 2 hours. After 2 hours, hot water poured to the bagasse to remove the NaOH. The raw material was incubated in oven at 103℃ for 24 hours. The incubated bagasse allowed to react with dilute sulfuric acid of 1.25% (w/v) H_2SO_4 solution for 90 min. The mixture was then filtered to separate insoluble solids from aqueous solution. Finally the hydrolyzed sample was obtained for enzymatic hydrolysis.

.

3.5 Enzymatic Hydrolysis

Xylanase enzyme was used in enzymatic hydrolysis process. Xylanase (45 FGU/g substrate) was added into 50 mM of sodium acetate buffer (pH 4.8) plus a supplement of 0.02% (w/v) sodium azide to inhibit microbial contamination and then mixed to the substrate in a concentration of 2% (w/v). The experiment was then carried out in 125-ml Erlenmeyer flasks which containing 25 ml total reaction volume (the buffer–enzyme mixture). The mixture was then incubated in a rotary shaker at 150 rpm for 180 min. Then, the reaction mixture centrifuged at 4000 rpm for 10 min. The solid phase was removed and the liquid phase (hydrolyzate) was obtained for enzyme assay (Xu *et al*., 2007).

3.6 Effect of Agitation Time on Xylose Production

The enzymatic hydrolysis method was conducted at different agitation time (2, 4, 6, 8, 10, 12 and 24 hr) and all the other parameters such as pH (4.8), temperature (45 °C), agitation rate (150rpm) and substrate concentration (50mg/ml) were remain constant.

3.7 Effect of pH on Xylose Production

The experiment was repeated at optimal temperature value obtained but in different pH value (2, 4, 6, 8, 10 and 12) and all the other parameters such as temperature (45 °C), agitation rate (150rpm) and substrate concentration (50mg/ml) were remain constant.

3.8 Effect of Substrate Concentration on Xylose Production

The experiment was repeated again using optimal agitation time and optimal pH value obtained at different substrate concentration (10, 30, 50, 70, 90 and 110 mg/ml). The other parameters such as temperature (45 °C) and agitation rate (150rpm) remain constant.

3.9 Enzyme Assay

Xylose (reducing sugar) production was measured and determined using dinitrosalicyclis acid (DNS) method (Miller, 1959) by analyzed the hydrolyzate concentration after enzymatic hydrolysis process. The supernatant from centrifugation was added with 1 mL of DNS reagent. The mixture was then boiled at 100˚C for 10 minutes. Next the sample was cooled down to room temperature before determination of the OD using uv-vis spectrophotometer. The absorption of samples was then measured by using spectrophotometer with the OD_{540nm} after boiling. A blank sample of 0.2 M sodium phosphate buffer (pH 6.5) with lacking of enzyme used as control and tested simultaneously with each batch of samples.

3.10 Optimization Using Response Surface Methodology

The program Design Expert (State- Ease Inc, Statistic Made Easy, Minneapolis, MN, USA, Version 6.0.8) was applied for the response surface methodology. A central composite design (CCD) was employed in this study. According to the central composite design, the total number of experimental combinations is $2^k + 2k + n_0$, where k is the number of independent variables and n_0

is the number of repetitions of the experiments at the centre point. For statistical calculation, the experimental variables X_i have been coded as x_i according to the following transformation equation:

$$x_i = \frac{X_i - X_0}{\delta X} \qquad \textbf{(Equation 3.1)}$$

where x_i is the dimensionless coded value of the variable X_i, X_0 is the value of X_i at the center point, and δX is the step change. In this study, the central composite design with three factors and five levels, including three replicates at the center point, was used for fitting a second order response surface. This methodology allows the modeling of a second order equation that describes the process. The accuracy and general ability of the above polynomial model could be evaluated by the coefficient of determination R^2 (Li *et al.*, 2007).

Response Surface Methodology (RSM) was used to determine the optimum agitation time, pH and substrate concentration to maximize the xylose production in hemicelluloses hydrolyzate. This method also used to establish the independent variables of agitation time, pH and substrate concentration with codifies three levels as -1, 0 and +1. The design experimental using RSM will vary as shown in Table 3.1.

Table 3.1: Experimental range of the independent on xylose production

Independent variables	**Symbol**	**Range and levels**				
		-1.414	-1	0	+1	+1.414
Agitation Time (min)	X_1	t1	t2	t3	t4	t5
pH	X_2	p1	p2	p3	p4	p5
Substrate Concentration	X_3	s1	s2	s3	s4	s5

By using the data that obtained from response surface methodology (RSM), the experiment was carried out at various combinations of different parameter values. The two-dimensional graphical representation of the system behaviour, called the response surface, was used to describe the individual and the cumulative effects of the variables as well as the mutual interactions between the independent variables and the dependent variables (Cruz *et al.*, 2010)

CHAPTER 4

RESULT AND DISCUSSION

4.1 Effect of Enzymatic Hydrolysis on Xylose Production

Xylose (reducing sugar) production is effect by several parameters. The parameters involved in these studies were agitation time, pH and substrate concentration. Studies on these parameters that affect the production of xylose were carried out by using the one factor at a time method (OFAT). This method studies one factor at a time while keeping the others at a constant level. After obtain the optimum value of a parameter, the value was used to determine the optimum value of another parameter. This study carried out to determine the optimum range of parameters for further optimization process using response surface methodology (RSM). From the OFAT method, the low and high levels of every parameter were obtained before and after peak values.

4.1.1 Effect of Agitation Time on Xylose Production

By conducting enzymatic hydrolysis, the optimum agitation time was determined. The agitation time is varied at 2, 4, 6, 8, 10, 12 and 24. The samples were incubated in a rotary shaker at 45°C and 150rpm. The other two parameters which are pH (4.8) and substrate concentration (50mg/ml) are remained constant. The effects of agitation time on the production of xylose are shown in figure 4.1.

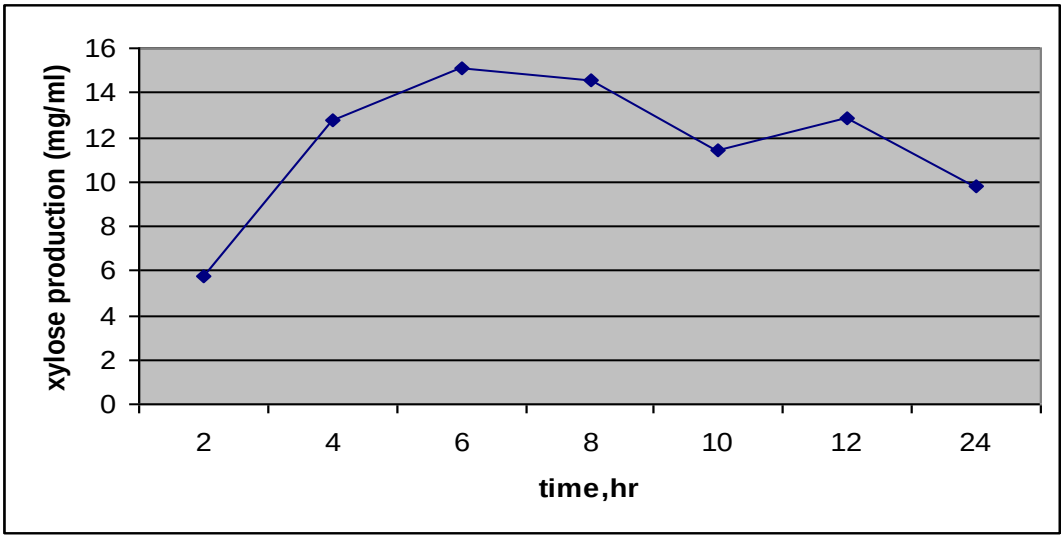


Figure 4.1: Effect of agitation time on xylose production

Based on the result in Figure 4.1, initially the xylose produced increased exponentially at the time of 2 – 6 hours. After 6 hour, the xylose concentration is start to decrease constantly. Then, there was a slight increase in the amount of xylose produced from 10 – 12 hours. However, after 12 hours the production started to decreased drastically.

Agitation time in enzymatic hydrolysis plays a major role expressing the reducing sugar from substrate (Karin *et al.*, 2007). As time increases, the reaction between delignified hemicellulose and xylanase enzyme increases. This interaction

causes xylanase to bound with hemicellulose rapidly to produce xylose. But after the peak time, the xylose production decreases. This behavior might due to the inhibition of the enzyme action by the accumulated hydrolysis products (Draude *et al*., 2001).

4.1.2 Effect of pH on Xylose Production

Based on the result from the effect of agitation time, the optimum time was used to study the effect of second parameter, pH. The pH level is varied at 2, 4, 6, 8, 10 and 12. All the samples were incubated for 6 hours in a rotary shaker at 45°C and 150 rpm using substrate concentration of 50mg/ml. The effects of pH on the production of xylose are shown in Figure 4.2

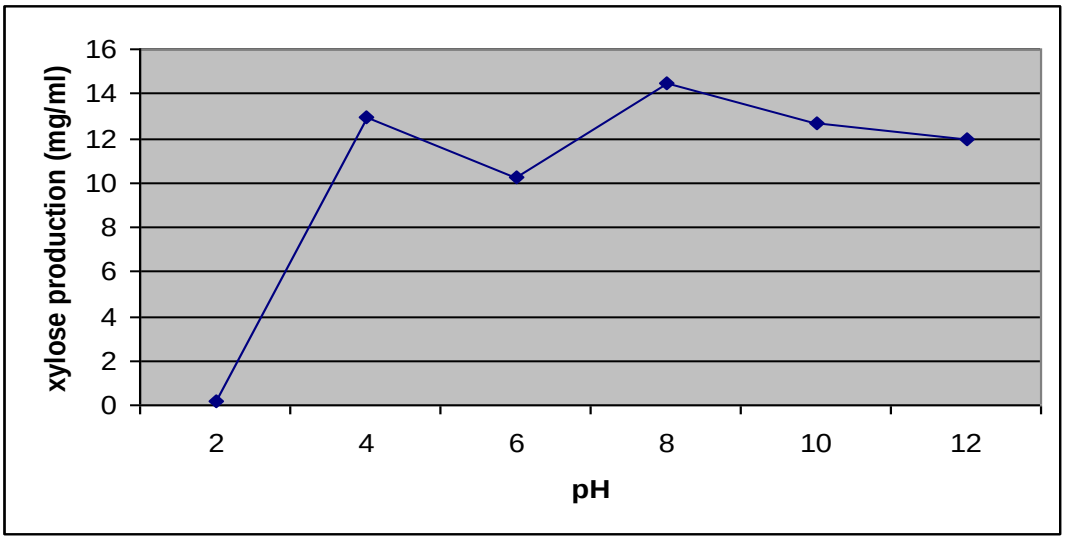


Figure 4.2: Effect of pH on xylose production

Based on the result in Figure 4.2, initially the reducing sugar produced increased exponentially at the pH level of 2 - 4. From pH 4 – 6, there is a slight decrease in xylose production before it increases back at pH 8 where it reach the peak before decreases again constantly.

Draude *et al*, (2001) reported that pH can have an effect of the state of ionization of acidic or basic amino acids of a enzyme in enzymatic hydrolysis. Acidic amino acids have carboxyl functional groups in their side chains. Basic amino acids have amine functional groups in their side chains. This can lead to altered protein recognition or an enzyme might become inactive. In general, enzyme has a pH optimum. However the optimum is not the same for each enzyme. Changes in pH not only affect the shape of an enzyme but it may also change the shape or charge properties of the substrate so that either the substrate cannot bind to the active site or it cannot undergo catalysis. This lead to the inhibition of reaction in enzymatic hydrolysis (Xu *et al*., 2007).

4.1.3 Effect of Substrate Concentration on Xylose Production

The effect of substrate concentration (10 – 110mg/ml) on production of xylose was shown in figure 4.3. The increasing amount of xylose production was recorded started from 10 – 70mg/ml. The highest amount of xylose was recorded at 70mg/ml substrate concentration and at 90mg/ml, lowest amount of xylose production was recorded. However, at concentration of 90 – 110mg/ml, it showed a little increase in amount of xylose production.

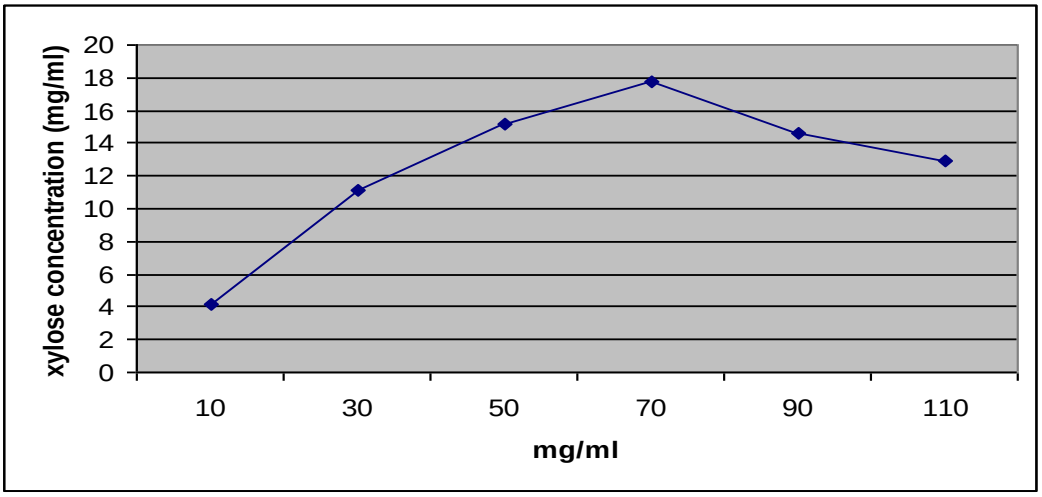


Figure 4.3: Effect of substrate concentration on xylose concentration

When there were higher concentration of substrate, the more the interaction between enzymes and substrates. Therefore the production of xylose increases at the initial stage. Nevertheless, excessive amount of substrate, cause the inhibitors to inhibit the xylanase enzymes that caused by protein-protein interaction. This caused the results of xylose production decreases after it reach the optimum level (Corredor *et al*., 2006).

Lignin removed hemicellulose is a valuable substrate for production of xylose. (Roberto *et al*., 2003). After the optimum substrate concentration the further increase in the substrate concentration decelerated the rate of hydrolysis. Reduction of the aqueous movable phase and end product inhibition might hinder the enzymatic hydrolysis at higher substrate concentration. The mechanism behind enzyme catalysis involves the binding of substrate to the active site of the enzyme, which proceeds to form an enzyme-substrate complex. Inhibition causes a restriction part where enzyme cannot bind with substrate to produce the end product (Xu *et al*., 2007).

4.2 Determination of the Optimum Condition for Xylose Production Using Response Surface Methodology (RSM)

As an important subject in the statistical design of experiments, the Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. When treatments are from a continuous range of values, then a Response Surface Methodology is useful for developing, improving and optimizing the response variable (Myers *et al*., 1988). In this study, parameter of agitation time, pH and substrate concentration were selected for RSM and central composite design (CCD) was applied to identify the optimum concentration of xylose production. The low level and high level of the optimum parameters conditions are determined from the previous result. Table 4.1 shows the low level and high level of each parameter.

Table 4.1: Low level and high level of parameter conditions

Parameters	**Low Level**	**High Level**
Agitation Time (hr)	5	7
pH	7	9
Substrate Concentration (mg/ml)	60	80

Based on rotatable central composite design (CCD), a total of 17 experimental trials including 3 center points were designed between these three parameters. The result was analyzed by using the analysis of variance (ANOVA) as appropriate to the experimental design used. The full quadratic second-order polynomial equation was found to explain the xylose production by applying multiple regression analysis on the experimental data. Experiments arranged by Design Expert Software and the xylose production (reducing sugar) are listed Table 4.2.

The result was analyzed by using the analysis of variance (ANOVA) as appropriate to the experimental design used. The full quadratic second-order polynomial equation was found to explain the xylose production by applying multiple regression analysis on the experimental data. All terms regardless of their significance were included in the following equation:

$$Y = 18.58 - 1.95A + 0.18B + 0.20C - 1.3A^2 - 0.18B^2 - 0.94C^2 - 0.40AB - 0.25AC - 0.81BC \quad \textbf{(Equation 4.1)}$$

where Y is the predicted response, A is the coded value for agitation time, B is the coded value for pH and C is the coded value for substrate concentration.

Table 4.2: Central composite design matrix, the predicted and experimental value obtained for the for the production of xylose

Run	Agitation Time (hr)	pH	Substrate Concentration (mg/ml)	Predicted Value (mg/ml)	Actual Value (mg/ml)
1	6	9.68	70	18.54	18.80
2	5	7	60	16.30	16.16
3	6	8	70	18.58	18.21
4	6	8	53.18	15.57	15.45
5	4.32	8	70	18.00	17.86
6	7.68	8	70	11.44	11.63
7	6	8	70	18.58	18.83
8	6	6.32	70	18.02	17.81
9	5	7	80	18.81	19.25
10	5	9	60	19.04	19.40
11	7	9	60	14.82	14.35
12	7	7	80	15.23	14.83
13	5	9	80	18.31	17.85
14	7	8	86.82	16.34	16.41
15	6	9	80	13.11	13.22
16	6	7	60	13.70	14.13
17	7	8	70	18.58	18.68

Table 4.2 showed that Run 10 gave the highest production of xylose which produced 19.40 mg/ml. The optimum conditions of Run no. 10 were 5 hours of agitation time, pH value of 9 and substrate concentration of 60mg/ml. The lowest production of xylose found at Run 6 with the parameter conditions of agitation time, pH and substrate concentration were 7.68 hour, 8 and 70mg/ml respectively.

Table 4.3 shows the ANOVA analysis for the production of xylose sugar. The precision of a model can be checked by the determination coefficient (R^2) and correlation coefficient (R) (Yin *et al.*, 2007). The determination coefficient (R^2) implies that the sample variation of 98.24% for xylose production was attributed to the independent variables and only about 1.76% of the total variation cannot be explained by the model. Normally, a regression model having an R^2 value higher than 0.9 is considered to have a very high correlation. The closer the value of R (correlation coefficient) to 1, the better the correlation between the experimental and predicted values (Liu *et al.*, 2003). Here, the value of R (0.9917) indicates a close agreement between the experimental results and the theoretical values predicted by the model equation. The value of the adjusted R^2 (coefficient of determination) was calculated to be 95.98%, indicating that a good agreement existed between the experimental and predicted values of xylose production. The adequate precision value, which measured the signal to noise ratio is 21.031, which indicates an adequate signal.

Table 4.3: ANOVA for response surface quadratic model

Model Terms	**Values**
R^2	0.9824
Adj R^2	0.9598
Pred R^2	0.8614
Adeq precision	21.031

Table 4.4 shows the *P*-values obtained were small, <0.0001 compared to a desired significance level, 0.05. This means the regression model was accurate in predicting the pattern of significance to the production of xylose. The *P*-values are used as a tool to check the significance of each of the coefficients which, in turn, are necessary to understand the pattern of the mutual interactions between the best variables. The smaller the *P*-values, the bigger the significance of the corresponding coefficient (Liu *et al*., 2003).

Table 4.4: ANOVA for response surface quadratic model for the production of xylose

Sources	**Sum of Square**	**Degree of Freedom**	**Mean Square**	**F-Value**	**P-Value (Prob>F)**	
Model	86.61	9	9.62	43.46	<0.0001	Significant
A-Agitation Time	51.84	1	51.84	234.10	<0.0001	
B-pH	0.33	1	0.33	1.48	0.2633	
C-Substrate Concentration	0.54	1	0.54	2.45	0.1612	
A^2	20.94	1	20.94	94.54	<0.0001	
B^2	0.12	1	0.12	0.55	0.4818	
C^2	10.04	1	10.04	45.35	0.0003	
AB	1.30	1	1.30	5.89	0.0456	
AC	0.49	1	0.49	2.19	0.1824	
BC	5.23	1	5.23	23.63	0.0018	
Residual	1.55	7	0.22			
Lack of Fit	1.34	5	0.27	2.56	0.3041	Not Significant
Pure Error	0.21	2	0.10			
Correlation Total	88.16	16				

Based on the table, independent variable, A (agitation time), squared terms of agitation time (A^2) and substrate concentration (C^2) are significant model. The interaction term of agitation time and pH (AB) and the interaction term of pH and substrate concentration (BC) also have a significant effect on xylose production. Values greater than 0.1 indicate the model terms are not significant. The independent variables, B (pH), C (substrate concentration) are not significant as well as the interaction term of agitation time and pH (AC). If there are many insignificant model terms, model reduction may improve the model.

Three-dimensional plot were drawn to investigate the effect of different parameters towards xylose production. The purpose of this plotting is to convince and comprehends the interaction between three parameters and also to locate their optimum levels. Figure 4.4, 4.5 and 4.6 shows the response surface curves for the three variables in the production of xylose. The response surface representing the xylose production activity was a function of two parameters with the other one parameter being at their optimal levels.

Figure 4.4 concluded that the agitation time gave a significant effect on the xylose production. Production of xylose increases as agitation time increases. The maximal production of 19.40 mg/ml xylose was obtained at the agitation time of 5 hours. After the optimum time obtained, the xylose production start decreases as agitation time increases. This shows both higher and lower agitation time decreases the xylose production.

Lee *et al*. (1982) through the research of kinetic studies of enzymatic hydrolysis on insoluble cellulose stated that high agitation time may reduce the production of reducing sugar. Time initially increases the rate of reaction between enzyme and substrate. The reducing sugars as well as percent hydrolysis rate decreased as soon as

prolong the time after optimum. This behaviour might due to the inhibition of the enzyme action by the accumulated hydrolysis products.

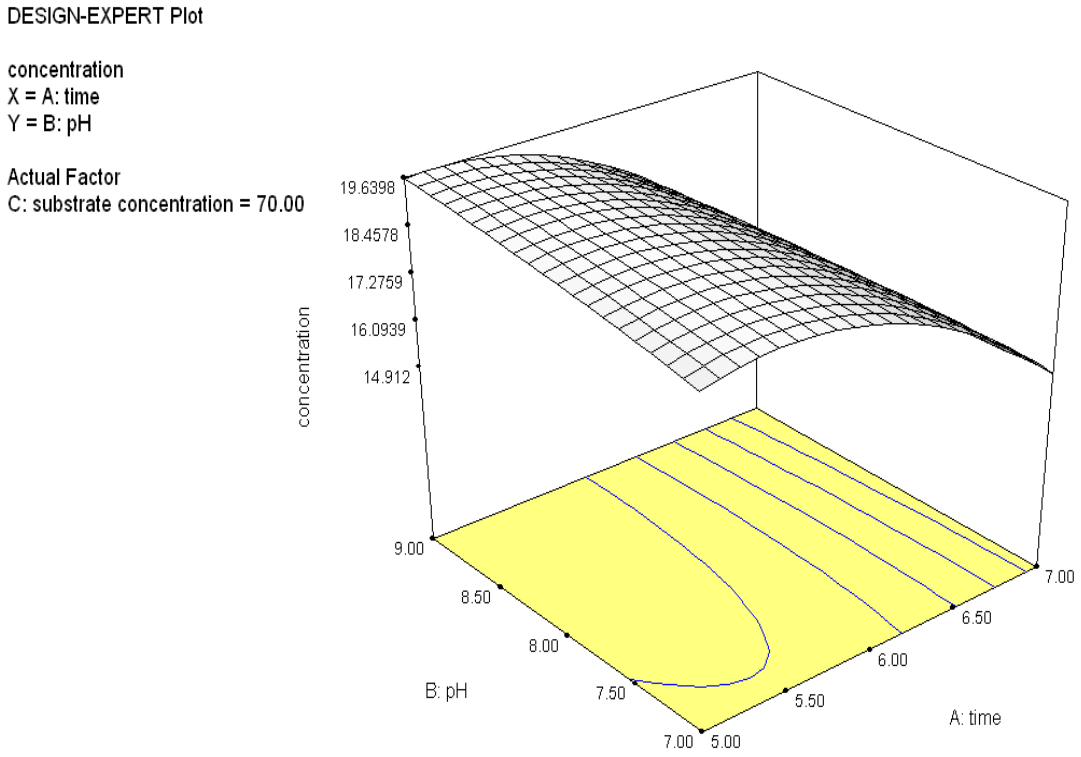


Figure 4.4: Response surface plot of xylose production: Agitation time vs pH

Figure 4.5 showing the effects of pH on the xylose production. This 3D plot indicates the xylose production increases as the pH increases. The optimal range of pH for xylose production was at 9 with the production rate of 19.40 mg/ml xylose. This suggests that increasing the concentration of pH within the tested range was beneficial to the accumulation of xylose production.

Based on Lee *et al*. (1982) when the initial pH of the cultivation medium was near optimum, the accumulation yield maximum desired product. All enzymes have optimum pH. Most enzymes are sensitive to pH and have specific range of activity. To be more precise, pH indicates the concentration of dissolved hydrogen ions (H^+) in the particular solution. An increase or decrease in the pH changes the ion concentration in the solution. These ions alter the structure of the enzymes and at times, the substrate either due to formation of additional bonds or breakage of already existing bonds. Ultimately, the chemical makeup of the enzyme and substrate are changed. The pH can stop enzyme activity by denaturing the three dimensional shape of the enzyme by breaking ionic and hydrogen bonds. It also decreases the concentration of peptide bonds available for hydrolysis.

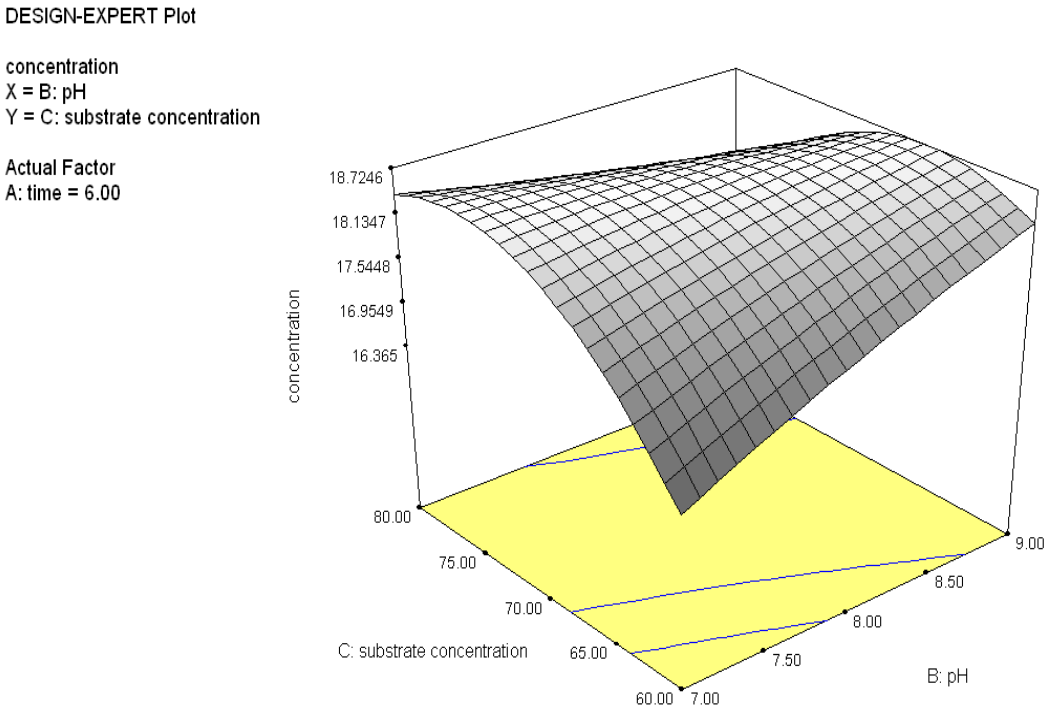


Figure 4.6: Response surface plot of xylose production: pH vs substrate concentration

Figure 4.6 presents 3D plot and showing the effects of substrate concentration on the xylose production, while the initial pH value was fixed at its middle level (pH 8). It is evidence that the yield of xylose production steadily increased as xylose concentration increases at initial stage. By analysis this figure using the Design-Expert software, the maximum xylose of 19.40 mg/mL was obtained at substrate concentration of 60mg/ml. However, the xylose production gradually decreased when substrate concentration exceeded the optimal conditions. This indicated that, increase in substrate concentration would not further increase the yield of xylose production.

Similar result reported in the study of improvement of xylanase production by *Penicillium oxalicum* ZH-30 using response surface methodology by Li *et al*. (2007). Further increase in the substrate concentration after its optimum level, decelerated the rate of hydrolysis. This is due to the end product inhibition that hinder the enzymatic hydrolysis at higher substrate concentration. Reduction in the reaction rate may also due to the limitation of the enzyme activity by formation of reaction products at high degrees of hydrolysis when the substrate concentration increases. Inhibition causes a restriction part where substrate cannot bind with the enzyme thus not producing the desired end product.

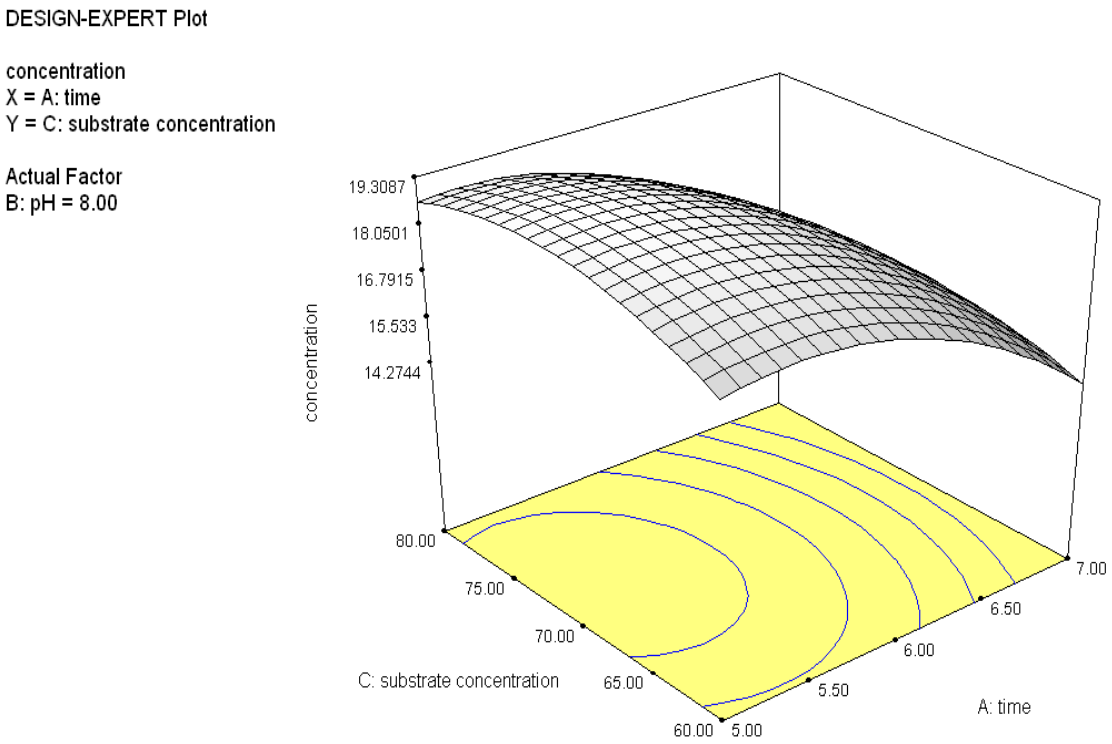


Figure 4.5: Response surface plot of xylose production: time vs substrate concentration

4.3 Optimization of Sugar Production using Response Surface Methodology (RSM)

The effect of the different parameters toward the production of xylose was analyzed and the optimum value for each factor investigated was predicted. Based on the Table 4.2, the production of xylose was successfully optimized after the optimization process was carried out using Design Expert. By using these optimized values, the model predicted that the production of xylose could reach 19.49 mg/ml.

Experimental rechecking proposed by the model was performed in order to confirm the predicted optimized xylose concentration. Rechecking enables the predicted value is precise and accurate. The maximum value of xylose concentration produced after optimization is 19.64 mg/ml. The model was acceptable since the value of xylose produced in experiment was close to predicted value. Table 4.5 shows summary of the optimized conditions for xylose production.

Table 4.5: The summary of the optimized conditions for xylose production.

Parameter	**Before optimization**		**After optimization**		
	Value	**Xylose concentration (mg/ml)**	**Value**	**Xylose concentration (mg/ml)**	
				Predicted	**Actual**
Agitation Time (hr)	6	13.22	5.36	19.64	19.49
pH	9.0		8.73		
Substrate concentration	80		70.6		

Based on the result obtained, it is found that optimization of xylose production able to achieved at agitation time of 5.36 hours, pH value of 8 and substrate concentration of 70.6 mg/ml which yield 19.64 of xylose. After optimization process, the agitation time and substrate concentration able to reduce from 6 hour and 80 mg/ml respectively which only produce 13.22 mg/ml of xylose.

$$Increment = \frac{19.64mg/ml - 13.22mg/ml}{13.22mg/ml} \times 100\% = 49\%$$

There is increment about 49% on xylose production after optimization. Optimum condition for xylose production at lower agitation time and substrate concentration will be more economical. Lower agitation time can reduce the cost of xylose production. The consumption of substrate to produce xylose also can be minimized.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study on the optimization xylose production from sugarcane bagasse using Response Surface Methodology (RSM) was successfully carried out. By using conventional method, one factor at a time (OFAT), the best range of agitation time, pH and substrate concentration was able to be determined. The optimum range of agitation time was within 5.0 to 7.0 hours, pH range was within 7 to 9 and substrate concentration range was within 60 to 80 mg/ml. All of the values were used for further optimization using RSM.

The optimum condition for the maximal production of xylose were obtain at agitation time of 5.36 hour, pH level of 8.73 and substrate concentration of 70.6 mg/ml using RSM. Meanwhile, the highest value of xylose achieved after optimization was 19.64 mg/ml. This improved about 49% after the optimization was carried out compare to initial experiment which only produced 13.22 mg/ml of xylose. The combination between these parameters enhanced the production of xylose.

Effect of parameters such as agitation time, pH, and substrate concentration on xylose production from hemicellulose has been successfully carried out in this study. Besides that, the objective of this experiment which to optimize the xylose production from sugarcane bagasse also has been achieved by using Response Surface Methodology (RSM).

5.2 Recommendation

Field burning is the major practice for removing sugarcane bagasse, but it increases the air pollution and consequently affects the public health. Many countries in western Europe has already banned open field burning. The increasing interest in biotechnological processes employing lignocellulosic residues is quite justifiable because these materials are cheap, renewable and widespread sugar sources. Hemicellulosic hydrolysates made from such residues have been frequently utilized in studies for developing a technically and economically viable bioprocess for obtaining xylose.

There are other pretreatment methods available to remove lignin in plant besides acid hydrolysis. Alkaline peroxide method also used to remove lignin from plant. Recently, fractionation and solubilization studies of lignocellulosic materials by hydrothermal treatments have shown the efficiency of this technology to improve the yields of extraction of hemicelluloses. These treatments allow the selective obtaining of hydrolysates composed essentially of hemicellulose derivatives and solid pulp composed of cellulose and lignin residue. The advantage of this treatment is the prevention of equipment corrosion observed for acid hydrolysis and the absence of neutralization and acid recycling steps, which simplifies process conduction. Economic estimations show that autohydrolysis can be a useful and advantageous technique as compared to other alternative methods (Boussarsar *et al*., 2009).

Research on other parameters that effect the xylose production can be carried out. Parameters such as temperature, rpm rate and enzyme concentration can be investigated to optimized the reducing sugar production. Raw material other than sugarcane bagasse such as rice straw, rice hull, corn cob, oat hull, corn fiber, woodchip and cotton stalk could be rich with xylose. Research on different kind of raw material provides vital opportunity in the production of xylose in future.

It is also recommended to scale-up the optimal value of parameters to a continuous pilot production of xylose from sugarcane bagasse. From this, further research could be done to investigate more on the production of xylose from sugarcane bagasse and the parameters affecting it at larger scale. Production of xylose in plant scale is beneficial because of its high commercial demand.

REFERENCES

Alén. R, Basic chemistry of wood delignification, in: *Papermaking Science and Technology: Forest Products Chemistry* (Book 3), P. Stenius (Ed.), J. Gullichsen, H. Paulapuro (Series Eds.), Fapet, Helsinki, Finland, 2000, pp. 58–106.

Akpinar, O., Erdogan, K. and Bostanci, S. (2009). Enzymatic production of Xylooligosaccharide from selected agricultural wastes. *Food and Bioproducts Processing*. 87: 145-151.

Boussarsar, H., Rogé, B. and Mathlouthi, M. (2009). Optimization of sugarcane bagasse conversion by hydrothermal treatment for the recovery of xylose. *Bioresource Technology*.100: 6537-6542.

Brienzo, M., Siqueira, A.F. and Milagres, A.M.F. (2009). Search for optimum conditions of sugarcane bagasse hemicellulose extraction. *Biochemical Engineering.* 46, 199-204.

Charles, M. and Shuichi, F. (2003). Electricity from bagasse in zimbabwe. *Biomass and Bioenergy*. 25:197-207.

Chen, Y., Dong, B., Qin, W.and Xiao, D. (2010). Xylose and cellulose fractionation from corncob with three different strategies and separate fermentation of them to bioethanol. *Bioresource Technology*. 101:6994–6999.

Coreddor, D.Y., Bean, S. and Wang, D. (2006). Pretreatment and Enymatic Hydrolysis of Sorghum Bran. *Biosource Technology*. 84: 61-66.

Cruz, A. J., Faria, J.A.F., Walter, E.H.M., Andrade, R.R., Cavalcanti, R.N., Olivera, C.A.F. and Granato, D. (2010). Processing optimization of probiotic yogurt containing glucose oxidase using response surface methodology. *Dairy Science.* 93: 5059-5068.

Derriche, R. and Berrahmoune, K.S. (2007) Valorisation of olive oil cake by extraction of hemicelluloses. *Journal of Food Engineering.* 78: 1149–1154.

Draude, K.M., Kurniawan, C.B. and Duft, S.J.B. (2001). Effect of oxygen delignification on the rate and extent of enzymatic hydrolysis of lignocellulosic material, *Bioresource Technology* 79**:** 201-214.

Gray, K.A., Zhao, L. and Emptage, M. (2006). Bioethanol Production. *Chemical. Biology.* 10: 141–146.

Gunda, K. Peter, B and Walteir, L. (1970). Enzymatic Degradation of Cellulose, Cellulose Derivatives and Hemicelluloses in Relation to the Fungal Decay of Wood. *Journal of Wood Science and Technology*. 14: 273-283.

Houjg, J.Y., Chen, K. C. and Hsu, W.H. (1989). Optimization of Cultivation medium Composition for Isoamylase Production. *Journal of Applied Microbiology and Biotechnology*. 39: 61-64.

Ishihara, M., Matsunaga, M., Hayashi, N. and Tišler, V. (2002). Utilization of d-xylose as carbon source for production of bacterial cellulose. *Enzyme and Microbial Technology* 31:986–991.

Karimi, K., Kheradmandinia, S., and Taherzadeh, M.J. (2006). Conversion of rice straw to sugars by dilute-acid hydrolysis, *Biomass and Bioenergy*. 30: 247 – 253.

Lavarack, B.P., Griffin, G.J. and Rodman, D. (2002). The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products. *Biomass and Bioenergy*. 23: 367-780.

Lee, Y.H. and Fan, L.F. (1982). Kinetic studies of enzymatic hydrolysis of insoluble cellulose: analysis of the initial rates. *Biotechnology and Bioengineering*. 24:2383–2406.

Li, Y., Cui, F., Liu., Xu, Y. and Zhao H. (2007). Improvement of xylanase production by *Penicillium oxalicum* ZH-30 using response surface methodology. *Enzyme and Microbial Technology*.40:1381-1388.

Liu, J.Z., Weng, L.P., Zhang, Q.L., Xu, H. and Ji, L.N. (2003). Optimization of glucose oxidase production by *Aspergillus niger* in a benchtop bioreactor using response surface methodology. *World J Microbiol Biotechnol.* 19:317–323.

Marion, C., Seran, A.L. , Denux, D., Lasnier, J.M., Pichavant, F.H., Cansell. F. and Cyril Aymonier. C. (2009). Thermogravimetric analysis as a new method to determine the lignocellulosic composition of biomass. *Biomass and Bioenergy*. 96:1-10.

, E.A., Silva**,** S.S., Silva, J.B.A., Solenzal**,** A.I.N. and Felipe, M.G.A**.** (2003). The influence of pH and dilution rate on continuous production of xylitol from sugarcane bagasse hemicellulosic hydrolysate by *C. guilliermondii*. *Process Biochemistry*. 38: 1677-1683.

Miller, G.L., (1959). Use Of Dinitrosalicylic Acid Reagent For Determination Of Reducing Sugar. *Analitical Chemistry* 31: 426.

Murthy, G.S., Sridhar, S., Shyam, M., Shankaraiah, B. and Ramakrishna, M. (2005). Concentration of xylose reaction liquor by nanofiltration for the production of xylitol sugar alcohol. *Separation and Purification Technology*. 44: 205–211.

Myers, R.H and Montgomery, D.C. (2002). Response Surface Methodology, Process and Product Optimization Using Designed Experiments (2nd ed.), John Wiley and Sons Inc.

Neureiter, M., Danner, H., Thomasser, C., Saidi, B. and Braun, R. (2002). Dilute acid hydrolysis of sugar cane bagasse at varying conditions. *Appl. Biochem. Biotechnol.* 98: 49–58.

Öhgren, K., Bura, R., Saddler, J. and Zacchi, G. (2006).Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. *Biochemical Engineering* 98:2503 – 2510.

Psomas, S.K., Kyriakides, M.L. and Kyriakidis, D.A. (2007*)***.** Optimization study of xanthan gum production using response surface methodology. *Biochemical Engineering*. 35: 273-280.

Rahman, S.H.A., Choudhury, J.P. and Ahmad, A.L. (2006). Production of xylose from oil palm empty fruit bunch fiber using sulfuric acid. *Biochemical Engineering*. 30: 97-103.

Saha, B. C., Iten, L. B., Cotta, M. A., and Wu, Y. V. (2005). Dilute acid pretreatment, enzymatic saccharification, and fermentation of rice hulls to ethanol. *Biotechnol Technology*. 21:816-822.

Sjoman, E., Mantarri M., Nyström, M., Koivikko, H. and Heikkila, H. (2008). Xylose recovery by nanofiltration from different hemicellulose hydrolyzate feeds. .*Membrane Science*. 310: 268-277.

Sun, J.X., Sun, X.F., Sun, R.C. and Su, Y.Q. (2004) Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. *Carbohydrate Polymers*. 56: 195-204.

Taherzadeh, M.J., Niklasson, C., and Lidén, G. (1997). Acetic acid—friend or foe in anaerobic batch conversion of glucose to ethanol by *Saccharomyces cerevisiae*, *Chemical Engineering Scienc.e* 52: 2653–2659.

Xu, Z., Wang, Q., Jiang, Z., Yang, X. and Ji, Y. (2007). Enzymatic hydrolysis of pretreated soybean straw. *Biomass and Bioenergy*. 31: 162-167.

Yin, L., Cui, F., Liu, Z., Xu, Y. and Zhao, H. (2007).Improvement of xylanase production by *Penicillium oxalicum* ZH-30 using response surface. *Enzyme and Microbial Technology*. 40:1381-1388.

Zabihi, S., Alinia, R., Esmaeilzade, F. and Kalajahi, J.F. (2010). Pretreatment of wheat straw using steam, steam/acetic acid and steam/ethanol and its enzymatic hydrolysis for sugar production. *Biosystems Engineering*. 105: 288-297.

Zhu, S., Wu, Y., Yu, Z., Zhang, X., Li, H. and Gao, M. (2006). The effect of microwave irradiation on enzymatic hydrolysis of rice straw, *Bioresource Technol* .97: 1964–1968.

APPENDIX A

Preparation of Buffer Solution

Sodium Acetate Buffer (pH 4.8)

50Mm Sodium Acetate = 0.05M Sodium Acetate

$$0.05 mol\ dm^3 = \frac{mol}{1000 ml}$$

$$0.05 mol\ dm^3 = \frac{mol}{1\ dm^3}$$
$$= 0.05\ mol$$

$$0.05 mol = \frac{mass}{JMR}$$
$$= \frac{mass}{82.03}$$
$$mass = 4.1015 g$$

- 4.1015g + 1000mg distilled water

50mM Acetic Acid

0.05 Acetic Acid = 250ml

$M_1V_1 = M_2V_2$

$(0.05)(250ml) = (1)V_2$

$V_2 = 12.5$ ml

- 12.5ml Acetic Acid + 237.5 distilled water

1 M Sodium Hydroxide (250ml)

$$1\ M\ dm^3 = \frac{mol}{0.25 dm^3}$$
$$= 0.25\ mol$$

$$0.25 mol\ = \frac{mass}{40}$$

Mass = 10g + 250ml distilled water

APPENDIX B

Preparation of Standard Curve

Table B: Result of xylose standard curve

xylose(mg/ml)	OD (540nm)
0	0
2	0.221
4	0.257
6	0.496
8	0.543
10	0.811
12	1.176
14	1.295
16	1.316
18	1.481
20	1.869

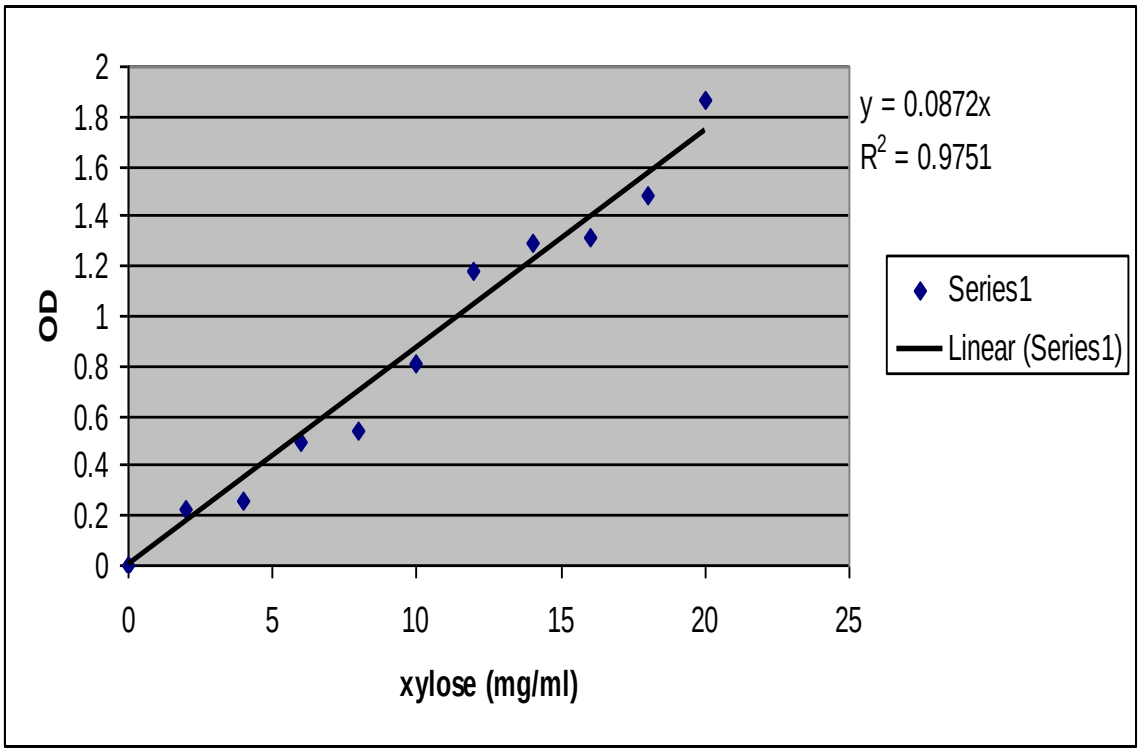


Figure B: Xylose concentration vs OD

APPENDIX C

OPTIMIZATION PROCESS

Result for One Factor at Time Method (OFAT)

Table C1: Effect of agitation time on xylose production

time,hr	OD	xylose (mg/ml)
2	0.5	5.734
4	1.116	12.798
6	1.313	15.057
8	1.267	14.53
10	0.997	11.434
12	1.121	12.856
24	0.858	9.839

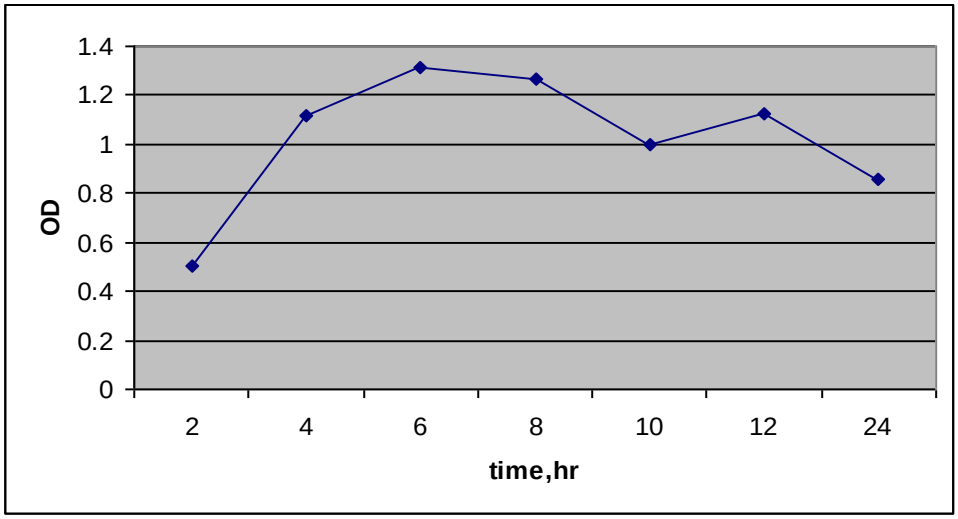


Figure C2: Effect of Ph on xylose production

Table C2: Effect of pH on xylose production

pH	OD	Xylose (mg/ml)
2	0.018	0.206
4	1.131	12.97
6	0.897	10.287
8	1.264	14.495
10	1.105	12.672
12	1.039	11.915

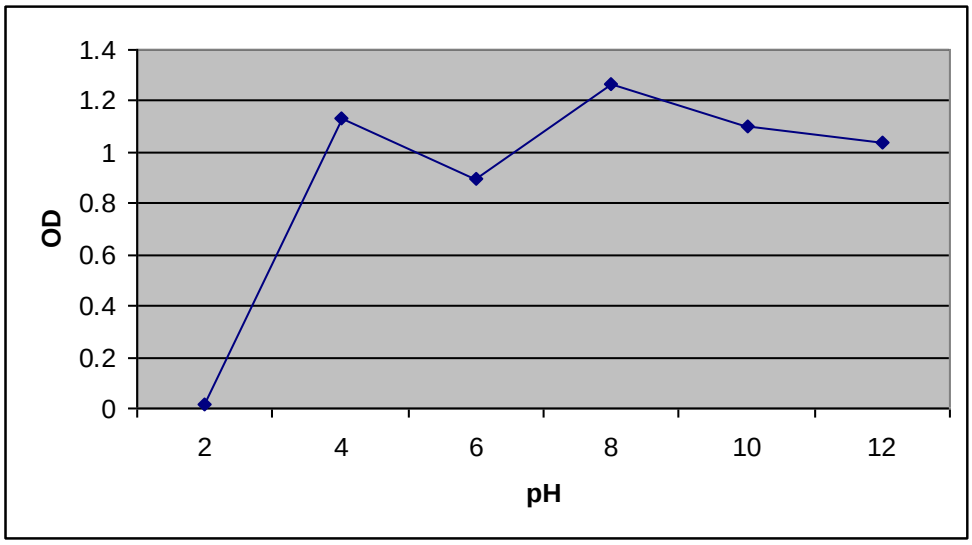


Figure C2: Effect of pH on xylose production

Table C3: Effect of substrate concentration on xylose production

Substrate Concentration (mg/ml)	OD	Xylose (mg/ml)
10	0.365	4.186
30	0.968	11.101
50	1.326	15.206
70	1.55	17.775
90	1.271	14.576
110	1.302	12.936

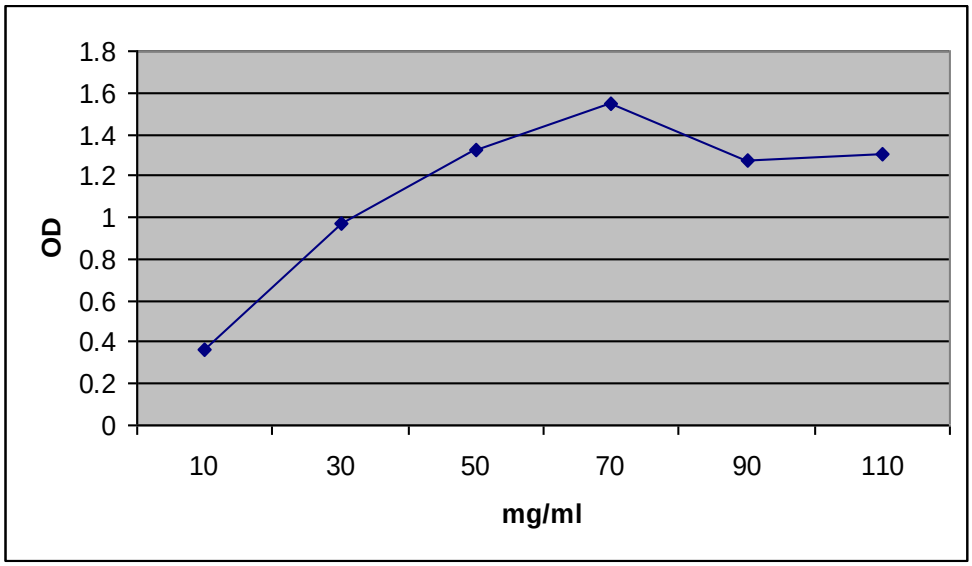


Figure C3: Effect of substrate concentration on xylose concentration

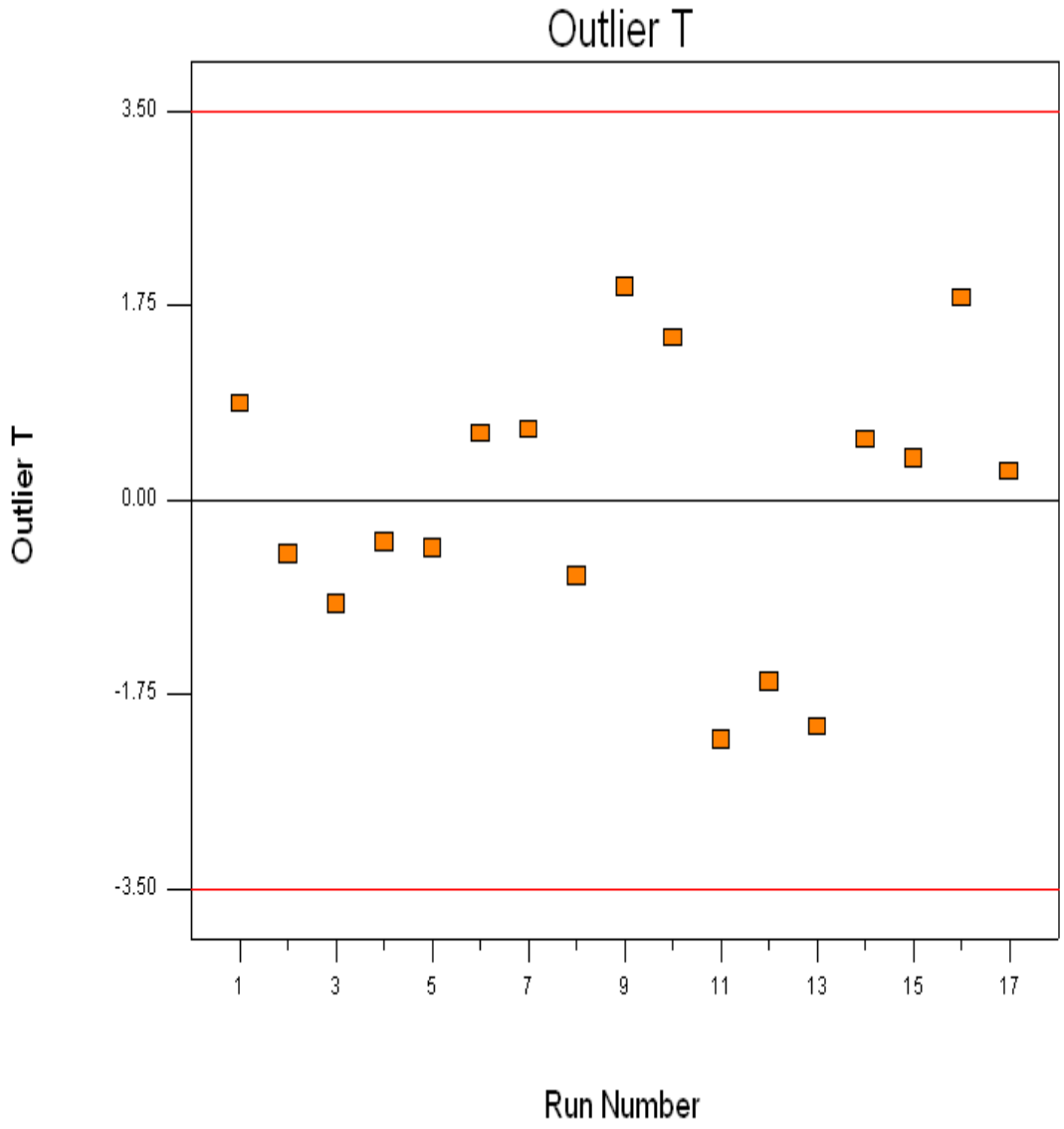
APPENDIX D

ANOVA Diagnostics Case Statistics

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Student Residual	Cook's Distance	Outlier t
1	16.16	16.30052	-0.14052	0.669865	-0.51971	0.054804	-0.49071
2	14.13	13.70393	0.42607	0.669865	1.575814	0.503856	1.816204
3	19.4	19.03525	0.364751	0.669865	1.349026	0.369264	1.451863
4	14.35	14.82366	-0.47366	0.669865	-1.75183	0.622702	-2.16427
5	19.25	18.80952	0.440485	0.669865	1.629127	0.538526	1.914205
6	14.83	15.22793	-0.39793	0.669865	-1.47173	0.439493	-1.63964
7	17.85	18.30925	-0.45925	0.669865	-1.69852	0.585378	-2.05097
8	13.22	13.11266	0.107342	0.669865	0.397003	0.03198	0.371763
9	17.86	17.99817	-0.13817	0.607498	-0.46866	0.033995	-0.44086
10	11.63	11.44491	0.185087	0.607498	0.627803	0.061003	0.598321
11	17.81	18.02109	-0.21109	0.607498	-0.716	0.079347	-0.68858
12	18.8	18.54199	0.258008	0.607498	0.875148	0.11854	0.858558
13	15.45	15.57103	-0.12103	0.607498	-0.41051	0.026083	-0.38472
14	16.41	16.24206	0.167944	0.607498	0.569658	0.050226	0.540068
15	18.21	18.57602	-0.36602	0.33203	-0.95168	0.04502	-0.94429
16	18.68	18.57602	0.103983	0.33203	0.270368	0.003634	0.251629
17	18.83	18.57602	0.253983	0.33203	0.660384	0.021678	0.631381

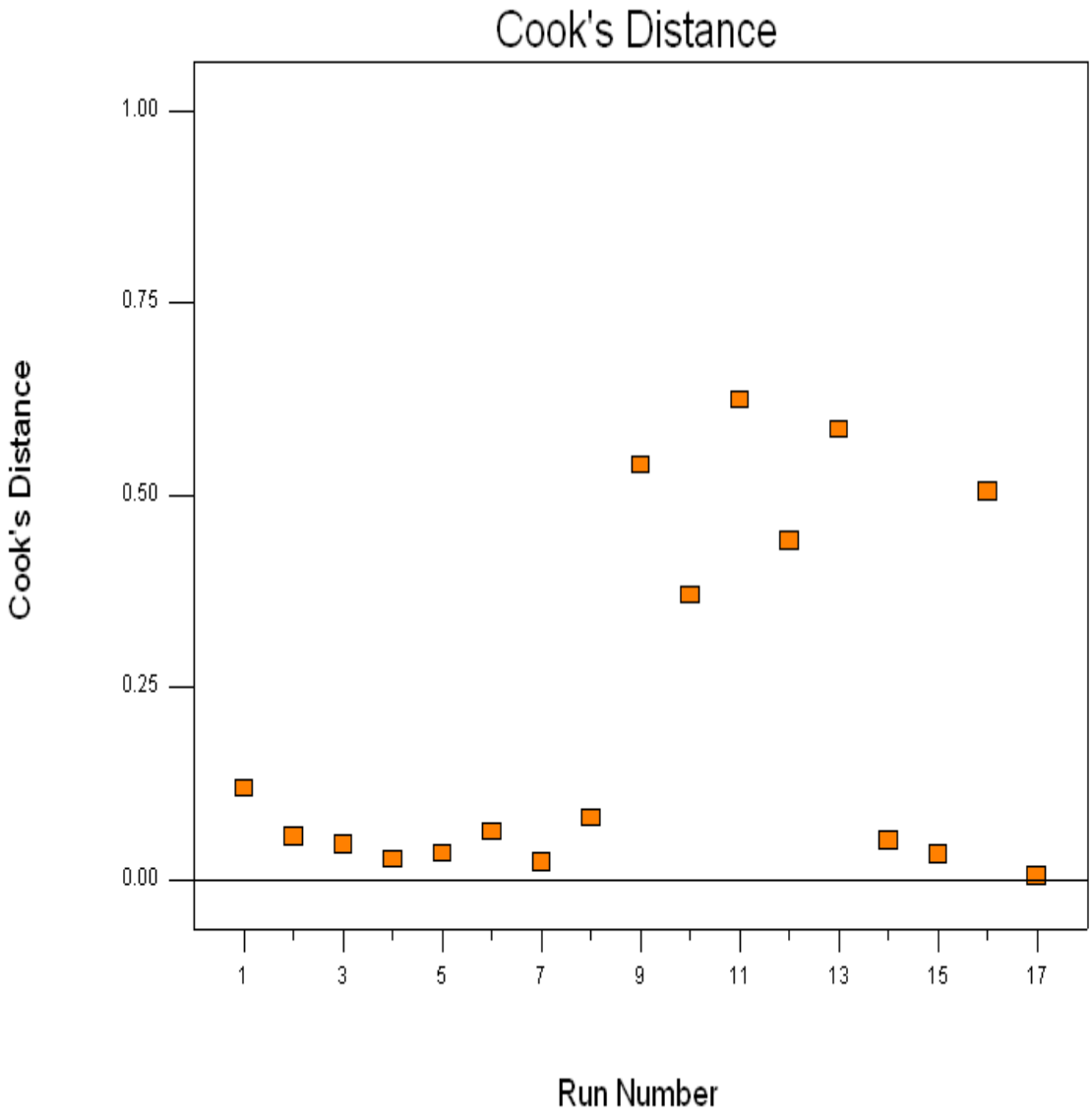
APPENDIX E

Outlier T Analysis



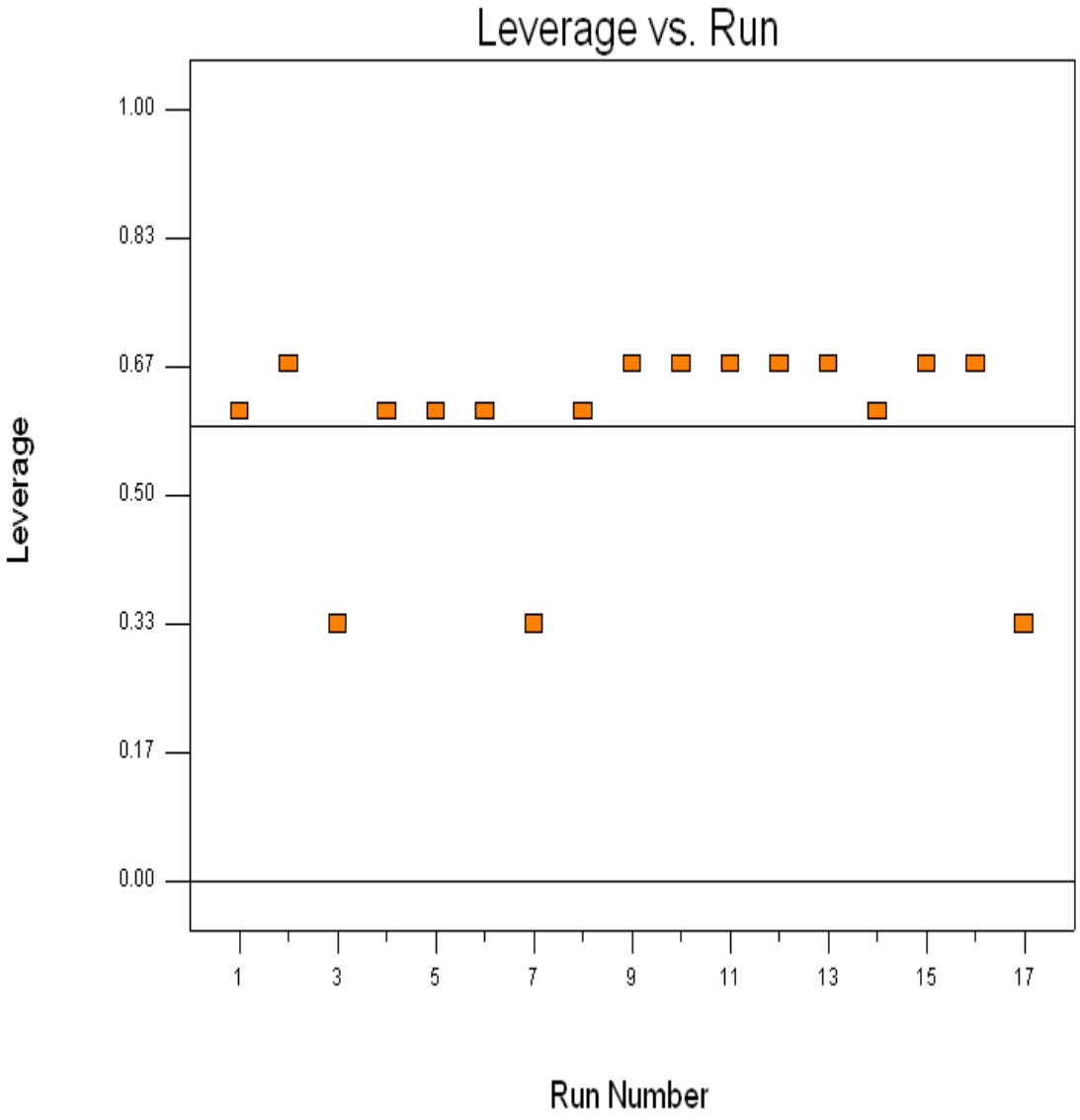
APPENDIX F

Cook's Distance Analysis



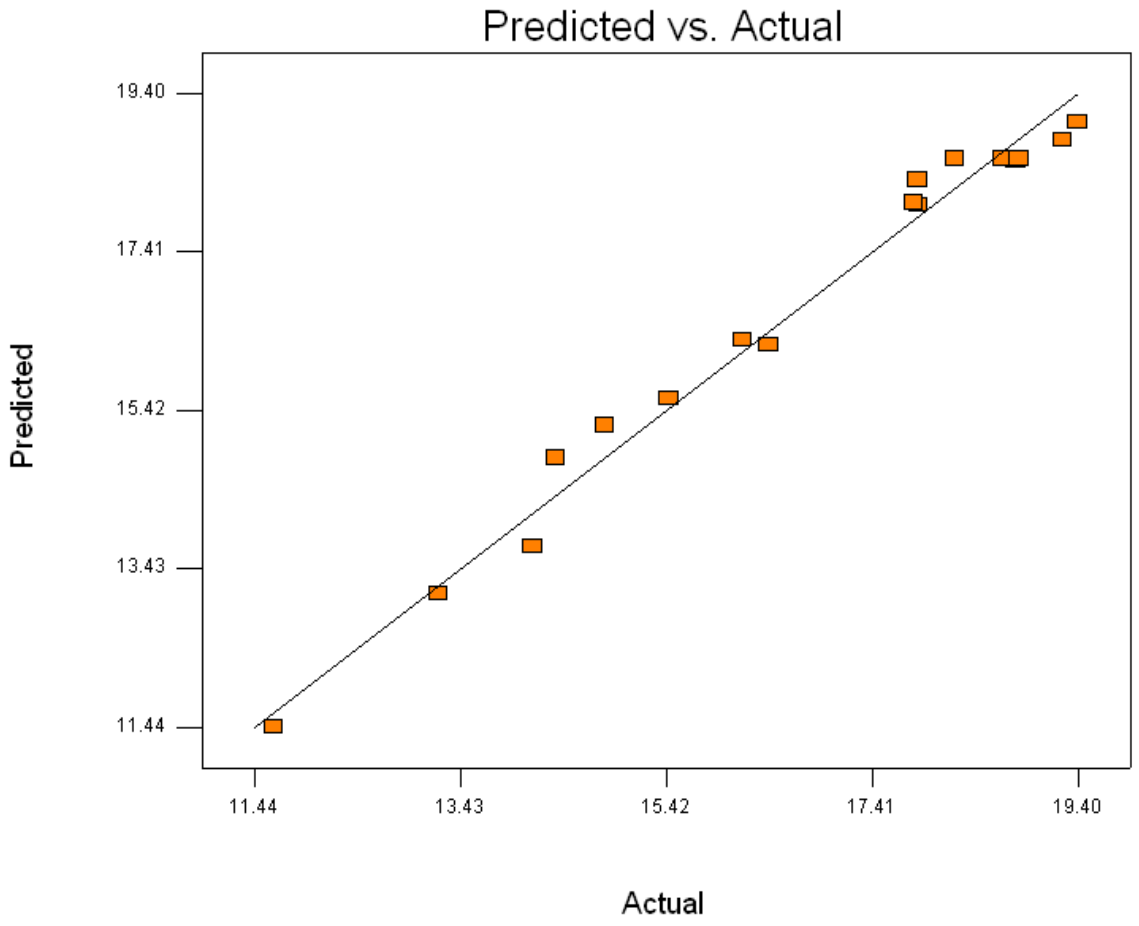
APPENDIX G

Leverage Versus Run Aanalysis



APPENDIX H

Predicted versus Actual Value



APPENDIX I

Box Cox Analysis