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CHEMICAL PRETREATMENT OF RICE HULL AND COCONUT HULL USING RESPONSE SURFACE METHODOLOGY

By
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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours

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DECLARATION

I hereby declare that the work embodied in this Report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the Report of this final year project entitled “Chemical pretreatment of rice hull and coconut hull using Response Surface Methodology” by Lee Rui Ying, matric number F14A0112 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with the Honours, Faculty of Agro-Based Industry, University Malaysia Kelantan.

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Chemical pretreatment of rice hull and coconut hull using Response Surface Methodology (RSM)

ABSTRACT

Nowadays, the demand for ruminant in the livestock industry is rapidly expanded and the demand for livestock feed supply was hardly fulfill. In this research, rice hull and coconut hull from agriculture waste was investigated. Before feed the rice hull and coconut hull to ruminant, the lignin content within the rice hull and coconut hull were determined before and after pretreatment. Response Surface Methodology (RSM) and Central Composite Designs (CCD) helped to get the optimum condition for alkali treatments by using sodium hydroxide (NaOH) to carry out. Fourier Transform Infrared (FTIR) helped to identify the lignin content in both hulls. The interaction of 3 parameters which are NaOH concentration, contact time, and weight of sample was investigated to optimise the lignin removal percentage (%). The parameters range were NaOH (1 M to 10 M), contact time (1 hour to 12 hours), and weight of sample (0.5 g to 5.0 g). The correlation coefficient, R^2 for quadratic model of rice hull lignin removal (%) was 0.8863 while for coconut hull lignin removal (%) in linear model was 0.7998 as well as 2FI model was 0.8892. Three-dimensional (3D) response surface graph and two dimensional (2D) contour plots used to find out the relationship of the variables on the lignin removal. The optimum condition for rice hull lignin removal predicted by RSM were10 M NaOH concentration, 1 hour contact time, 0.5 g sample weight with 32.45% rice hull lignin removal percentage. The optimum condition for coconut hull lignin removal predicted by RSM were10 M NaOH concentration, 12 hours contact time, 0.5 g sample weight with 59.47% coconut hull lignin removal percentage. This shows pretreated rice hull and coconut hull able to be used as an effective alternative ruminant feed. This study improved utilization of agriculture waste as well as alternative feed for gradually expands feed cost.

Keywords: Rice Hull, Coconut Hull, Lignin Removal, Pretreatment, Response Surface Methodology (RSM)

Pra-Rawatan Kimia Sekam Padi Dan Sabut Kelapa Menggunakan Kaedah Gerak Balas Permukaan (RSM)

ABSTRAK

Pada masa kini, keperluan untuk ruminan dalam industri ternakan berkembang dengan pesat dan permintaan untuk bekalan makanan haiwan sukar dipenuhi. Dalam kajian ini, sekam padi dan sabut kelapa dari sisa pertanian merupakan cara telah disiasat. Kandungan lignin yang kumpul dalam sekam padi dan sabut kelapa telah ditentukan sebelum dan selepas pra-rawatan. kaedah gerak balas permukaan (RSM) and reka bentuk komposit berpusat (CCD) membantu mendapatkan keadaan optimum untuk rawatan alkali dengan menggunakan natrium hidroksida (NaOH) untuk dilaksanakan. Fourier Transform Infrared (FTIR) membanru mengenal pasti kandungan lignin dalam kedua-dua sekam. Interaksi antara 3 parameter iaitu kepekatan NaOH, masa sentuhan, dan berat sampel telah disiasat untuk mengoptimumkan penyingkiran lignin (%). Julat parameter iaitu kepekatan NaOH (1M kepada 10M), masa sentuhan (1 jam kepada 12 jam), dan berat sampel (0.5 g kepada 5.0 g). Pekali korelasi, R^2 bagi model kuadratik untuk penyingkiran lignin sekam padi (%) adalah 0.8863 manakala untuk penyingkiran lignin sabut kelapa (%) dalam model linear adalah 0.7998 sertai dengan model 2FI adalah 0.8892. Graf gerak balas permukaan tiga dimensi dan plot kontur dua dimensi telah digunakan untuk mencari hubungan antara pembolehubah dalam penyingkiran lignin. Keadaan optimum bagi penyingkiran lignin sekam padi (%) yang telah diramalkan oleh RSM adalah 10 M kepekatan NaOH, 1 jam masa sentuhan, 0.5 g berat sampel dengan 32.45% penyingkiran lignin sekam padi. Keadaan optimum bagi penyingkiran lignin sabut kelapa (%) yang telah diramalkan oleh RSM adalah 10 M kepekatan NaOH, 12 jam masa sentuhan, 0.5 g berat sampel dengan 59.47% penyingkiran lignin sabut kelapa. Ini membuktikan bahawa sekam padi dan sabut kelapa yang telah dirawat boleh digunakan sebagai makanan alternatif yang berkesan untuk ruminan. Kajian ini telah meningkatkan penggunaan sisa pertanian dan makanan alternatif bagi menyelesaikan masalah harga makanan haiwan yang semakin meningkat.

Kata kunci: Sekam Padi, Sabut kelapa, Penyingkiran Lignin, Pra-Rawatan, Kaedah Gerak Balas Permukaan (RSM)

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis Of Variance
CCD	Central Composition Design
DOE	Design Of Experiments
FTIR	Fourier Transform Infrared
IUPAC	International Union Of Pure And Applied Chemistry
RSM	Response Surface Methodology
rpm	Rotation Per Minutes
3D	Three Dimensional
2D	Two Dimensional
NDF	Neutral Detergent Fiber
ADF	Acid Detergent Fiber
OECD	Organisation For Economic Co-Operation And Development
FAOSTAT	Food And Agriculture Organization Corporate Statistical Database
TDN	Total Digestible Nutrient
DM	Dry Matter
LCC	Lignin – Phenolic Carbohydrate Complex
ICR	Institute For Cancer Research
FCR	Feed Conversion Ratio
DE	Digestible Energy
AOAC	Association Of Official Agricultural Chemists
AHP	Alkaline Hydrogen Peroxide
g	Gram
min	Minute
mL	Milliliter
pH	Acidity

^0C	Degree Celcius
%	Percentage
M	Molar

CHAPTER 1

INTRODUCTION

1.1 Research Background

As the human population growing annually, rice has become a staple food among Asia countries due to its economic and it has become a dietary habit. Other than rice, maize, potato, sugarcane and others are the major crop plants for human consumption. According to Hegde and Hegde (2013), there are 95% of the total rice production are developing country originated with China as the world largest producer among other countries. For 7000BC rice existed (OECD, 1999) and Mekong rivers in Southeast Asia and Niger River in Africa are the two places of rice origin (Porteres, 1956; OECD, 1999).

Since there is an unmilled rice or commonly known as paddy, there is a rice by-product (rice bran, hull, and germ). Heuzé and Tran (2015) stated that the proportion of rice and rice by-product are hulls (20%); bran (10%); polishing (3%); broken rice (1-17%); and polished rice (50-66%). Although most countries lack to make use of the rice hull but according to Hicks (1999), some countries like Egypt, Myanmar, and Bangladesh introduce rice hull as a ruminant feed, agriculture (medium for mushroom and enzyme), industries (concrete blocks and ceramic), fuel (biomass fuel), and energy (electricity and heat).

Meanwhile, coconut or the fruit of *Cocos nucifera* stays the forth important role in the industrial crop according to Main *et al.* (2014). Among the total world production of coconut, almost 90% of them are from the Asia Pacific region where the producer from India, Indonesia, and the Philippines occupy 75% of the world production (Warner *et al*., 2007). Coconut related products can be easily spotted in the market as feed for the domestic animal to local dishes and to ropes. When there is need for consumption and utilisation of coconut, there will be coconut waste and by-product production.

Since the coconut hulls or exocarp or coir are readily and easily available as waste from green coconut production in the hawker stall, thus numerous coconut hull fiber could be obtained. Sivapragasam (2008) acknowledged that there are 5 major types of coconut in Malaysia which include 92.2% of Malayan Tall, 4.3% of hybrid Matag, 1.7% of Mawa, 1.7% of aromatic type (Pandan), and 0.2% of Malayan Dwarfs. Zafar (2015) mentioned that there are 30% coconut fibers out of 40% of the coconut hulls. The composition of the coconut hull for lignin is 32.8%, holocellulose is 56.3%, and cellulose is 4.2% according to Khalil *et al*. (2006). Coconut hulls are usually preferable for making the non-edible product like strings, mats, brushes, and stuffing for cushions but less focused as ruminant feed.

Lawrence (2010) stated that the major issue in the livestock industry is the feed cost, which occupies 60-70% of the total production cost. Besides, the rising cost in feed, as well as the feed shortage also gives rise to chaos among Asian farmers (Ahuja, 2012) and FAOSTAT (2010) stated that Asia has heavily import tonnes of maize as livestock feed 20 years previously. These troubles the farmer and they had to search for alternative feed to cope with this problem.

Rough rice bran (RRB), palm kernel meal (PKM) and cassava pulp (CP) are the alternative feedstuff farmers usually apply in the feed but since the composition of rice hull is double of the rice bran, double waste product after milling process will be produced. The composition of the rice hull for cellulose is 38%, hemicellulose is 20%, and lignin is 22% based on Ludueña *et al.* (2011). Countries like Malaysia treat rice hull as an agricultural waste and rice milling company did not take further action to manage the rice hull but just left it to decompose in the field or burnt it in open space. The decomposition process for rice hull takes a long period of time and within the process, methane gas will be generated and it is a huge problem for the environment as well as open burning which cause pollution (Rozainee *et al.*, 2009). Some universities in Malaysia, for instance Universiti Teknologi Malaysia (UTM) and Universiti Teknologi MARA (UiTM) had been researching on the rice hull.

Thus, it is a chance for us as Malaysian to works this alternative method out by determining the suitability of the rice hull and coconut hull as ruminant feed although some research showed that rice hull is not appropriate as the feed of animal but inversely, rice bran has better potential as animal feed. The reason for this is the high level of cellulose and hemicellulose that cannot be digested by monogastric animal and will lower the digestibility of ruminant is coated and sheltered on the surface of the rice by the rice hull. However, rice hull and coconut hull does undergone pretreatment technology and the result was able to be used by some farmers as animal feedstuff.

The pretreatment method includes mechanical, chemical, biological, and physicochemical methods but it can be also a combination of several of it. Lignocellulosic biomass mainly consists of three polymeric components, hemicellulose which its role is to connect lignin and cellulose fibers, cellulose, the main component of cell walls, and lignin, which holds together cellulose and hemicellulose fibers and gives support, resistance and impermeability to the plant. Pretreatment which meant to enhance digestibility will somehow affect the fraction of the cellulose, hemicellulose, and lignin (Harmsen & Huijgen, 2010). Physical treatment includes contaminant elimination as well as structural cut down (Gupta &

Polach, 1985). Chemical treatment involves heating chemical to isolate contaminant shelter inside the product. Biological treatment involves microorganism to process lignocellulose.

1.2 Problem Statement

Rice hull or husk is a major problem of agriculture waste disposing and most people tend to solve this problem by giving rice hull a second life to turn into and the toothpaste, compost, fuel, and animal feed were produced. On the other hand, the majority of the coconut structure, on the other hand, face almost the same problem which the waste from it do not fully utilise by the agro-industrial chain to generate recyclable product but to burn the coconut waste to solve the problem. Farmers especially small-scale farmers are facing burdensome feed cost thus they intended to search for another alternative feedstuff for livestock especially ruminant. Due to the low quality composition of the rice hull and coconut hull, to make both hulls as feed, it needs to undergo several processes and some deduced so that the quality of the rice hull and coconut hull escalated but it is under expectation. However, if the rice hull is left untreated, it will raise the methane level and bring a problem to the environment which also causes pollution. Thus, why not make use of the abundant resources. Even though these hulls fitted farmers selection as the alternative feed but the lignocellulose becomes a barrier to them which affect the digestive ability of ruminant and this impact their performance and indirectly affect the livelihood of farmers. Therefore, other alternative, effective, low cost and environmental friendly method must be applied for the removal of the unwanted substance obtained inside the rice hull and coconut hull as well as determine the suitability of it as an alternative feed.

1.3 Objectives

The objectives of this study are:

1. To determine the efficiency of rice hull and coconut hull towards lignin removal using chemical pretreatment.
2. To optimize the process variables (in terms of concentration, contact time, and weight of sample) on the removal of lignin content within rice hull and coconut hull.
3. To analyse the relationship between process variables on the lignin removal with the aid of CCD model.

1.4 Scope of Study

The scope of the study is to investigate the removal of lignin content in rice hull and coconut hull using alkaline extraction method as an alternative low cost ruminant feed. The parameters investigated in this study were NaOH concentration (1 M to 10 M), contact time (1 hours to 12 hours), and weight of sample (0.5 g to 5.0 g) where these experimental data obtained were analysed for optimization study aided by Response Surface Methodology (RSM) in Design Expert Software (Version 10.0).

1.5 Significance of Study

Rice hull and coconut hull could be an alternative low cost feed to be used in the animal from the agricultural by-product. As the human population keeps on increasing which decrease in arable land for the crop production to feed the livestock, readily available agricultural waste give a new life for feed and at the same time strengthen our livestock industry without dependence on other countries. It has a potential to be a feed constituents after pretreatment process to improve the nutritive value of rice hull and coconut hull. The contribution of this study may help and improve the environmental protection as it fully utilizes the rice hull and coconut hull.

CHAPTER 2

LITERATURE REVIEW

2.1 Lignin Breakdown

Lignocelluloses component is a plant biomass which consists of carbohydrate polymers (cellulose and hemicellulose) and an aromatic polymer (lignin). In ruminant, the barrier for the plant cell wall degradation is the lignin-carbohydrate complexes mediated by phenolic compounds (PCLCC) which prevent the attack by the rumen microbes that eventually reduce the digestibility of ruminant (Cornu *et al.,* 1994). Lignin is bounded chemically to carbohydrate and protein in cell wall which eventually forms a macromolecule that causes lignin to be problematic to extract (Moore & Jung, 2001). There are several cross-linkage structures between lignin and cell wall components which are the α-ether linkage between lignin to polysaccharides (Baumberger *et al.,* 2001). The plant cell wall structure is shown in Figure 2.1.

Srivastava *et al.* (2012) stated that the problem that affects the ruminant digestibility is the lignin content which bound to cellulose and hemicellulose. The study also mentioned that the energy source of ruminant depends on fiber in their diet with the aid of rumen microbes. Their complicated structure cause they are ignored by the industrial use (Pouteau *et al.*, 2003). It has been proven by Jung *et al*. (1994) that the digestibility of lignin in *in vivo* and *in vitro*, there was the adverse impact of the lignin concentration and cell wall digestibility. The microbe that naturally presents in the rumen has difficulty to access the cell wall will cause the low digestibility of the feed in ruminant (Metha *et al.,* 2015). The lignin content in the rice hull is about 26 to

31% (Ludueña *et al.*, 2011) which is undigestable fiber with the negative effect to dry matter intake and digestible energy (DE) of ruminant (Moore *et al*., 1994).

It is acknowledged that the role of sodium hydroxide in pretreatment proven to break the lignin structure (by degenerate both ester and glycosidic chains and modify the lignin structure) cause cellulose to enlarge and the crystalline structure in cellulose and hemicellulose interrupted (Mcintosh & Vancov, 2010; Sills & Gossett, 2011). Pretreatment also able to damage the biomass surface thus causing asymmetrical cracks and exposure to porosity (Zhu *et al.,* 2008). Sukri *et al.* (2014) pointed out that the specific condition in the alkali pretreatment parameters are still a deficit in order to get the maximum removal of lignin and improve both cellulose and hemicellulose quantity. NaOH also separate natural fats, waxes as well as low-molecular weight lignin compounds from the samples surface in order to expose reactive functional groups such as hydroxyl groups (Papita *et al*., 2012). The pretreatment of plant cell wall structure is shown in Figure 2.1.

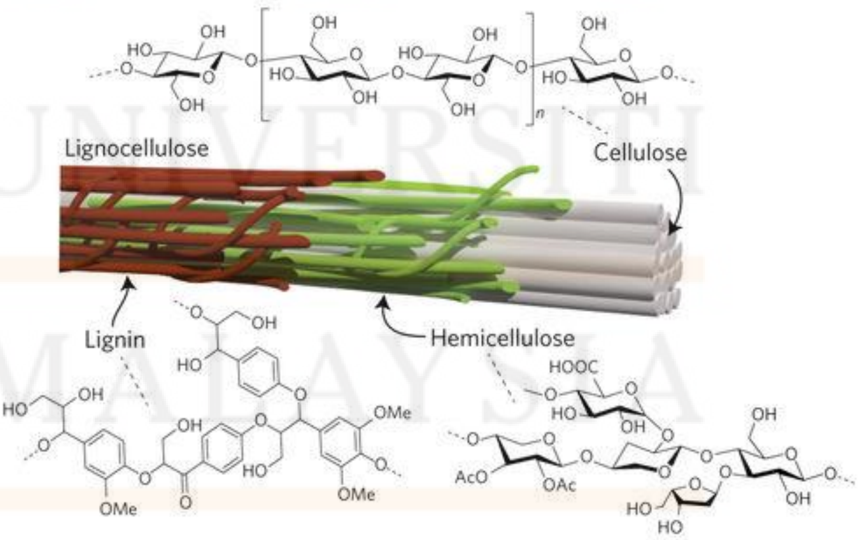


Figure 2.1: Structure of plant cell wall (Wakerley *et al.,* 2017).

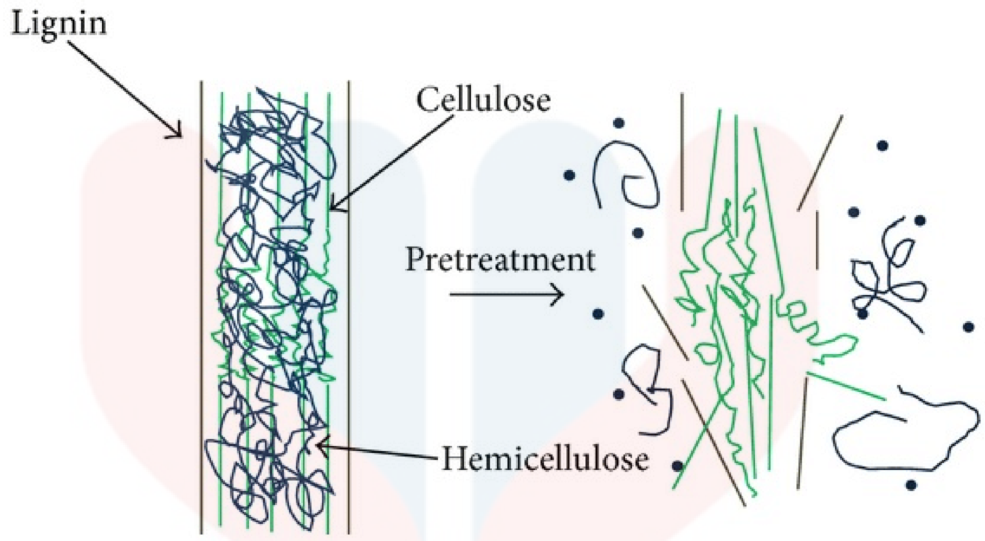


Figure 2.2: Structure of pretreatment of plant cell wall (Lee *et al.,* 2014).

2.2 Rice Hull

Rice hull is one of the major agriculture waste that is plentiful in amount and its cellulose level is reachable within rice husk (Deschamps *et al*., 2013; Draman *et al*., 2014) and the quantity is over redundancy even though it has been transform into other benefited substance but still it is ended up as a waste (Giddel & Jivan, 2007; Ludueña *et al*., 2011). The cell wall where the lignocellulose which consist of hemicellulose, cellulose, and lignin that is present covered the surrounding of the rice hull which acts as a protection which is stubborn (Mussatto & Teixeira, 2010). The hydrogen bond that makes microfibril structure allows rice hull to be undissolvable and non-degenerable (Carpita & McCann, 2000).

Unfortunately, the rice hull has minimal digestibility, low denseness in volume, prominent in silica content, and coarse surface (Saha & Cotta, 2008) which on the other hand rice bran after mix in animal feed tend to be more nutritious than rice hull (Heuzé & Tran, 2015). Vadiveloo *et al*. (2009) mentioned that although few experiments were implemented, the nutritive value of rice hull is still under performance as animal feed. Hendriks & Zeeman (2009) also do not encourage as low protein and high lignocellulose content.

2.3 Coconut Hull

Young drinking coconut can be easily available throughout the tropical country and it is a cheap and hydrated drink for the locals. This indicates coconut is a readily available market for the local economy. In 2009, the coconut related industry gain economic support for RM 29 million to boost this agricultural sector (Razak *et al*., 2010). Coconut waste from the stalls which sell coconut water majority will reach the landfill which is wasted (Tahir, 2012). According to Ding (2015), Malaysia did use coconut trunk as an alternative to furniture production other than using timber to promote green technology.

India and Sri Lanka are the only two vital coir (coconut hull fiber) producer which 10% from all the coconut hull are utilise while the left remains as waste (Warner *et al*., 2007). It was stated that coconut planting countries neglected the capability of the coconut hull (Warner *et al*., 2007). It has been estimated that coconut meal may be another alternative feed but it is still underutilise due to the lack of nutritional facts and processing facilities (Hutagalung, 1981; Wilson & Brigstocke, 1981).

The major components in coconut hulls are lignin and cellulose. Green coconut hull fiber was chemically treated in order to remove pectin, waxy material as well as natural oil surrounding the fibre cell wall which all considered as lignin-related composition. The chemical composition of the coconut hulls are water soluble

(5.25%), pectin and related compounds (3.30%), hemicellulose (0.25%), cellulose (43.44%), lignin (45.84%), and ash (2.22%) (Jayabal *et al.,* 2011).

The coconut ripening process normally starts from the 6 months where the coconuts are used for drinking purposes but there was no coconut meat produced. At the 7 months, the coconut water getting sweeter and meat start to thickening until the 10 months. Starts from the 11 months, the coconut hulls begin to dry out and become brown in colour (Chan & Elevitch, 2006).

2.4 Various Pretreatment Methods

Vadiveloo *et al.* (2009) mentioned that the maximum nutritional value can be reached when the rice hull undergoes pretreatment. The aim of pretreatment process is to alter the linkage in the lignocellulose into a more approachable for further action (Alonso *et al.*, 2013; Barakat *et al.,* 2013) but each of them has their own pros and cons. By combining several pretreatment processes are said to be more economic (Saha, 2005). Microwave, sulphur dioxide, alkaline hydrolysis, humid oxidation, dilute and concentrated-acid hydrolysis, steam explosion, milling and others are some of the common pretreatment method (Taherzadeh & Karimi, 2008).

Wang *et al.* (2016) selected alkaline and peroxide treatment to isolate cellulose from the rice husk. In their experiment, sodium hydroxide (NaOH) was selected as the alkali treated agents to prune polymerization and crystallization as well as disconnect the ester bonds tie on the xylan hemicellulose and lignin (Tarkow & Feist, 1969). For the peroxide treatment, it is expensive to carry out so another alternative and economic method are by applying lower hydrogen peroxide (H_2O_2) concentrations in alkaline hydrogen peroxide (AHP) treatment (Nigam *et al.*, 2009).

Bensah and Mensah (2013) mentioned that by applying the chlorite method the lignin content reduced after various pretreatment, which are basic (Chang & Holtzapple, 2000), chloride and peracids (Kim & Lee, 2005), biodelignification using lignin microorganism (Han & Anderson, 2002), and photochemical pretreatment were tested (Durh *et al.,* 2008).

Jackson (2008) applying grinding and steam processing in the physical treatment, alkali treatment, and microbiological treatment to improve digestibility and nutritive value. Streaming able to improve energy level (Nour, 2006). Two outstanding NaOH methods are applied by following Bender *et al.* (2000) and Boliden (as mentioned by Homb *et al.*, 2011) method. Nikzad (2013) concluded that among dilute sulfuric acid (1% v/v, 121°C, 30 minutes), dilute-NaOH (3% w/v, 121°C, 30 minutes) and heat treatment (121°C, 30 minutes), dilute-NaOH was the most suited method to apply in rice hull pretreatment as more lignin were eliminated.

Aderolu *et al.* (2007) show the fungus, *Trichoderma viride* had maximised the nutritional value. White-rot fungi also use by Villas-Boas *et al.* (2002); Vadiveloo (2003) in biological treatment. The use of biological pretreatment is an energy conserve and moderate production situation that participated by white, brown and soft rot-fungi (Harmsen & Huijgen, 2010). This also supported by Chen *et al.* (2010) that prefer to use white rot fungi to break lignin down as it is eco-friendly with few damage to the environment. *Phanerodontia chrysosporium* microorganism is used by Potumarthi *et al.* (2013) in the pretreatment process. Besides, combination pretreatment showed a promising result. By joining mild either physical or chemical with biological pretreatment, it shorter the fungal pretreatment time in rice hull treatment (Yu *et al.,* 2009). With the biological and liquid hot water pretreatment cooperate, Wang *et al.* (2012) stated that 92.33% hemicellulose were removed which is the highest result among others pretreatment test.

2.5 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is a method by using FTIR spectrometer to collect an infrared spectrum of solid, liquid or gas absorption or emission (Griffiths & Hasseth, 2007). It interprets functional groups in material and contains molecular bond structure which covers from 4000 cm^{-1} to 400 cm^{-1} (Bakri & Jayamani, 2016). It is broadly apply in practically every field of science quantitatively and qualitatively. The sample was placed inside the spectroscopy and a molecular fingerprint of the sample was customized through infrared projection from the laser onto the sample. Infrared radiation either absorbed by sample or transmitted (passed through) it. Hence, it is more prominent and application compared to dispersive infra-red technology (Sawant *et al.,* 2011).

2.6 Optimisation Studies

Optimisation in science indicates using less resource to modify a system to get the effective result. Optimisation in analytical chemistry means to review the impact of the variable on the experimental feedback (Kamsonlian & Shukla, 2013). Classically, the optimum condition of the parameter needed to be determining repeatedly through the experimental work until the optimum condition of each parameter was determined (Breitkopf & Coelh, 2010). To test the feasibility of a design, several variables needed to be adjusted while knowing the particular limits. Since it is not viable to test the entire configuration, thus less number of trials (which are not the exact predictive model) was employed to determine the optimal configuration so that the result can form the model before conducting optimisation. Fortunately, advanced technology creates an effortless and effective optimisation design to substitute the experimental method with computer simulation (Breitkopf & Coelh, 2010).

2.6.1 Response Surface Methodology (RSM)

In the 50s, Response Surface Methodology (RSM) was created by Box and colleagues (Bruns *et al.,* 2006). RSM means the accumulation of techniques in statistical and mathematical for experimental design, modeling, optimising the variables for the preferable response as well as assess the parameters in the appearance of complex interaction. It will create a response to variable related polynomial function with the particular point of variables (-1, 0, 1). It is agreed that RSM allows work to be done in a more productive and resources saving ways since it keeps the experimental frequency to a minimum by determining the relationship between various study parameters (Jain *et al.,* 2011).

The objective of RSM is concurrently optimised among the range of variables to achieve favourable performance. There were two form of experimental design that needed attention before employing the RSM methodology which are the first-order model and the second-order model. The first-order models are suitable to utilise without the occurrence of curvature and used to examine the relation of two parameters while the second-order model is to utilise with the occurrence of curvature and used suitably to examine the relation of more than two parameters (Hanrahan *et al.,* 2006). Khuri and Mukhopadhyay (2010) mentioned that the preferable model frequently implemented is the second-order model where the second-order model in this experimental design adopted is Central Composition Design (CCD).

There were six points that apply in RSM as the optimisation method which are: (1) using the screening studies to pick on the dominant factor that affects the variable as well as recognise the binderies of the experimental region according to research studies; (2) experimental design selection and conducting experiment after experimental matrix were fixed; (3) utilise the experimental data to carry out mathematic-statistical treatment which is polynomial function suited; (4) model's qualification measurement by conducting analysis of variance (ANOVA); (5) demand of the displacement demonstration in direction to the optimal region confirmation; and (6) efficient values acquiring for each tested variable (Almeida *et al.,* 2008 and Roosta *et al.,* 2014).

2.6.2 Central Composition Design (CCD)

Central Composition Design (CCD) is the most favourable design among the second-order model created by Box and Wilson (Box & Wilson, 1951). This design contains three principles which are:

1) A fractional factorial design, 2^n, where n serve as the factor number;
2) An additional design, mostly a star design where the experimental points are at an interval α from its central; and
3) A central point (c_p), serve as the replicate number of the central point.

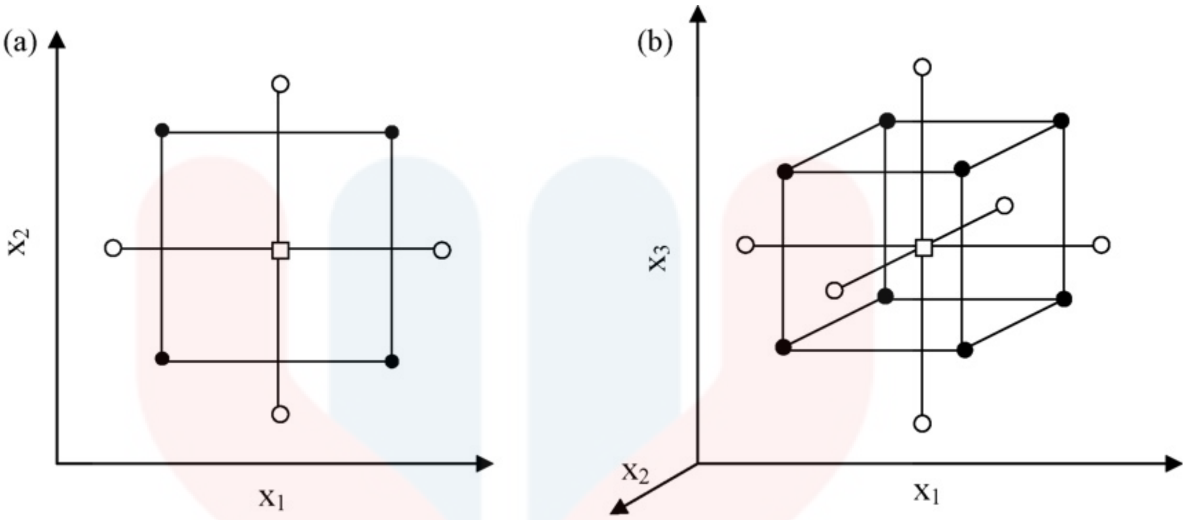
This design has three input factors responsible for the designing objective as well as the selected value according to the preliminary study which are diverged over five levels: minimum value (-1), middle value (0), maximum value (+1), and two outer points (-α and α) (Cho & Zoh, 2007). Three levels which are -1, 0, and +1 were applied in this study and depend on the equation of the total number of experiments, N and the equation 2.1 was used to aid the calculation.

$$N = 2^n + 2n + c_p \tag{2.1}$$

where N: the total number of experiments

n: the number of the point factors

c_p: the central points



(a) (b)

Figure 2.3: CCD for (a) two variables and (b) three variables where (○) factor points, (●) points of axial and (□) point of central (Bezerra *et al.,* 2008).

CHAPTER 3

METHODOLOGY

3.1 Material and Chemicals

The material used in this study was rice hull which was collected from rice hull supplier and green coconut hull was collected in the coconut stall in Sungai Petani, Kedah. The chemicals that used for the study include hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide (KOH), and calcium hydroxide ($Ca(OH)_2$) (Li, 1997).

3.2 Equipment and Apparatus

The equipment used included electronic balance, oven, vacuum pump, pH meter, and hot plate with a stirrer. The apparatus used were airtight zip bag, filter paper, filter funnel, glass, beaker (250 mL, 1 L, and 5 L), conical flask (250 mL and 500 mL), spatula, aluminium foil, dropper, and gloves (Pouteaua *et al,* 2003).

3.3 Methodology

3.3.1 Preparation of Rice Hull and Coconut Hull

The rice hull used in this study was collected from rice hull supplier and coconut hull was collected in the coconut stall in Sungai Petani, Kedah. The rice hull and coconut hull were washed with tap water and dried in an oven at 70°C for 24 hours and stored in airtight zipper bag under dry environment. 0.5 g to 5.0 g range of rice hull and coconut hull were weight using electronic balance and used in this experiment (Dong *et al,* 2011).

3.3.2 Preparation of Sodium Hydroxide (NaOH) Solution

Three different concentration of NaOH solution (1 M, 5.5 M, and 10 M) were used in this study and these concentration can be referred in Appendix A. The 4 g of NaOH pellets were measured and dissolved in 500 mL beaker with 100 mL distilled water to produce 1 M standard NaOH solution. Next, the solution was mixed well until all the NaOH pellets completely dissolved to prepare standard NaOH solution. To produce 5.5 M standard NaOH solution, 22 g of NaOH pellets were measured and mixed well until all the NaOH pellets completely dissolved in 500 mL beaker with 100 mL distilled water. To produce 10 M standard NaOH solution, 40 g of NaOH pellets were measured and mixed well until all the NaOH pellets completely dissolved in 500 mL beaker with 100 mL distilled water (Dong *et al,* 2011). Different concentration of the NaOH solution prepared when needed according to the experimental design by Design Expert software (Version 10.0).

3.3.3 Lignin Removal Studies

Lignin removal studies were carried out using rice hull and coconut hull. Three parameters were studied which are NaOH concentration, contact time, and sample weight. NaOH at concentrations of a range 1-10 molar (M) were used to pretreat 0.5-5.0 gram rice hull and coconut hull samples in a range of 1-12 hours contact time (Pouteaua *et al,* 2003).

The determined minimum (-1) and maximum (+1) value of each parameter were inserted into Design Expert software (Version 10.0). The experimental design that will be used in this study is response surface methodology (RSM) by employing central composite design (CCD). Analysis of variance (ANOVA) was used to support the relationship between the process parameters and the responses.

After the optimum condition of lignin removal was identified, other alkali solution which include potassium hydroxide (KOH) and calcium hydroxide ($Ca(OH)_2$) were used to replace sodium hydroxide (NaOH) to confirm the alkali solution choose in this study was the best solution to remove lignin contain in rice hull as well as coconut hull (Gonçalves *et al*, 2016).

3.3.4 Characterization of Rice Hull and Coconut Hull Using Fourier Transform Infrared spectroscopy (FTIR)

FTIR technique was practised to determine and recognise the chemical functional groups by identifying the peak between the particular gap and band contained in rice hull and coconut hull after the pretreatment process. For rice hull and coconut hull sample, powdered form of the sample after pretreatment and dried, the powder samples were used for FTIR analysis. Transmission for FTIR spectra of rice hull and coconut hull were recorded using Perkin Elmer spectrum in the 400-4000 cm^{-1} wavelength region (Bakri & Jayamani, 2016).

3.3.5 Experimental Design Using Response Surface Methodology (RSM)

The CCD in Design Expert software (Version 10.0) contains the setting of the ‘numeric factors’ and ‘categoric factors which are set at three and zero. In this study, since three parameters were analysed, thus the numeric factors are set as three. The specified information about each parameter along with its minimum (low) and maximum (high) level by applying CCD model is shown in Table 3.1.

Table 3.1: Experimental design with Central Composite Design (CCD) application.

Variables	**Name**	**Units**	**Low Level (-1)**	**High Level (+1)**
A	NaOH Concentration	molar (M)	1	10
B	Contact time	hours	1	12
C	Sample weight	gram (g)	0.5	5

Next, the CCD model followed by the faced centered choices of alpha, α equal to 1 is chosen. The face centered option shows the minimum (low), medium (middle), and maximum (high) levels. The response in this experimental design will be the removal percentage of lignin and the total numbers of experimental runs are 20 runs with different operating conditions, as shown in table 3.2. Each experiment will be run once and the final weight of the pretreated sample will be measured using electronic balance.

Table 3.2: Total experimental runs generated using CCD model.

Run	**A** **NaOH concentration (molar, M)**	**B** **Contact time (hours)**	**C** **Weight of sample (gram, g)**
1	5.5	6.5	5
2	1	12	0.5
3	5.5	6.5	2.75
4	5.5	6.5	2.75
5	5.5	6.5	0.5
6	1	1	5
7	10	6.5	2.75
8	5.5	6.5	2.75
9	5.5	6.5	2.75
10	1	1	0.5
11	5.5	12	2.75
12	10	12	0.5
13	1	6.5	2.75
14	5.5	6.5	2.75
15	10	1	0.5
16	1	12	5
17	5.5	6.5	2.75
18	5.5	1	2.75
19	10	12	5
20	10	1	5

3.4 Optimisation Studies

Optimisation studies of lignin removal were conducted by comparing all the experimental results with the predicted experimental data with the aid of Design Export software 10.0 before the experimental data were analysed for the experimental response. Next, the response of the pretreatmented rice hull and coconut hull were evaluated by ANOVA to compare the result with the actual values. 2D contour plot, 3D surface plot, and interaction plot generated by Design Export software 10.0 were used to observed and analysed the experimental data (Khuri & Mukhopadhyay, 2010).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Lignin Removal Study

There were 20 experimental runs were performed by using specific conjugation of three parameters with the guidance of statistical experimental design (Design Expert software version 10.0) to obtain the combined and individual outcome of a different parameter regarding the lignin removal effect. In this study, Response Surface Methodology (RSM) by employing central composite design (CCD) was used and since the setting is alpha distance to one ($\alpha = 1$), it implies that the design model involves three level design of low (-1) middle (0), and high (+1) for each factor. Table 4.1 displays the experimental design parameters using CCD.

Table 4.1: Experimental design parameters using CCD.

Factor	**Name**	**Units**	**Actual Factors**			**Coded Factors**		
			Low	**Middle**	**High**	**Low**	**Middle**	**High**
A	NaOH concentration	M	1	5.5	10	-1	0	+1
B	Contact time	hours	1	6.5	12	-1	0	+1
C	Weight of sample	g	0.5	2.75	5.0	-1	0	+1

In this experiment, the constant variables including agitation speed of the hot plate magnetic stirrer, room temperature (28^0C), and the volume of NaOH solution (100 mL). Zahoor (2011) mentioned that agitation speed has directly proportional to the removal process. This can be explained by high turbulence speed will lower the boundary layer of resistance around the sample and enhance removal rate. In the other words, the agitation cause kinetic movement effect in adsorbate (NaOH solution) and absorbent (rice hull or coconut hull) which aids collision effect for better lignin removal rate (Biglari *et al.*, 2016). Still, if the optimum agitation speeds are exceeded, it will enhance sufficiently kinetic energy which causes a rapid collision in the adsorbate and absorbent where the unsteadily bound adsorbate molecule will detach (Kusmierek *et al.,* 2015).

Theoretically, the temperature is directly proportional to lignin removal rate as temperature raises the surface of adsorbent pore size and activation as well as the movement of the chemical molecule towards the site of active adsorption (Salleh *et al., 2011*). In the other words high temperature will let the adsorbate diffuse to the adsorbent surface and internal adsorbent pores (Chowdhury *et al.,* 2011). Still, the active adsorbent surface and adsorbent particles will be destroyed if the excessive temperature were applied which weaken the efficiency or optimisation of the adsorption process (Khattri & Singh, 2009).

Besides, the volume of NaOH solution remains constant at 100 mL. A higher quantity of the solution indicates more adsorbent demanded to separate in a large quantity of the adsorbate to maintain the optimum adsorption process. At the same time, higher quantity of the NaOH solution is required, utilised, and then wasted.

Table 4.2: Experimental responses using CCD model

Std Run	A: NaOH concentration	B: Contact time	C: Weight of sample	Lignin removal in rice hull (%)	Lignin removal in coconut hull (%)
1	0	0	0	13.31	19.96
2	0	0	0	20.58	20.91
3	0	0	-1	19.4	47.8
4	-1	1	-1	16.6	44.4
5	-1	1	1	19.44	19.54
6	0	0	0	16.33	20.69
7	1	-1	1	10.72	11.16
8	0	0	0	15.60	23.82
9	0	0	0	15.53	23.31
10	0	-1	0	24.98	21.75
11	-1	0	0	17.96	24.91
12	1	-1	-1	33.2	30.2
13	-1	-1	1	9.88	10.12
14	1	0	0	8.98	16.18
15	-1	-1	-1	10	31
16	0	0	1	5.34	9.4
17	0	0	0	16.4	22.44
18	1	1	1	5.76	7.6
19	0	1	0	28.29	28.51
20	1	1	-1	28	63.6

4.1.1 Effect of NaOH Concentration

Figure 4.1 represents the relationship of NaOH concentration and percentage of lignin removal in rice hull and coconut hull. From the results, it was observed that the average percentage removal of lignin in both samples are different with a favourable percentage of lignin removal in coconut hull compared to rice hull. Lignin content in coconut hull (32.8%) (Khalil *et al*., 2006) is higher than rice hull (22%) (Ludueña *et al.*, 2011), thus more lignin removed from coconut hull.

After the rice hull being treated with 1 M NaOH solution, the lignin decreased by 14.78%. Meanwhile, with 5.5 M NaOH solution of delignification, lignin content shifted to 17.57%. Moreover, after delignification by NaOH 10 M lignin content decreased by 17.33%. From this data, it could be observed that the lignin content decreased by increasing the NaOH concentration. Wang *et al*. (2016) proved similar trend related to rice hull which reported that delignification results are better in lower NaOH concentration.

After the coconut hull being treated with 1 M NaOH solution, the lignin decreased by 25.99%. Meanwhile, with 5.5 M NaOH solution of delignification, lignin content shifted to 23.86%. Moreover, after delignification by NaOH 10 M lignin content decreased by 25.75%. From this data, it could be observed that the lignin content decreased by increasing the NaOH concentration. Jayabal *et al*. (2012) and Akbarningrum *et al*. (2013) reported less lignin obtained when increasing the alkali concentration which is opposite from this trend. This may be due to the different preparation method in this experiment, which only cut coconut hull into smaller pieces with Akbarningrum *et al*. (2013) which soaked coconut coir for 24 hours followed by cut, milled and sieved it to optimised the adsorption of NaOH thus aided the lignin removal.

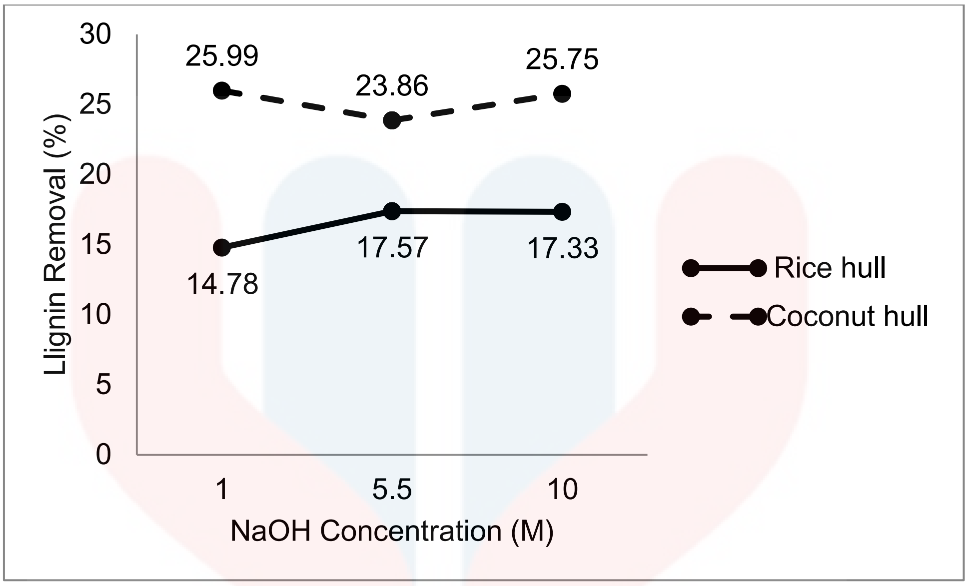


Figure 4.1: Effect of NaOH concentration on the lignin removal percentage in rice hull and coconut hull.

4.1.2 Effect of Contact Time

Figure 4.2 represents the relationship of contact time and percentage of lignin removal in rice hull and coconut hull. From the results, it was observed that the average percentage removal of lignin in both samples increased. After the rice hull being treated with NaOH solution for 1 hour, it showed 17.76% lignin removed from the rice hull. Meanwhile, for 6.5 hours of pretreatment process, lignin content decrease to 14.94% and in 12 hours delignification by NaOH solution, lignin content decrease by 19.62%. From this data, it could be observed that the lignin content decreased by increasing the contact time between NaOH and rice hull.

After the coconut hull being treated with NaOH solution for 1 hour, it showed 17.76% lignin removed from the coconut hull which is the same result with rice hull at the same pretreatment condition. Meanwhile, for 6.5 hours of pretreatment process, lignin content dramatically drops to 22.94% and in 12 hours delignification by NaOH, lignin content decrease by 32.73%. From this data, it could be observed that the lignin content decreased by increasing the contact time between NaOH and coconut hull.

Nikzad *et al*. (2013) mentioned that 30 minutes of contact time with NaOH solution allows highest lignin removal among 15-45 minutes of the test while Dong *et al*. (2011) mentioned that among 20 minutes till 120 minutes test, 120 minutes showed the best lignin removal result. Wang *et al*. (2016) on the other hand fixed 24 hours in all the lignin removal experiment. This indicated that there was various NaOH pretreatment contact period but most of the articles reported that better result in longer contact time.

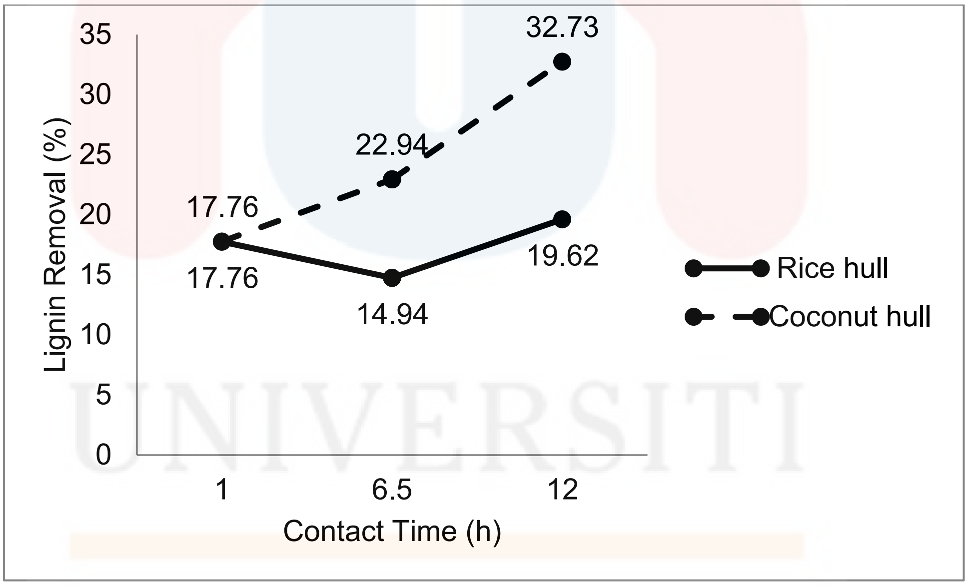


Figure 4.2: Effect of contact time on the lignin removal percentage in rice hull and coconut hull.

4.1.3 Effect of Weight of Sample

Figure 4.3 represents the relationship of the weight of sample and percentage of lignin removal in rice hull and coconut hull. From the results, it was observed that the average percentage removals of lignin in both samples are decreased.

After 0.5 g rice hull being treated with NaOH solution, it showed 21.44% lignin removed from the rice hull. Meanwhile, after 2.75 g rice hull being treated with NaOH solution of pretreatment process, lignin content dramatically drops to 17.8% and with 5 g rice hull being treated with NaOH solution, lignin content decrease by 10.23%. From this data, it could be observed that the less lignin content removed from rice hull by increasing the weight of rice hull.

After 0.5 g coconut hull being treated with NaOH solution, the result of lignin removal in coconut hull (43.2%) is double up to the result of lignin removal in rice hull (21.44%). Meanwhile, after 2.75 g coconut hull being treated with NaOH solution of pretreatment process, lignin content dramatically drops to 22.25% and with 5 g rice hull being treated with NaOH solution, lignin content decrease by 11.56%. From this data, it could be observed that the less lignin content removed from rice hull when the weight of rice hull is increased.

Biglari *et al.* (2016) mentioned that agitation cause kinetic movement effect in adsorbate (NaOH solution) and absorbent (rice hull or coconut hull) which aids the collision effect for better lignin removal rate but if excessive sample were added in the NaOH solution, it may reduce the effectiveness of the colliding process which causes less lignin removal. Ávila-Lara *et al.* (2015) supported in the study that 13.1% solid among 3% to 30% solid perform the best in alkali pretreatment which also supported by previous studies which obtain the optimum results (Wang *et al*., 2010; Xu *et al.,* 2010).

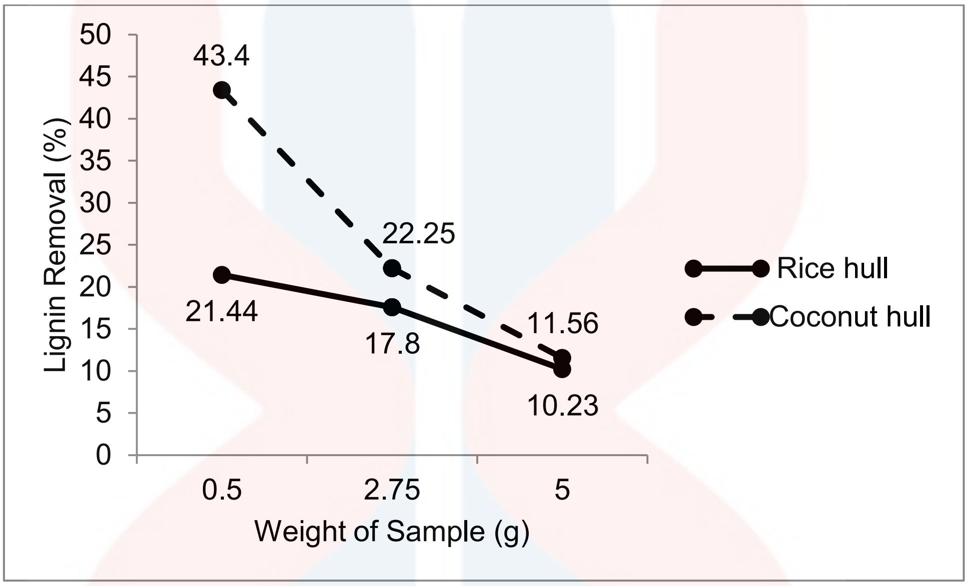


Figure 4.3: Effect of the weight of sample on the lignin removal percentage in rice hull and coconut hull.

4.2 Development of Regression Model Equation for Rice Hull (R1)

In this study, central composite design (CCD) was adopted in order to study the relationship of the independent variable individually and the interactive effect of on the percentage of lignin removal with 20 runs of the experiment. These independent variables comprised of NaOH concentration, contact time, and weight of the sample. Table 4.3 shows the model equation for rice hull lignin removal produced by the Design Expert Software Version 10.0.

Among the four sources of the model which include linear, 2FI, quadratic and cubic model suggested by the Design Expert Software Version 10.0, the quadratic model was the fittest model to be utilised in this study for lignin removal. Quadratic model equals to a second-order polynomial model which encompass linear as well as the two-factor term. On the other hand, the model remark aliased which is the cubic model was interpreted as not fitted for the responses.

Table 4.3: Model summary statistics for rice hull (R1).

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	REMARKS
Linear	6.82	0.3130	0.1842	-0.2951	1403.66	
2FI	5.37	0.6537	0.4938	0.3214	735.47	
Quadratic	3.51	0.8863	0.7839	0.2879	771.81	Suggested
Cubic	2.34	0.9697	0.9042	-4.0731	5498.21	Aliased

According to the model summary statistics in Table 4.3, the standard deviation of the quadratic model was 3.51 while R-squared (R^2) value was 0.8863. R^2 or correlation coefficient was used to determine the reliability of the model generated. Theoretically, if the R^2 value was proximally 1.00, this indicates the developed model is said to be valid and able to forecast a fruitful feedback (Narayana *et al*., 2011). In other words, this study has 88.63% of the variability in the response which predicted by the model and it indicated the predicted value was approximate to the actual experimental value, hence this fitted the response desirably.

Model summary statistics provided adjusted R^2 and predicted R^2 which both should be about 0.20 differences between them for reasonable agreement but this study showed 0.7839 for adjusted R^2 and 0.2879 for predicted R^2 which exceeded the reasonable range. It means that either the data or the model part had problem or block effect emerged. Frost (2013) reported that both types of R^2 contribute by evaluating the predictors number in the model. The contrast between adjusted R^2 and predicted R^2 is the adjusted R^2 was the adjusted or modified predictors' number in the model while the predicted R^2 determined the capability of the model forecast the response values.

Table 4.4: Standard deviation and the quadratic model for R^2 of lignin removal (R1).

Std. Dev.	3.51	R-Squared	0.8863
Mean	16.81	Adj R-Squared	0.7839
C.V. %	20.88	Pred R-Squared	0.2879
PRESS	771.81	Adeq Precision	11.193

Table 4.4 shows a more thorough quadratic model for R^2 for lignin removal which involved the standard deviation and quadratic model. The coefficient of variation (C.V. %) measures the dispersion of variables which compare the variables or standard deviation on the same relative scale or ratio. If CV is less than 10% indicates it is low with high precision. Meanwhile, 10 to 20% CV is evaluated as the medium, which indicated in good precision while 20-30% CV is evaluated as high, indicating low in precision. Excess 30% CV is evaluated as very high, which has very low precision (Gomes, 2009). In Table 4.4, the value of CV shown was 20.88% which located within the range showing nearly medium to low in precision. Adequate precision in this study is 11.193 which according to Ghafari *et al*. (2009), adequate precision means the signal to noise ration measurement and greater than 4 is preferable value. This ratio in this study indicated that this model is qualified to be used.

The empirical polynomial equation is a type of polynomial analysis that examines the relationship between different variables towards the lignin removal which generated by the RSM. Equation 4.1 demonstrated a complete empirical polynomial equation for lignin removal.

$$\text{RH lignin removal, } Y\ (\%) = 16.76 + 1.28A + 0.93B - 5.61C - 3.29AB - 5.93AC + 0.40BC - 3.98A^2 + 9.18B^2 - 5.08C^2 \quad (4.1)$$

where A: NaOH concentration

B: Contact time

C: Weight of sample

According to Equation 4.1, there were positive and negative sign before each coefficient which the positive sign represented as a synergistic effect whereas the negative sign represented as an antagonistic effect (Alkhatib *et al.,* 2015). The equation showed coefficient with one and two factors which indicated the influence of individual factor as well as the interaction effect among the factors. The coefficient with second-order term indicated the factors quadratic effect.

This equation also showed that among all three factors, factor A (NaOH concentration), factor B (contact time), and interaction factors of BC (contact time and weight of sample) have a positive response towards the lignin removal percentage. Factor A contributes the most towards the lignin removal due to it is the largest positive coefficient and the individual variation has more influence on the response apart from the interaction among the variation.

4.3 Statistical Analysis for R1

In order to convince the significance of the model, analysis of variance (ANOVA) was utilised as shown in Table 4.5. ANOVA is a statistical technique measuring contrast among varieties or in this study it analyses three independent variables. To get the mean square in the quadratic model, the total of the squares of individual variation associated with the degree of freedom were divided. This analysis able to measure the parameter hypothesis in the model (Jaikumar & Ramamurthi, 2009).

According to Ghafari *et al.* (2009), there were F-test and p-value in the ANOVA table where F-test examine the statistical importance contain in the model while the p-value examines the importance as well as the style of experimental parameters relationship. Statistically, the larger value of F-test and smaller p-value were demanded a better reputation in this model as reported by Arau *et al.* (2005) that below 0.0500 p-value is important for the model.

Smaller p-value also supported the rejection of null hypothesis hence favour the alternative hypothesis (Dorey, 2010). Table 4.5 present 8.66 F-value and 0.0011 p-value which means there was only 0.11% chance for large F-value to noise. Smaller p-value than 0.05 (95% confidence level) in this study supported the statistical significance of the model terms.

Table 4.5 shows non-significant in lack of fit in this model which represent this model fit the experiment data well. Lack of fit represents data variability surrounding the fitted model. The model does not fit the data if lack of fit in the ANOVA model is significant (Ghafari *et al*., 2009). There were 3.35 F-value in the lack of fit showed pure error was not significant and 0.1054 p-value in the lack of fit showed only 10.54% chance for F-value this large to appear because of noise. Hence, this model is appropriate for this experiment.

Table 4.5: ANOVA table for response surface quadratic model of R1

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	960.53	9	106.73	8.66	0.0011*
A-NaOH Dosage	16.33	1	16.33	1.32	0.2765
B-Contact Time	8.67	1	8.67	0.70	0.4213
C-Gram	314.27	1	314.27	25.49	0.0005*
AB	86.59	1	86.59	7.02	0.0243
AC	281.32	1	281.32	22.82	0.0007*
BC	1.28	1	1.28	0.10	0.7539
A^2	43.61	1	43.61	3.54	0.0894
B^2	231.89	1	231.89	18.81	0.0015
C^2	71.03	1	71.03	5.76	0.0373
Lack of Fit	94.92	5	18.98	3.35	0.1054

* represents that the value is significant

4.4 Predicted Values versus Actual Values for R1

The predicted values of the response were foreseen by the CCD model of the Design Expert Software Version 10.0 whereas the actual value is practically run by experiment. The fitness of the model according to the selective experimental data for parameters evaluated is the one that differentiates the predicted values and actual values (Chakraborty *et al.*, 2005). By employing error equation percentage, the normal plot of residual and scatter plot of predicted versus actual values could be analysed to determine the appropriateness and fitness of both predicted and actual values in a model (Pineiro *et al.*, 2008).

Based on Table 4.6, the most outstanding percentage of the lignin removal was 28.29% with the optimum condition of 5.5 M of NaOH concentration, 12 hours of contact time, and 2.75 g of sample weight in the actual value whereas the predicted value, 26.87% lignin removal were slightly differ from the actual value.

Furthermore, Figure 4.7 showed the analysis for the normality of the residuals in order to further evaluate the error terms are distributed normally. It can be stated that the residuals descended approximately to the straight which means that the errors distributed normally. Noordin *et al.* (2004) supported that if most of the residues lie on the straight means the proposed model for lignin removal can be accepted hence no discussion needed for the independence variance deduction.

Apart from that, Figure 4.8 showed the interaction of the predicted and actual values of the response of lignin removal percentage. It can be stated that the residuals descended approximately to the straight which means that the errors distributed normally. Kansal *et al*. (2005) supported that the quadratic model developed was moderately well fitted with the observed values.

Table 4.6: Results for actual values and predicted values lignin removal (R1).

Run Order	Coded Factors			Lignin Removal (%)	
	A (NaOH concentration)	B (Contact time)	C (Weight of sample)	Actual Value	Predicted Value
1	0	0	0	13.31	16.76
2	0	0	0	20.58	16.76
3	0	0	-1	19.4	17.28
4	-1	1	-1	16.6	19.09
5	-1	1	1	19.44	20.54
6	0	0	0	16.33	16.76
7	1	-1	1	10.72	8.58
8	0	0	0	15.60	16.76
9	0	0	0	15.53	16.76
10	0	-1	0	24.98	25.01
11	-1	0	0	17.96	11.50
12	1	-1	-1	33.2	32.45
13	-1	-1	1	9.88	11.30
14	1	0	0	8.98	14.05
15	-1	-1	-1	10	11.45
16	0	0	1	5.34	6.07
17	0	0	0	16.4	16.76
18	1	1	1	5.76	4.66
19	0	1	0	28.29	26.87
20	1	1	-1	28	26.93

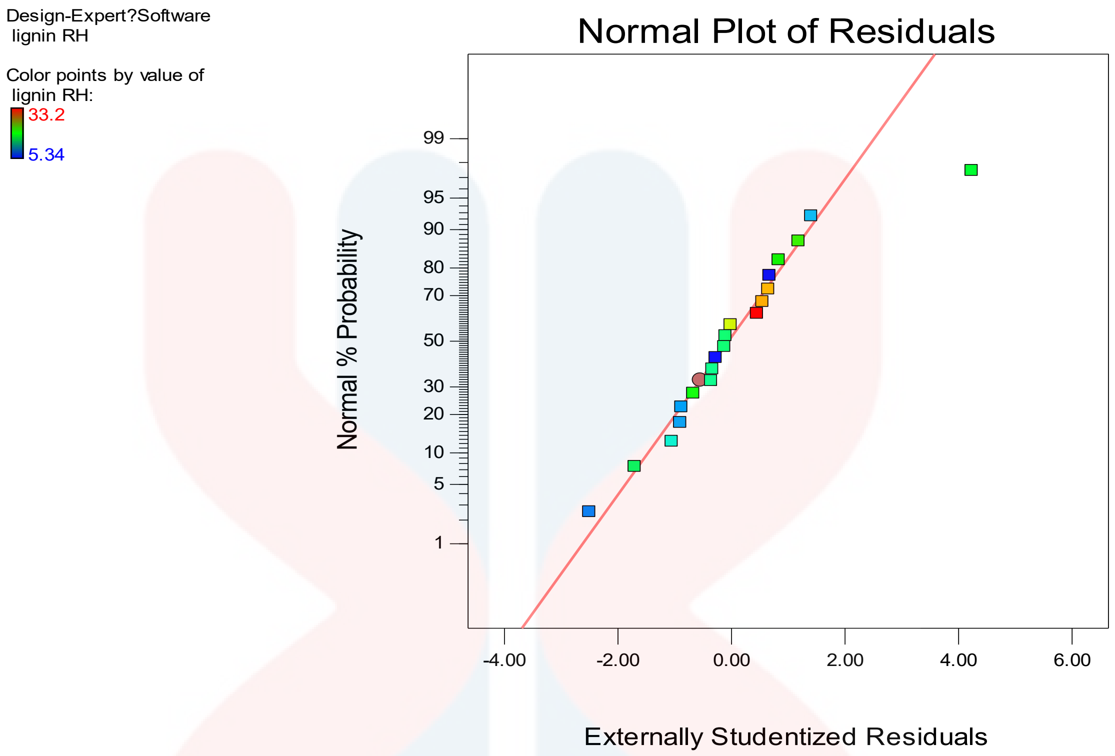


Figure 4.4: Plot of normal % probability versus residual error of lignin removal (R1).

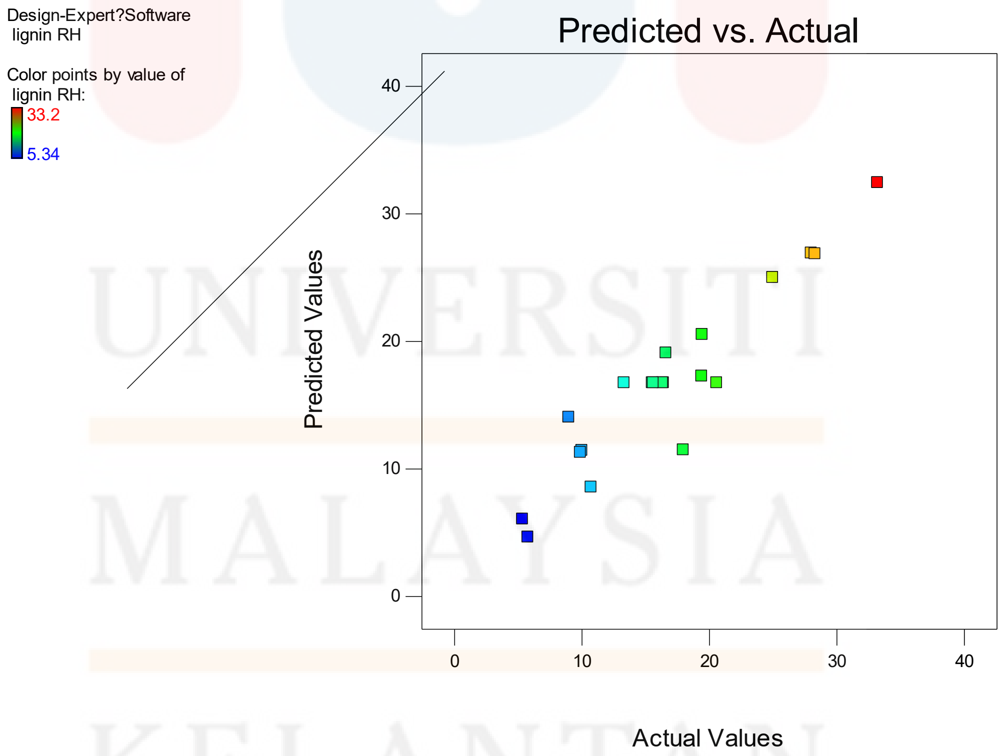


Figure 4.5: Diagnostic plot for predicted versus actual values for lignin removal (R1).

4.5 Optimisation of Adsorption Variables of Lignin Removal (R1)

Three-dimensional (3D) response surface graph and two dimensional (2D) contour plots are the visual inspection for the graph which intent to find out the relationship of the variables on the lignin removal at the same time keeps the other variables constant. It objectively utilised to search for the optimum response from optimum variables.

4.5.1 Effect of NaOH Concentration and Contact Time on Lignin Removal (R1)

Figure 4.6 (a) and 4.6 (b) displayed the integrated result of NaOH concentration and contact time while maintaining the constant weight of the sample at 2.75 g. Referring to the design point, the rice hull lignin removal percentage increased with increase of both NaOH concentration and contact time. From the graph, there were blue to the red colour zone where the colour around red zone is preferable and favourable response performance while colour around blue zone is the opposite meaning.

According to Figure 4.6 (a) and 4.6 (b), the NaOH concentration increase from 1 M to 5.5 M provide more chances to exposed adsorption site in the rice hull interact with NaOH solution which eventually increase the rice hull lignin removal percentage (Ofomaja, 2008). The figure showed 33.20% optimum response value in the red zone. Still, above 5.5 M NaOH concentration fails to remove rice hull lignin percentage which indicated equilibrium system had achieved at 5.5 M thus further higher concentration gradually retarded (Banerjee & Chattopadhyaya, 2013).

Meanwhile, the contact time increases continuously from 1 hour to 12 hours as the equilibrium was not reached throughout this period of time. The maximum rice hull lignin removal percentage for both NaOH concentration and contact time interaction effect was 28.29% at the optimum condition of 5.5 M NaOH concentration and 12 hours contact time with 2.75 g constant weight of the sample.

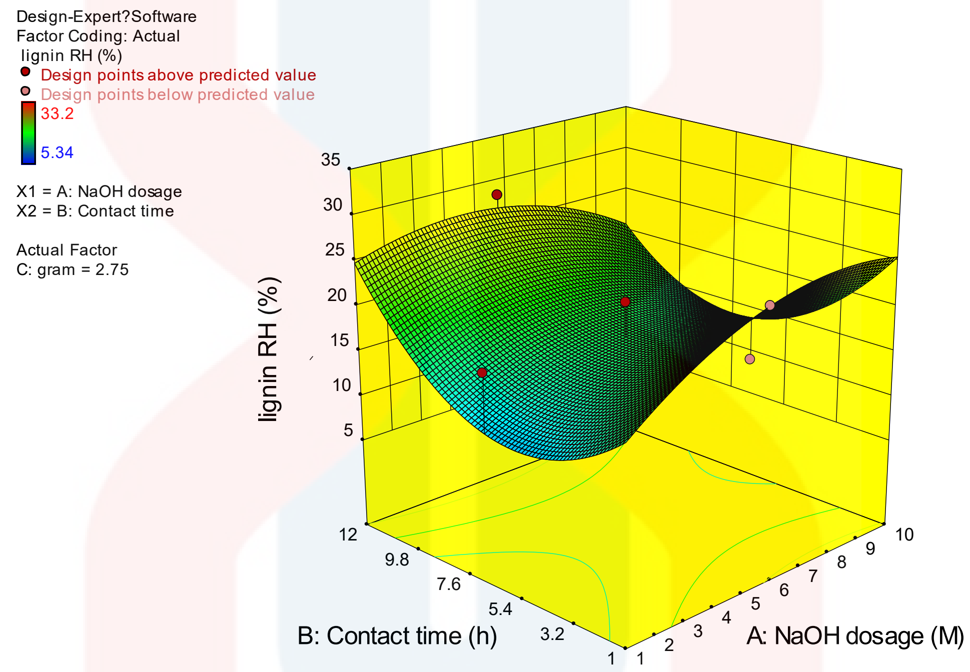


Figure 4.6: (a) 3D response surface graph of the interaction effect of NaOH concentration (M) and contact time (hours) on lignin removal (%).

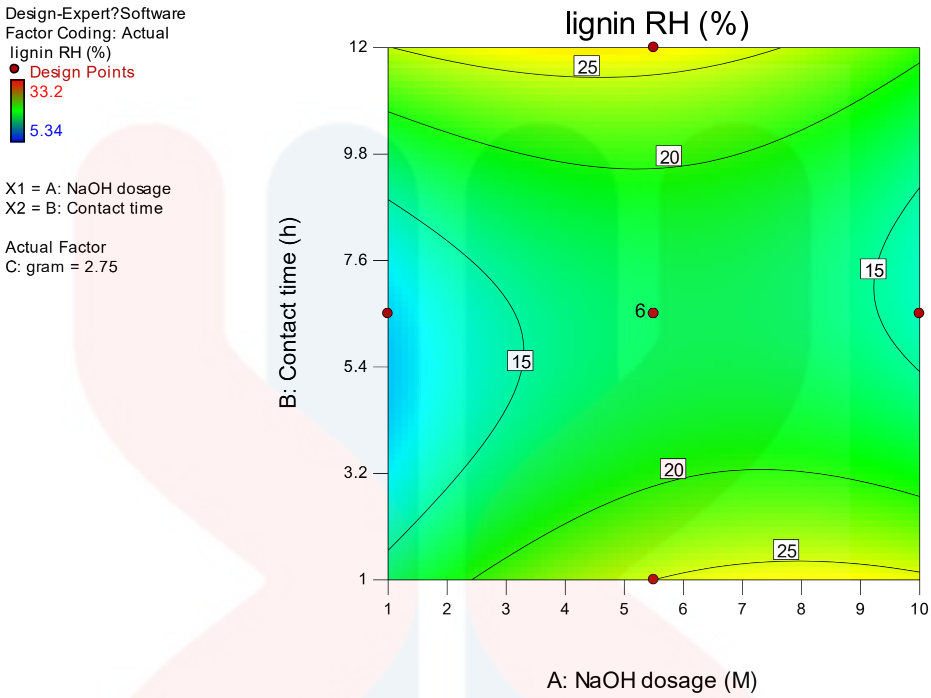


Figure 4.6: (b) 2D contour surface plot of the interaction effect of NaOH concentration (M) and contact time (hours) on lignin removal (%).

4.5.2 Effect of NaOH Concentration and Weight of Sample on Lignin Removal (R1)

Figure 4.7 (a) and 4.7 (b) demonstrated the interactive response of the NaOH concentration (M) and sample weight (g) on lignin removal (%) where the contact time was maintained constant at 6.5 hours. Figure 4.7 (a) and 4.7 (b) displayed the colour from blue to green which means the rice hull lignin removal percentage gradually increases along the increase of NaOH concentration (M) from 1 M to 5.5 M and sample weight (g) from 0.5 g to 2.75 g, respectively. NaOH concentration after 5.5 M does not show an increase in the lignin removal percentage indicated the alkali solution had saturated the rice hull binding site eventually become a barrier for free chemical particles in the alkali solution to be absorbed which lead to low lignin removal (El-Wakil *et al.*, 2015).

On the other hand, the sample weight (g) after 2.75 g do not have shown the increase in lignin removal percentage indicated there is more vacant adsorption site in total surface area obtain in rice hull than provided by the adsorbate in NaOH solution (Nuengmatcha *et al.,* 2014). The maximum rice hull lignin removal percentage for both NaOH concentration and sample weight (g) interaction effect was 20.58% at the optimum condition of 5.5 M NaOH concentration and 2.75 g sample weight with 6.5 hours constant contact time.

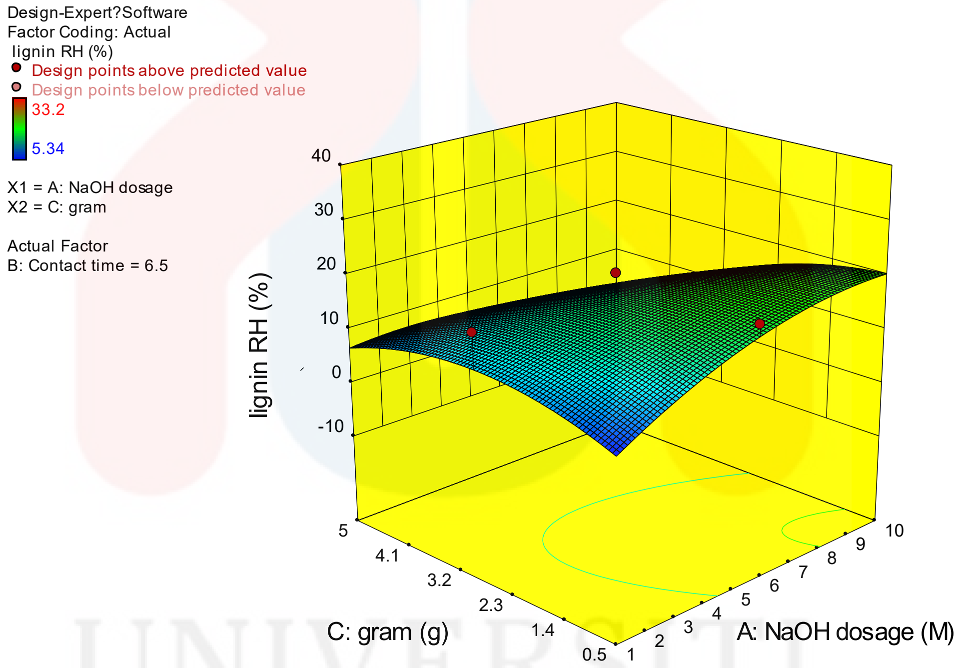


Figure 4.7: (a) 3D response surface graph of the interaction effect of NaOH concentration (M) and sample weight (g) on lignin removal (%).

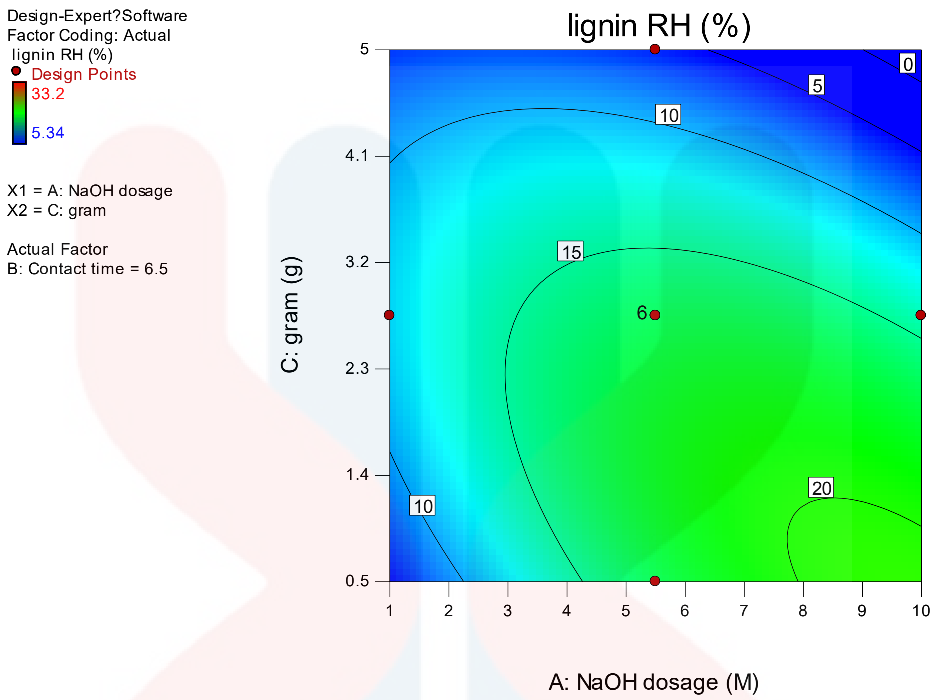


Figure 4.7: (b) 2D contour plot of the interaction effect of NaOH concentration (M) and sample weight (g) on lignin removal (%).

4.5.3 Effect of Contact Time and Weight of Sample on Lignin Removal (R1)

Figure 4.8 (a) and 4.8 (b) demonstrated the interactive response of the contact time (hours) and sample weight (g) on lignin removal (%) where the N was maintained constant at 5.5 M. The figures displayed the rice hull lignin removal percentage increase gradually along the increase of contact time (hours) from 1 hour to 12 hours and sample weight (g) from 0.5 g to 2.75 g, respectively. This indicated that at 2.75 g of the sample had reach equilibrium even in longest contact time in this study and further contact time may be needed to obtain better lignin removal. The maximum rice hull lignin removal percentage for both contact time (hours) and sample weight (g) interaction effect was 28.29% at the optimum condition of 12 hours contact time and 2.75 g sample weight with 5.5 M constant NaOH concentration.

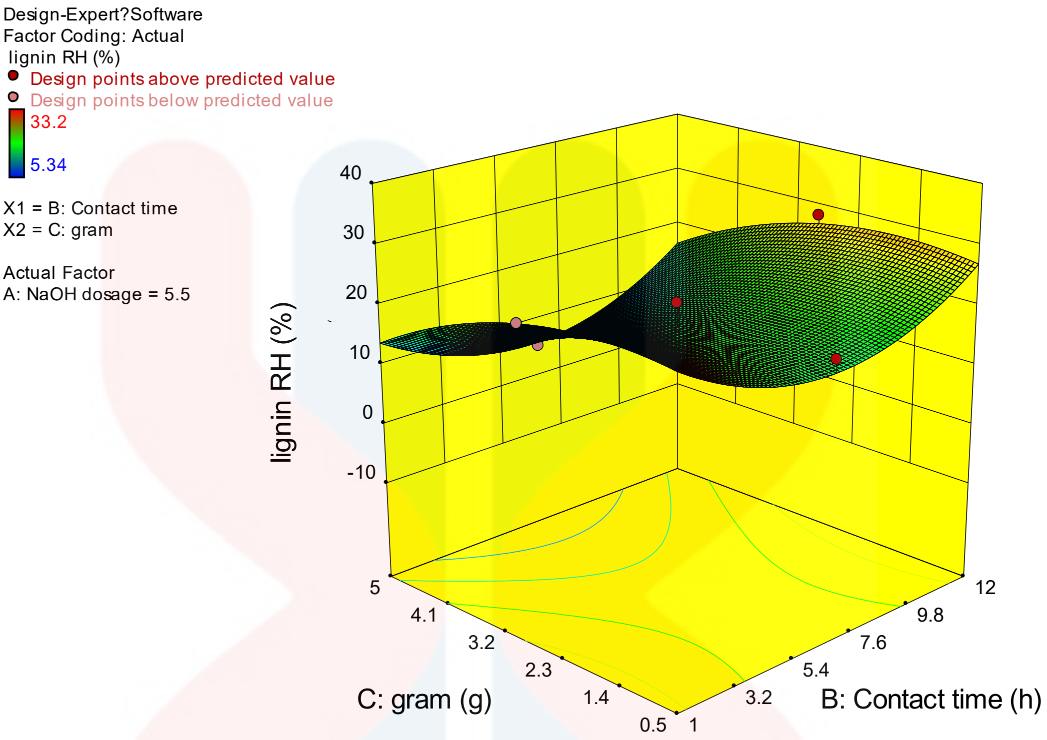


Figure 4.8: (a) 3D response surface graph of the interaction effect of contact time (hours) and sample weight (g) on lignin removal (%).

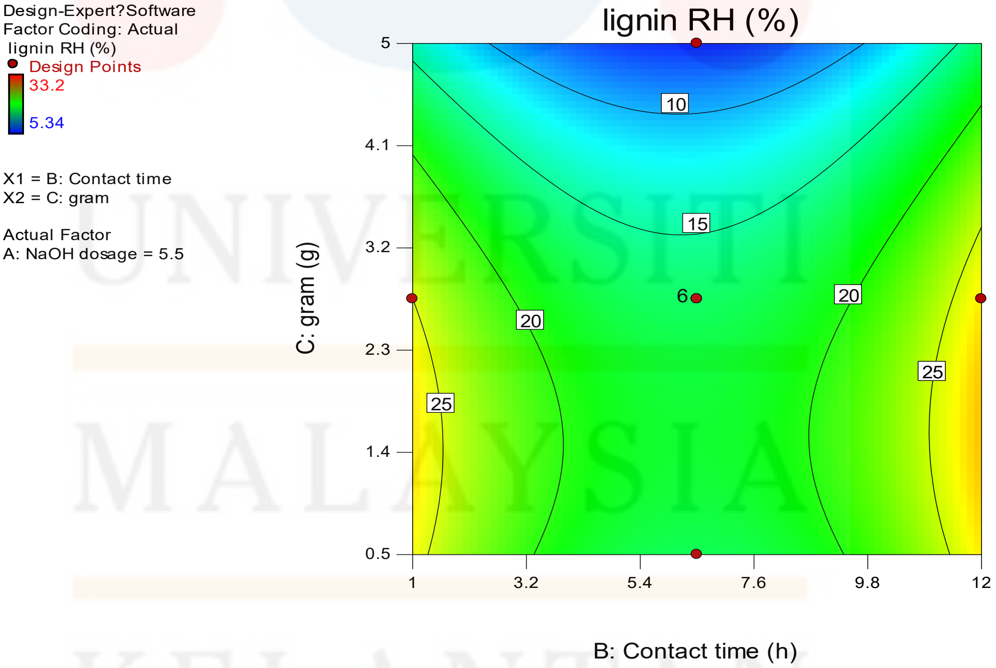


Figure 4.8: (b) 2D contour plot of the interaction effect of contact time (hours) and sample weight (g) on lignin removal (%).

4.6 Numerical Optimisation of Rice Hull using the Desirability Function of R1

Numerical optimisation objectively applied to familiarise with the compromise solution with features accompanied by parameters in the experiment mixed with bias, prediction and solidity compositions (Costa *et al.,* 2011). Desirability function, on the other hand, aided in optimisation process which randomly begins at any starting point thus continue to maximum (JMP Statistical Discovery, 2016). When one response positively shifted, it negatively compromises another response which desirability function might minimise this situation by providing the overall desirable function with the blend and reorganised the responses.

According to Jahani *et al.* (2008), desirability function has a range of 0 to 1 which d = 0 means completely intolerant response or less desirability while d = 1 means favourable response or more desirability. This indicated the value'd' is directly proportional to the desirability response. Experimentally, the best optimum values from the variables are 10 M NaOH concentration, 1 hour contact time, and 0.5 g sample weight with 33.2% rice hull lignin removal percentage. On the other hand, based on the Design Expert Software Version 10, the predicted rice hull lignin removal percentage which is 32.45% share the same condition operate under this set of the environment with the 0.973 desirability near to 1.

Figure 4.9 (a) and 4.9 (b) demonstrated the interactive response of the NaOH concentration (M) and contact time (hours) on lignin removal (%) with 0.5 g weight of sample remain constant. Figure 4.9 (a) which is a 3D response surface graph demonstrated the optimum point of the lignin removal lied near to desirability of 1.0. Figure 4.9 (b) which is a 2D response surface graph demonstrated the desirability extended from blue colour to the red colour as the range of desirability increase zero to one. The highest value of desirability represented by red colour is more than 0.8 desirability which is approximately to 1.

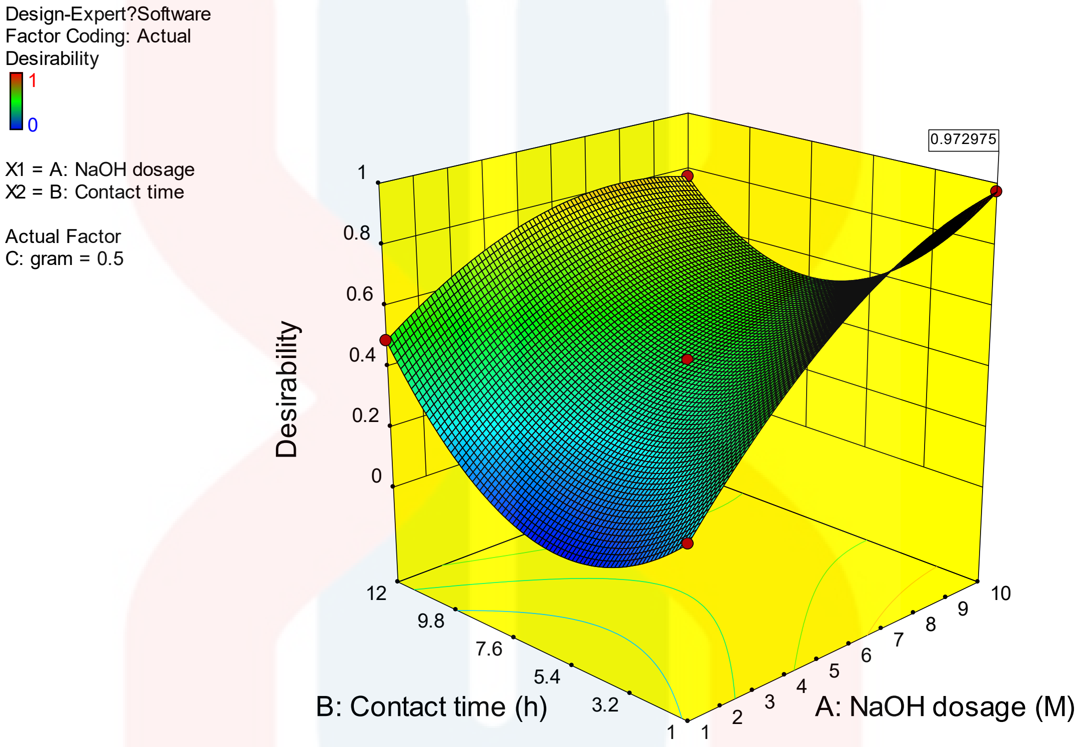


Figure 4.9: (a) 3D response surface graph of the interaction effect of NaOH concentration (M) and contact time (hours) on lignin removal (%).

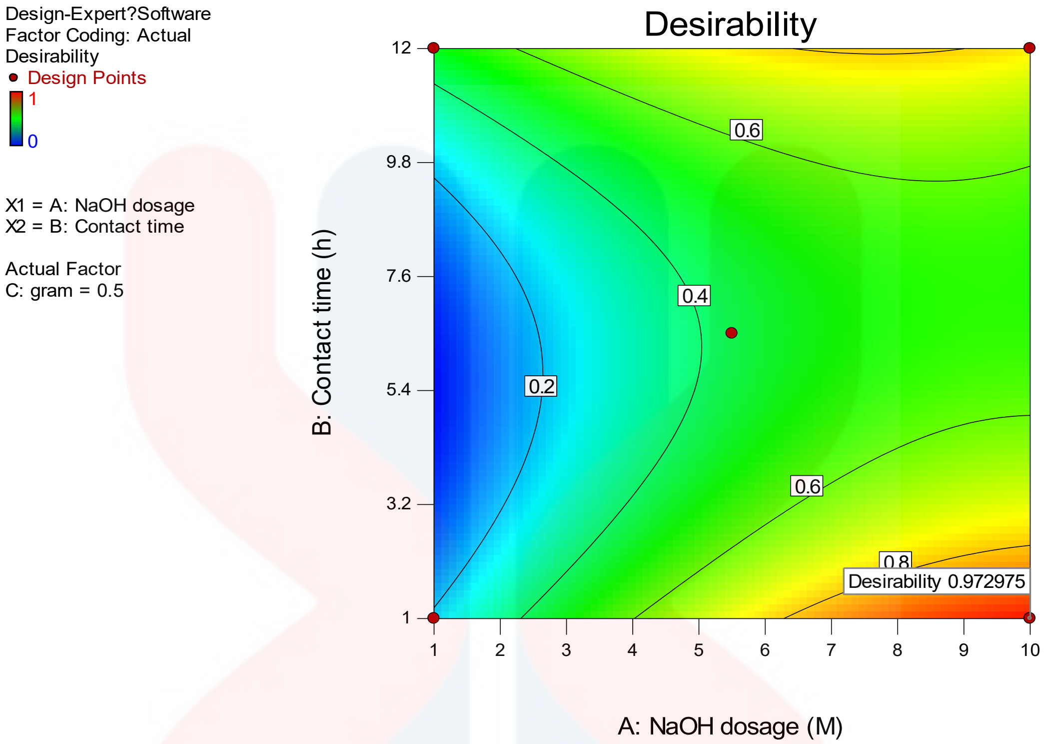


Figure 4.9: (b) 2D contour plot of the interaction effect of NaOH concentration (M) and contact time (hours) on lignin removal (%).

4.7 Development of Regression Model for Coconut Hull (R2)

According to the model summary statistics in Table 4.7, the best model recommended fitting the response among four sources of the model were linear and two-factor interactions (2FI) model. The cubic model was determined as an outlier model as it was labeled aliased.

Table 4.7: Model summary statistics for coconut hull (R2).

Source	**Std. Dev.**	**R-Squared**	**Adjusted R-Squared**	**Predicted R-Squared**	**PRESS**	**Remarks**
Linear	6.72	0.7998	0.7622	0.5863	1493.50	Suggested
2FI	5.55	0.8892	0.8381	0.2046	2871.38	Suggested
Quadratic	4.91	0.9331	0.8730	-0.0805	3900.44	
Cubic	1.69	0.9953	0.9850	-0.6836	6077.72	Aliased

The standard deviation in Table 4.7 for the linear model was 6.72 while the 2FI model was 5.55. The squared (R^2) value for linear model was 0.7998 while 2FI model was 0.8892 which this study indicates proximal to 1.00. The linear model was 79.98% while the 2FI model was 88.92% of response variability can be explained by the model. As mentioned both adjusted R^2 and predicted R^2 desirably have 0.20 differences. Table 4.7 showed 0.7622 for adjusted R^2 and 0.5863 for predicted R^2 in the linear model while 2FI model showed 0.8381 for adjusted R^2 and 0.2046 for predicted R^2 which nearly exceed the reasonable range. Hence, this model demonstrated reasonable agreement among linear as well as 2FI model and the experimental data.

Table 4.8: Standard deviation and quadratic model for R^2 for lignin removal (R2).

Std. Dev.	5.55	R-Squared	0.8892
Mean	24.86	Adj R-Squared	0.8381
C.V. %	22.31	Pred R-Squared	0.2046
PRESS	2871.38	Adeq Precision	16.092

Table 4.8 represents the standard deviation and quadratic model for R^2 for lignin removal (R2) from coconut hull. The value of CV shown was 22.33% which is evaluated as high as it is in the range of 20-30%, indicating low in precision. This indicated the model is reliably fit to the experimental data. Table 4.8 also represents 16.092 for adequate precision value which the value is greater than 4. This indicated the model is qualified to be used.

The empirical polynomial equation also produced by RSM regarded to coded factor. Equation 4.3 displayed as a completed empirical polynomial equation for lignin removal which is vital among the variables relationship towards of lignin removal.

$$\text{CH lignin removal, } Y\ (\%) = 24.87 - 0.12A + 5.94B - 15.92C + 0.88AB - 3.66AC - 5.12BC \quad (4.3)$$

where A: NaOH concentration

B: Contact time

C: Weight of sample

According to Equation 4.1, the positively sign coded of factor B (contact time) and factor AB (NaOH concentration and contact time) has significance positive impact towards the lignin removal. Among the positive sign factors, individual coded factor B has more influence on the response apart from interaction among the variation.

4.8 Statistical Analysis of R2

Table 4.9 shows ANOVA table for the response surface quadratic model of R2. ANOVA was suitable to be used to determine the significance of the model shown in Table 4.9. Smaller p-value than 0.05 (95% confidence level) in this study supported the statistical significance of the model terms. Besides, factor B, C, and BD less than 0.05 show that the model terms were significant. The value excess 0.1000 is insignificant for the model.

Table 4.9: ANOVA table for response surface quadratic model of R2

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	3210.03	6	535.01	17.39	< 0.0001*
A-NaOH dosage	0.15	1	0.15	4.918E-003	0.9452
B-Contact time	353.07	1	353.07	11.48	0.0049
C-gram	2533.83	1	2533.83	82.37	< 0.0001*
AB	6.16	1	6.16	0.20	0.6619
AC	107.31	1	107.31	3.49	0.0845
BC	209.51	1	209.51	6.81	0.0216
Lack of Fit	387.72	8	48.47	19.93	0.0022*

* represents that the value is significant

Table 4.9 presented 17.39 F-value and less than 0.0001 p-values which means there was less than 0.01% chance for large F-value to noise. From the table, factor C (gram) has the largest F value which is 82.37 indicated this was the most vital factor in this model.

4.9 Predicted Values versus Actual Values of R2

Table 4.10 represented the results for actual values, predicted values and standard error of coconut hull. Based on Table 4.10, the most outstanding percentage of lignin removal was 63.60% with the optimum condition of 10 M of NaOH concentration, 12 hours of contact time, and 0.5 g of sample weight In the actual value whereas the predicted value, 56.26% lignin removal were slightly differ from the actual value.

Table 4.10: Results for actual values, predicted values of R2.

Run Order	Coded Factors			Lignin removal (%)	
	A (NaOH concentration)	B (Contact time)	C (Weight of sample)	Actual Value	Predicted Value
1	0	0	0	19.96	24.87
2	0	0	0	20.91	24.87
3	0	0	-1	47.8	40.78
4	-1	1	-1	44.4	47.43
5	-1	1	1	19.54	12.68
6	0	0	0	20.69	24.87
7	1	-1	1	11.16	3.46
8	0	0	0	23.82	24.87
9	0	0	0	23.31	24.87
10	0	-1	0	21.75	18.92
11	-1	0	0	24.91	24.99
12	1	-1	-1	30.2	32.39
13	-1	-1	1	10.12	12.79
14	1	0	0	16.18	24.74
15	-1	-1	-1	31	27.06
16	0	0	1	9.4	8.95
17	0	0	0	22.44	24.87
18	1	1	1	7.6	6.86
19	0	1	0	28.51	30.81
20	1	1	-1	63.6	56.26

Furthermore, Figure 4.10 showed the analysis for the normality of the residuals in order to further evaluate the error terms are distributed normally. It can be stated that the residuals descended moderately around and normally distributed to the straight line which means that the errors distributed normally.

Apart from that, Figure 4.11 showed the interaction of the predicted and actual values of the response of coconut hull lignin removal percentage. It can be stated that the residuals descended approximately to the straight which means that the errors distributed normally. It defined that the predicted value foreseen by CCD model of the Design Expert Software Version 10.0 was sufficiently fitted to the actual experimental run value.

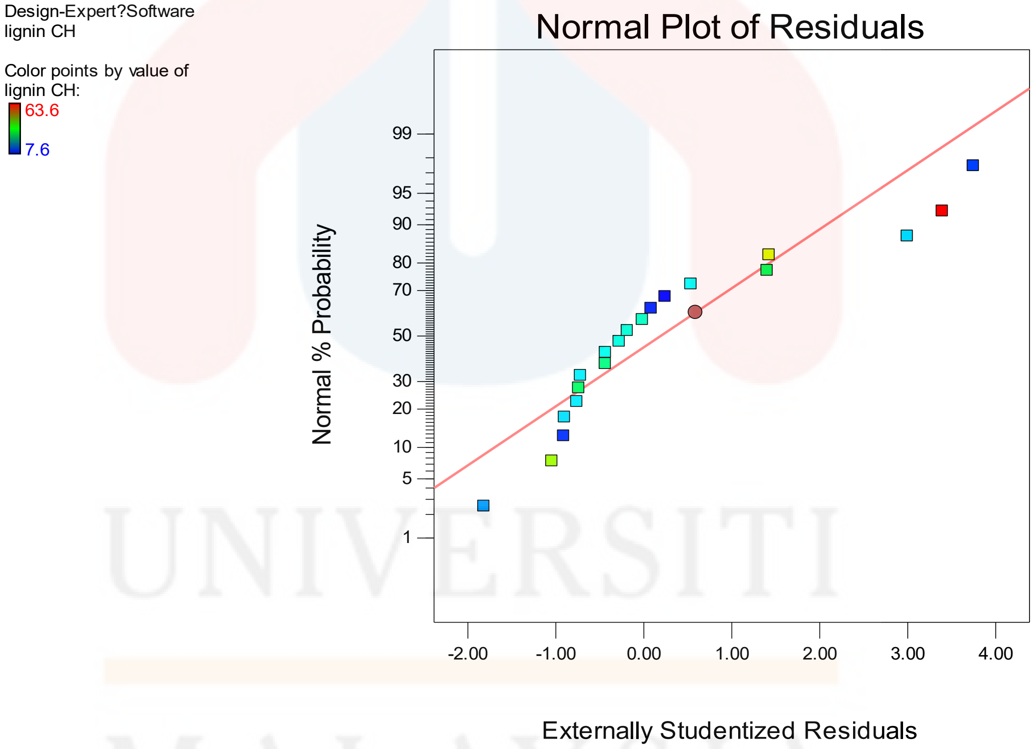


Figure 4.10: Plot of normal % probability versus residual error of lignin removal (R2).

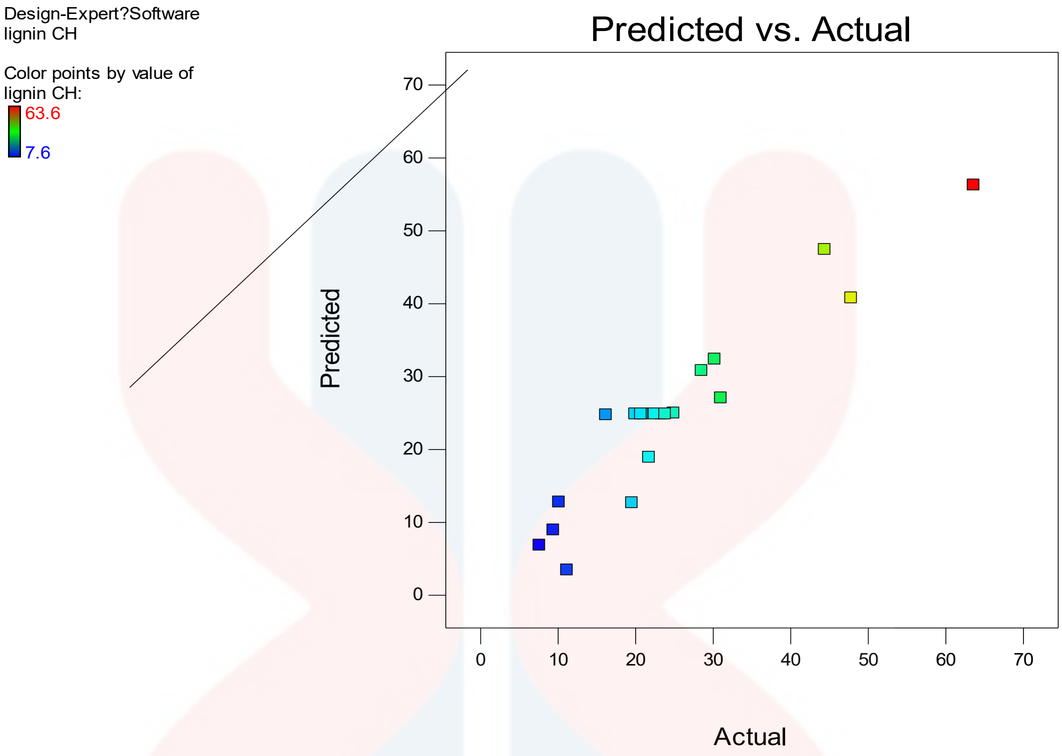


Figure 4.11: Diagnostic plot for predicted versus actual values of lignin removal (R2).

4.9.1 Effect of NaOH Concentration and Contact Time on Lignin Removal (R2)

Figure 4.12 (a) and 4.12 (b) demonstrated the interactive response of the NaOH concentration (M) and contact time (hours) on lignin removal (%) where the sample weight was maintained constant at 2.75 g. This design point also displays from blue zone to red zone which the red zone act as the optimum response value of 63.6%. The figures displayed the coconut hull lignin removal percentage increase gradually along the increase of contact time (hours) from 1 hour to 6.5 hours and NaOH concentration (M) remain constant at 1 M respectively. This means that NaOH concentration possessess less significant on lignin removal percentage if it interacted with the contact time. This is also due to in long contact time at 6.5 hours the lignin removal had reach equilibrium even in the lowest NaOH concentration in this study. Hence, further increases more than 1 M NaOH concentration do not response to the increase in lignin removal percentage.

Saha *et al.* (2010) reported that longer contact time allows the stubborn lignin boundary layer of the sample to degrade which allow high coconut lignin removal percentage. The maximum rice hull lignin removal percentage for both NaOH concentration (M) and contact time (hours) interaction effect was 24.91% at the optimum condition of 1 M NaOH concentration and 6.5 hours contact time with 2.75 g constant weight of the sample.

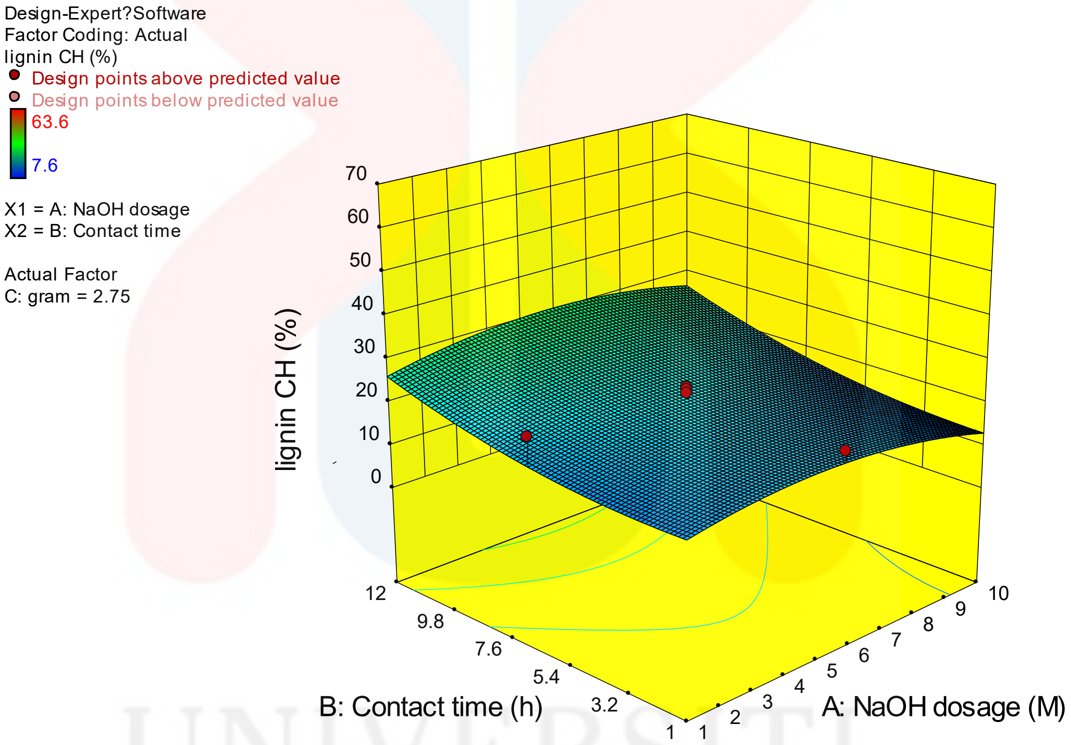


Figure 4.12: (a) 3D response surface graph of the interaction effect of NaOH concentration (M) and contact time (hours) on lignin removal (%).

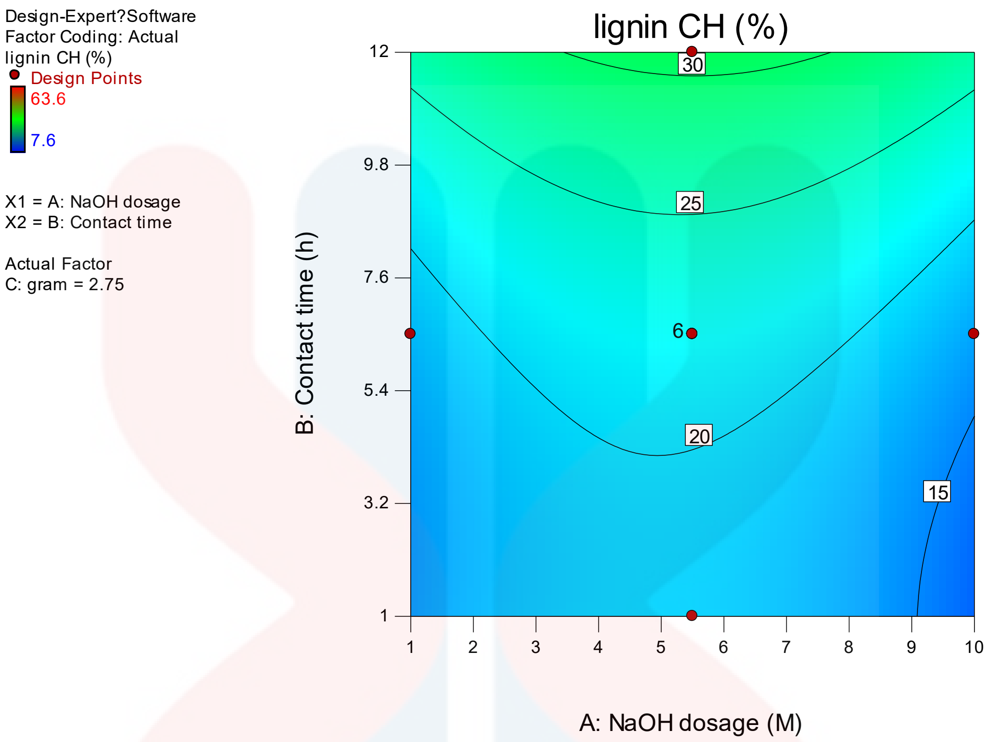


Figure 4.12: (b) 2D contour plot of the interaction effect of NaOH concentration (M) and contact time (hours) on lignin removal (%).

4.9.2 Effect of NaOH Concentration and Weight of Sample on Lignin Removal (R2)

Figure 4.13 (a) and 4.13 (b) demonstrated the interactive response of the NaOH concentration (M) and weight of the sample (g) on lignin removal (%) where the contact time was maintained constant at 6.5 hours. The figures displayed the coconut hull lignin removal percentage increase gradually along the increase of NaOH concentration (M) from 1 M to 5.5 M and sample weight remain constant at 0.5 g respectively. This means that sample weight possess less significant on lignin removal percentage if it interacted with NaOH concentration. Further increase of sample weight above 0.5 g failed to promote higher lignin removal percentage as this may due to NaOH cause the negative effect on the coconut hull binding site which prevents further lignin removal to happened. The maximum rice hull lignin removal percentage for both NaOH concentration (M) and weight of sample (g) interaction effect was 47.80% at the optimum condition of 5.5 M NaOH concentration and 0.5 g weight of sample with constant 6.5 hours contact time.

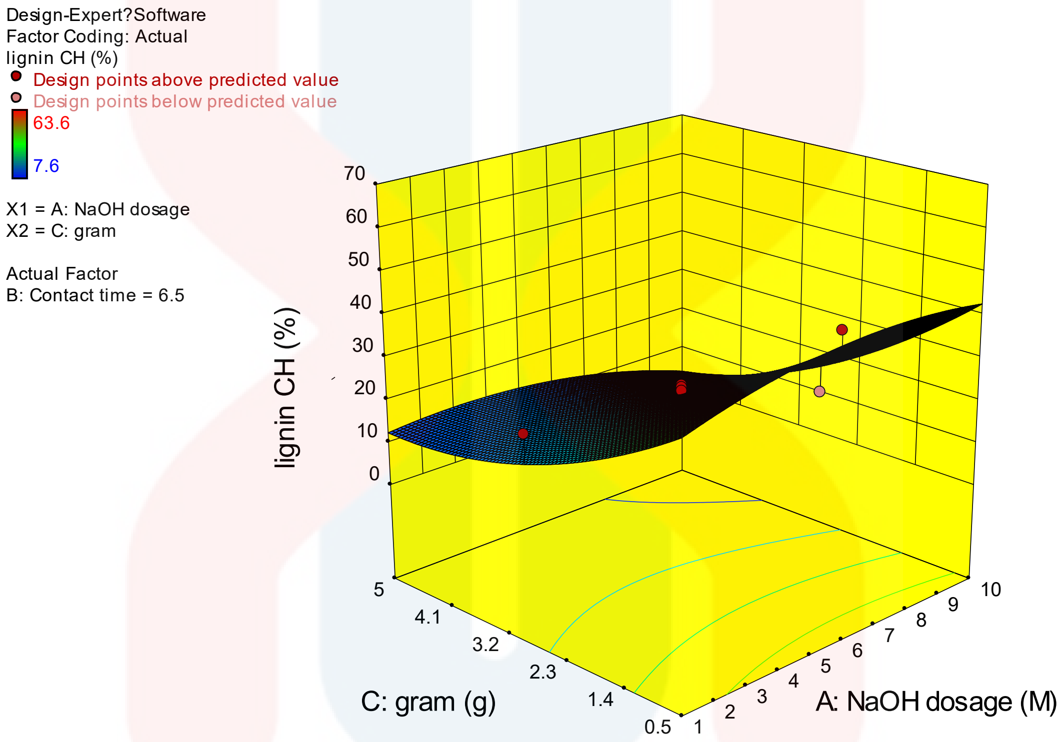


Figure 4.13: (a) 3D response surface graph of the interaction effect of NaOH concentration (M) and sample weight (g) on lignin removal (%).

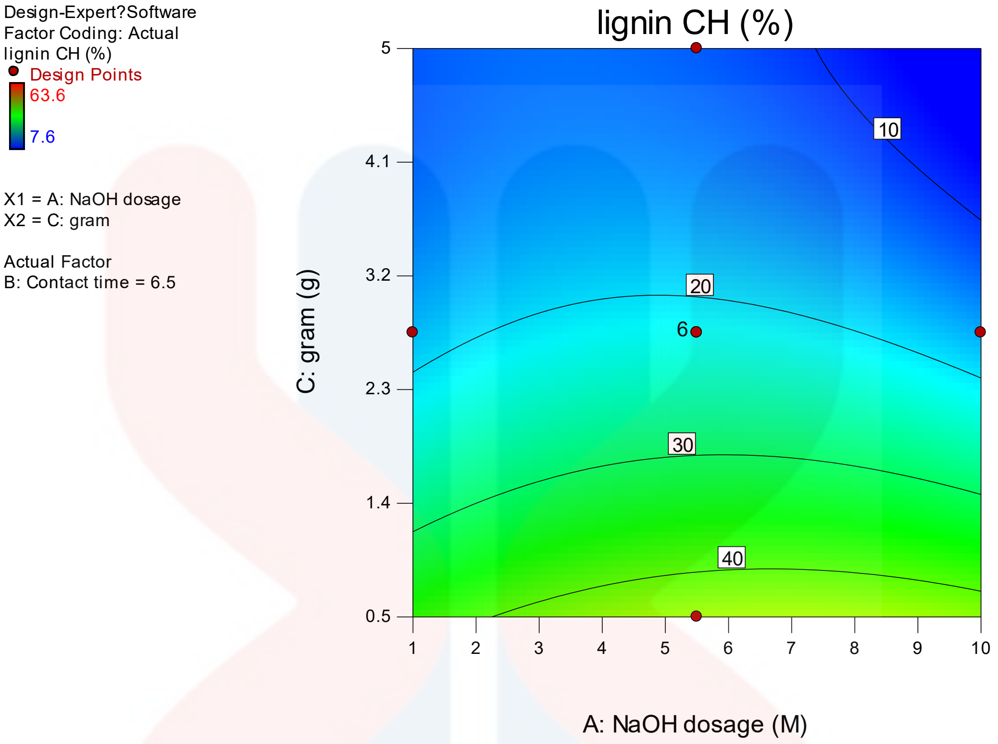


Figure 4.13: (b) 2D contour plot of the interaction effect of NaOH concentration (M) and sample weight (g) on lignin removal (%).

4.9.3 Effect of Contact Time and Weight of Sample on Lignin Removal (R2)

Figure 4.14 (a) and 4.14 (b) demonstrated the interactive response of the contact time (hours) and weight of sample (g) on lignin removal (%) where the NaOH concentration was maintained constant at 5.5 M. The figures displayed the coconut hull lignin removal percentage increase gradually along the increase of contact time from 1 hour to 6.5 hours and sample weight remain constant as 0.5 g respectively. This means that sample weight possess less significant on lignin removal percentage if it interacted with contact time. Longer contact time cause ascending in lignin removal percentage. Hence, contact time act as the driving force to overcome the resistance when lignin transfer out from coconut hull to solution (Hameed & Ahmad, 2009). The maximum rice hull lignin removal percentage for both contact time (hours) and weight of sample (g) interaction effect was 47.80% at the optimum condition of

6.5 hours contact time and 0.5 g weight of sample with constant 0.5 M NaOH concentration.

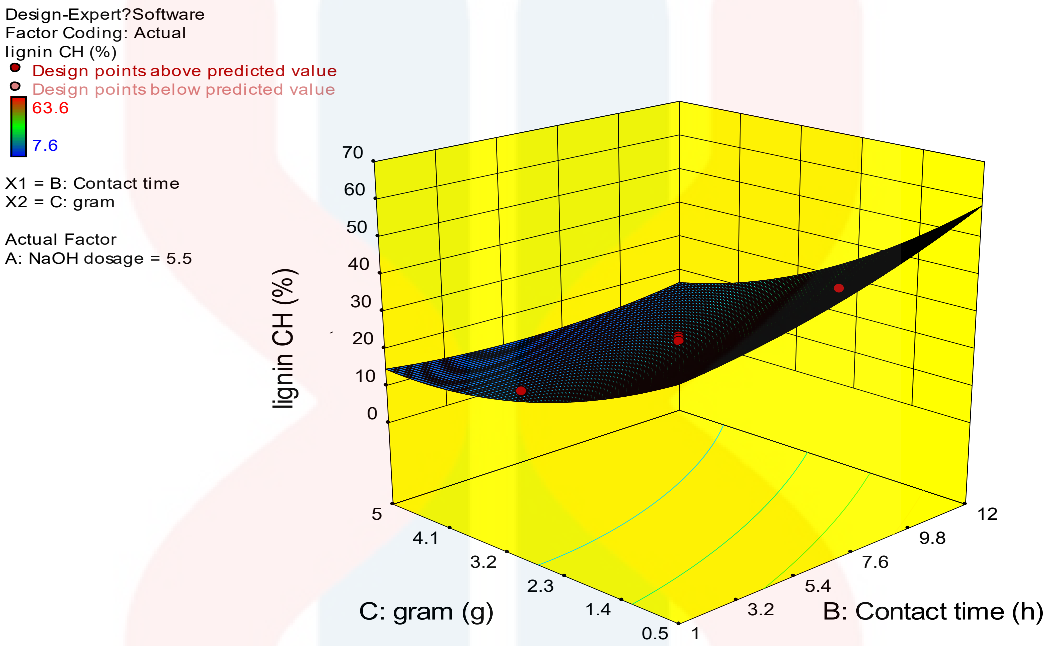


Figure 4.14: (a) 3D response surface graph of the interaction effect of contact time (hours) and sample weight (g) on lignin removal (%).

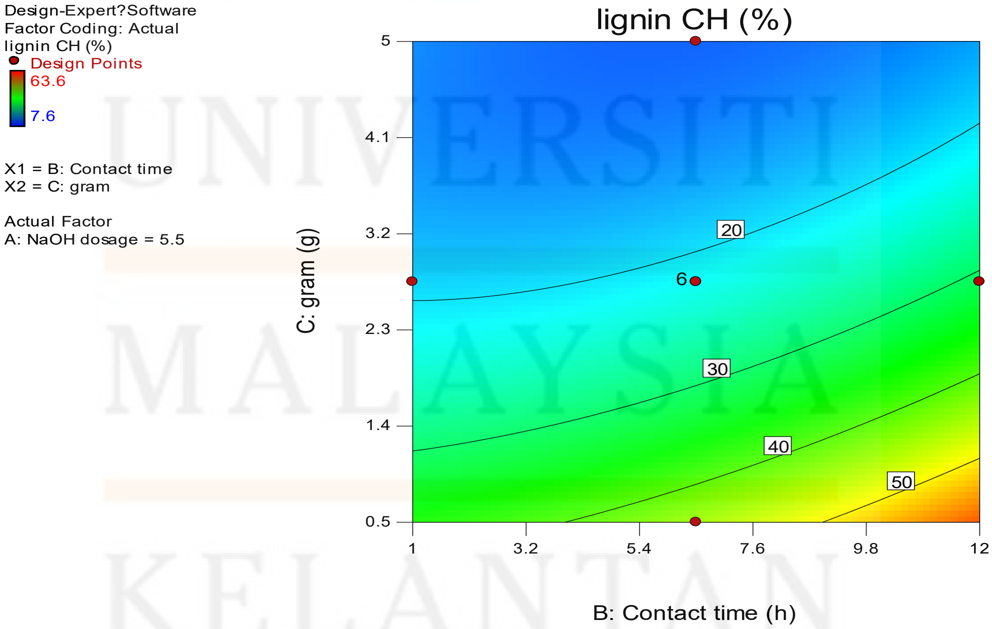


Figure 4.14: (b) 2D contour plot of the interaction effect of contact time (hours) and sample weight (g) on lignin removal (%).

4.10 Optimisation of Rice Hull using the Desirability Function of R2

Experimentally, the best optimum values from the variables are 10 M NaOH concentration, 12 hours contact time, and 0.5 g sample weight with 63.6% coconut hull lignin removal percentage. On the other hand, based on the Design Expert Software Version 10, the predicted coconut hull lignin removal percentage which is 59.47% under the predicted optimum condition operate under this set of environment except for the NaOH concentration of 7.42 M with the 0.926 desirability near to 1. This indicates NaOH concentration is a significant variable in this coconut lignin removal.

Figure 4.15 (a) and 4.15 (b) demonstrated the interactive response of the NaOH concentration (M) and contact time (hours) on lignin removal (%) with 0.5 g weight of sample remain constant. Figure 4.15 (a) which is a 3D response surface graph demonstrated the optimum point of the lignin removal lied near to the desirability of 1.0. Figure 4.15 (b) which is a 2D response surface graph demonstrated the desirability extended from blue colour to the red colour as the range of desirability increase from zero to one. The highest value of desirability represented by red colour is more than 0.9 desirability is approximate to 1.

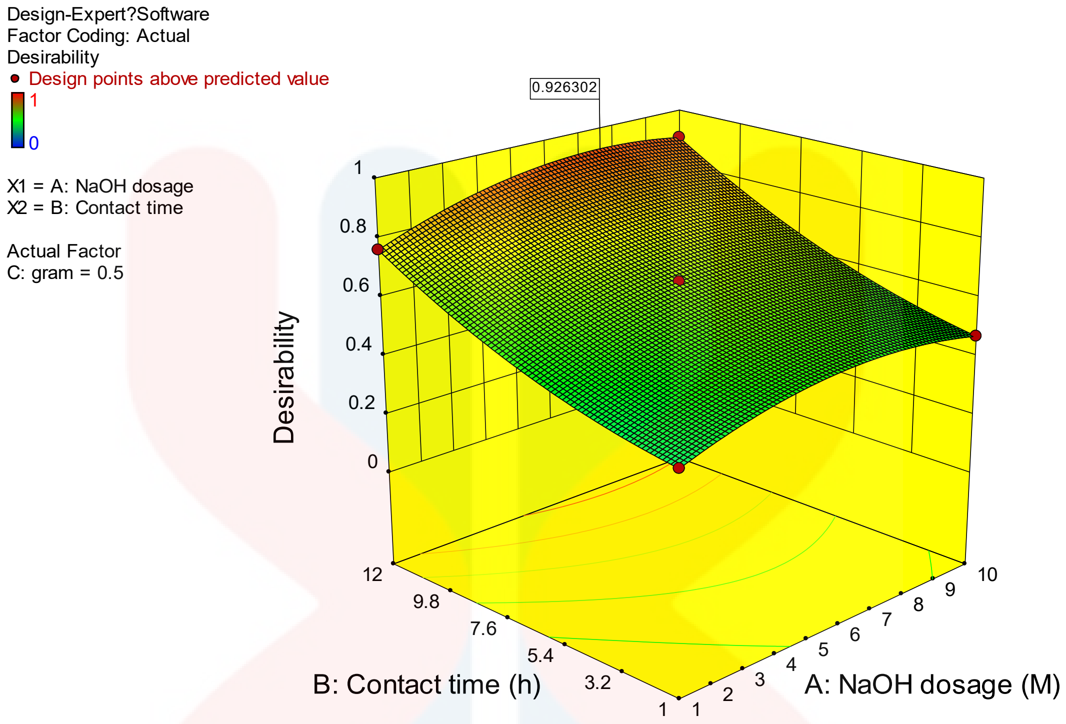


Figure 4.15: (a) 3D response surface graph of the interaction effect of NaOH concentration (M) and contact time (hours) on lignin removal (%).

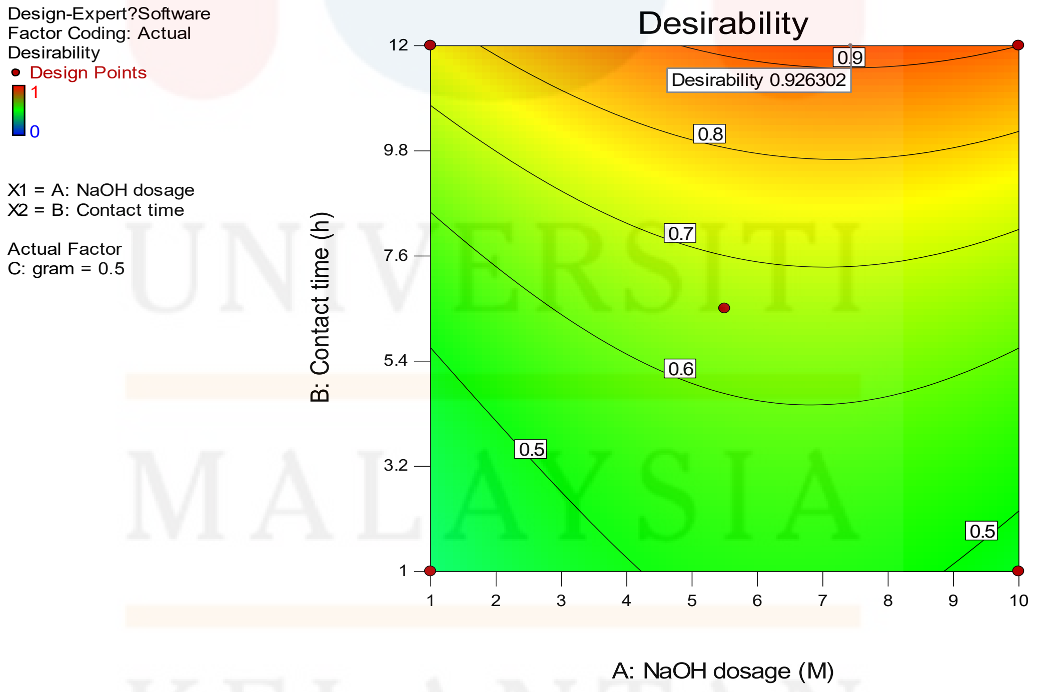


Figure 4.15: (b) 2D contour plot of the interaction effect of NaOH concentration (M) and contact time (hours) on lignin removal (%).

4.11 Physical Characteristic by FTIR Spectra Analysis

The FTIR result confirmed the bond structure of certain functional groups (hemicellulose, cellulose, and lignin) in rice hull and coconut hull which were removed and changed due to the alkaline pretreatment. The functional group existed at the peak between the frequencies was observed. Table 4.11 showed the FTIR absorbance of typical lignin component in biomass. Among the functional group, the significance chemical functional group to determine the accuracy of the lignin existence were hydroxyl (-OH), methoxyl ($O-CH_3$), carboxyl (-COOH), and carbonyl (C=O) groups (Gosseling *et al.,* 2004; Shamsuri & Abdullah, 2010). Mansouri and Salvad´o (2007) mentioned that the lignin functional group include phenolic and aliphatic hydroxyl, carbonyl, methoxyl, carboxyl and sulfonate groups. Chemical pretreatment able to minimise the content (hemicellulose, lignin, and cellulose) in rice hull so that the specific area of the surface on rice hull can be improved (Daffalla *et al.*, 2010).

Table 4.12 showed the FTIR spectra identification of the untreated rice hull and Figure 4.16 showed FTIR spectra identification of the treated rice hull. Pretreated rice hull showed absorption peak decrease after pretreatment process from standard untreated rice hull, 3403.31 cm^{-1} to around 3334.06 cm^{-1} indicated the presence of stretched -OH group and the amine group in pretreated rice hull. This means there was the reaction of NaOH with either phenolic or aliphatic functional group in the fiber that enhances free hydroxyl that produces free hydroxyl bond structure.

The stretching of C=C vibration of 1632.49 cm^{-1} indicates alkenes and aromatic functional groups. The peak in 475.10 cm^{-1} showed the presence of -Si-H group. The peak in 1026.49 cm^{-1} indicates the presence of C=O ester group, a C-O ether group, and an alkyl halide group. Hinterstoisser *et al.* (2001) and Xu *et al.* (2013)

agreed that among 1100 cm^{-1} to 1000 cm^{-1} there was glycosides linkage in a C-O stretching bond structure in the functional group which this linkage can be found in the lignin. The peak around 456.48 cm^{-1}, 447.76 cm^{-1}, 438.38 cm^{-1}, 429.08 cm^{-1}, 418.10 cm^{-1}, and 409.75 cm^{-1} showed the presence of -OCH_3 which have lower wavelength than untreated rice hull (580 cm^{-1}) which could be found in the lignin (Ralph *et al.,* 1992).

Table 4.11: FTIR absorbance of typical lignin component in biomass (Sills & Gossett, 2012; Xu, 2016).

Wavenumber (cm^{-1})	**Functional group**	**Component**
1035	C-O, C=C, and C-C-O stretching	Cellulose, hemicellulose, lignin
1215	C-C+C-O stretching	Lignin
1270	Aromatic ring vibration	Guaicyl lignin
1327	C-O stretching of syringyl ring	Lignin
1335	C-H vibration, O-H in-plane bending	Cellulose, hemicellulose, lignin
1380	C-H bending	Cellulose, hemicellulose, lignin
1425	C-H in-plane deformation	Lignin
1440	O-H in-plane bending	Cellulose, hemicellulose, lignin
1465	C-H deformation	Lignin
1500	Aromatic ring vibration	Lignin
1595	Aromatic ring vibration +C=O stretching	Lignin
1682	C=O stretching (unconjugated)	Lignin
2840,2937	C-H stretching	Lignin
3421	O-H stretching	Lignin

Table 4.12: FTIR spectra identification of the untreated rice hull (Daffalla *et al.*, 2010).

Wavelength Number (cm^{-1})	**Functional Group**
3403.31	-OH and Si-OH
2925.81	C-H stretching of alkanes
1641.31-1737.74	C=O stretching of aromatic groups
1546.8-1652.88	C=C stretching of alkenes and aromatic
1461.94	CH_2 and CH_3
1379.01	Aromatic CH and carboxyl-carbonate
1238.21	CHOH stretching of alcohol group
1153.35-1300	CO group in lactones
1080-1090	Si-O-Si
935.41	C-C
469-800	Si-H
580-34	$-OCH_3$

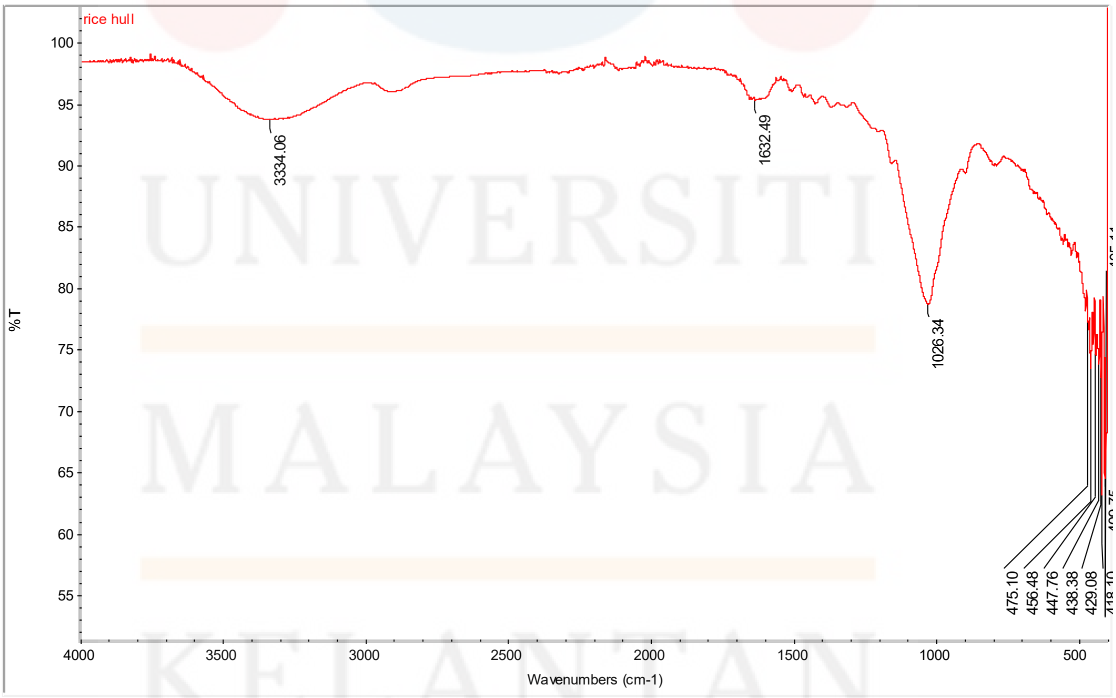


Figure 4.16: FTIR spectra identification of the treated rice hull.

Table 4.13 showed the FTIR spectra identification of the untreated coconut hull and Figure 4.17 showed the FTIR spectra identification of the treated coconut hull. The peak around 3630 cm^{-1} to 2980 cm^{-1} for untreated coconut hull were reduced to 3288.61 cm^{-1} in treated coconut hull indicates the presence of alcohol OH stretching were undergo bond structure break down after pretreatment thus there was carbohydrate from hemicellulose and cellulose (Ramadevi *et al.,* 2012). The C-H stretching vibration peak at 2900 cm^{-1} for untreated coconut hull to around 2922.47 cm^{-1} for treated coconut hull confirmed the alkane (cellulose and lignin) functional group emerged. The peak around C-H stretching region suggested the presence of methyl (CH_3), methylene (CH_2), as well as aliphatic saturated (CH) functional group (Khalil *et al.,* 2013).

The untreated coconut hull peak at 1700 cm^{-1} reduced to the treated coconut hull of C=O stretching vibration around 1605.74 cm^{-1} indicate the presence of carbonyl group with stretching of an ester linkage between carboxylic groups of lignin and hemicellulose. This decrease indicates the partly eradication of lignin and hemicellulose in this effective pretreatment process. The peak around 1412.70 cm^{-1} indicates the presence of C=C stretching aromatic functional group and a -C-H bending bond from alkane functional group which consist of cellulose, hemicellulose, and lignin. The peak at 1015.19 cm^{-1} C-F alkyl halide group and C-O ether group indicates the presence of glycosides linkage functional group in lignin structure (Hinterstoisser *et al.,* 2001). The peak around 525.74 cm^{-1} and 479.85cm^{-1} indicates the presence of Si-H group.

Table 4.13: FTIR spectra identification of the untreated coconut hull (Torres *et al.*, 1992).

Wavelength Number (cm^{-1})	**Functional Group**
3630-2980	-OH stretching vibration
2900	C-H stretching vibration
1690	C=O stretching vibration
1600, 1500, 1440	Aromatic ring
1370	C-H wagging vibration
1260	Aromatic ring guaiacyl unit stretching vibration
1210	C-O stretching vibration
1110	C-O-C stretching vibration
1020	C-O wagging vibration

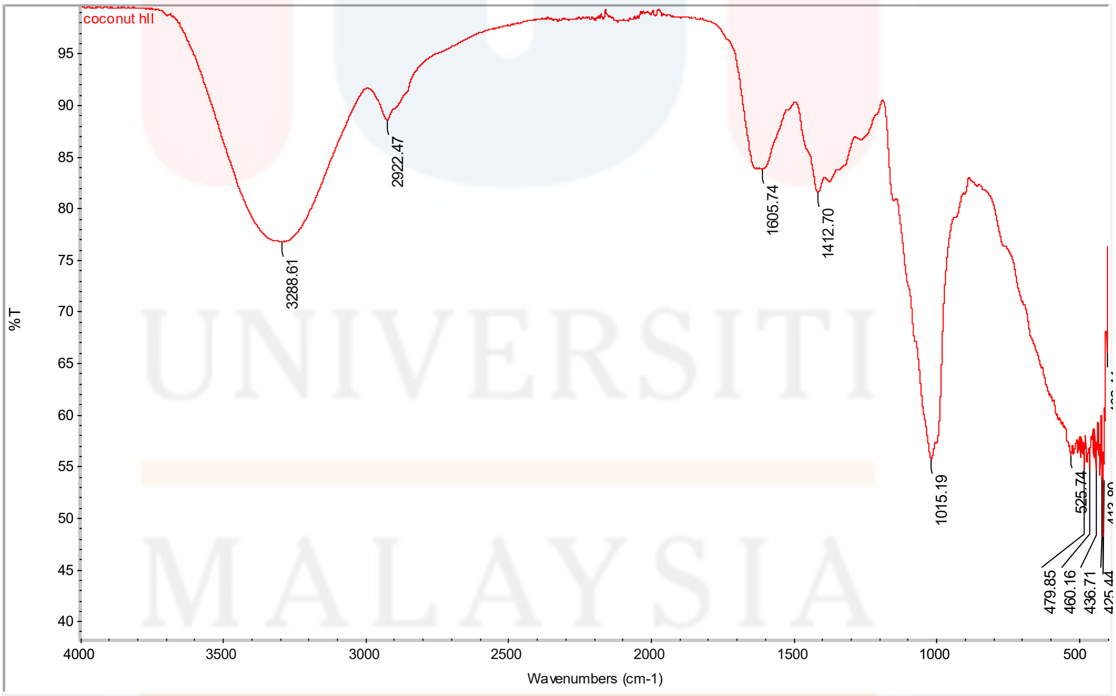


Figure 4.17: FTIR spectra identification of the treated coconut hull.

4.12 Comparison of Different Types of Alkali Solution in Alkali Pretreatment

Table 4.14 showed results for lignin removal of different alkali solution in R1 (rice hull removal) and Table 4.15 showed results for lignin removal of different alkali solution in R2 (coconut hull removal). Both Table 4.14 and Table 4.15 showed that NaOH solution was the optimum alkali solution to used in this lignin removal pretreatment among KOH and Ca $(OH)_2$ as the lignin removal percentage in rice hull using NaOH is 33.2%, KOH is 31.8%, and $Ca(OH)_2$ is 20.2% while in coconut hull lignin removal percentage using NaOH is 63.6%, KOH is 58.6%, and $Ca(OH)_2$ is 25.8%.

According to Cheng *et al.* (2010), Ca $(OH)_2$ is less preferable compared NaOH as it needs more water during the pretreatment and performed poorly in pretreatment process to remove lignin. Chang *et al.* (2017) supported that compared to NaOH, Ca $(OH)_2$ was less preferable due to it lignin recovered is less easily at room temperature. Among the hydroxyl reagent such as potassium, calcium, as well as the ammonium salt, NaOH solution was the most effective mild-alkali in alkali pretreatment (Kumar & Wyman, 2009).

Table 4.14: Results for lignin removal of different alkali solution in R1.

Types of alkali solution	Alkali concentration (M)	Contact time (h)	Sample weight (g)	Lignin removal in rice hull (%)
NaOH	10	1	0.5	33.2
KOH	10	1	0.5	31.8
$Ca(OH)_2$	10	1	0.5	20.2

Table 4.15: Results for lignin removal of different alkali solution in R2.

Types of alkali solution	Alkali concentration (M)	Contact time (h)	Sample weight (g)	Lignin removal in coconut hull (%)
NaOH	10	12	0.5	63.6
KOH	10	12	0.5	58.6
$Ca(OH)_2$	10	12	0.5	25.8

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Rice hull and coconut hull were served as a functional low cost and high accessibility alternative feed after being pretreated using NaOH pretreatment. This study revealed rice hull and coconut hull from agricultural waste showed high performance for lignin removal of rice hull and coconut hull from alkali solution.

The lignin contained in rice hull and coconut hull was investigated by the application of experimental design known as response surface methodology (RSM) with the help of two level three factor full factorial central composite design (CCD). The fitness of the model was confirmed using correlation coefficient R^2 which were 0.8863 for rice hull lignin removal and 0.8892 for coconut hull lignin removal.

The lignin removal from solution show preferences when increasing the NaOH concentration where more adsorption site provided for adsorbate to bind. The lignin removal from solution also favours increase of the contact time which allows all the adsorption process completed. The lignin removal from the solution also prefers low amount of sample weight so that more absorption chances were provided but not be shielded.

For rice hull lignin removal percentage, the result showed that the quadratic model affects the removal the most while for the coconut hull lignin removal percentage, the linear and 2FI model showed better lignin removal. The individual effect of factor A and B, as well as interaction effect of factor BC more significant for percentage of lignin removal in rice hull while Individual effect of factor B as well as interaction effect of factor AB more significant for the percentage of lignin removal in coconut hull.

Based on the experimental data, the optimum percentage of rice hull lignin removal was 33.2% under optimum conditions of 10 M of NaOH concentration, 1 hour of contact time, and 0.5 g sample weight. For the coconut lignin removal the percentage was 63.6% under optimum conditions of 10 M of NaOH concentration, 12 hours of contact time, and 0.5 g sample weight.

Based on the predicted model generated by Design Expert Software Version 10.0, the optimum percentage of rice hull lignin removal was 32.45% under optimum conditions of 10 M of NaOH concentration, 1 hour of contact time, and 0.5 g sample weight. For the coconut lignin removal was 56.26% under optimum conditions of 10 M of NaOH concentration, 12 hours of contact time, and 0.5 g sample weight. Thus, the optimum condition suggested by the predicted model is as same as the optimum condition in experimental data.

In conclusion, the rice hull and coconut hull could be considered as the alternative ruminant feed materials after lignin removal by pretreatment using NaOH solution.

5.2 Recommendation

Extensive studies could be implemented for thorough the understanding the adsorption mechanism and rice hull as well as coconut hull effectiveness. Scanning Electron Microscope (SEM) able to scans the electrons beam gives the surface morphological image of the rice hull and coconut hull. The untreated rice and coconut hull should also be tested through FTIR in order to compare with the treated results more specifically. Next, every run in the experiment should be triplicate in order to get the average value as well as to minimise the experimental error.

Besides, other pretreatment style could be applied for instance different mechanical pretreatment (milling and ultrasound), chemical pretreatment (liquid hot water, weak acid hydrolysis, alkaline hydrolysis, organosolv, oxidative delignification, and room temperature ionic liquid), combined chemical and mechanical pretreatment (steam explosion, ammonia fibre explosion, CO_2 explosion), and biological pre-treatment worth to be tried.

Other parameters for instance effect of temperature, sample size, as well as pH towards efficiency of lignin removal can be investigated. Other agricultural waste like banana stem, coconut front, coconut trunk, pineapple crown can be studied instead of rice hull and coconut hull.

In order to reduce the error obtained in the study, it is important to have standard sample size before conducting the experiment. The inconsistent size of the sample can be cut and blend into the standard size. This may ensure the hot plate to agitation smoothly with constant speeds without the barrier to stop the agitation process throughout the experimental process.

REFERENCES

Ahuja, V. (2012). Asian livestock. *Agriculture Group.* Thailand. Regional Office for Asia and the Pacific.

Al-Khatib, S. M., Arshad, A., Balk, E. M., Das, S. R., Hsu, J. C., Joglar, J. A., & Page, R. L. (2015). Risk Stratification for arrhythmic events in patients with asymptomatic pre-excitation: a systematic review for the 2015. *ACC/AHA/HRS Guideline for the Management of Adult Patients With Supraventricular Tachycardia, 133*(14), 575-586.

Almeida, M.B., Erthal, R.S., & Padua, E.O. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, 76, 965–977.

Alonso, D. M., Wettstein, S. G., & Dumesic, J. A. (2013). Gamma-valerolactone, a sustainable platform molecule derived from linocellulosic biomass. *Green ChemIstry,15*(3), 584-595.

Arau, P. D., Ferreira, J., Aguiar, C., & Seabra-gomes, R. (2005). TIMI, PURSUIT , and GRACE risk scores : sustained prognostic value and interaction with revascularization in NSTE-ACS. *European Heart Journal*, *26*, 865–872.

Ávila-Lara, A. I., Camberos-Flores, J. N., Mendoza-Pérez, J. A., Messina-Fernández, S. R., Saldaña-Duran, C. E., Jimenez-Ruiz, E. I., & Pérez-Pimienta, J. A. (2015). Optimization of alkaline and dilute acid pretreatment of agave bagasse by response surface methodology. *Frontiers in Bioengineering and Biotechnology*, *3,* 146.

Bakri, M. K. B., & Jayamani, E. (2016). Comparative study of functional groups in natural fibers: fourier transform infrared analysis (FTIR). *Faculty of Engineering, Computing and Science*, *3*(1), 2394-0697.

Banerjee, S., & Chattopadhyaya, M. C. (2013). Adsorption characteristics for the removal of a toxic dye , tartrazine from aqueous solutions by a low cost agricultural by-product. *Arabian Journal of Chemistry. 2*(3), 734.

Barakat, A., de Vries, H., & Rouau, X. (2013). Dry fractionation process as an important step in current and future lignocellulose biorefineries: a review. *Bioresour Technology, 134,* 362–373.

Baumberger, N., Ringli, C., & Keller, B. (2001). The chimeric leucine-rich repeat/extensin cell wall protein LRX1 is required for root hair morphogenesis in *Arabidopsis thaliana*. *Genes & Development, 15*(9):1128-39.

Bender, F., Heaney, D. P., & Bowdess, A. (2000). Potential of steamed wood as a feed for ruminants. *Forest Production Journal 20,* 364.

Biglari, H., Javan, N., Khosravi, R., & Zarei, A. (2016). Direct blue 71 removal from aqueous solutions by adsorption on pistachio hull waste:equilibrium, kinetic and thermodynamic studies. *Iranian Journal of Health Sciences*, *4*(2), 55–70.

Box, G. E. P., & Wilson, K. B. (1951). On the experimental attainment of optimum conditions. *Journal of the Royal Statistical Society. Series B (Methodological), 13* (1), 1-45.

Breitkopf, P., & Filomeno, C. R. (2010). Multidisciplinary design optimization in computational mechanics. *ISTE/Wiley, Chippenham*, *1,*549.

Bruns, R.E., Scarminio, I.S., & Neto, B.B. (2006). Statistical design – chemometrics, *Organic Process Research and Development, 10*(5), 422.

Carpita, N., & McCann, M. (2000). The cell wall, in: Biochemistry and molecular biology of plants. *American Society of Plant Physiologists,* Rockville, MD, 61.

Chakraborty, S., De, S., Dasgupta, S., & Basu, J. K. (2005). Adsorption study for the removal of a basic dye: experimental and modeling. *Chemosphere, 58,* 1079–1086. http://doi.org/10.1016/j.chemosphere.2004.09.066

Chan, E., & Elevitch, C.R. (2006). *Cocos nucifera* (coconut)-Species profiles for Pacific Island agroforestry. *Permanent Agricultural Resources, 2*(1), 1-27.

Chang, V. S. & Holtzapple, M.T. (2000). Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochemistry and Biotechnology*, 5(37), 84-86.

Chang, M. L., Li, D. N., Wang, W.,Chen, D. C., Zhang, Y. Y., Hu, H. W., & Ye, X. F. (2017). Comparison of sodium hydroxide and calcium hydroxide pretreatments on the enzymatic hydrolysis and lignin recovery of sugarcane bagasse. *Bioresource Technology,* 224.

Chen, S., Zhang, X., Singh, D., Yu, H., & Yang, X. (2010). Biological pretreatment oflignocellulosics: potential, progress and challenges. *Biofuels, 1,* 177–199.

Cheng, Y. S., Zheng, Y., Yu, C. W., Dooley, T. M., Jenkins, B. M., & VanderGheynst, J. S. (2010). Evaluation of high solids alkaline pretreatment of rice straw. *Applied Biochemistry and Biotechnology*, *162*(6), 1768–1784.

Chowdhury, S., Mishra, R., Saha, P., & Kushwaha, P. (2011). Adsorption thermodynamics, kinetics and isosteric heat of adsorption of malachite green onto chemically modified rice husk. *Journal of Desalination, 265*,159-168.

Chun, S., Hui, T., Keat, T., & Brosse, N. (2011). Evaluation and optimization of organosolv pretreatment using combined severity factors and response surface methodology. *Biomass and Bioenergy, 35,* 4025–4033.

Cornu, A., Besle, J. M., Mosoni, P., & Grenet, E. (1994). Lignin-carbohydrate complexes in forages: structure and consequences in the ruminal degradation of cell-wall carbohydrates. *Reproduction Nutrition Development, EDP Sciences*, *34* (5), 385-398.

Costa, N. R., Lourenço, J., & Pereira, Z. L. (2011). Desirability function approach: a review and performance evaluation in adverse conditions. *Chemometrics and Intelligent Laboratory Systems*, *107*(2), 234–244.

Daffalla, S.B., Mukhtar, H., and Shaharun, M.S. (2010). Characterization of adsorbent developed from rice husk: effect of surface functional group on phenol adsorption. *Journal of Applied Sciences*, *10*, 1060-1067.

Deschamps, G., Caruel, H., Borredon, M. E., Bonnin, C., & Vignoles, C. (2003). Oil removal from water by comparison with other cotton fiber- based sorbents. *Environmental Science and Technology, 37,* 1013–1015.

Dikeman, C. L., Murphy, M. R., & Fahey, G. C. (2006). Dietary fibers affect viscosity of solutions and simulated human gastric and small intestinal digesta. *Journal of Nutrition, 136*, 913-919.

Ding Y. M. (2015, May 11). Malaysia Go Green-Wood and Sustainability [Blog post]. Retrieved from http://www.fdmasia.com/index.php/wood-sustainability/item/316-malaysia-goes-green

Dong, X., Dong, M., Lu, Y., Turley, A., Jin, T., & Wu, C. (2011). Antimicrobial and antioxidant activities of lignin from residue of corn stover to ethanol production. *Industrial Crops and Products, 34*(3), 1629–1634.

Dorey, F. (2010). In brief-the P value. *Clinical Orthopaedics and Related Research*, 468, 2297–2298.

Draman, S. F. S., Daik, R., Latif, F. A., & El-Sheikh, S. M. (2014). Characterization and thermal decomposition kinetics of kapok (Ceiba pentandra L.)-Based Cellulose, *Bioresources, 9,* 8-23.

Durh, N., Rodriguez, J., Gomez, E., Campos, V., & Baeza, J. (2008). Dynamic FTIR study on native cellulose. *Carbohydrate Research*, *334*(1), 27-37.

El-Wakil, A., Abou, E.-M. W., & Oudah, A. A. A.-R. O. (2015). methylene blue dye removal from aqueous solution using several solid stationary phases prepared from papyrus plant. *Analytical & Bioanalytical Techniques*, *1*(13), 1–7.

FAOSTAT (2010). FAO database, Rome. Available at: http://faostat.fao.org/ retrieved from March 11 2017.

Fatmawati, A., Agustriyanto, R., & Liasari, Y. (2013). Enzymatic hydrolysis of alkaline pre-treated coconut coir. *Bulletin of Chemical Reaction Engineering & Catalysis, 8* (1), 34-39.

Feedipedia. (2016). Animal feed resources information system, http://www.feedipedia.org.

Garg, U. K., Kaur, M. P., Sud, D., & Garg, V. K. (2009). Removal of hexavalent chromium from aqueous solution by adsorption on treated sugarcane bagasse using response surface methodological approach. *Journal of Desalination, 249*(2), 475-479.

Ghafari, S., Abdul, H., Hasnain, M., & Akbar, A. (2009). Application of response surface methodology (RSM) to optimize coagulation – flocculation treatment of leachate using poly-aluminum chloride (PAC) and alum. *Journal of Hazardous Materials*, *163,* 650–656.

Giddel, M. R., & Jivan, A. P. (2007). Waste to wealth, potential of rice husk in India a literature review, *International Conference on Clear Technologies and Environmental Management PEC, Pondicherry, India.*

Gomes, F.P. (2009). Curso de estatística experimental. *Piracicaba: Esalq*, *15*, 477.

Gonçalves, F. A., Ruiz, H. A., Santosa, E. S.,Teixeira, J. A., & Macedo, G. R. (2016). Bioethanol production by Saccharomyces cerevisiae, Pichia stipitis and Zymomonas mobilis from delignified coconut fibre mature and lignin extraction according to biorefinery concept. Renewable Energy, 94, 353-365.

Griffiths, P., & de Hasseth, J. A. (2007). Fourier Transform Infrared Spectrometry. *Journal of the American Chemical Society, 129* (43), 13358-13358

Hameed, B. H., & Ahmad, A. A. (2009). Batch adsorption of methylene blue from aqueous solution by garlic peel, an agricultural waste biomass. *Journal of Hazardous Materials*, *164,* 870–875.

Han, Y. W., & Anderson, A. W. (1975). Semi-solid fermentation of rye grass straw. *Journal of Applied Microbiology 27,* 159–165.

Hanrahan, G., & Lu, K. (2006). Application of factorial and response surface methodology in modern experimental design and optimization. *Critical Reviews in Analytical Chemistry, 36,* 141–151.

Harmsen, P., & Huijgen, W. (2010). Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. *Technical Representative, 1184, Biosynergy, Wageningen UR Food and Biobased Research, 2013,* 21.

Hatfield, R. D., & Marita, J. M. (2010). Enzymatic processes involved in the incorporation of hydroxycinnamates into grass cell walls, *Phytochemistry Reviews, 9,* 35-45.

Hegde, S., & Hegde, V. (2013). Assessment of global rice production and export opportunity for economic development in Ethiopia*. International Journal of Science and Research 2*(6), 257–260.

Hendriks, A., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology, 100*(1), 10-15.

Heuzé, V., & Tran G. (2015). Rice bran and other rice by-products. *Feedipedia*, a programme by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/750

Hicks, P. A. (1999). ERIKA-JACK - The potential for rice products and by-products.pdf. FAO Regional officer for Asia and the Pasific Bangkok. Thailand. https://doi.org/AU J. T. *2*(3), 105-117.

Hinterstoisser, B., Åkerholma, M., & Salména, L. (2001). Effect of fiber orientation in dynamic FTIR study on native cellulose. *Carbohydrate Research*, *334*(1), 27-37.

Homb, T., Sundstol, F., & Arnason, J. (2005). Chemical treatment of straw at commercial and farm levels. *Agricultural University of Norway, 12*(4), 295-309.

Hosseini, S., Aziz, H., Syafalni, C., & Kiamahalleh, M. (2011). Optimization of NaOH thermochemical pretreatment for enhancing solubilisation of rice straw by Response Surface Methodology. 11th edition of the World Wide Workshop for Young Environmental Scientists , Arcueil, France.

Hutagalung, R. I. (1981). The use of tree crops and their by-product for intensive animal production. *BSAP Occasional Publication*, *4,* 151-188.

Jaikumar, V., & Ramamurthi, V. (2009). Effect of biosorption parameters kinetics isotherm and thermodynamics for acid green dye biosorption from aqueous solution by brewery waste. *International Journal of Chemistry. 1*(1), 1-12.

Jain, M., Garg, V. K., & Kadirvelu, K. (2011). Investigation of Cr (VI) adsorption onto chemically treated Helianthus annuus: optimisation using Response Surface Methodology. *Journal of Biosource Technology, 102*(2), 600-605.

Jayabal, S., Sathiyamurthy, S., Loganathan, K. T., & Kalyanasundaram, S. (2012). Effect of soaking time and concentration of NaOH solution on mechanical properties of coir–polyester composites. *Bulletin of Materials Science*, *35*(4), 567–574.

JMP Statistical Discovery. (2016). Desirability Profiling and Optimization. Retrieved November 5, 2017, from http://www.jmp.com/support/help/Desirability_Profiling_and_Optimization.shtml

Jung, H. G., Smith, R. R., & Endres, C. S. (1994). Cell wall composition and degradability of stem tissue from lucerne divergently selected for lignin and in vitro dry-matter disappearance. *Grass Forage Science, 49*, 295–304.

Kamsonlian, K., & Shukla, B. (2013). Optimization of process parameters using response surface methodology (RSM): removal of cr (vi) from aqueous solution by wood apple shell activated carbon (WASAC). *Research Journal of Chemical Sciences, 3*(7), 31-37.

Kansal, H. K., Singh, S., & Kumar, P. (2005). Parametric optimization of powder mixed electrical discharge machining by response surface methodology. *Journal of Materials Processing Technology*, *169,* 427–436.

Karunanithy, C., & Muthukumarappan, K. (2011). Optimization of alkali, big bluestem particle size, and extruder parameters for maximum enzymatic sugar recovery using response surface methodology, *BioResources, 6*(1), 762–790.

Khalil, A., Alwani, S., & Omar, M., (2006). Chemical composition, anatomy, lignin distribution, and cell wall structureof malaysian plant waste structure. *Biological Resource, 1*(2), 220-232.

Khalil, A.S., Rahim, A.A., Taha, K.K., & Abdallah, K.B. (2013). Characterization of methanolic extracts of agarwood leaves. *Journal of Applied and Industrial Science*, *1*(3), 78-88.

Khattri, S. D., & Singh, M. K. (2009). Removal of malachite green from dye wastewater using neem sawdust by adsorption. *Journal of Hazardous Materials*. 167(1-3), 1089-94.

Khuri, A. I., & Mukhopadhyay, S. (2010). Response surface methodology. *WIREs Computational Statistics*, *2,* 128–149.

Kim, T. H. & Lee, Y. Y. (2005). Pretreatment of corn stover by soaking in aqueous ammonia. *Applied Biochemistry and Biotechnology,* 124(1-3), 1119-1131.

Knowles, T. A., Southern, L. L., Bidner, T. D., Kerr, B. J., & Friesen, K. G. (1998). Reactive oxygen species as agents of wood decay by fungi. *Animal Science Journal, 76*, 2818 -32.

Kumar, R., & Wyman, C. E. (2009). Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnology Progress*, *25*, 302–314.

Kuśmierek, K., Sankowska, M., Skrzypczyńska, K., & Świątkowski, A. (2015). The adsorptive properties of powdered carbon materials with a strongly differentiated porosity and their applications in electroanalysis and SPME-GC. *Journal of Colloid and Interface Science, 446*, 91–97.

Lawrence, J. D., Mintert, J., Anderson, J. D., & David, P. A. (2010). Feed grains and livestock impacts on meat supplies and prices, *American Agricultural Economics Association, 23*(2), 1–5.

Lee, H. V., Hamid, S. B. A., & Zain, S. K. (2014). Conversion of lignocellulosic biomass to nanocellulose: structure and chemical process. *Nanotechnology and Catalysis Research Centre (NANOCAT).*

Li Y, Mlynar J, Sarkanen S. The first 85% Kraft lignin-based thermoplastics. *Journal of Polymer Science, 35*, 1899–910.

Ludueña, L., Fasce, D., Alvarez, V. A., & Stefani, P. M. (2011). Nanocellulose from rice husk following alkaline treatment to remove silica, *Bioresources. 6,* 1440-1454.

Main, N. M., Talib, R. A, Ibrahim, I., Rahman, R. A., & Zuriyati, A. M. (2014). Suitability of coir fibers as pulp and paper. *Agriculture and Agricultural Science Procedia,* 2, 304 – 311.

Mansouri.,N. E., & Normally, J. S. (2007). Analytical methods for determining functional groups in various technical lignins. *Industrial Crops and Products. 26*, 116–124.

Metha, W., Anusorn, C., Phesatcha, K., & Kang, S. H. (2015). Dietary sources and their effects on animal production and environmental sustainability. *Chinese Association of Animal Science and Veterinary Medicine, 1*(3), 96-103.

Mcintosh, S., & Vancov, T. (2010). Enhanced enzyme saccharification of Sorghum bicolor straw using dilute alkali pretreatment. *Bioresource Technology,101,* 6718-6727.

Moore, K. J., & Hatfield, R .D. (1994). Carbohydrates and forage quality. In G. C. Fahey, Jr. (ed.) Forage Quality, Evaluation, and Utilization. ASA, CSSA, SSSA, Madison, Wisc. 229-280.

Moore, K. J., & Jung, C. G. (2001). Lignin and fiber digestion. *Journal of Range Management. 54*, 420–430.

Mussatto S. I., & Teixeira, J. A. (2010). Lignocellulose as raw material in fermentation processes, in: current research, technology and education, topics in applied microbiology and microbial biotechnology, A.Mendez-Vilas, Eds. *Formatex Research Center, Badajoz*, 897–907.

Narayana, S., King, P., Gopinadh, R., & Sreelakshmi, V. (2011). Response surface optimization of dye removal by using waste prawn shells. *International Journal Chemistry Science Applied, 2*(3), 186-193.

Nigam, P. S., Gupta, N., & Anthwal, A. (2009). Pre-treatment of agro-industrial residues, *Biothechnology for agro-industrial residues utilization*, Nigam, P.S., Pandey, A., Eds. Netherlands. Springer Netherlands, 13-33.

Nikzad, M., Movagharnejad, K., Najafpour, G. D., and Talebnia, F. (2013). Comparative studies on the effect of pretreatment of rice husk on enzymatic digestibility and bioethanol production. *International Journal of Engineering, 26*(5), 455-464.

Nikzad, M., Movagharnejad, K., Talebnia, F., Aghaiy, Z., & Mighani, M. (2015). Modeling of alkali pretreatment of rice husk using response surface methodology and artificial neural network. *Chemical Engineering Communications, 202*(6), 728–738.

Noordin, M. Y., Venkatesh, V. C., Sharif, S., Elting, S., & Abdullah, A. (2004). Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel. *Journal of Materials Processing Technology*, *145,* 46–58.

Nour, A. M. (2006). Rice straw and rice hulls in feeding ruminants in Egypt. ARNAB. *Proceedings Workshop.* Blantyr. Malawy, October (1986). 53-61.

Nuengmatcha, P., Mahachai, R., & Chantai, S. (2014). Thermodynamic and kinetic study of the intrinsic adsorption capacity of graphene oxide for malachite green removal from aqueous solution. *Oriental journal of chemistry*, *30*(4), 1463-1474.

OECD. (1999). Consensus document on the biology of Oryza sativa (Rice). ENV/JM/MOMO *Organization for Economic Co-operation and Development. (99) 26.*

Ofomaja, A. E. (2008). Sorptive removal of Methylene blue from aqueous solution using palm kernel fibre: Effect of fibre dose. *Biochemical Engineering Journal*, *40*, 8–18.

Organisation for Economic Co-operation and Development. (2004). Consensus document on compositional considerations for new varieties of rice *(Oryza sativa): key food and feed nutrients and anti-nutrients*. Annual Report 2004. Paris.

Papita, D. S., Chakraborty, S., & Subhabrata, D. (2012). Optimization of hazardous crystal violet by chemically treated rice husk: Using central composite response surface methodology. *Archives of Environmental Science, 6*, 57-61.

Perry, R. & Don, G. (1997). Perry's chemical engineer's handbook, 7th Edition, McGrow hill Publication, New York.

Porteres, R. (1956). Taxonomic agrobotanique der riz cultives O. sativa Linne. et O. glaberrima Steudelo, *Journal of Agricultural Tropical Bot App, 3*, 341-384.

Potumarthi, R., Baadhe, R. R., Nayak, P., & Jetty, A. (2013). Simultaneous pretreatment and saccharification of rice husk by Phanerochete chrysosporium for improved production of reducing sugars. *Bioresource Technology, 128*, 113–117.

Pouteau, C., Dole, P., Cathala, B., Averous, L., & Boquillon, N. (2003). Antioxidant properties of lignin in polypropylene. *Polymer Degradation and Stability, 81*(1), 9–18.

Ralph, J., Helm, R.F., Quideau, S., & Hatfield, R.D. (1992). Lignin-feruloyl ester cross-links in grasses. Part 1. Incorporation of feruloyl esters into coniferyl alcohol dehydrogenation polymers. *Journal of the Chemical Society, Perkin Transactions 1*, *1*, 2961–2969.

Ramadevi, P., Sampathkumar, D., Srivasa, C.V., & Bennehalli, B. (2012) Effect of alkali treatment on water absorption of single cellulosic abaca fiber. *Bioresources*, *7*(3), 3515-3524.

Roosta, N., Wong, M.K., & Woodley, D.T. (2012). Utilizing hairdressers for early detection of head and neck melanoma: an untapped resource. *Journal of the American Academy of Dermatology*, *66* (4), 687-688.

Rozainee, M., Ngo,S. P., Johari, A., Salema, A. A., & Tan, K. G. (2009). Utilisation of rice husk waste and its ash (part 1). *The Ingenieur, 54.* Retrieved from http://www.academia.edu/438079/Utilisation_Of_Rice_Husk_Waste_And_Its_Ash_Part_1_

Ryan, E. P. (2011). Bioactive food components and health properties of rice bran, *Journal of the American Veterinary Medical Association, 238* (5), 593-600.

Sah, B. C. (2005). Enzymes as biocatalysts for conversion of lignocellulosic biomass to fermentable sugars. *In Handbook of Industrial Biocatalysis*, *24*, 1-12.

Saha, B., & Cotta, M. (2008). Lime pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol. *Biomass and Bioenergy 32*, 971-977.

Saha, P., Chowdhury, S., Gupta, S., Kumar, I., & Kumar, R. (2010). Assessment on the removal of malachite green using tamaring fruit shell as biosorbent. *Journal of Clean-Soil, Air, Water, 38* (5-6), 437-445.

Salleh, M. A. M., Mahmoud, D. K., Azlina, W., & Idris, A. (2011). Cationic and anionic dye adsorption by agricultural solid wastes: A comprehensive review. *Desalination, 280*(2011), 1-13.

Sawant, A., Mankeshwar, R., & Shah, S. (2011). Prevalence of metabolic syndrome in urban India. *Cholesterol*, *2011*(10), 7.

Sills, D. L., & Gossett, J. M. (2011). Assessment of commercial hemicellulases for saccharification of alkaline pretreated perennial biomass. *Bioresource Technology. 102*, 1389-1398.

Sills, D. L., & Gossett, J. M. (2012). Using FTIR to predict saccharification from enzymatic hydrolysis of alkali-pretreated biomasses. *Biotechnology and Bioengineering, 109*, 353-62.

Sivapragasam, A. (2008). Coconut in Malaysia-current developments and potential for re-vitalization. *Rice and Industrial Crops Center MARDI*, 1, 1-9.

Srivastava, S., Mudgal, V., & Kumar J. (2012). Lignin - its role and importance in animal nutrition. *International Journal of Livestock Research, 2*(1), 7-23.

Sukri, S. S. M., Rahman, R. A., Illias, R. M., & Yaakob, H. (2014). Optimization of alkaline pretreatment conditions of oil palm fronds in improving the lignocelluloses contents for reducing sugar production. *Romanian Biotechnological Letters*, *19*(1), 9006-9018.

Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A Review. *International Journal of Molecular Sciences, 9,* 1621-1651.

Tahir, T. A. (2012). Waste audit at coconut-based industry and vermicomposting of different types of coconut waste (Doctoral dissertation, University of Malaya). Retrieved from http://studentsrepo.um.edu.my/3868/1/1._Title_page%2C_abstract%2C_content.pdf.

Tarkow, H., & Feist, W. C. A. (1969). A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid NH3, in: cellulases and their applications, Hajny, G. J., Reeses, E. T., Eds, Advance Chemistry Series 95, *American Chemical Society, Washington, DC,* 197-218.

Taylor, P., Street, M., Wt, L., Draper, N. R., & John, J. A. (1988). Response-surface designs for quantitative and qualitative variables. *Journal of Technometrics, 30*(4), 423–428.

Tejinder, S. (1969).The Tribun,Industrial Uses of Rice Husk, Chandigarh (Nov. 2000).

Torres, H. V., Escamilla, G. C., and Ramos, C. A., (1992). Coconut husk lignin. II.Characterization by infrared and nuclear magnetic resonance spectroscopy. *Journal of Applied Polymer Science, 45*, 645-653.

Vadiveloo, J., Nurfariza, B., & Fadel, J. G. (2009). Nutritional improvement of rice husks. *Animal Feed Science and Technology, 151*(3–4), 299–305.

Wakerley, D.W., Kuehnel, M.F., Orchard, K.L. Khoa, H.L., Rosser, T.E., & Reisner, E. (2017). Solar-driven reforming of lignocellulose to H_2 with a CdS/CdO$_x$ photocatalyst. *Nature Energy*, 2(10), 1038.

Wang, W., Yuan, T., Wang, K., Cui, B., & Dai, Y. (2012). Combination of biological pretreatment with liquid hot water pretreatment to enhance enzymatic hydrolysis of Populus tomentosa. *Bioresource Technology, 107*, 282–286.

Wang, Z., Keshwani, D. R., Redding, A. P., & Cheng, J. J. (2010). Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass. *Bioresource Technology*, *101,* 3583–3585.

Wang, Z., Li, J., Barford, J. P., Hellgradt, K., & Mckay, G. (2016). A comparison of chemical treatment methods for the preparation of rice husk cellulosic fibers. *International Journal of Environmental and Agriculture Research, 2*(1), 67–77.

Warner, B., Quirke, D., & Longmore, C. (2007). A review of the future prospects for the world coconut industry and past research in coconut production and product. *Australian Centre for International Agricultural Research*. ACIAR GPO Box 1571 Canberra ACT 2601, Australia.

Wilson, P. N., & Brigstocke, I. D. A. (1981). Improved feeding of cattle and sheep. *Livestock Research for Rural Development, 24*(5), 1-9.

Xu, F., Sun, J. X., Sun, R., Fowler, P., & Baird, M. S. (2016). Comparative study of organosolv lignins from wheat straw, Industry Crop Production*, 23*, 180-93.

Xu, G., Wang, L., Liu, J., & Wu, J. (2013). FTIR and XPS Analysis of the changes in bamboo chemical structure decayed by white-rot and brown-rot fungi. *Applied Surface Science, 280*(1), 799-805.

Xu, J., Cheng, J. J., Sharma-Sivappa, R. R., & Burns, J. (2010). Sodium hydroxide pretreatment of switchgrass for ethanol production. *Energy Fuels*, 24, 2113–2119.

Yu, J., Zhang, J., He, J., Liu, Z., & Yu, Z. (2009). Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull. *Bioresource Technology, 100*, 903–908.

Zafar, S. (2015). Energy potential of coconut biomass. bioenergy consult powering clean energy future. *Renewable and Sustainable Energy Reviews*, *44,* 530-542.

Zahoor, M. (2011). Effect of agitation speed on adsorption of imidacloprid on activated carbon. *Journal of the Chemical Society of Pakistan*, *33*(3), 305–312.

Zhao Y., Wang Y., Zhu J. Y., Ragauskas, A., & Deng, Y. (2008). Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature, *Biotechnology and Bioengineering, 99*(6), 1320–1328.

Zhu, L., O'dwever, J. P., Chang, V. S., Granda, C. B., & Holtzapple, M. T. (2008). Biological pretreatment of lignocellulosics: potential, progress and challenges. Biofuels. *Bioresource Technology*, 99, 3817-3828.

APPENDIX A

A. 1 Preparation of different concentration of NaOH solution.

Solution 1: Preparation of 1 M NaOH solution:

Mass (g) = Concentration (mol/L) ×Volume (L) × Formula Weight (g/mol)

= 1 mol/L × 0.1 L × 40.00 g/mol

= 4 g

Hence, using 4 g of NaOH solution diluted up to 100 mL to produce 1 M NaOH solution.

Solution 2: Preparation of 5 M NaOH solution:

Mass (g) = Concentration (mol/L) ×Volume (L) × Formula Weight (g/mol)

= 5 mol/L × 0.1 L × 40.00 g/mol

= 20 g

Hence, using 20 g of NaOH solution diluted up to 100 mL to produce 5 M NaOH solution.

Solution 3: Preparation of 10 M NaOH solution:

Mass (g) = Concentration (mol/L) ×Volume (L) × Formula Weight (g/mol)

= 10 mol/L × 0.1 L × 40.00 g/mol

= 40 g

Hence, using 40 g of NaOH solution diluted up to 100 mL to produce 10 M NaOH solution.

Solution 4: Preparation of 10 M KOH solution:

Mass (g) = Concentration (mol/L) ×Volume (L) × Formula Weight (g/mol)

= 10 mol/L × 0.1 L × 56.12 g/mol

= 56.12 g

Hence, using 56.12 g of NaOH solution diluted up to 100 mL to produce 10 M NaOH solution.

Solution 5: Preparation of 10 M $Ca(OH)_2$ solution:

Mass (g) = Concentration (mol/L) ×Volume (L) × Formula Weight (g/mol)

= 10 mol/L × 0.1 L × 47.10 g/mol

= 47.10 g

Hence, using 47.10 g of NaOH solution diluted up to 100 mL to produce 10 M NaOH solution.

APPENDIX B

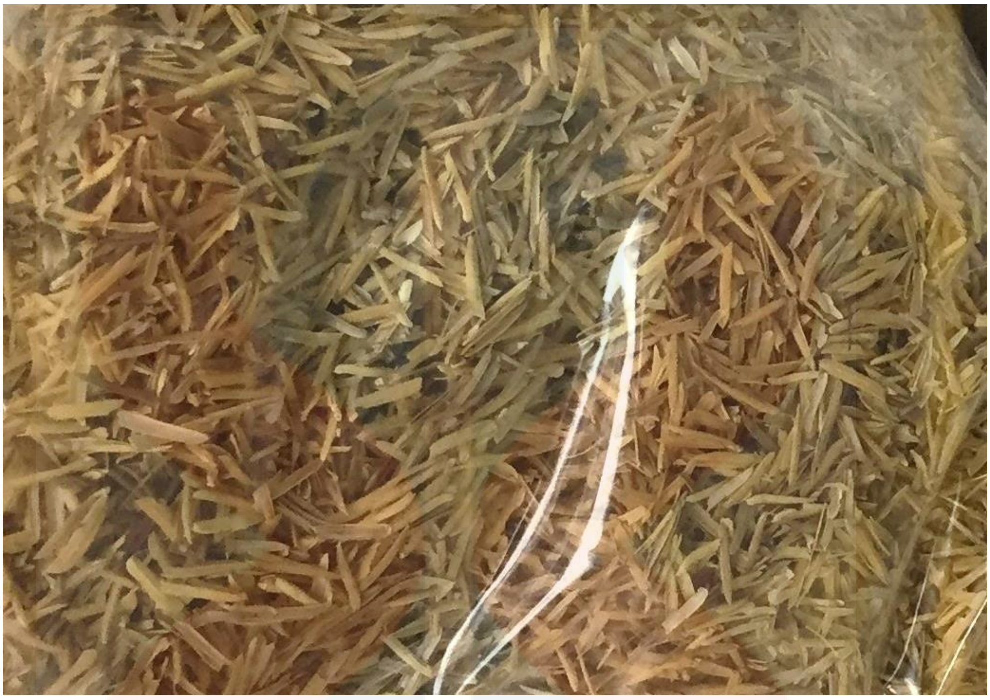


Figure B.1: Raw rice hull before pretreatment.

Figure B.2: Rice hull after pretreatment.

Figure B.3: Raw coconut hull before pretreatment.

Figure B.4: Coconut hull after pretreatment.