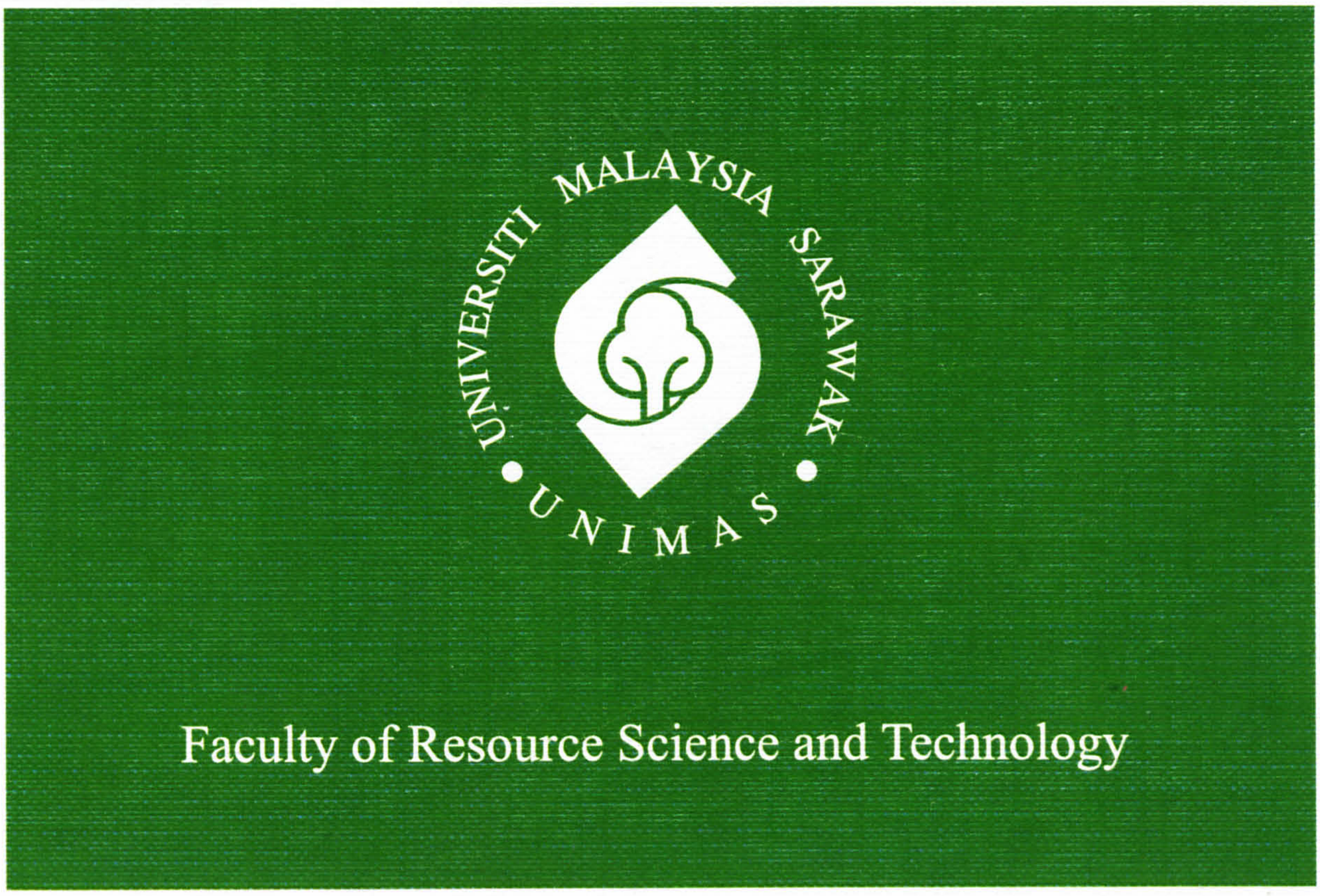

ENHANCEMENT OF SUGARS RECOVERY FROM RESIDUAL STARCH OF SAGO HAMPAS THROUGH RETROGRADATION REACTION

Nurul Nakhiah Rubian

(40200)

Bachelor of Science with Honours
(Resource Biotechnology)
2016

ENHANCEMENT OF SUGARS RECOVERY FROM RESIDUAL STARCH OF SAGO HAMPAS THROUGH RETROGRADATION REACTION

NURUL NAKHIAH RUBIAN (40200)

A final project report submitted in partial fulfilment of the

Final Year Project (STF 3015)

(Resource Biotechnology)

Supervisor: Dr. Dayang Salwani Bt Awang Adeni

Resource Biotechnology
Department of Molecular Biology

Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2016

APPROVAL SHEET

Name of Candidate : Nurul Nakhiah binti Rubian

Title of Dissertation : Enhancement of sugars recovery from residual starch of sago hampas through retrogradation reaction.

Dr. Dayang Salwani Binti Awang Adeni

Supervisor

Dr. Ho Wei Seng

Programme Coordinator

Department of Molecular Biology

Faculty of Resources Science and Technology

ACKNOWLEDGEMENT

Alhamdulillah, all praises to Allah S.W.T., The Most Greatest and The Most Merciful for his guidance and blessing, because without it I can't finished this research. First and foremost I offer my sincerest gratitude to my supervisor, Dr. Dayang Salwani Awang Adeni, who has supported me from the beginning for the completion of this final year project research with her patience and knowledge. Without her, this final year project research would not have been completed or written and one simply could not wish for a friendlier supervisor.

A special thanks to my lab mate, Mhd. Shaifuddin bin Safiee , for giving me support and helpful guidance to complete my research. Thank you to all post graduate students in the Biochemistry laboratory who have helped a lot. Thank you for sharing knowledge to accomplish this study. I also would like to thank to all my friends who have helped me in conducting my research.

Last but not least, I am sincerely indebted to my parents for their love, guidance, and support throughout my life. I am also thankful to my siblings for their friendship and love.

DECLARATION

I, hereby declare that I have completed this thesis on the 'Enhancement of sugars recovery from residual starch of sago hampas through retrogradation reaction' on my own original work except for citations which have been properly acknowledged in this thesis. This thesis is written for my Final Year Project in completing my Bachelor of Science with Honours in 'Resources Biotechnology'.

This thesis has not been submitted for evaluation to any other institute, university or institution other than Faculty Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS). All parts of the thesis which are cited literally from journals are acknowledged. I further confirm that I have submitted to my supervisor, Dr. Dayang Salwani Awang Adeni this thesis in both hardcopy and softcopy in the form of CD.

.....

Nurul Nakhiah binti Rubian

Department of Molecular Biology

Programme Resource Biotechnology

Universiti Malaysia Sarawak

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

DNS	Dinitrosalicyclic acid
g	gram
g/L	gram per Litre
HPLC	High Performance Liquid Chromatography
KH_2PO_4	Potassium dihydrogen phosphate
ml	millilitre
NaOH	Sodium Hydroxide
OD	Optimal Density
rpm	revolution per minute
SEM	Scanning Electron Microscope
w/v	weight over volume

LIST OF TABLES

LIST OF FIGURES

Enhancement of Sugars Recovery from Residual Starch of Sago Hampas through Retrogradation Reaction

Nurul Nakhiah binti Rubian

Programme Biotechnology Resource
Department of Molecular Biology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Sago hampas which contain mostly starch is potentially for production of reducing sugars which able to be used as fermentable sugar. In this study, the yield of reducing sugars from sago hampas after undergo the retrogradation reaction (24 h, 48 h and 72 h) at 4°C was examined. The substrate loading is fixed at 7% (w/v). The retrograded starch was further hydrolysed into reducing sugars utilising liquozyme and spirizyme enzymes. From the result obtained, there is not much different of reducing sugars concentration at different period of retrogradation reaction. However, after 48 h of retrogradation reaction 55.71% of reducing sugar was obtained higher compared to 24 h and 72 h which was 36.60 %, and 48.80 % respectively. This result indicates that the retrogradation reaction can enhance the starch recovery from sago hampas, thus able to increase reducing sugars concentration.

Keywords: sago hampas, retrogradation reaction, reducing sugars concentration, fermentable sugar

ABSTRAK

Hampas sagu mengandungi kanji berkeupayaan bagi menghasilkan gula terkurang yang boleh digunakan sebagai gula fermentasi. Dalam kajian ini, gula daripada hampas sagu terhasil setelah menjalani reaksi retrogradasi (24 h, 48 h, and 72 h) pada suhu 4°C diuji. Pengisian substrat ditetapkan 7% (w/v). Kanji yang mengalami retrogradasi kemudian dihidrolisasi kepada gula terkurang menggunakan enzim liquozyme dan spirizyme. Keputusan mendapati tiada banyak perbezaan kepekatan gula pada tempoh masa reaksi retrogradasi yang berbeza. Walaubagaimanapun, selepas 48 jam reaksi retrogradasi, 55.71 % gula didapati tinggi berbanding 24 jam dan 72 jam sebanyak 36.60 % dan 48.80 % setiapnya. Keputusan ini menandakan bahawa reaksi retrogradasi mempengaruhi pendapatan semula kanji daripada hampas sagu dan meningkatkan kepekatan gula.

Kata kunci: *hampas sagu, reaksi retrogradasi, kepekatan gula penurunan, gula fermentasi*

1.0 Introduction

In recent years, production of bioethanol as a renewable energy source attracted worldwide attention as it can be used as alternative source of energy for fuel industry and also reduce greenhouse emissions. Malaysia is well known country for its agricultural activities and the existence of huge amount of agriculture waste is undeniable. Agriculture waste has great potential feedstock in the production of bioethanol.

Sago palm is belonging to Family Palmae. Scientifically it is known as *Metroxylon Sagu.* The palm are crops that can tolerate wet growing condition (Karim et al., 2008) such swampy area or peats soil. In Malaysia sago palm is found grow well in Sarawak and more than 90% of sago palm is cultivated. Due to high contain of starch, sago palm has great potential to utilize as food stuff for the local people.

Currently, the sago hampas which left behind after starch extraction process has been recognised as promising agriculture residue in industry for conversion into value added product. According to Awg-Adeni (2010) sago hampas can be utilize as alternative substrate for solid substrate fermentation as it has highly contents of starch and lignocellulosic materials. However, production of glucose is result in lower concentration after undergo enzyme hydrolysis process due to complexity of lignocellulosic structure (Awg-Adeni et al., 2013).

As stated by Zahid et al (2014) lignocellulosics are comprised of 10-25% lignin, 20-30% hemicellulose and 40-50% cellulose. Hence, the raw material must be treated before, to enhance cellulose activity in order to improve enzymatic hydrolysis.

Through pretreatment, it gives huge impact as it helps in breakdown the lignin barrier to recover cellulose and easily converted into sugar through enzyme hydrolysis and continues with the conversion into value added product at the end. The aim of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity and increase the porosity of the materials (Sun & Cheng, 2002) before proceed for enzymatic hydrolysis.

Presently, no work has been reported yet on the production of reducing sugar through retrogradation reaction and thus there is a need to test that retrogradation reaction pretreatment as one of the potential and economical for enhance sugars recovery from residual starch of sago hampas.

Hence, this research study is carried out to yield sugars from sago hampas production after undergoing steaming and retrogradation reaction pretreatment and followed by enzymatic hydrolysis. In this experiment the substrate load is fixed to 7% (w/w).

The aim for this study includes:

1. To determine the sugars released from residual starch of sago hampas after enzymatic hydrolysis of pre-treated stage.
2. To identify the parameters during heating and retrogradation process in which higher starch can be recovered from sago hampas.

2.0 Literature Review

2.1 Bioethanol Production

As another countries, currently Malaysia also have established target to produce an alternative renewable fuel from biomass (Ong et al., 2011). There are several different agriculture biomass can be converted into bioethanol by technology. The use of agriculture biomass in the ethanol production gives several impacts as it can use as alternative fuel and environmental friendly. According to Balat et al. (2008) bioethanol which has higher octane number, broader flammability limits, higher flame speeds and higher heats vaporization allow for shorter burn time and leaner burn engine. Lignocellulosic biomass is one of feedstock can be used for bioethanol production. For several years, lignocellulose has been recognised as world's largest bioethanol renewable resource (Limayem & Ricke, 2012).

2.2 Sago Palm

Malaysia is one of countries rich with many commercial production plant resources such as sago palm. Sago palm is one of the most abundant palms distributed in South East Asia region and due to regular temperature of 25°C and humidity of 70%, in these tropical areas suitable for growth of the palm (Bujang, 2011). The Malaysia sago palm industry is the most important sago exporter in the world and every year there are 25, 000 mt sago starches are exported. Due to high needs for sago production the amount of waste from the industry is keep increasing that lead to the waste management problem

and environmental pollution. The by-product produced including sago hampas, sago bark and sago wastewater (Jenol et al., 2014).

2.2.1 Sago Hampas

In Malaysia, the Sarawak state is recognised as the largest sago palm growing area. Sago hampas is an example of agriculture biomass. Sago hampas also called as sago pith waste. This agriculture biomass is inexpensive and as stated by Rahman et al. (2013) the generated sago hampas is in the range of 1,511 ktones in 2005 and 1734 ktonnes in 2010 left behind after extraction process of grinded pith.

Sago hampas is a starchy lignocellulosic biomass generated from the pith of sago palm after starch extraction process. It is widely utilised as animal feed, compost for mushroom culture, manufacture of particleboard and also hydrolysis for confectioner's syrup (Singhal et al., 2008). Apart from that, hampas that containing starch and lignocellulose make it potential to be converted into sugar through enzymatic hydrolysis.

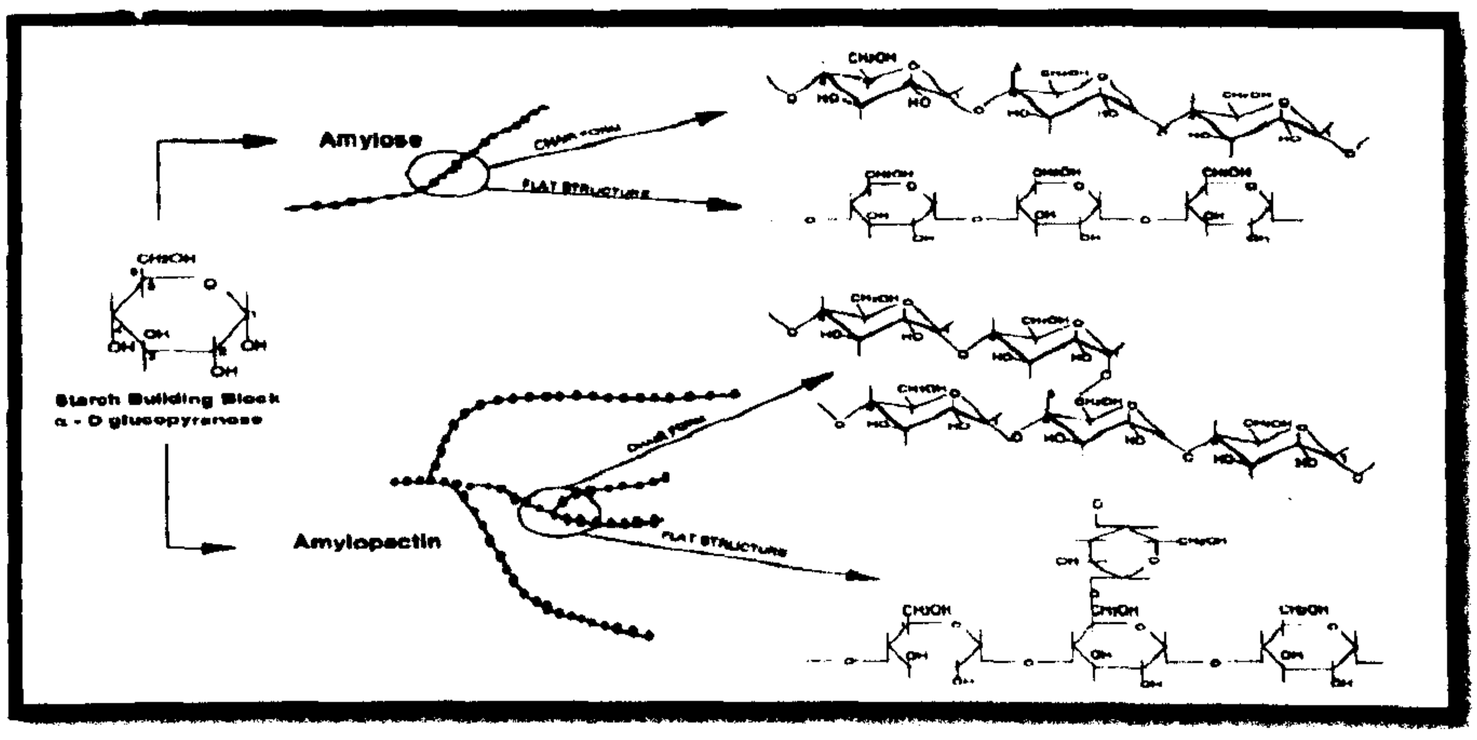

Figure 1: Linear and branch of starch polymer (Murphy, 2000)

Starch comprise of two polysaccharides which are amylose and amylopectin polymer. Amylose is a linear chain of glucose linked by α-1, 4 bonds while amylopectin is a branched polymer of glucose consisting branch point which is α-1, 6 bonds. On a dry basis, sago hampas contains 58% starch, 23% cellulose, 9.2% hemicellulose and 4% lignin (Awg-Adeni et al., 2013).

Through technology improvement todays, the bioconversion of sago hampas into value added products make it such valuable by-product as it can generate additional income in the future. In the meantime, bioconversion of this by product has been seen such a good way in reducing environmental problems.

2.3 Starchy Lignocellulic Biomass

Towards into the future, lignocellulosic biomass is a renewable source that will help in the production of alternative fuel due to high demand of the fossil fuel. Lignocellulosic biomass comprise of cellulose, hemicellulose and lignin. Cellulose is a major component of plant cell wall and its highly stable polymer properties. It is a long chain polysaccharide formed by D-glucose units linked by β-1, 4 glycosidic bonds. Hemicellulose consist repeated polymers of pentose and hexoses. The existing of non-covalent attraction making both cellulose and hemicellulose binds tightly between each other (Anwar et al., 2014). The most complex is lignin with long-chain polymer of phenyl propane units which linked by ether bond. Lignin holds together cellulose and hemicellulose fibers.

The lignocellulosic biomass can be hydrolysed into sugar which will be further used for bioethanol production. The bioconversion of lignocellulosic biomass into higher value added must undergo several process including pretreatment, enzymatic hydrolysis and fermentation process. There are several types of lignocellulosic of biomass have been used before such cassava root fiber and wheat straw.

2.3.1 Cassava Root Fiber

Cassava waste pulp is the by-product of cassava starch production. The residual solid of lignocellulosic cassava root fiber after extraction is 11.2% (w/w) which composed of 59.9% of cellulose, 20% of hemicellulose and 10.7% of lignin.

The steam explosion pretreatment was performed which in result the optimum condition 6 % (w/v) substrate loading, 180°C, 5 min) able to released maximum reducing sugar (3.73 g/100 g) after enzymatic hydrolysis (Kerdlaew & Akaracharanya., 2014).

2.3.2 Wheat Straw

The chemical compositions of wheat straw according to Sarkar et al. (2012) are 35-45% cellulose, 20-30% hemicellulose and 8-15% of lignin. The cell wall of wheat straw mainly consists of cellulose fibers rather than hemicellulose and lignin. High content of cellulose, 34 % (w/v) make the wheat straw as one promising biomass for ethanol production. Through steaming pretreatment, the maximum yield for sugar is 96% at 200°C for 10 minutes (Jakobsson, 2002).

2.4 Pretreatment of Lignocellulosic Biomass

In the process of sugar recovery, pretreatment is required to enhance the efficiency during enzymatic hydrolysis. The main reason of pretreatment is to break the recalcitrant structures of lignocellulosic (Huang et al., 2011). Besides that is also to diminish cellulose crystallinity and increase the porosity of materials (Sun & Cheng, 2002).

According to Huang et al. (2011) and Anwar et al. (2014) the pretreatment process should meet several criteria which are (1) the lignin barrier is remove, (2) cellulose crystallinity is disrupt (3) minimize the formation of toxic degradation product (4) lessen the loss of sugar components and (5) cost-effective.

Through pretreatment, when the biomass structure is altered and disrupted will cause cellulose in the plant fibres to be exposed. At this stage, the cellulose becomes easily accessible to enzyme. Generally process can be used for lignocellulosic materials can be done physically, chemically, biologically and physic-chemical (Sun & Cheng, 2002).

2.5 Steaming Pretreatment

Steam pretreatment is method used in pretreatment of agriculture waste with saturated steam. According to Agbor (2011), as stated by Chandra et al (2007) the term autohydrolysis indicate the changes that occur during steam pretreatment. Steam pretreatment is one of the technologies used and it is the most extensively study for biomass pre-treatment.

In this method physicochemical is applied which both chemical and physical technique are used in the process of breaking down lignocellulosic materials.

The process involves the raw material treated with high pressure saturated steam. The high pressure helps in the hydrolysis of hemicellulose and causes hemicellulose degradation and lignin transformation. The degradation of hemicellulose occurs when the hydro ions is released from the dissociation of water molecules (Zhu, 2011). During steaming process, the fibril is disrupted and causes cellulose more accessible to the enzyme. In addition, the particle size is a factor affecting the production of sugars. The larger particle size the higher yield sugar is released. This method highly prefers due to its ability to enhance sugar recovery with a low capital investment and limited chemical is used (Brodeur et al., 2011).

2.6 Retrogradation Reaction of Starch

As stated by Tako et al. (2014) the starch retrogradation reaction is consequence from prolong of cooling storage of gelatined solution. This causes the gelatinized solution changes to gel and rearrange itself again to a crystalline structure. The reaction takes place in gelatinization and cooling of starch where it is not in a state of equilibrium (Farhat & Ottenhof, 2004). This reaction occur when the native starch is heated and dissolves in water causes the crystallizatine structure of amylose and amylopectin is lost and hydrate then viscous solution is formed. The linear molecule, amylose and linear parts of amylopectin molecule retrograde and rearrange to a more crystalline structure when the viscous solution is cooled between -8 and +8 °C for long period.

Upon the retrogradation reaction the gelatinized state of starch tends to recrystallize which involve crystallization of amylose and amylopectin. (Vandeputte et al., 2000). Retrogradation occurs in both amylose fraction and amylopectin gelatinized granules. Amylose retrogrades first before amylopectin due to the linear structure of amylose molecules. The retrogradation process depends on amylose content, the structure of amylopectin, the storage temperature presence of non-starch components. (Sobolewska-Zielinska & Fortuna, 2010).

2.7 Enzymatic Hydrolysis

The pretreated lignocellulosic further undergo enzymatic hydrolysis as to degrade cellulose into glucose by action of enzyme. Enzymatic hydrolysis widely used because it is cost-effective compared to acid or alkaline hydrolysis (Sun & Cheng, 2002).

The hydrolysis is carried out by cellulase enzyme. As reported by Maitan-Alfenaset al. (2015) endoglucanase, cellobiohydrolases, exoglucanase and β-glucosidase are necessary groups of cellulases involved in hydrolysis process in order to complete the cellulose degradation.

According to Sun et al. (2002), cellulose is degraded by cellulases in this process in order to produced simple sugar which further fermented and converted into value-added products. All the factors such substrates, cellulase activity, pH, and temperature need to be monitor in order to improve the yield product.

2.8 Starch Enzymatic Hydrolysis

Generally starch is form of carbohydrate in plants. Starch molecules are polysaccharides and it composed of amylose and amylopectin. Amylose in a linear polysaccharide with α-1, 4 linked D-glucopyronase units while amylopectine is highly branched molecules with α-1, 4 glycosidic-linked short linear chains connected by α-1, 6 glycosidic linkages.

In the most plants, starch granule is found as the storage of energy molecule. The physical properties of starch are influenced by the composition of amylose and amylopectin polymers. Heating of starch, make the starch granules swell naturally and in high state of heat temperature lead the starch granules burst and amylose is released and enriched with amylopectin. This process called as gelatinization. During gelatinization, the crystalline structure lost and turned starch molecule into liquid.

Through enzymatic hydrolysis, starch tends to convert into glucose which further altered for production of value-added products. A research on the hydrolysis of sago hampas able to produce sugar was done and as reported in (Bujang, 2011), sugar yield is 40% and the amount rise up as subsequent enzymatic hydrolysis is performed.

In order to achieve an efficient enzymatic hydrolysis, pretreatment need to be done to open up the structure of lignocellulosics as well as starch to enhance rate of hydrolysis (Ramos, 2003).

3.0 Material and Methods

3.1 Pretreatment of Substrate

The sago hampas used in this study was obtained from Herdson Sago Mill in Sarawak The substrate was treated via steaming process and retrogradation reaction.

3.1.1 Steaming Pretreatment

The sago hampas load was fixed at 7% (w/v) for all this trials done in this study. The substrate was initially weighed and then mixed with KH_2PO_4 buffer pH 5 with the ratio of 35 g hampas with 500 ml of buffer. The mixture was steamed at 121°C for 20 minutes.

This step was conducted to disrupt the lignocellulosic compound to make more starch to be released. Next, the mixture was left to at room temperature in order to cool it down before filtered.

Figure 2a: Sago hampas before steaming

Figure 2b: Sago hampas after steaming

3.1.2 Retrogradation Reaction Pretreatment

Once cooled, the mixture was then filtered in order to separate solid and liquid residues. The solid residue was dried in an oven for 24 h while the liquid residual was stored at 4°C for retrogradation reaction under various periods of time (24 h, 48 h and 72 h) respectively before undergo enzymatic hydrolysis process.

Figure 3: The dried solid residue

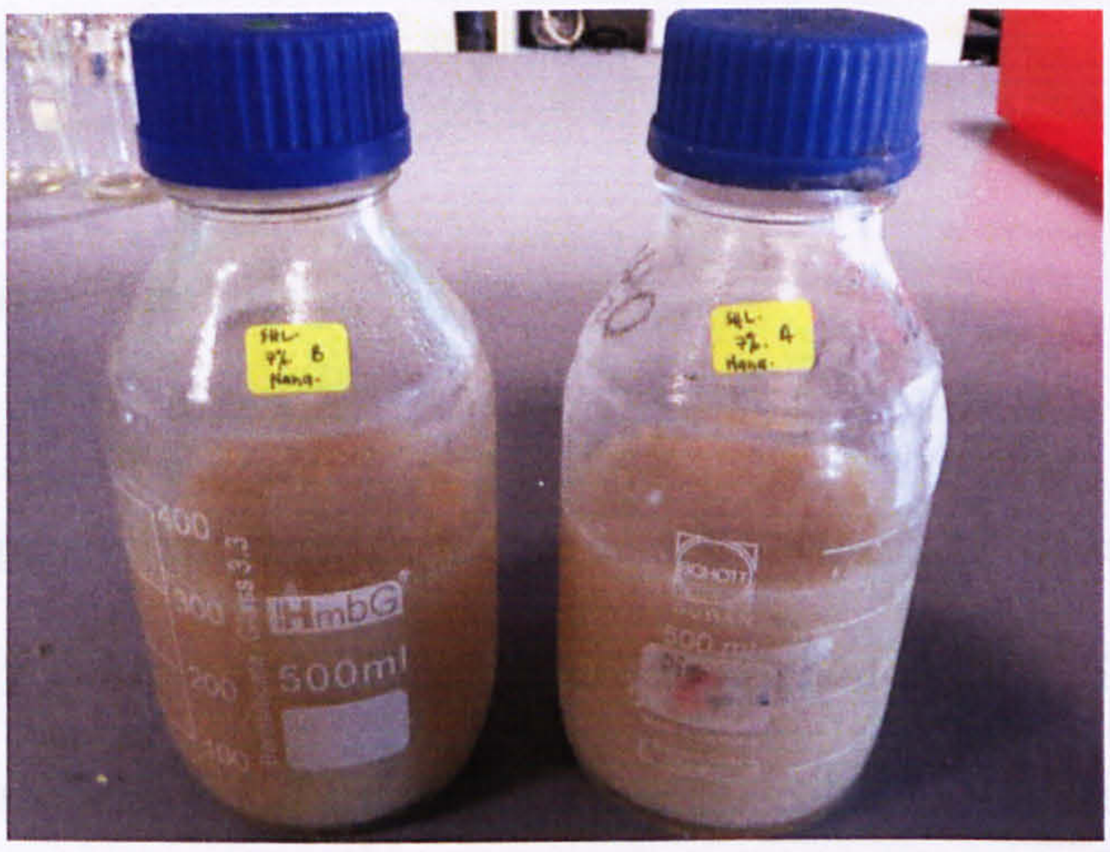

Figure 4: The appearance of liquid residue after retrogradation reaction
(Stored at 4°C for 24 h, 48 h and 72 h)

3.2 Enzymatic Hydrolysis of liquid Residue

Liquid residue which contains starch was further hydrolysed into reducing sugars through liquefaction and saccharification process. The mixture was initially heated at 90°C. The enzyme 14 µl Liquozymes SC DS (per 7 gram of substrate) was added for liquefaction purpose.

The mixture was stirred manually for 20 minutes and next the mixture was left to cool until 50°C before enzyme Spirizyme Fuel was added. The reaction was incubated by placed it in the orbital shaker at 50°C for 2 hours. An aliquot of 2 ml of sample is taken from the bottle every 30 minutes manually and stored at 4°C before further analysis.

3.3 Analytical Methods

The analysis performed was reducing sugar analysis.

3.3.1 Reducing Sugar Analysis

The resulting sugar hydrolysate from enzymatic hydrolysis of sago hampas was quantified by using Dinitrosalycyclic acid (DNS) method (Miller, 1959). The ratio of sample to ratio of reagent, 1:2. 1 mL of the sugar hydrolysate sample added to 2 mL of DNS reagent. The reaction mixture was boiled for 5 minutes. The mixture was allowed to cool at room temperature before the absorbance of the reaction mixture was read at 540 nm using spechtrophotometer (model UV Mini-1240, Shimadzu UV-Vis