Faculty of Resource Science and Technology

EFFECTS OF YEAST EXTRACT (YE) AND PEPTONE (P) ON SAGO HAMPAS HYDROLYSATE (SHH) FOR BIOETHANOL PRODUCTION

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Effects of Yeast Extract (YE) and Peptone (P) on Sago Hampas Hydrolysate (SHH) for Bioethanol Production.

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A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science with Honours (Biotechnology)

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree celcius
M	Meter
Mm	Millimeter
Min	Minute
H	Hour
Ml	Millilitre
μL	Microlitre
mL/min	Millilitre per minute
g/L	Gram per litre
mM	Millimolar
(w/v)	Weight per volume
Rpm	Revolution per minute
SHH	Sago hampas hydrolysate
HPLC	High Performance Liquid Chromatography
OD	Optical Density

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Effects of Yeast Extract and Peptone on Sago Hampas Hydrolysate (SHH) for Bioethanol Production.

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ABSTRACT

Nitrogen sources are very crucial in the fermentation process in order to provide nutrients to the yeast cell so that it can produce high amount of bioethanol. This study is focuses on batch ethanol fermentation utilizing 50 g/L glucose of sago hampas hydrolysate (SHH) with the supplementation yeast extract (3 g/L) or peptone (1 g/L). The fermentation was conducted in 100 mL working volume, agitated at 100 rpm, initial pH of 5.5-5.6 and maintains at (30 ℃ ± 0.5). Based on the results, it was observed that glucose from SHH was found to be the good carbon source for production of bioethanol compared with commercial glucose. Furthermore, out of two nitrogen sources (peptone and yeast extract) tested, peptone was preferable as less amount (1 g/L) was used compared to yeast extract (3 g/L). However the bioethanol production was comparable (21.42 g/L) for both nitrogen sources. From the present study, it can be concluded that glucose from SHH can be an attractive feedstock for bioethanol production from both economic stand point as well as environment friendly.

Key words: yeast extract, peptone, Sago Hampas Hydrolysate (SHH), nitrogen sources, bioethanol

ABSTRAK

Sumber nitrogen adalah sangat penting dalam proses penapaian untuk membekalkan nutrien kepada sel yis supaya ia boleh menghasilkan jumlah bioethanol yang tinggi. Kajian ini menumpukan kepada kaedah proses fermentasi kelompok dengan menggunakan 50 g/L glukosa daripada hidrolisat hampas sagu dengan menggunakan dua sumber nitrogen tambahan sama ada ekstrak yis (3 g/L) atau pepton (1 g/L). Proses fermentasi telah dijalankan dengan menggunakan 100 mL isipadu kerja, agitasi dengan kelajuan 100 rpm, permulaan pH dalam likungan 5.5-5.6 dan kekalkan dalam suhu (30 ℃ ± 0.5). Berdasarkan keputusan yang telah diperoleh, glukosa daripada SHH adalah yang terbaik sebagai sumber glukosa dalam proses penapaian untuk pengeluaran bioetanol berbanding glukosa komersial. Tambahan pula, daripada dua sumber nitrogen (pepton, ekstrak yis) yang dikaji, pepton dipilih sebagai sumber nitrogen yang terbaik dengan jumlah yang digunakan adalah sedikit (1 g / L) berbanding yis ekstrak (3 g/L). walaubagaimanpun, kepekatan pengeluaran bioethanol untuk kedua-dua sumber nitrogen adalah sama (21.42 g / L). Dari kajian ini, ia boleh disimpulkan bahawa glukosa daripada SHH boleh menjadi bahan mentah menarik untuk pengeluaran bioetanol jika dilihat daripada sudut pendirian ekonomi dan mesra alam.

Kata kunci: ekstrak yis, pepton, hidrolisat hampas sagu, sumber nitrogen, biethanol

1.0 INTRODUCTION

Fossil energy sources such as oil, natural gas, coal and others are highly used for the fuel production, electricity and other goods (Uihlein *et al.*, 2009). According to the Tortora *et al.*, (2010), fossil fuel is the non- renewable energy and the price would be more expensive over the years. Hence, there are drastically increase in the greenhouse gasses level in the earth's atmosphere due to the over consumption of fossil fuel to overcome the increasing of the generation population (Ballesteros *et al.*, 2006).There also has been interest to finds out the alternative sources energy to replace fossil fuel as it getting lesser over the years. New renewable source of energy has been found by production of ethanol from renewable agro waste by fermentation sugars and in a few countries of the world used ethanol as partial gasoline replacement (Sharma *et al.*, 2007). Brazil, USA, China, India and Pakistan are the example of the countries that have used bioethanol as alternative fuels (Rubio-Arroyo *et al.*, 2005).

Lin and Tanaka (2006) studies that, the component of agriculture waste mostly contain starch which is it can acts as substrate for the ethanol production through microbial fermentation process. According to Bujang (2008), sago palm was abundantly planted in Malaysia especially in Sarawak. Approximately, this sago palm contain high amount of starch and released 25 tons of dried sago starch per day by local sago mill. Hence, the production of sago waste also increased and this lead to environmental pollution. In order to minimize the pollution, nowadays sago fiber also known as sago hampas was fully utilized as the alternative raw material in order to produce fermentable sugars and bioethanol (Awg-Adeni *et al.* 2012).

Yeast are eukaryotic microorganism that has been widely used in biofuel industry in order to produce ethanol from sucrose or starch-derived glucose by fermentation process (Madhavan *et al.,* 2012). Yeast strains were exposed in variety of stresses such as oxygen concentration, osmotic pressure, pH, end product, nutrient availability and increasing temperature during the fermentation process whether for traditional beverage or bioethanol production (Kumar *et al.,* 2015). Glycolysis process by Embden-Meyerhof (EM) pathway explained that *S. cerevisiae* able to catalyze sugar (Lin & Tanaka, 2006). Tortora *et al.,* (2010) also stated that, alcoholic fermentation started when a molecule of glucose produced two molecules of pyruvic acid and two molecule of ATP. Then, two molecule of pyruvic acid converted into two molecules of acetaldehyde and two molecules of carbon dioxide Lastly, two molecules of acetaldehyde reduced by two molecules of NADH and catalyzed by alcohol dehydrogenase to form two molecules of ethanol.

Fermentation process commonly used yeast, *Saccharomyces cerevisiae* in order to produce ethanol and carbon dioxide. The nitrogen sources are important in the fermentation process which needed by yeast in order to get nutrients for growth and metabolic activity (Adding nitrogen to fermentation, 2014). Therefore, research needed to be done which none nitrogen content was observed in sago hampas, thus, in order to ensure efficient bioethanol fermentation by yeast, nitrogen need to be supplemented. Thus, the aims of this study were to:-

1. Study the effects of yeast extract and peptone on sago hampas hydrolysate (SHH) for bioethanol production.
2. Determine the correlation of yeast growth with bioethanol production utilizing glucose from sago hampas hydrolysate (SHH) and commercial glucose.

This research study was focused on effect of yeast extract and peptone in Sago Hampas Hydrolysate (SHH) at 50 g/L and commercial glucose was used as act as control and the ability to produce high amount of bioethanol through fermentation process.

2.0 LITERATURE REVIEW

2.1 Bioethanol Industry

The demand on green biofuel which is ethanol from fermentation process has increased due to increase of environmental issues such as global warming, petroleum crude oil shortage, the very high cost of oil and political instability of some crude oil producing countries (Saifuddin *et al,* 2011). In the year of 2050, the production of global oil is expected to decline from 25 billion barrels to 5 billion barrels (Campbell & Laherree, 1998).

Hence, the renewed interest in the potential use of renewable sources such as lignocellulosic materials to produce a variety of liquid biofuels such as biodiesel and bioethanol has developed when the situation of oil prices shows unstable or continual fluctuation (Vincent *et al.*, 2014). The production of bioethanol in USA and Brazil are the current leading nation compared to Asian countries altogether for about 14% of world's production (Carere *et al.*, 2008; Vincent, 2010). In the year of 2015, the bioethanol market is expected to reach 100 x 10^9 liters where the biggest producers of bioethanol are the USA which is used corn as feedstock for producing 35.5 x 10^9 liters of ethanol, next Brazil using sugarcane for produced 30x 10^9 liters and China is the most producer of ethanol and nowadays is the largest producer (Ivanova *et al.* 2011).

2.2 Applications of Bioethanol

Bioethanol is known as a renewable biomass fuel and also environmentally friendly which used for alternative energy sources, in addition, the physiological state and the microorganism fermentation will be influenced by the composition of the media (Hahn-Hagerdal *et al.,* 2005). Acording to Dhillon *et al.,* (2007) study that, there are several benefit about using bioethanol which are oxygen contain in the ethanol is 35% which is the fuel has completely combustion so that it will reduce the emission of harmful gases.

According to the Prasad *et al.,* (2007), the world total ethanol production was almost 100 billion liters during year of 2009-2010 and according previous research, ethanol consumption for fuel is the highest percentage as 68% followed 21% for industries and 11% for potable purpose. Besides, ethanol was able to replace gasoline by making gasoline-ethanol mixtures which are E15 (15% ethanol and 85% gasoline) or E85 (85% ethanol and 15% gasoline). Based on research study of Lin and Tanaka (2006), three main types of raw materials can be used to produce bioethanol. Firstly, sugar from sugarcane, sugar beets molasses and fruits which these sugars can be converted directly into ethanol. Secondly, cellulose from wood, agriculture residues, wastes sulfite liquor from pulp and paper mills. Generally, mineral acids are needed to convert cellulose to sugar. Thirdly, starches from corn, cassava, potatoes and root crops which need the action of enzyme to hydrolyzed the fermentable sugars.

2.3 Lignocellulosic Waste

Lignocellulosic occurs within plant cell walls, which consists of cellulose microfibrils embedded in lignin, hemicellulose and pectin. Wheat straw, corn stalk and cobs, ground nut shell and alfafa fiber are the example of the crops residues rich in lignocellulosic which have been exploited for ethanol production. **Table 1** below shows the importance of some agricultural waste for ethanol production (Prasad et *al.*, 2007).

Table 1: The importance of some agricultural waste for ethanol production

Agricultural waste	Importance
Corn fiber	Rich in hemicellulose, produced from corn hull during the wet milling of the corn
Sunflower hulls	Low commercial value, disposal problem due to their low bulk density, contained 53% cellulose, 17.5% hemicellulose and 11.4% lignin
Rice straw	Easily available agricultural waste in bulk, contained 40% cellulose, 18% hemicellulose and 5.5% lignin
Alfafa fiber	Consist of cellulose, hemicellulose, lignin, small amount of pectin and protein. Potent in the production of juice derived co-protein.

Source from Prasad *et al.* (2007)

2.4 Sago Hampas

Sago hampas is a starchy lignocelluloses, generated by- product during starch extraction process from pith of Metroxylon sago (sago palm). The quality of the extraction process could affect the amount of sago hampas released from the sago processing (Awg-Adeni *et al.*, 2010). Awg-Adeni *et al.*, (2010) adds that about 50-110 t of sago hampas are produced daily especially in Sibu and Mukah, Sarawak. The component of dry basis on sago hampas comprised of 58% starch, 23% cellulose, 9.2% hemicellulose and 4% lignin respectively (Linggang *et al.*, 2012). Ground sago hampas, which is in powder form, can be hydrolyzed into fermentable sugars through enzymatic hydrolysis (Awg-Adeni *et al.*, 2010).

2.5 Enzymatic Hydrolysis

Enzymatic hydrolysis of starch is the process to convert the starch into glucose and maltose. There are several processes under enzymatic hydrolysis which are gelatinization, liquefaction and saccharification. Gelatinization is the process to form viscous suspension by dissolution of starch granules while liquefaction is starch have to undergo partial hydrolysis with associated loss in viscosity and production of the glucose and maltose were through gelatinization process where further hydrolysis occurred (United State, 2008).

2.6 Sago Hampas Hydrolysate (SHH)

Sago hampas hydrolysate (SHH) was produced when the sago hampas undergoes process of starch saccharification. According to Awg-Adeni *et al.,* (2012), after gelatinization process, the Dextrozyme enzyme was added to aid saccharification of residual starch in sago hampas. Concentration of the glucose can be obtain at high amount when the three cycles of enzymatic hydrolysis where the first and the second cycle have the same method of enzymatic hydrolysis compared to the third cycle the centrifugation procedure is used which the hydrolysate have to centrifuge once (Awg-Adeni *et al.,* 2012), refer **Table 2** below. From the report, it show that, 40.30 g/L of ethanol was produced from the 84.75 g/L of glucose for 16 hours of fermentation which correspond 93.29% of fermentation efficiency.

Table 2: Concentration of glucose produced during the cycles

Enzymatic Hydrolysis	Glucose (g/L)
Cycle I	27.79
Cycle II	73.00
Cycle III	138.45

Source from Awg-Adeni *et al.,* (2012)

2.7 Batch fermentation

Ethanol can be produced by fermentation process which can be conducted in various types of operations such as batch, continuous, fed batch and semi continuous. In the batch fermentation, the process was carried out by putting the substrate and the yeast culture into the bioreactor together with nutrients. According to the Prasad *et al.*, (2007), batch fermentation is the most commonly method used in industry in order to produce ethanol because the operation cost is low; do not require much control and can be accomplished with unskilled labour.

2.8 Roles of yeast extract and peptone

During the fermentation process, the nitrogen sources are needed by the yeast so that yeast was able to get nutrients for growth and metabolic activity (Adding Nitrogen to fermentation, 2014). Based on the previous research that has been done by Jumoi @ Jumoi (2015), it indicates that the yeast extract and peptone were the best nitrogen sources for fermentation in the production of bioethanol. It also supported by Sridee *et al.* (2011) study which state that yeast extract and peptone shows more positive result than others nitrogen sources such as urea and ammonium sulfate. Based on study of Bergman (2001), yeast extract and peptone were nitrogen sources that provide many of metabolites which yeast cell would synthesize when growing under minimal growth conditions. Yeast extract is an excellent source because it containing peptides, amino acids, vitamin, nucleotides and essential elements where those components provide multi-functional nutritional supplement such as peptides and amino acid are important in cell growth. Peptone also consists of natural sources of amino acids and protein which get through enzymatic hydrolysis of animal tissue (Protein Sources, 2015).

2.9 Yeast

S. cerevisiae or known as baker yeast is able produce high concentration of ethanol at suitable nutrition condition and environment (Thomas *et al.*, 1996; Bafrncova *et al.*, 1999). Others previous study shows on some nutrients that give protective effect either on growth and fermentation or viability of yeast, which can stimulate fermentation rate and production of ethanol such as yeast extract (Casey *et al.*, 1984; Thomas and Ingledew, 1996; Jones *et al.*, 1994; Bafrncova *et al.*, 1999), ammonium (Jones *et al.*, 1994), urea (Jones and Ingledew, 1994a), calcium and magnesium (Dombek & Ingram, 1986). According to Lin and Tanaka (2006), yeast also able to produce the high amount of ethanol as high concentration up to 18% of fermentation broth, beside that this microorganism can survive on simple sugar such as glucose and disaccharide sucrose. In addition, yeast cell can metabolize the sugar directly to form degradable sugar;hence, it will reduce the amount of cost preparation (Lin & Tanaka, 2006).During industrial fermentations that exploit yeast, cells are confronted with a multitude of chemical, physical and biological stresses that may impair cell function and thus fermentation progress. Cells adapt to such stresses by generating responses designed to maintain cell growth and survival (Albertyn *et al.*, 1994). According to the Khongsay *et al.*, (2010) experiment that have been done on growth and batch fermentation of *S. cerevisae* on sweet sorghum stem juice, the results showed that under normal gravity conditions, final ethanol concentration and ethanol yield at the initial cell concentrations of $1x10^7$ and $1x10^8$ cells mL^{-1}were not significantly different and were slightly higher than those of $1x10^6$ cells mL^{-1}.Yeast cells are able to grow on a minimal medium containing dextrose (glucose) as carbon source and rapidly growth in presence of rich medium such as yeast extract (Bergman, 2001). During the period of log phase cell densities are $<10^7$ cell/mL and yeast divide once approximately every 90 min. after that, in the mid-log phase densities of cell are between 1 and 5 $x10^7$cells/mL and late log phase between 5 $x10^7$and 2 x 10^8 cells/ mL. There were two analyses that could be used on the yeast growth rate which are optical density (OD) reading at 600 nm and dry cell weight calculation.