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THE EFFECTS OF IONIC COMPOUNDS ON VERY HIGH GRAVITY FERMENTATION OF SAGO STARCH BY *SACCHAROMYCES CEREVISAE*

Unwaniah Binti Abdull Rahim

Bachelor of Science with Honours
(Resource Biotechnology)
2014

UNIVERSITI MALAYSIA SARAWAK

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Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
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The Effects of Ionic Compounds on Very High Gravity Fermentation of Sago Starch by *Saccharomyces cerevisae*

Unwaniah Binti Abdull Rahim
(33355)

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

Supervisor: Madam Dayang Salwani Awang Adeni

Co-Supervisor: Assoc. Prof Dr. Cirilo Nolasco Hipolito

Resource Biotechnology
Department of molecular Biology

Faculty of resource Science and Technology
University Malaysia Sarawak
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LIST OF ABBREVIATIONS

AMG	Amyloglucosidase
°C	Degree celcius
g/l	Gram per litre
HSS	Hydrolysed Sago Starch
kg	Kilogram
μl	Microliter
μ/g	Micro per gram
ml	Milliliter
mM	Millimolar
min	Minute
%	Percentage
Rpm	Revolutions per minute
VHG	Very High Gravity
v/v	Volume per volume
w/v	Weight per volume

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The Effects of Ionic Compounds on Very High Gravity Fermentation of Sago Starch by *Saccharomyces cerevisae*

Unwaniah Binti Abdull Rahim

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

ABSTRACT

Bioethanol is a technology implement by scientists for replacing non-renewable sources of energy. In this study, sago starch hydrolysate acted as the source of glucose was used as the sugar substrate (carbon sources) which converted into sugar prior to Very High Gravity (VHG) fermentation process. Highly concentrated glucose of 400 g/l was used for VHG fermentation to generate high final biethanol production which was stated to consume less energy and time. The production of bioethanol from VHG fermentation was carry out through batch fermentation process by the baker's yeast, *S. cerevisiae* at initial pH 5.5 to 5.6, temperature of 30 °C in 100 mL working volume. Two type metal ionic compounds, magnesium and calcium work as nutrient supplementations were added into fermentation concentrated 0.3 g/l each. Rate of fermentation were observed through yeast growth profile, glucose consumption and ethanol production. Supplementation of calcium ion into media fermentation has shown to produce highest ethanol level with 125.06 g/l at 72 hours. Compared to magnesium, calcium has lead to greater yeast cell growth and metabolic activity during VHG ethanol fermentation.

Key words: Sago starch, Very High Gravity (VHG) Fermentation, *Saccharomyces cerevisiae,* metal ionic compound, magnesium, calcium, bioethanol.

ABSTRAK

Bioetanol merupakan satu teknologi yang dijalankan oleh saintis untuk menggantikan sumber tenaga yang tidak boleh diperbaharui. Dalam kajian ini, kanji sagu hidrolisat bertindak sebagai sumber glukosa telah digunakan sebagai substrat gula (sumber karbon) yang ditukar menjadi gula sebelum proses fermentasi Graviti Sangat Tinggi (VHG). Glukosa yang likat, 270 g/l telah digunakan untuk fermentasi VHG bagi menghasilkan bioetanol yang tinggi untuk mengurangkan tenaga kerja dan menjimatkan masa. Yis roti, Saccharomyces cerevisae telah dipilih untuk proses penapaian dalam kajian ini. Pengeluaran bioetanol daripada fermentasi oleh S. cerevisiae dijalankan pada pH 5.5 hingga 5.6, pada suhu 30 °C untuk 100 mL. Sebatian dua jenis logam ionik, magnesium dan kalsium bertindak sebagai nutrisi tambahan ditambah ke dalam fermentasi sebanyak 0.3 g/l setiap komponen. Kadar fermentasi telah dicerap menerusi profil pertumbuhan yis, penggunaan glukosa dan pengeluaran etanol. Penambahan kalsium ion kedalam media fermentasi menunjukkan produksi etanol tertinggi sebanyak 125.06 g/l pada jam 72. Berbanding dengan magnesium, kalsium berupaya mengasilkan profil pertumbuhan yis dan aktiviti metabolisma yang tertinggi untuk fermentasi etanol menggunakan glukosa pada 270 g/l.

Kata kunci: Kanji sagu, fermentasi graviti sangat tinggi, *Saccharomyces cerevisia,* sebatian logam ionik, magnesium, kalsium, bioetanol.

CHAPTER 1

INTRODUCTION

1.1 Background Study

Over the time, the world oil crisis has been a serious issue. Climate changes, too much depending on oil and rapid usage of petroleum have led to the depletion of resources, the fossil fuels. Therefore the fossil fuels are substitute with the usage of biofuels as an alternative way for generating energy. Biofuels are type of renewable source potential to become sustainable energy source especially for transportation sector and other developments. Biofuels are obtained from bio-renewable or renewable feedstock. Compared to petroleum, biofuels are nonpolluting as they have higher oxygen content providing a better combustion, eventually minimize the emission of hydrocarbon and carbon monoxide. Basically, biofuels are classified in either gaseous or liquid type. Bioethanol is one of the liquid biofuels produced from cellulosic or hemi cellulosic biomass. It was derived from sugar or starch crops and lignocelluloses-based materials through the process of fermentation and acid/enzymatic hydrolysis respectively.

Most commercial food starches come from cereals, legumes, tubers and roots. Meanwhile, sago starch which is less commercial is derived from the stem of sago palm. However, sago, also known as *Metroxylan sagu* Rottb able to yield starches higher compare to that rice, wheat or corn (Flores, 2009). Composing of 88% carbohydrates, 0.5% protein while the rest are fat and vitamin B. Sago starch is beneficial in production of food and beverages such as for high fructose glucose syrup, monosodium glutamate and others. Studies proved that sago starch able to be converted into highly valued products. For instances the conversion of sago starch into ethanol as the source of fuel, or lactic acid, as an expensive raw material for biopolymer industry.

There were series of studies has been conducted regarding usage of sago starch for ethanol production. According to Sunaryanto *et al.* (2007), the sago starch was hydrolysate using 2.5% sulfuric acid and amylases enzymes. The hydrolyzed sago starch concentrations used were varies among another; 5, 10, 15, 20 and 30% (w/v). Highest hydrolysate composed of sugar was then used as substrate for ethanol production through fermentation by *Saccharomyces cerevisae.* Using 20% (w/v) sago starch has produce the highest sugar concentration which is 17.1% (w/v). The final production gives out ethanol concentration of 7.98% (v/v) (Sunaryanto *et al.*, 2013). However, higher yield of ethanol can be achieved above 15% (v/v) if Very High Gravity (VHG) fermentation is applied. According to Chunkeng *et al.*, (2011), the potential of VHG to increase the ethanol productivity has been proven better compared to general observation in distilleries that is only 10-12% (v/v). Improvement of VHG fermentation has been studied using an optimal medium of 300 g/l glucose. The result summarized final product of ethanol by 30% higher, 29% decrease in fermentation time, 80% increase in biomass formation and 26% increase in glucose consumption. VHG fermentation has shortened the time required to end the fermentation, which is from 84 hours to 60 hours (Chunkeng *et al.*, 2011).

Medium supplement supplied into fermentation are found able to enhance fermentation effectiveness eventually improve final ethanol productivity. A research results in higher ethanol level as much as 20% through optimization of medium (Wang *et al.*, 2007). Therefore, to improve VHG ethanol fermentation the media supplemented requires medium optimization

Metal ions, such as calcium and magnesium believed to effects the yeast cell growth and fermentation eventually yield higher ethanol level. Consequently, implementation of VHG ethanol fermentation benefits by lower the energy consumption and more costs saving would have a greater economic.

1.2 Problem Statement

Which metal ionic compound in medium supplementation able to improve most the final bioethanol concentration in Very High Gravity fermentation of Sago starch Hydrolysate by *S. cerevisiae.*

1.3 Objectives

- To evaluate the effects of the selected metal ionic compound; magnesium and calcium on VHG fermentation utilizing sago starch hydrolysate by *S. cerevisiae* for bioethanol production
- To optimized media composition with selected metal ionic compound to suit with VHG fermentation utilizing sago starch hydrolysate by *S. cerevisiae* for bioethanol production

CHAPTER 2

LITERATURE REVIEW

2.1 Sago Starch

Starch is basically a polysaccharide of plant origin that unable to be altered into sugars easily. Sago starch is made from the processed pith which is inside the trunk of Sago Palm (Shafie, 2009). The stem of sago palm possesses high concentration of starch as it can reach up to 250 kg (dry weight per plant). Still, starch yield varies depends on the soil condition where it growth. For example, is it around 180-385 kg starch/trunk in Sarawak. Sarawak covers wild sago around 21,000 hectares. However, 1.69 million hectares of Sarawak land believed to be suitable for sago cultivation, especially in Mukah (Flores, 2009).

Glucose obtain from sago starch is widely used as the substrate in fermentation industry and the main component in ethanol production industry. Table 2.1 shows high composition of amylose and amylopectin in the sago starch. An early research has been conducted on increasing the ethanol yield from sago starch using submerged fermentation. The method of simultaneous saccharification and fermentation (SSF) using coimmobilized amyloglucosiade (AMG) and *Zymomonas mobilis* was conducted and maximum ethanol concentration obtained was 55.3 g/l from 150 g/l of sago starch (Bandaru *et al.*, 2006).

Theoretically, conversion of starch into glucose by hydrolysis produces 60% of glucose. There are two methods of hydrolysis of starch namely enzymatic and acid hydrolysis. Commonly enzymes used during enzymatic hydrolysis are α-amylase and amyloglucosidase and dextrozyme. *S. cerevisae* does not possess amylolytic enzyme preventing it from directly convert starch into ethanol. The starch is hydrolysed into fermentable sugar via liquefaction and saccharification process prior to fermentation.

Table 2.1: Chemical composition of sago starch (Swinkles, 1985).

Constituents	Dry weight basis (%)
Amylose	27
Amylopectin	73
Lipids	0.1
Protein (nitrogen content 6.25)	0.1
Ash	0.2
Phosphorus	0.02

2.2 Very High Gravity Bioethanol

In recent years, Very High Gravity technology in fermentation has been applied as it conventional and capable to enhance ethanol productivity without consumption of much energy compared to general fermentation. Early definition of VHG is fermentation of mash composes of 27 g or more dissolve solids per 100 g mash. VHG fermentation doubles the ethanol production of fermentor by increasing from 7-10 to 15-18% (v/v) from highly dissolved solids of carbohydrates (Ingledew, 2005). The medium consist of starch hydrolysates containing 250 g or more of dissolved solids per liter of mash. Due to the high concentration of carbohydrates within substrates, yeast will undergoes ethanol stress during the VHG fermentation. At the same time, the extracellular ethanol will cause water stress towards the yeast. Thus, one of the factors for ethanol tolerance in yeast is the potency of osmotolerence. Higher osmotic pressure exerted on yeast the higher intracellular ethanol accumulation. The simultaneous effects of ethanol tolerance and osmotolerence have already been in physiological and molecular biological studies. Besides, the nutrients limitations, toxic product from fermentation may also decrease the process efficiency. Some factors should be considered for VHG fermentation such as nutrients supplementation and optimization of pH, temperature and aeration strategy.

2.3 Metal Ionic Compound

It is generally known the fermentation performance (shorter lag phase, fast and complete fermentation) can be enhance with the presence of nutrients provided in medium. It has been well documented of the effects of metal ions (potassium, magnesium, calcium, zinc, etc) on yeast cell growth and fermentation. The metal ions studied are capable to increase the sugar conversion rate and biomass accumulation especially magnesium and calcium which have widely studied on their influence against ethanol stress. Metal ions such as cobalt, manganese and ferrous are ineffective in promoting ethanol tolerance (Xue *et al.*, 2008). An addition of 0.02 g/l of cobalt does not influence on ethanol tolerance although increase the ethanol productivity. Xue *et al.* (2008) stated that magnesium and potassium are generally required by growing yeast cells in the minimolar (i.e. hundreds of ppm) concentration range, while calcium and zinc are regarded as trace metals being required in the micromolar (sub-ppm) range.

During VHG fermentation, magnesium ions protect the yeast cells by a mechanism that results in decreased permeability of plasma membrane under ethanol stress conditions (Chunkeng *et al.*, 2011). The effects of both magnesium and calcium ionic compound in medium supplementation against ethanol stress has been studied earlier. Moreover, the ions is required for several glycolytic enzymes activation and fatty acid biosynthesis enzymes in yeast, namely phosphofructokinase, phosphoglycerate kinase, pyruvate kinase and enolase.

Calcium ions on the other hand potential to increasee plasma membrane stability by decreasing ethanol-induced passive protons influx or by stabilizing the ATPase activity inhibited by ethanol. Effect of calcium ions also increasement of plasma membrane stability thus preventing the release of cytoplasmic compounds.

2.4 Yeast for Bioethanol

Saccharomyces cerevisiae is the most employed microorganism has the capability of producing ethanol using starch and sugar substrate. It is the most significant yeast and primarily used in bioethanol production. Single yeast cell capable to ferment glucose of its own weight in an hour time. *S. cerevisiae* can generate ethanol up to 18% volume compared to the standard, only 15 to 16% (Shafie, 2009).

During fermentation, the tank is possible to raise temperature over 40 °C due to exothermic reaction from yeast. According to Araque *et al.,* (2008), bioethanol production when conducted at high temperature could cause yeast cells to will result in inhibition of ethanol production hence leads to lower final ethanol concentration, meanwhile optimum temperature for best productivity happens at 32 °C. Hence it is important to specify an optimum temperature and proper yeast for maximum productivity of the yeast to ferment sugars from carbohydrates. The reason for using S. *cerevisae* because it is known able to convert carbohydrate sugars into biethanol at temperature range 25 to 30 °C.

Furthermore, *S. cerevisae* is predictable to be high tolerance towards bioethanol and able to maintain high viability. Besides, under optimal condition, the yeast is capable to perform the glycolysis at high rates and produce over 50 mmol of ethanol per hour per gram of cell protein. During VHG ethanol fermentation, low oxygen levels and sufficient amount of nitrogen is observed to promote and prolong the *S. cerevisae*.

CHAPTER 3

METHODS AND MATERIALS

3.1 Sago starch

Sago starch was obtained from Herdsen Sago Industries Sdn Bhd. from Pusa, Sarawak (Figure 3.1).

Figure 3.1: Herdsen sago starch.