IDENTIFICATION AND OPTIMIZATION OF PROCESS PARAMETERS FOR THE PRODUCTION OF BIOETHANOL FROM THE FERMENTATION OF OIL PALM TRUNKS SAP

NORHAZIMAH BINTI ABDUL HALIM

Thesis submitted in fulfillment of the requirements
for the award of the degree of
Master of Engineering (Bio-process)

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

MARCH 2012

ABSTRACT

Oil palm trunk (OPT) is generated from the replantation of oil palm trees at every 25-30 years interval, left as troublesome waste as it becomes the source of infection to young oil palm trees. This OPT contains a high amount of ready-to-use sugar in the form of sap which can be directly fermented to the most fermentation products. The fermentation process for ethanol production from OPT sap was evaluated in order to produce larger amount of bioethanol.

The feasibility of yeast *S. cerevisiae* to produce bioethanol from OPT sap was investigated along with the effect of different strains of *S. cerevisiae* and different pretreated mediums. It was proven that yeast *S.cerevisiae* was able to produce bioethanol even though the OPT sap undergoes less pretreatment compared to the previous works done by the other reseachers. The highest bioethanol yield and productivity had been obtained by using *S. cerevisiae* Kyokai no.7 in heat sterilized sap.

The effects of temperature, initial pH, agitation rate, percentage inoculum and time of incubation were explored using 2-level full factorial design in order to find out the main factor that affecting bioethanol fermentation from the OPT sap. The factors of temperature, initial pH and agitation rate were chosen for optimization study based on the higher percentage contribution (>5 %) and lower p-values (<0.05)

The influential factor was then optimized using rotatable central composite design under response surface methodology (RSM). The validation experiment in shake flask were also carried out and compared with lab-scale bioreactor (2 L). The bioethanol concentration improves with the temperature around 30-32 ^{o}C, agitation rate 60-90 rpm and initial pH of 5.00 to 5.50. The optimum condition for obtaining highest bioethanol production was after 36 h of fermentation at; temperature of 31 ^{o}C, initial pH 5.42 and agitation rate 80 rpm. The bioethanol production of 25.98 /L was obtained using this optimal condition, which is 97.93 % of the value predicted by the models. In 2 L of bioreactor, about 26.60 g/l of bioethanol concentration was obtained which is 2.39 % deviation from the result in shake flask experiment.

Analysis on the production capacity of the bioethanol fermentation from OPT sap showed that this OPT sap was comparable with other biomass in term of bioethanol production per hectare (944 liter of bioethanol per hectare of oil palm replantation) and annual production capacity. This can be improved by further study on optimization of nutrient and follow the recommended improvements. In conclusion, the process parameter for the production of bioethanol from OPT sap was successfully identified and optimized in this study.

ABSTRAK

Batang pokok kelapa sawit (OPT) yang terhasil daripada penanaman semula pokok kelapa sawit pada setiap 25-30 tahun menjadi bahan buangan bermasalah apabila ia menjadi punca jangkitan terhadap pokok kelapa sawit muda. OPT mengandungi amaun gula tersedia yang tinggi dalam bentuk jus yang mana ia boleh di tapai secara terus kepada produk fermentasi. Proses penapaian bagi penghasilan bioetanol dari jus OPT dinilai untuk menghasilkan bioetanol yang banyak.

Kebolehan yis *S. cerevisiae* untuk menghasilkan bioetanol dari jus OPT disiasat bersama dengan kesan strain yang berlainan dan jus berlainan rawatan. Adalah terbukti bahawa yis *S. cerevisiae* mampu menghasilkan bioetanol walaupun kurang rawatan diperlukan oleh jus OPT berbanding dengan hasil kerja terdahulu yang dilakukan oleh penyelidik lain. Penghasilan bioetanol dan produktiviti tertinggi dicapai menggunakan *S. cerevisiae* Kyokai no.7 dalam jus OPT dirawat dengan haba.

Kesan suhu, pH permulaan, kadar goncangan, peratus inokulum dan masa penapaian disiasat menggunakan "2-level full factorial design" untuk memilih faktor berpengaruh yang memberi kesan kepada penapaian bioetanol daripada jus OPT. Faktor suhu, pH permulaan dan kadar goncangan telah dipilih untuk kajian pengoptimuman berdasarkan "percentage contribution" yang tinggi (>lima %) dan "p-values' yang rendah (<0.05).

Faktor yang berpengaruh dioptimumkan menggunakan "rotatable central composite design" dibawah "response surface methodology (RSM)". Validasi eksperimen di dalam kelalang goncang dijalankan dan dibandingkan dengan bioreaktor berskala makmal (2 L). Kepekatan bioetanol ditingkatkan dengan suhu sekitar 30-32 ^{o}C, kadar goncangan 60-90 rpm dan pH permulaan 5.00-5.50. Keadaan optimum untuk penghasilan bioetanol tertinggi adalah semasa 36 jam penapaian pada; suhu 31 ^{o}C, pH permulaan 5.42 dan kadar goncangan 80 rpm. Penghasilan bioetanol sebanyak 25.98 /L telah diperolehi menggunakan keadaan optimum ini, yang mana merupakan 97.93 % dari nilai yang dijangkakan oleh model. Di dalam 2 L bioreaktor, bioetanol sebanyak 26.60 g/l diperolehi, yang mana merupakan 2.39 % sisihan dari hasil di dalam kelalang goncang.

Analisis ke atas kapasiti penghasilan bioetanol daripada jus OPT menunjukkan ia setanding dengan bio jsim lain dalam istilah penghasilan bioetanol per hektar (944 liter bioetanol per hektar penanaman semula pokok kelapa sawit) dan kebolehan penghasilan tahunan. Ini dapat ditingkatkan dengan kajian tambahan terhadap pengoptimuman nutrien dan mengikut cadangan-cadangan penambahbaikkan. Secara konklusinya, proses parameter untuk penghasilan bioetanol daripada jus OPT telah berjaya dikenalpasti dan dioptimumkan dalam kajian ini.

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LIST OF SYMBOLS

°C	degree celcius
µm	micrometer
g/cm	gram per centimeter
g mol^{-1}	gram per mol
$g.g^{-1}$	gram per gram
g/l	gram per liter
g/l.h	gram per liter per hour
ha	hectare
kPa	kiloPascal
h	hour
L	liter
min	minute
ml	mililiter
nm	nanometer
Pa.s	Pascal per second
rpm	rotation per minute
R_t	retention time
v/v	volume per volume
w/v	weight per volume

LIST OF ABBREVIATIONS

2FI	two factor interaction
ANOVA	analysis of variance
ATCC	American Type of Culture Collection
B-B	Box-Benhken
CDW	cell dry weight
CFU	colony forming unit
CPO	crude palm oil
DM	dried matter
DOE	design of experiment
EFB	empty fruit bunch
FFD	full factorial design
GC-FID	gas chromatography flame ionized detector
HPLC	high performance liquid chromatography
OD	optical density
OFAT	one-factor-at-a-time
OPT	oil palm trunk
P-B	Plackett-Burman
RSM	response surface methodology
TRS	total reducing sugar

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

The natural energy resources such as petroleum and coal have been consumed at rapid rates over the last decades due to heavy reliance of modern economy on these fuels thus, this leads to the diminishing of this nonrenewable supply (Siqueira et al., 2008). Extreme usage of these resources such as fossil fuels resulting global warming, which affects human lives and causing an environmental problem due to the alleviation of earth's temperature (Goh et al., 2010; Shuit et al., 2009). Substitution of fossil fuels with renewable energy or bioenergy is important to sustain energy demand and reduce the emission of greenhouse gases to our environment.

Bioenergy is a special form of chemical energy that involves any kind of chemical energy accumulated through recent photosynthetic processes. In general, natural resources that contained bioenergy and can be processed to obtain more complex energy carriers suitable for end-uses are called biomass (BNDES and CGEE, 2008). Examples of sources of bioenergy include wood and sawmill waste, charcoal, biogas resulting from the anaerobic decomposition of waste, as well as liquid biofuels, such as bioethanol and biodiesel. Bioethanol can be directly used as a fuel or can be blended with petrol or gasoline to form blend fuel. It has been long considered as a suitable alternative to fossil fuels either as a sole fuel in cars with dedicated engines or as an additive in fuel blends with no engine modification requirement when mixed up to 30 % (Talebnia et al., 2010).

1.2 PROBLEM STATEMENT

In the 21st century, the production of fossil fuel is expected to reach the peak and afterwards decline when oil fields are depleted. However, global demand for energy continues to grow as human population rapidly expand and also due to the increase of the industrial prosperity in developing countries (Talebnia et al., 2010). As a consequences, global transport fuel demand forecast to be rise by 45 % from 2006 to 2030 (Rashid and Ibrahim, 2009). This phenomenon will cause increasing of production costs for fossil fuels as well as their selling price. It is expected that production of ethanol becomes important to fulfill increasing demands.

The process of ethanol production is well established for some sugars, such as glucose from cornstarch or sugarcane, and now become an emerging industry. However, the established commercial ethanol production relies on the fermentation of food sugar or starch causes food 'insecurity'. Besides, human food, which is relatively expensive, is often used as the feedstock; hence, the cost of the ethanol production by fermentation is relatively high (Kun, 2003). Biofuels have been in the spotlight recently owing to the surges in food and agricultural commodity prices for which biofuel production has often been held largely responsible (BNDES and CGEE, 2008).

There is a growing interest to find alternate bioresources apart from sugar cane and starchy crop for bioethanol production such as biomass (Swain et al., 2007). However, the production of fuel ethanol from the sugars in lignocellulosic biomass becomes challenging with many opportunities for enhancement. The production costs are still the key impediment to the widely use of ethanol as fuel, even though the fermentative process for ethanol production is well known (Siqueira et al., 2008). The barrier for the production and recovery of valuable materials from lignocellulosic waste is mostly related with the structure of lignocellulose, which has evolved to resist degradation due to rigid cross-linking between the polysaccharides (cellulose and hemicellulose) and lignin (Yan and Shuzo, 2006; Xiao et al., 2007). In order to overcome this problem, a lot of research has been carried out to obtain the most efficient fermentative strain utilizing carbon sources in biomass, abundant yet easy-to-ferment biomass and also optimal fermentation conditions in order to reduce the costs (Pinilla et

al., 2010; Swain et al., 2007; Chin et al., 2010; Rocha et al., 2010; Lim[b] et al., 2011, Cazetta et al., 2007; Alam et al., 2009; Yingling et al., 2010).

The utilizing of waste especially biomass can reduce the impact of energy demands on food-derived-ethanol prices and environmental without having to compete with the food requirement. The cheaper but plentiful substrates for ethanol production become requirement to increase the production capacity and to reduce the production costs (Sumphanwanich et al., 2008). The raw materials itself can contributed up to 70 % of the cost of ethanol production (Shuler and Kargi, 2002). Selection of inexpensive raw materials has an important impact on process economic (Shuler and Kargi, 2002). Locally grown agricultural crops are a good choice since transportation cost can be reduced and guaranteed continuous supply of biomass.

There are abundant waste sources generated from palm oil mill and plantation in Malaysia includes fronds, empty fruit bunch (EFB), oil palm trunk (OPT) and also palm-oil-mill-effluent (POME). In specific, about 10827 thousand tons of trunks were generated from 4304914 hectares (ha) of total cultivation area in 2007, where each ha of oil palm cultivation can generate 2.515 tons of trunks per year when trees are chopped at every 25 years (Goh et al., 2010). Recently, a lot of research on the production of bioethanol has been studied for EFB (Piarpuzan et al., 2011; Lau et al., 2010), OPT fiber (Chin et al., 2010; Kaida et al., 2009) and POME (Alam et al., 2009) while limited researches have been done for production of bioethanol from OPTs sap (Kosugi et al., 2010; Murai and Kondo, 2010). Preliminary studies done by Kosugi et al. (2010) shown that OPTs sugar can be used for bioethanol production, however, the method used was sluggish and consume a lot of energy. Simple and easy approaches can reduce a lot of steps and production cost.

During replantation stage, OPT usually smashed into small pieces and left to be rotten naturally in plantation area. Generally, high sugar content in OPTs will attract microflora and microfauna, which increase the possibility of plant diseases (Sulaiman et al., 2009). Although oil palm lumber has been successfully utilized as a main material in the production of blackboard, but not all part can be used for plywood manufacturing because only the outer part is relatively strong (Sumathi et al., 2009; Shuit et al., 2009;

Kosugi et al., 2010). Moreover, OPT cannot be used as building structure due to its low specific density. Generally, the inner part will be discarded as a waste due to its weak physical properties thus, can be considered as important biomass in Malaysia. OPT have a lot of liquid (sap) that naturally contains lower lignin percentage and ready fermentable sugars compared to the other parts of oil palm trees (Denmark's Technical University, 2009). By this means, less or no chemical or biological pretreatment is needed to delignify or convert lignocellulose to readily fermentative sugar, as compared with the other part of oil palm tree.

There are many types of ethanol fermentative organisms that is being used currently. Yeast are the preferred organisms for industrial-scale ethanol production, where wide variety of yeast species can be utilized depending on the composition of raw material used (Shuler and Kargi, 2002). Even though *S. cerevisiae* is the most chosen organism for ethanol production, however, a wide variety of *S. cerevisiae* is available particularly between the namely industrial and laboratory strain. On average, industrial strain such as *S. cerevisiae* Kyokai no.7 or also known as *S. cerevisiae* var. *ellisideuous* have greater ability in term of ethanol production. But, in term of cost, *S. cerevisiae* Kyokai no.7 is quite expensive compared to the laboratory strain such as baker's yeast *S. cerevisiae.*

Fermentation that can takes place in the non-treated medium was favorable due to simple procedures as well as to reduce the cost. However, the sterilization become compulsory if the strain used cannot survive in that environment or unable to produce desire product. Also, it was well known that not all methods are suitable for certain biomass since it can generate secondary product that can inhibit production. Therefore, selection of an appropriate sterilization method is an important step for the bioethanol production.

In the research area of bioethanol derived from biomass, response surface methodology (RSM) had been applied for bioethanol production from sago starch, cassava mash and carob pod (Bandaru et al., 2006; Yingling et al., 2010; Turhan et al., 2010; Vaheed et al., 2010). To the best of our knowledge, there is no research has been

done by any researcher to optimize the bioethanol production from OPTs sap especially using statistically design of experiment (DOE).

1.3 OBJECTIVES

The aim of this research is to produce higher production of bioethanol by optimizing the overall process conditions for bioethanol production from OPT sap. The objectives are:

(i) To identify process parameters for the production of bioethanol from the fermentation of OPT sap
(ii) To identify the optimum condition for the production of bioethanol from the fermentation of OPT sap

1.4 SCOPES

In order to achieve the stated objectives, the following scopes have been identified:

(i) To evaluate the feasibility of OPT sap as a direct substrate for bioethanol production by using yeast *Saccharomyces cerevisiae*
(ii) To investigate the effect of sampling methods on bioethanol production
(iii) To investigate and justify the performance of different strains for bioethanol production (*S. cerevisiae* Kyokai no.7 and baker's yeast *S. cerevisiae*)
(iv) To carry out a comparative study of bioethanol production in different pretreated OPT sap (heat sterilization, cold sterilization and nonsterile sap)
(v) Five main factor affecting bioethanol productions from literature studies are screened out using 2-level full factorial design. Experiments are carried out as a function of temperature, initial pH, agitation rate, inoculums size and time of incubation

(vi) The important variables are optimized using rotatable central composite design RSM. Experiments are carried out to validate the result from response surface methodology and compared with lab-scale bioreactor. The capability of ethanol production per ha of replantation and annual production capacity are calculated.

CHAPTER 2

LITERATURE REVIEW

2.1 ETHANOL

The ethanol (ethyl alcohol), CH_3CH_2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group (–OH), bonded a carbon atom. It is volatile and flammable. The characteristics and properties of ethanol are shown by Table 2.1.

Table 2.1: Characteristic and properties of ethanol

Parameter	Units	Properties/values
Molecular formula	-	C_2H_6O
Molar mass	g mol^{-1}	46.07
Exact mass	g mol^{-1}	46.041864814
Density	g/cm	0.789
Melting point	°C	-114
Boiling point	°C	78
Flash point	°C	13-14
Autoignition temperature	°C	362
Vapor pressure	kPa	5.95 (at 20 °C)
Viscosity	Pa.s	0.0012 (at 20 °C)
Refractive index	-	1.36
Dipole moment, D	-	1.69
Log P	-	-0.18
Acidity (pK_a)	-	15.9
Basicity (pK_b)	-	-1.9
Appearance	-	Colorless liquid

The properties of the ethanol (i.e combustion energy) enable it to be used as energy sources. Complete combustion of ethanol produces only carbon dioxide and water which are not harmful to the environment and that became one of the advantages

to accelerate the research momentum. It can be used as an automotive fuel by itself or can be mixed with petrol to form an ethanol-petrol blend, increase octane levels and extend the supply of gasoline. Due to this fact, it is widely used by major oil companies and distributors (Okamoto et al., 2011; Trummer, 2006). Apart from that, it is used as indicator in thermometers, as alcoholic beverages and also as industrial solvent. In hospital and laboratory, it is consumed for sterilization purposes.

2.1.1 Production of Bioethanol

Ethanol can be produced from two main processes which are via synthetic process such as hydration of ethene, and via biological pathway such as fermentation. From ethene, ethanol is manufactured by reacting ethene with steam as in Eq.2.1.

$$\underset{\text{(ethene)}}{C_2H_4 + H_2O} \rightarrow CH_3CH_2OH \qquad \text{(Eq.2.1)}$$

Compared to the synthetic processes, ethanol is commonly produced by fermentation of hexose sugar by employing ethanol fermentative organism such as yeast *Saccharomyces cerevisiae*. Under certain condition, yeast will consumed available sugar to the ethanol. The equation for the fermentation of glucose is as Eq.2.2.

$$\underset{\text{(in the presence of yeast)}}{C_6H_{12}0_6} \rightarrow 2CH_3CH_2OH + 2CO_2 \qquad \text{(Eq.2.2)}$$

The economic between both process are depending on the price of raw material. For example, ethanol produced via synthetic pathway will be influenced by the petroleum price while ethanol produced via fermentation will be influenced by 70 % cost of the raw material (Shuler and Kargi, 2002). Conventional ethanol production from fermentation is based on sugar (glucose). For instance, fruits, sugar cane, or grains such as corn and wheat, potatoes and soy starches are used as raw materials for the ethanol production in many full-scale plants around the world (Trummer, 2006). Generally, the ethanol that derived from edible sources is called first-generation while second generation ethanol derived from non-edible sources such as lignocellulosic

biomass. The ethanol produces from renewable sources is called as bioethanol. In western Asia, drink-alcohol or wine-making can be dated as early as 5400-5000 BC at a site in what is today northern Iran and, further south in Iran, at a site from 3500 to 3000 BC (Kamm et al., 2007). Ripe grapes supply the abundant sugar and other nutrients necessary for rapid microbial fermentations as well as the causative yeasts themselves, usually as passengers on the skins of the fruit (Mousdale, 2008). Simply crushing the grapes initiates the fermentation process that produces ethanol at 5-10 %v/v in the unstirred vessel. Grape wines, beers from cereals and alcoholic drinks made from honey, dates, and other fruits grown in the Fertile Crescent are likely to have ethanol concentrations below 10 %v/v (Mousdale, 2008).

In Malaysia, there is currently no large-scale production of bioethanol and biomethanol as the demand for this alternative fuel is low since most of the vehicles in Malaysia are still running on petrol (Shuit et al., 2009). Moreover, bioethanol from lignocellulosic biomass is still a relatively new idea in Malaysia as the development of lignocellulose-related technologies is also not very well-established in the world (Goh et al., 2009). The Fuel Diversification Policy in Malaysia has been continuously reviewed to avoid from over depending on a single source of energy. As a signatory to the UN Convention on Climate Change and the Kyoto Protocol which commits to take steps to reduce green house gaseous emissions, Malaysia has the responsibility to diversify the energy mix with more sources of renewable energy (Goh et al., 2009). The global fuel mandates for Malaysia by 2020 are 10-15 % of bioethanol. From the total 11 billion liters of petrol used in 2007, it was estimated that 10 to 15 % of bioethanol blends requires 1.10-1.65 billion liters of bioethanol per year (Rashid and Ibrahim, 2009).

2.1.2 Biomass for Bioethanol Production

Typically, researches on the biomass are depending on the availability of biomass in that place. Table 2.2 showed the researches on bioethanol from the differrent biomass.

Table 2.2: Research on bioethanol from biomass

Biomass	Pre-treatment*	Initial raw material	Maximum yield of ethanol (g/l)	Time (h)	References
Acacia dealbata	Dilute acid pretreatment and enzymatic saccharification	31.1 g/l of glucose	10.31	24	Ferreira et al., 2011
Cashew apple bagasse	Dilute acid pretreatment and pH adjustment	25.1 g/l of glucose	12.44	48	Rocha et al., 2010
Carob pod extract	Five nutrients added	115.3 g/l sugar	42.60	48	Turhan et al., 2010
Cassava stem	Dilute acid pretreatment and enzymatic hydrolysis	9.5 g/l wheat bran (15.5 g/l glucose)	7.55	24	Han et al., 2011
Corncob residues	Sulfite pretreatment and enzymatic hydrolysis	15 % w/v of glucan substrate loading	60.08	72	Cheng et al., 2011
Forest residue *Pterospartum tridentum*	Dilute acid pretreatment	9.8 g/l sugar	3.20	24	Ferreira et al., 2010
Hazelnut shell	Partial synthetic xylose supplementation	50.0 g/l of TRS	16.79	90	Arslan and Eken-Saracoglu, 2010
Jerusalem artichoke juices	Acid and enzymatic hydrolysis	-	104.20	36-48	Onsoy et al., 2007
Kinnow waste and banana peels	Steam exploded, enzymatic saccharification and SSF	63.0 g/l reducing sugar	26.84	24	Sharma et al., 2007
Mahula flowers	Immobilized cells. Steam cooked and pH adjustment	9 ^{0}Brix	33.99	96	Swain et al., 2007
OPT sap	No	55 g/l	30.00	48	Kosugi et al., 2010
OPT sap	Hot water extract, saccharification and liquefaction	-	25.25	-	Murai and Kondo, 2010
Potato starch residue	$ZnCl_2$ added	10.0-20.0 g/l starch	5.52	36	Hashem and Darwish, 2010

Table 2.2: Continued

Biomass	Pre-treatment*	Initial raw material	Maximum yield (of ethanolg/l)	Time (h)	References
Red sage	Dilute acid pretreatment, detoxification and delignification	34.8 g/l sugar	17.70	16	Kuhad et al., 2010
Rice straw	No	20.0 g/l rice straw	3.00	96	Okamoto et al., 2011
Rice straw	Acid hydrolysis, hydrolysate concentrated and detoxification	16.8-31.0 g/l sugar	12.00	36	Yadav et al., 2011
Sorghums fibers	Ammonia hydroxide treatment	46.9 g/l of glucose	24.53	48	Salvi et al., 2010
Soybean molasses	Media concentrated	116.6 g/l sugar	58.60	6	Siqueira et al., 2008
Sugar maple chips	Hot water extract	50.0 g/l total sugar	20.57	120	Xu[a] and Liu, 2009
Sweet sorghums juice	Yeast extract and peptone added	280.0 g/l total sugar	121.22	72	Laopaiboon et al.,
(sucrose /molasses added)	No	280.0 g/l total sugar	117.42	72	2009
Sweet sorghums stalk juice	Immobilization	187.7 g/l total sugar	90.00	-	Yu et al., 2009
Sugar cane bagasse	Sodium chlorite, acetic acid and SSF	180.0 g/l sugar cane	32.60	72	Sasikumar and Viruthagiri, 2008
Wheat bran	No	20.0 g/l wheat bran	4.30	96	Okamoto et al., 2011
Wood (*Eucalyptus globules)*	Deliglinification, simultaneous saccharification and fermentation (SSF)	-	35.00	48	Munoz et al., 2011
Wood waste (lumber)	Two stage of acid hydrolysis and pH adjustment	20.0 g of wood	25.60	72	Cho et al., 2011
Wood waste (plywood)	Two stage of acid hydrolysis and pH adjustment	20.0 g of wood	19.90	72	Cho et al., 2011
Wood waste (particleboard)	Two stage of acid hydrolysis and pH adjustment	20.0 g of wood	19.20	72	Cho et al., 2011

*Comminuted, milling and sterilization was excluded from the table (basic pre-treatment)

Refer to the Table 2.2, there are different biomass for bioethanol production has been studied such as bagasses (Rocha et al., 2010), soybean molasses at the different scale (Siqueira et al., 2008), carob pod (Turhan et al., 2010), hazelnut shell (Arslan and Eken-Saracoglu, 2010), sorghum (Liu and Shen, 2008; Salvi et al., 2010; Yu et al., 2009) and mahula *Madhuca latifolia L*. flowers (Swain et al., 2007). Even though studies on converting lignocellulosic biomass to sugar for bioethanol production became interest among researchers, however, it was found that this conversion has many technical and economic challenges that delayed its commercialization (Sindhu et al., 2011).

Specific biomass need to be treated depending on its nature. Pre-treatment of the biomass is a crucial process since it is essential for the fermentable sugars to be release whereas available for the fermentation process (Harun and Danquah, 2011). It is necessary to have a pretreatment step to break the lignin and to expose cellulose and hemicelluloses for enzymatic saccharification in order to convert biomass into fermentable sugars if there are no ready sugar was available. The most common methods of pretreatment in commercial use are steam explosion and dilute acid hydrolysis (as in Table 2.2), but both methods having the drawback due to the formation of inhibitor that affects fermentation process (Sindhu et al., 2011). On the other hand, efficiency of enzymatic hydrolysis still requires improvement even though it has several advantages over acid hydrolysis including lowering environmental impact and reduce by-product yield. In fact, cost of the enzymatic hydrolysis of the biomass become a major bottleneck in the biomass-to-ethanol conversion (Okamoto et al., 2011).

The economic of a hydrolysis process depend on the yield of the usable component such as glucose (Chin et al., 2011). Xu et al. (2009) clarified that some options proposed to reduce costs of conversion of lignocellulosic to the ethanol are including eliminating pretreatment, increasing cellulose hydrolysis yield, enhancing enzyme activity and improving the fermentation yield. Nevertheless, each pretreatment method has its own advantages and disadvantages that must be considered to be proceed for large scale commercial production. It is beneficial if it already contain a lot of fermentable sugar such as OPT sap.

2.1.3 Microorganism for Bioethanol Production

Changes in the operational conditions are quite common in ethanol fermentation plants not only due to variation in raw material's quality but also because of yeast variations (Rivera et al., 2006). The yeast commonly used in industrial alcohol production include *Saccharomyces cerevisiae* (ferment glucose, fructose, maltose, maltoriose), *Saccharomyces uvarum* (carlbergensis), *Saccharomyces diataticus* (ferment dextrins), *Kluyveromyces fragilis* and *Kluyveromyces lactus* (ferment lactose) (Kun, 2003).

Kluyveromyces fragilis or *Candida* sp. can be utilized when there are available of lactose and pentoses. Another pentose and hexose fermenting organism such as *Clostridium hermosaccharolyticum* and *Thermoanaerobacter ethanolicus* are the thermophilic organisms that grant significant advantages for ethanol fermentation and separation. Unfortunately, its can gain undesirable end product and produce dilute ethanol (Shuler and Kargi, 2002). Apart from that, a Gram-negative bacterium, *Zymomonas mobilis*, is also considered as alternative organism for the large scale ethanol production fuel due to higher sugar uptake, higher ethanol yield and lower biomass production (Maiti et al., 2010). This bacteria is able to utilize glucose, fructose and sucrose as the substrates for the ethanol production. Development of genetic engineering also has succesfully transformed bacterium *E. Coli* to an ethanol producer where it was able to reach 43 %v/v of the ethanol concentration (Shuler and Kargi, 2002).

Alternatively, simultaneous saccharification and fermentation (SSF) as one of the direct bioconversion was favorable for the fermentation of lignocellulosic biomass in order to reduce dependency on chemical pretreatment. White rot basidiomycetes, *Trametes hirsuta*, is a fungi that suited for biological pretreatment of the lignocellulosic biomass and it was being used by Okamoto et al. (2011) to directly ferment wheat bran and rice straw for bioethanol production. Similarly, Alam et al. (2009) used mixed cultures including *Phanerochaete chrysoporium*, *Thrichoderma harzianum*, *Mucor hiemalis* and *S. cerevisiae* for direct ethanol bioconversion from POME. In addition, Chandel et al. (2010) works on combination of *Pichia stiptis* with *S. cerevisiae* found