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# A Review Article :
# Ethanol Fermentation by Saccharomyces cerevisiae using Agricultural Waste

**Siti Maftukhah[1)] dan Mutia Amyranti[2)]**

*Jurusan Teknik Kimia, Fakultas Teknik, Universitas Islam Syekh Yusuf, Jl. Mulana Yusuf No.10 Tangerang Banten 15118, Indonesia*

[1)] sitimaftukhah@unis.ac.id
[2)] mutiaamyranti@unis.ac.id

**Abstrak.** *Bioetanol adalah salah satu alternatif yang paling menjanjikan dan ramah lingkungan untuk bahan bakar fosil, yang diproduksi dari sumber terbarukan. Bioetanol dapat diproduksi dari berbagai jenis bahan baku. Tanaman konvensional seperti jagung dan tebu tidak dapat memenuhi permintaan global produksi bioetanol karena nilai makanan dan pakan utamanya. Limbah pertanian berbiaya efektif, terbarukan, dan berlimpah. Untuk melakukan ini, fermentasi gravitasi sangat tinggi (VHG) yang melibatkan penggunaan medium yang mengandung konsentrasi gula tinggi (>250 g/L) harus diterapkan untuk mencapai konsentrasi etanol yang tinggi. Namun, fermentasi VHG menyebabkan stres yang signifikan untuk Saccharomyces cerevisiae karena tekanan osmotik pada awal fermentasi dan kadar etanol yang tinggi pada akhirnya. Pada ulasan ini, jerami padi adalah limbah yang paling melimpah dibandingkan dengan limbah utama lainnya dan berpotensi menghasilkan 205 miliar liter bioetanol per tahun, yang merupakan yang tertinggi di antara keempat limbah pertanian tersebut.*

**Kata kunci:** *bioethanol, limbah pertanian, gravitasi tinggi, Saccharomyces cerevisiae*

***Abstract.*** *Bioethanol is one of the most promising and eco-friendly alternatives to fossil fuels, which is produced from renewable sources. Bioethanol can be produced from different kinds of raw materials. Conventional crops such as corn and sugarcane are unable to meet the global demand of bioethanol production due to their primary value of food and feed. Agricultural wastes are cost effective, renewable and abundant. To do this, very high gravity (VHG) fermentation which involves use of medium containing high sugar concentration(>250g/L) must be implemented to achieve high ethanol concentration. However, VHG fermentation leads to significant stress for Saccharomyces cerevisiae due to osmotic pressure at the beginning of the fermentation and high ethanol content at the end. At this review, rice straw is the most abundant waste compared to the other major wastes and potentially produce 205 billion liters bioethanol per year, which is the highest among these four mentioned agricultural wastes.*

**Keywords:** *agricultural waste,bioethanol,Saccharomyces cerevisiae,very high gravity*

## I. Introduction

Nowadays, the world is mostly dependent on fossil fuels for meeting its energy demand and more than 80% of the total global energy is obtained by burning fossil fuels. 58% of fossil fuels is consumed by the tranport sector (Escobar et al., 2009). There are 3 major challenges with the fossil fuels. First, the higher consumption of fossil fuels due to the growing industrialization and motorization has caused fast depletion of these nonrenewable fuels. Secondly, fossil fuels has the contribution to green house gas emissions and global warming that cause climate change,rise in sea level,and loss o fbiodiversity and urban pollution (Singh et al., 2010). Thirdly, political crisis, particularly in the Middle East countries, resulted an incidence of oil supply disruption by the major oil producer countries in the 1970s, which has also led to a rethink of our dependence on fossil fuels, since such crises are unsettling to the energy sector of both the developed and developing nations (Ogbonna et al., 2001). Therefore,it is necessary to find out an alternative energy source for our industrial economies and consumer societies by using renewable, sustainable, efficient and cost effective feed stocks with lesser emission of green house gases, where bioethanol would be an attractive alternative option

due to its ease of production and lack of toxicity (Zabed et al., 2014).

Very High Gravity (VHG) processes are very attractive and promising for bioethanol production allowing significant improvements in the overall productivity thus minimizing the production costs due to energy savings (Zhao et al., 2009).

The use of VHG technology imposes increased stressful conditions to the yeast cells, which have been associated with the loss of yeast viability during VHG fermentation, reduced fermentation rates and incomplete fermentations (Piddocke et al., 2009). Thus, the successful implementation of VHG technology in bioethanol production requires the development of yeast strains that efficiently ferment high sugar concentrations (>250 g l−1) (Bai et al., 2008). Such strains must be resistant to the multiple stresses found in the process, including the osmotic stress that results from the high sugar concentrations, the ethanol stress at the end of fermentation, the anaerobic conditions established in the large-scale bioreactors and the cell recycling procedures for utilization of the yeast biomass for several consecutive fermentation cycles (Mussatto et al., 2010).

S. cerevisiae has been widely used in industrial ethanol production. It is regarded as a safe microorganism, which can produce up to 20% (v/v) of ethanol under VHG conditions (Zaldivar et al., 2009). However, high ethanol concentrations are toxicto yeast cells, resulting in the reduction of cell viability. In theory,bioethanol is produced via the glycolysis pathway under anaerobic conditions. However, it has been found that appropriate aeration coupled with agitation, especially at the beginning of yeast growth, can markedly improve ethanol production (Khongsay et al., 2012).

High ethanol concentration is one of the goals of VHG fermentation. The yeasts used in VHG fermentations have to be highly ethanol tolerant strains. Ergosterol is a sterol that is an important component of plasma membranes. It plays an important role in ethanol tolerance in terms of membrane fluidity (Lei et al., 2007). Microaerobic conditions can improve the ethanol tolerance of yeasts, leading to an increase in yeast cell permeability and overall fermentation rate. Membrane fluidity depends on the environmental conditions to which yeast areexposed (Beney et al., 2001). During VHG ethanol fermentation, yeasts require a small amount of oxygen to synthesize sterols and unsaturated fatty, which are essential for plasma membrane integrity (Fornairon et al., 2002). Therefore, ethanol fermentation with appropriate aeration can promote ethanol production by S.cerevisiae, especially under VHG conditions.

It has been estimated that 442 billion liters of bioethanol can be produced from lignocellulosic biomass and that total crop residues and wasted crops can produce 491 billion liters of bioethanol per year, about 16 times higher than the actual world bioethanol production (Cadoche et al., 1989). It includes crop residues, grasses, sawdust, wood chips, etc. Extensive research has been carried out on ethanol production from lignocellulosics in the past two decades (Binod et al., 2010). Hence bioethanol production could be the route to the effective utilization of agricultural wastes. Rice straw, wheat straw, corn straw, and sugarcane bagasse are the major agricultural wastes in terms of quantity of biomass available (Duff et al., 1996). This review aims to present a brief overview of the available and accessible technologies for bioethanol production using these major agrowastes.

## II. Method and Results

### Agricultural Waste

The four major agrowastes mentioned in the preceding section are the most favorable feedstocks for bioethanol production due to their availability throughout the year. Worldwide production of these agrowastes is given in Table 1. Asia is the major producer of rice straw and wheat straw, whereas corn straw and bagasse are mostly produced in America (Table 1).

**Table 1.** Quantities Of Agricultural Waste (Million Tons) Reportedly Available For Bioethanol Production

| Agrowaste | Africa | Asia | Europe | America | Oceania |
|---|---|---|---|---|---|
| Rice straw | 20.9 | 667.6 | 3.9 | 37.2 | 1.7 |
| Wheat straw | 5.34 | 145.20 | 132.59 | 62.64 | 8.57 |
| Corn straw | 0.00 | 33.90 | 28.61 | 140.86 | 0.24 |
| Bagasse | 11.73 | 74.88 | 0.01 | 87.62 | 6.49 |

They also vary in chemical composition (Table 2), cellulose being the major component. These agro residues are also utilized as animal fodder, as domestic fuel, and as fuel to run boilers.

a. Rice Straw

The utilization fraction of wheat straw, rice straw and corn straw is too low and varies with geographic region. Each year a large portion of agricultural residues is disposed of as waste. For instance, approximately 600-900 million tons per year rice straw is produced globally. The options for the disposal of rice straw are limited by the great bulk of material, slow degradation in the soil, harboring of rice stem diseases, and high mineral content. Only a small portion of globally produced rice straw is used as animal feed, the rest is removed from the field by burning, a common practice all over the world, increasing air pollution and affecting human health.

b. Corn Straw

Open field burning is already banned in many countries in Western Europe and some other countries have considered it seriously. Less than 1% of corn straw is collected for industrial processing and about 5% is used as animal feed and bedding. More than 90% of corn straw in United States is left in the fields (Glassner et al., 1999).

**Table 2.** Chemical Composition of Agricultural Waste

| Substate | Cellulose (%) | Hemicellulose (%) | Lignin (%) | Protein (%) | Ash (%) |
|---|---|---|---|---|---|
| Rice straw | 32-47 | 19-27 | 5-24 | - | 12.4 |
| Wheat straw | 35-45 | 20-30 | 8-15 | 3.1 | 10.1 |
| Corn straw | 42.6 | 21.3 | 8.2 | 5.1 | 4.3 |
| Baggase | 65 ( total carbohydrate) | | 18.4 | 3 | 2.4 |

c. Wheat Straw

Wheat straw consists of D-xylose: 12.80 ±0.25 g/L and D-glucose: 1.70 ± 0.30 g/L. For processing wheat straw we have to do knife milling with 0.7-1.0 mm rejection screen then washed with water and dried. Hydrolisis of wheat straw optimally occurs st 90 0C with 1.85% (w:v) sulfuric acid for 18 h; liquid to solid ratio of 20:1. Then the suspension centrifuged and the residue is washed with hot water.

**Table 3.** Worldwide Potential Bioethanol Production from Agricultural Waste

| Agricultural Residu | Potential annual bioethanol production (globally) (giga liter) |
|---|---|
| Rice straw | 205 |
| Wheat straw | 104 |
| Corn straw | 58.6 |
| Sugarcane bagasse | 51.3 |

d. Bagasse

Sugarcane bagasse has its prominent use as a fuel for boilers and for cogeneration of electricity (Banerjee et al., 2010). It consists of 89.2 ±0.7% (glucose), 77.2 ±0.9% (xylose). It will be well processed by ball mill. Then hydrolyzed with Enzymatic (Acremonium cellulase at 5 FPU/g substrate of cellulase and 20 U/g substrate of xylanase from Optimash BG at 45 0C, pH 5.0 for 72 h.

**Table 4.** Carbohyddrate Content of Agricultural Waste (%)

| | Glucose | Xylose | Mannose | Galactose | Arabinose |
|---|---|---|---|---|---|
| Rice straw | 41-43.4 | 14.8-20.2 | 1.8 | 0.4 | 2.7-4.5 |
| Wheat straw | 38.8±0.5 | 22.2±0.3 | 1.7±0.2 | 2.7±0.1 | 4.7±0.1 |
| Corn straw | 39 | 14.8 | 0.3 | 0.8 | 3.2 |
| Bagasse | 38.1 | 23.3 | - | 1.1 | 2.5 |

Globally, bioethanol production from rice straw, wheat straw, corn straw and sugarcane bagasse is now a matter of interest (Table 3). Rice straw is the most abundant waste compared to the other major wastes (Table 1) and rice straw can potentially produce 205 billion liters bioethanol per year, which is the highest among these four mentioned agricultural wastes.

Lignocellulose is a complex carbohydrate polymer of cellulose, hemicellulose and lignin. Cellulose is linear and crystalline. It is a homopolymer of repeating sugar units of glucose linked by b-1,4 glycosidic bonds. Hemicellulose is a short and highly branched polymer. It is a heteropolymer of D-xylose, D-arabinose, D-glucose, D-galactose, and D-mannose. Lignin is hydrophobic in nature and is tightly bound to these two carbohydrate polymers. It thus protects these polymers from microbial attack (Peiji et al., 1997). Sugar compositions of various agrowastes (rice straw, wheat straw, corn straw, bagasse) are given in Table 4 (Lee et al., 1997). Lignocellulosics are processed for bioethanol production through three major operations: pretreatment for delignification is necessary to liberate cellulose and hemicellulose before hydrolysis; hydrolysis of cellulose and hemicellulose to produce fermentable sugars including glucose, xylose, arabinose, galactose, mannose and fermentation of reducing sugars. The non-carbohydrate components of lignin also have value added applications (Balat et al., 2008).

**Very High Gravity (VHG)**

Very high gravity (VHG) technology has been introduced toincrease the volumetric productivity and the cost effectivenessof the SSF process. In VHG technology, mash preparation contains at minimum of 270 g/l dry matter (Bayrock et al., 2001). This technology has a great deal of advantages in ethanolproduction: (i) increasing plant capacity and reduction in capitalcosts; (ii) increasing plant efficiency; (iii) reducing risk of contaminating bacteria (Thomas et al., 1996).

Nevertheless, VHG technology causes also some inconvenience, including the high viscosity of starch paste after liquefaction,which leads to the resistance to solid–liquid separation, difficulties in handling process, incomplete hydrolysis of starch tofermentable sugars and lower fermentation efficiency (Srikanta et al., 1992). Therefore, the success of its application depends on the preparation of mash with low viscosity. For instance, in order to reduce starch paste's viscosity, sweet potato was pretreated in a VHG process by using cell-walldegrading enzymes such as cellulases, pectinase, hemi-cellulasesand viscosity reduction enzyme (xylanase). As a result, the ethanolyield was achieved approximately 90% of the theoretical ethanol yield. Thomas et al. (1993) reported that in VHG (dissolved solids 300 g/l) of wheatmash fermentation at 20◦C for 200 h, maximal final ethanol concentration of 23.8% v/v was obtained. In another approach to VHG technology with cassava, optimization has been applied to study the effects of some key factorsthat influence ethanol production such as gravity, particle size, initial pH, liquefaction and fermentation temperature, liquefaction time and enzyme concentration. Under optimized conditions, high ethanol concentration (greater than 15%) and high starch utilization ratio (c.a. 90%) were obtained (Yingling et al., 2011). However,the investigation on VHG technology with cassava at a larger scale than that of laboratory has still been limited.

High ethanol concentration is one of the goals of VHG fermentation. The yeasts used in VHG fermentations have to be highly ethanol tolerant strains. Ergosterol is a sterol that is an important component of plasma membranes. It plays an important role inethanol tolerance in terms of membrane fluidity. Micro aerobic conditions can

improve the ethanol tolerance ofyeasts, leading to an increase in yeast cell permeability and overall fermentation rate (Lei et al., 2007). Membrane fluidity depends on the environmental conditions to which yeast areexposed (Hoppe et al., 2001). During VHG ethanol fermentation, yeasts require a small amount of oxygen to synthesize sterolsand unsaturated fatty acids, which are essentialfor plasma membrane integrity. Therefore, ethanol fermentation with appropriate aeration can promote ethanol production by S.cerevisiae, especially under VHG conditions.

**Microorganisms Saccharomyces cerevisiae**

Microorganisms for bioethanol fermentation can best be described in terms of their performance parameters and other requirements such as compatibility with existing products, processes and equipment. The performance parameters of fermentation are: temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability, and inhibitor tolerance. The characteristics required for an industrially suitable microorganism are summarized in Table 5 (Dien et al., 2003).

**Table 5.** Important Traits for Bioethanol Fermentation Process

| Trait | Requirement |
|---|---|
| Bioethanol yield | >90% of theoretical |
| Bioethanol tolerance | >40 g/l |
| Bioethanol productivity | >1 g/l |
| Robust grawer and simple growth requirement | Inexpensive medium formulation |
| Able to grow in undiluted hydrolysates | Resistance to inhibitors |
| Culture growth conditions retard contaminants | Acidic pH or higher temperature |

Traditionally, Saccharomyces cerevisiae and Zymomonas mobilis have been used for bioethanol fermentation. They are capable of efficiently fermenting glucose into bioethanol, but are unable to ferment xylose (Keshwani et al., 2009). Natural xylose-fermenting yeasts, such as Pichia stipitis, Candida shehatae, and Candida parapsilosis, can metabolize xylose via the action of xylose reductase (XR) to convert xylose to xylitol, and of xylitol dehydrogenase (XDH) to convert xylitol to xylulose. Therefore, bioethanol fermentation from xylose can be successfully performed by recombinant S. cerevisiae carrying heterologous XR and XDH from P. stipitis, and xylulokinase (XK) from S. cerevisiae In bacteria, a xylose isomerase (XI) converts xylose to xylulose, which after phosphorylation, is metabolized through the pentose phosphate pathway (PPP) (Zaldivar et al., 2001).

The most common and widely used microorganism for ethanol fermentation is a yeast (S. cerevisiae), which has been proved to be robust and well suited to the fermentation of lignocellulosic hydrolysates. It can efficiently ferment six carbon sugars, but hardly pentoses due to the lack of enzymes thatc onvert xylose to xylulose (Virupakshi et al., 2005). The common bacterial species used for ethanol fermentation is a gram negative bacterium, Z. mobilis (Saha, 2005). Some thermophilic anaerobic bacteria such as, Thermo anaero bacter ethanolicus, Clostridium thermo hydro sulfuricum, Thermo anaero bacter mathranii, Thermo anaerobium brockii, and Clostridium thermo saccharolyticum have been investigated for lignocellulosic ethanol production (Chang et al., 2001). Even though most bacteria have abroad substrate range, ethanol is rarely the single product of their metabolism that creates difficulties in the down stream processing of ethanol recovery (Zhang et al., 2009). The performance of any microorganism as ethanol fermenter can be evaluated based on their efficiency under different process conditions including wide temperature range, pH range, ethanol tolerance, growth rate, ethanol productivity, osmotic tolerance, specificity, ethanol yield, genetic stability, and inhibitor tolerance. The characteristics of an ethanologenic microorganism to be involved in lignocellulosic ethanol production include capability to utilize multiple sugars, high ethanol yield, tolerance to high ethanol concentration, high ethanol productivity, good growth in simple and inexpensive media, capability to grow in undiluted fermentation broth with resistance to inhibitors, and ability to retard contaminants under the growth condition.

## III. Conclusion

Agro residues biomass has been proposed to be one of the main renewable resources for cost-effectively attractive bioethanol production. The hypothetical ethanol yields from sugar and starch are superior compared to lignocelluloses agro residues; however, these conventional sources are not enough for worldwide bioethanol production. In that aspect, agro residues are renewable, less expensive and in large quantities available on earth crust. For the production of agro residues, there is no need of separate land, water, and energy requirements and also they do not have food value additionally. Rice straw is the most abundant waste compared to the other major wastes (Table 1) and rice straw can potentially produce 205 billion liters bioethanol per year, which is the highest among these four mentioned agricultural wastes.

Aeration during the ethanol fermentationunder VHG conditions significantly promoted ergosterol synthesisin the yeast cells, resulting in an increase in ethanol tolerance. Bothsufficient nitrogen and optimal aeration during the VHG ethanolfermentation affected ADH activity, resulting in higher levels ofethanol production..

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