Ethanol Biofuel Production from Lignocellulosic Biomass by Engineered *Saccharomyces cerevisiae*

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**Abstract**

Lignocellulosic biomass as raw materials played a great importance to produce fermentable sugars involved in bioethanol production. Global fuel ethanol production was predicted to arise up to 90 billion liters in 2014. Many years ago, ethanol has been considered as one type of alcohol found in alcoholic drinks, recently as long as technologies increasing, its application is expanding to satisfy the energy demands. In this review, four main steps: Pretreatment, enzyme hydrolysis, fermentation and separation/distillation involved in bioethanol production are discussed. Bioethanol production from lignocellulosic biomass often contains pentoses and inhibitory compounds such as furfural that are not well tolerated by *Saccharomyces cerevisiae*. Different recombinant yeast strains, particularly constructed inhibitors tolerant recombinant xylose-utilizing *Saccharomyces cerevisiae* strains to improve ethanol production from xylose are highlighted in this review. Some current results indicating the improvement of ethanol yields from lignocellulosic materials using recombinant *S. cerevisiae* strains through simultaneous saccharification and fermentation process are mentioned herein.

**Keywords:** Bioethanol, lignocellulosic biomass, pretreatment, enzymatic hydrolysis, fermentation, *Saccharomyces cerevisiae*.

**Introduction**

Energy is the basis of all human activity because people along the world need considerable energy to sustain and improve the quality of their life. In recent days, rapid increase in global energy demand and the availability of current energy sources lead to a highly exploitation of several forms of energy found in nature, such as fossil fuels, wind, solar, nuclear, large rivers and waterfalls, and burning trees (Moriarty and Honnery, 2012; Mathews, 2014). Therefore, the high cost of energy, environmental degradation, increasing population and industrialization and less supplies of fossil fuels and a huge amount greenhouse emissions encourage scientists to produce sustainable fuels using renewable sources (Samsudin and Mat Don, 2014; Gupta and Verma, 2015).

A high number of research projects to produce biofuels in the first generation were concentrated on the production of ethanol by fermentation of corn and sugar. This way of only using corn and sugar for producing biofuels, faces a big problem of not replacing the $5.4 \times 10^{20}$ J consumed worldwide every year and need a large quantity of food crops. Nowadays, in second generation bioethanol, lignocellulosic biomass play a great role in the production of bioenergy (especially biofuels) which is organic in composition and has the same property and energy content like petroleum based energy (Limayema, 2012).

Lignocellulose have been taken into consideration as one form of biomass suitable to be a renewable energy source because, it is obtained from non-food biomass which can avoid the conflict between food and fuel (Tan and Lee, 2014). Lignocellulosic wastes are composed of cellulose, hemicellulose and lignin. Other components such as ash, proteins, pectin etc. are also found in the lignocellulosic residues in different proportions (Egues et al., 2012; Behera et al., 2014). Those wastes are based on their sources of origin and they include residues of crops or forestry production (e.g. forest thinning, sawdust, etc.), dedicated energy crops (e.g. switchgrass, poplar and miscanthus), fraction of municipal and industrial solid waste and algal biomass (Ho et al., 2014). Consequently, as they are found in any location across the world, residues and wastes for bioenergy production stress the usage of lignocellulosic biomass for the production of energy. The production of energy of this way depends on the type, abundance and cost of biomass feedstocks, efficiency of the available processing technologies and the pattern of energy demand (Moe et al., 2012). The closeness of those main components (cellulose, hemicellulose and lignin) in the lignocellulosic wastes, induces the necessity of pretreatment process in order to make these carbohydrates available for enzymatic hydrolysis and fermentation during biofuels production (Kumar et al., 2009).
Their abundance comprised 30-40%, 30-35% and 11-25% respectively of floral cell walls (Kotarska et al., 2015). The presence of 25% of lignin among other lignocellulosic components is the major components that prevent plant cell destruction by fungi and bacteria for conversion to fuel. During conversion of biomass to fuel (e.g. ethanol), the cellulose and hemicellulose must be broken down into their corresponding monomers (sugars), so to be easily utilized by microorganisms. To achieve this, three major hydrolysis process were reported to facilitate the biofuel production: dilute acid, concentrated acid and enzymatic hydrolysis (Fig. 1) (Anwar et al., 2014). This process along with the high cost of enzymes increases the whole process cost. The thermochemical conversion route either gasification or pyrolysis in which high temperatures are employed is highly established technology, able to process a wide range of cellululosic material feed stocks. The major concern in this approach is the cost of a large quantity of biomass which combines the collection, transportation and deliverance to the plant gate. This high cost should be reasonable enough to meet the requirements of commercial biofuel production from plants. Generally, there are some key differences between the two conversion approaches. First, in the thermochemical conversion, the lignin component of biomass is converted to gas along with cellulose and hemicellulose. Whereas, the biochemical route can hardly break down the lignin component which constitutes 10-40% of biomass using fermentative or enzymatic reactions to fermentable compounds. Second, ethanol is the major fermentation product obtained through the biochemical pathway, while a wide variety of biofuels can be generated from syngas through the thermochemical approach. However, the biochemical conversion path is probably the most mature process to generate ethanol from the transformation of cellullosic materials (Mohammadi et al., 2011; Atsonios et al., 2015).

Hydrolysis and fermentation
Biochemical or thermochemical techniques can be used to transform lignocellulosic biomass into bioethanol. Those two techniques involve the degradation of the recalcitrant cell wall structure of lignocellulose into fragments of lignin, hemicellulose and cellulose. The sugars produced from hydrolysis of each polysaccharide is converted into bioethanol through a process of fermentation followed by product separation/distillation (Fig. 2) (Demirbas, 2007; Balat, 2011). Among three biomass component of wood, only two polysaccharide polymers cellulose and hemicellulose can be hydrolyzed into sugars then fermented to ethanol. Lignin and other smaller amount of extractives cannot be used for ethanol production. Before hydrolysis of cellulose which consists of approximately 40-60%, hemicelluloses and lignin have to be modified or removed (Oksman et al., 2011). The ethanol process consists of pretreatment and two stage acid hydrolysis to separate the cellulose, hemicellulose and lignin (Hamelinck et al., 2005; Sassner et al., 2008).

As reviewed by Olofsson et al. (2008), acid hydrolysis, however, produces hydrolyzates which are relatively toxic to the fermenting microorganisms strains and the maximum glucose yield is limited to approximately 60% in a batch process for kinetic reasons (Olofsson et al., 2008). This stage must optimize the biomass feedstock by exposing cellulose and hemicellulose for subsequent enzymatic hydrolysis, increasing the surface area of the substrate for enzymatic action to take place (Mabee et al., 2011).

Optimization of biomass for facilitating enzymatic hydrolysis is related to the different pretreatments techniques used. Therefore, a number of non-traditional pulping techniques have been examined by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI), including water-based, acidic, alkaline and organic solvent pulping systems (Wyman et al., 2005). Unless the pretreatment is successful, the resultant residue is not easily hydrolysable by cellulase enzyme and also the excessive pretreatment will result in the production of toxic compounds which inhibit the microbial metabolism.
The various pretreatments are usually designed to make biomass more susceptible to enzyme hydrolysis by removing lignin and/or hemicellulose, increasing surface area of hemicelluloses and cellulose for greater enzyme accessibility, reducing the cellulose crystalline structure, and increasing the substrate porosity with lignin redistribution. Pretreatment has been reported as one more expensive method among all steps within the conversion of biomass to fermentable sugar (Sridevi, 2011; Balat, 2011; Kim and Kim, 2014). The choice of the suitable pretreatment technology depends primarily on various factors such as the value of by-products, environmental, economical, the type of biomass, the process complexity and technological factors including energy savings, wastewater, recycling issues, substrate recovery along with a maximal solid loading yield and minimal use of chemicals (Talebnia et al., 2010). Four categories of pretreatment technologies namely physical pretreatment, chemical pretreatment, solvent fractionation and biological decomposition have been mentioned for efficient enzymatic hydrolysis of cellulose so to produce a good yield of ethanol (Mosier et al., 2005).

Enzymatic hydrolysis

Hydrolysis usually converts cellulose into reducing sugars (oligomers) including glucose molecule. There are two enzymes, Cellulases and hemicellulases which are often used to cleave cellulose and hemicellulose to monosaccharides (reducing sugars). Those enzymes are produced by fungi such as Trichoderma reesei and Aspergillus niger and sometimes bacteria like Clostridium cellulovorans can be used. Many filamentous fungi are more used than bacteria because of majority of relevant bacteria are anaerobes with a very low growth rates (Narra et al., 2012). Numerous characteristics such as the substrate type, composition and lignin content were reported to affect the enzyme choice. Cellulase enzyme loadings in hydrolysis vary from 7 to 33 FPU/g substrate which depends on the type and concentration of substrates (Sun and Cheng, 2002; Kinnarinen and Hakkinen, 2014). The degradation of cellulose by Cellulases (mixture of endoglucanases, exoglucanases and cellobiohydrolases) is processed following this mechanism: (1) endoglucanase cleaves β-1,4 glycosidic bonds in the long-chain polymer and free ends are created; (2) exoglucanases, such as b-glucosidases, act on the reducing and non-reducing ends to liberate oligosaccharides and (3) cellobiohydrolases cleave the polymer from the reducing ends to liberate cellobiose. The low cost of enzymatic hydrolysis compared to acid or alkaline hydrolysis is taken in consideration because enzyme hydrolysis is usually conducted at a relatively mild conditions (pH 4.8 and temperature 45-50°C) and this enables effective cellulose breakdown without the formation of byproducts that would otherwise strongly inhibit enzyme activity (Guo et al., 2012). The quality and efficiency of pretreatment in biofuel production from lignocellulosic biomass can directly affect the subsequent steps including enzyme hydrolysis and fermentation steps. Well treated substrates enhance enzymatic (cellulase) hydrolysis rates compared to untreated substrates and this can lead to enzyme inactivation (Yang et al., 2011; Vancov et al., 2012). The degree of crystallinity can play a crucial role on enzyme hydrolysis efficiency. Inefficacy of enzyme hydrolysis is directly related to the lower degree of crystallinity resulted from the steam explosion pretreatment. Also, alkaline pulping pretreatments can have an effect on conversion of sugars in agricultural biomass to ethanol (El-Zawawy et al., 2011).

The enzymatic hydrolysis can be influenced by substrate and end-product concentrations, enzyme activity and reaction conditions. Some hydrolysis products like glucose and especially cellobiose are strongly able to inhibit cellulase adsorption in a linear fashion adsorption. However, indirectly the cellobiohydrolases are even stronger inhibited by glucose. The glucose inhibits β-glucosidase and leads to the accumulation of cellobiose, which acts as a particularly strong end-product inhibitor of many cellulases including bothexo- and endoglucanases (Galbe, 2002; Rabinovich, 2002; Kristensen et al., 2009; Kim et al., 2012). Also, substrate concentrations have been considered as one of the main factor that affects the yield and initial rate of enzymatic hydrolysis of cellulose. It was reported that high substrate concentration can cause the loss of enzyme activity and substrate inhibition, which essentially lowers the rate of the hydrolysis and decreasing the yield of sugar (Gregg, 1996; Wang, 2011). Recently, depolymerization of lignocellulosic biomass into fermentable sugars for conversion to biofuels and biochemical through enzymatic hydrolysis process, focus on operating at high solid loadings. It has been suggested that enzymatic hydrolysis conducted at high-solids loadings will be necessary to render the lignocellulosic conversion process more economically feasible (Modenbach and Nokes, 2013).

Fermentation

Different processes including pretreatment and hydrolysis have been investigated to increase the optimization of fermentation process. This metabolic process requires raw materials and the presence of microorganisms which assist the conversion of reducing sugars to acids, gases, lactic acid or other end-products and/or alcohol (Wheals, 1999; Gamage, 2010; Chandel, 2007). Lignocellulosic fermentation is more complex compared to sugar/starch fermentation. Cellulosic materials often contain cellulose and hemicellulose. Cellulose is the largest component and composed of glucan, while hemicellulose is composed of arabinian, galactan, mannann and xylan. Through a process of acid or enzyme hydrolysis, those components can be broken down into two categories of sugar, hexoses and pentoses. Hexoses comprise a large group of sugars such as glucose, fructose, galactose and mannose and...
pentoses comprise xylose and arabinose (Keshwani, 2009). Many reports indicated that one ton of glucan, galactan or mannan yields 1.11 tons of six-carbon sugars (hexoses) and could be fermented theoretically into 172.0 gallons of bioethanol. One ton of arabinan or xylan yields 1.14 tons of five-carbon sugars (pentoses) and could be fermented theoretically into 176 gallons of bioethanol. Microorganisms involved in fermentation are used to consume and convert all lignocellulose-derived sugars to bioethanol (Szluczyn, 2010; Demirbas et al., 2011).

**Microorganisms involved in bioethanol production**

Microorganisms that can grow on pentose sugars and convert them to ethanol at high yields are highly desirable. Therefore, there is a great interest in the development and use of natural food and additives derived from microorganisms (Caplice, 1999). Thus, several microorganisms were reported to ferment reducing sugars to bioethanol and many of them show a higher hydrolytic and acidogenic activity when using lignocellulosic biomass as substrates (Yue et al., 2013). To find microorganisms that are able to ferment biomass sugars to ethanol can best be determined in terms of their performance parameters and other requirements such as compatibility with existing products, processes and equipment. Depending on the yield and production required, the fermentation process necessitates the precise control of a number of parameters such as: temperature and pH, alcohol and osmotic tolerance, productivity, growth rate, specificity, yield, inhibitor tolerance and genetic stability (Olmos et al., 2014; Mimitsuka et al., 2015).

**Strain improvement to enhance bioethanol production**

High cost associated with the large quantity of required enzymes for the complete hydrolysis of cellulose to ethanol induces engineering to provide efficient yeast cell biocatalysts able to convert lignocellulosic materials directly to ethanol by displaying cellulolytic and hemicellulolytic enzymes on the cell surface (Hasunuma and Kondo, 2012). Several technologies for strain development have been employed to bioethanol engineer *S. cerevisiae* capable of fermenting xylose efficiently and rapidly. These are (1) Optimization of xylose-assimilating pathways, (2) Perturbation of gene targets for reconfiguring yeast metabolism and *Saccharomyces cerevisiae*, (3) simultaneous co-fermentation of xylose and cellobiose (Kim et al., 2013).

Recently, many researches focused on combined approaches of genetic engineering, evolutionary adaptation and chemical mutagenesis by ethyl methane sulfonate (EMS) (Liu, 2010), rational metabolic engineering, inverse metabolic engineering, random engineering strategies (support inverse metabolic engineering), evolutionary engineering, transposon mutagenesis and gene overexpression libraries and global transcription machinery engineering (Nevoigt, 2008) were employed to obtain a recombinant *S. cerevisiae* strain with high-efficiency xylose fermentation. Evolutionary engineering experiments were successful in obtaining inhibitor tolerant strains, as well as to accelerate the co-utilization of glucose, xylose and arabinose mixtures by a recombinant strain which is *S. cerevisiae* strain. A combination of cell surface engineering and intracellular metabolic engineering was reported to be a very effective approach for development of cells with novel fermentation ability for industrial applications (Hasunuma and Kondo, 2012).

A high-tolerant *S. cerevisiae* mutant strain P6H9 tolerant to high concentrations of HMF was obtained by evolutionary engineering from the industrial strain P6 and has been reported to be used for second-generation bioethanol production (Sehnem et al., 2013). Hasunuma and Kondo (2012) demonstrated that *S. cerevisiae* strains with the capability of utilizing both glucose and xylose in the presence of inhibitors such as furfural are very important in industrial ethanol where the production by co-expressing the TAL1 and ADH1 genes, ethanol production could be improved by 127% in the presence of 70 mM furfural. The results of fermentation using undetoxified hydrolysates indicate that the synergistic effects of TAL1 and ADH1 were capable of improving ethanol production in the presence of 7.8 mM furfural, which mimics real industrial applications (Hasunuma et al., 2014). The gene PHO13 encoding p-nitrophenylphosphatase, was disrupted in a recombinant xylose-fermenting *S. cerevisiae* strain in order to improve ethanol production from xylose in the presence of three major inhibitors, acetic and formic acids, and furfural. The ethanol yield obtained by the DPHO13 mutant was 0.45 g-ethanol/g-xylose with 30 mM acetic acid where with 90 mM furfural, ethanol productivity of the mutant was fourfold higher than that of the control strain (Fujitomi et al., 2012).

In the presence of laccase which removes specifically lignin phenols from the overall inhibitory compounds present in the slurry, *K. marxianus* can produce ethanol concentrations and yields comparable to those obtained by *S. cerevisiae* (often used in industrial fermentation process) through both, simultaneous saccharification and fermentation (SSF) and presaccharification and simultaneous saccharification and fermentation (PSSF) (Moreno et al., 2013). Using *S. cerevisiae* NBRC 1440/B-EC3, the fermentation occurred in the presence of 10 FPU/g-biomass cellulase added in combination with the recombinant cellulolytic strain, high-titer ethanol production from high-solid lignocellulosic materials was achieved using strain NBRC1440/B-EC3 with an ethanol yield corresponding to 89% of the theoretical yield. The recombinant strain used showed a high capacity of hydrolyzing a portion of the cellulose material that was not hydrolyzed by commercial cellulose (Matano et al., 2012).
Khramtsov et al. (2011) engineered an industrial *Saccharomyces cerevisiae* yeast strain capable of fermenting ethanol from pretreated lignocellulosic material where through a simultaneous saccharification and fermentation process was performed without addition of exogenously produced cellulases. The recombinant strain 590.E1 fermented 63% of the cellulose in 96 h and this strain achieved yields in excess of 94% of the theoretical maximum on pretreated Whatman paper (Khramtsov et al., 2011). Coexpression of TAL and FDH genes in a recombinant xylose fermenting *S. cerevisiae* strain improved ethanol production from xylose in the presence of both 30 mM acetate and 20 mM formate. A mixture of glucose, fructose and xylose as carbon source and fermentation inhibitors, formate and acetate, was performed for five cycles without any loss of fermentation capacity (Sanda et al., 2011). Several reductases and dehydrogenases were tested to identify 2-furaldehyde (furfural) and 5-hydroxymethyl furaldehyde (HMF) detoxifying enzymes in *S. cerevisiae*. Among them, overexpression of ALD6 enhanced cell growth and ethanol production in batch fermentation containing furfural and HMF. Batch fermentation of *S. cerevisiae* D452-2/pHALD6 in the presence of 2 g/L furfural and 0.5 g/L HMF resulted in 20-30% increase in specific growth rate, ethanol concentration and ethanol productivity (Sanda et al., 2011). Khattab et al. (2013) constructed a recombinant *S. cerevisiae* carrying a novel set of mutated strictly NADPH-dependent XR and NADP+-dependent XDH genes with overexpression of endogenous xylulokinase (XK) to study the effects of complete NADPH/NADP+ recycling on ethanol fermentation and xylitol accumulation. Comparing to the control strain, 34.4-54.7% was demonstrated as reduced xylitol accumulation using mutated strains and the later showed 20% and 10% improvement in ethanol production (Khattab et al., 2013).

Another recombinant *S. cerevisiae* strain (SK-NY) overexpressing GRE3-encoded NADPH-dependent aldose reductase and xylulokinase with a mutated strictly NADP+–dependent *Pichia stipitis* xylitol dehydrogenase have been developed and reported that its fermentation efficiency was compared with that of an isogenic constructed reference strain expressing *P. stipitis* xylose reductase instead of the GRE3 gene where the ethanol production by SK-NY was 21.4% higher than that of the reference strain. The yield of ethanol production by SK-NY strain increased from 0.395 g ethanol/g sugar to 0.435 g ethanol/g sugar after glucose depletion (Khattab and Kodaki, 2014). Walhomb et al. (2003) used a metabolic engineering and random mutagenesis to mutagenize a recombinant strain of *S. cerevisiae* to improve xylose utilization and bioethanol production (Walhomb et al., 2003). Using ethyl methane sulfonate (EMS), mutant strains of *S. cerevisiae* was reported for a higher bioethanol production than wild-type strains. Mutagenized strains produced bioethanol 17.3% more than the wild type (Mobini-Dehkordi et al., 2008). The engineered *Saccharomyces cerevisiae* strain MA-R5 produced the highest ethanol yield (0.48 g/g) from non-sulfuric acid hydrolysate of wood chips (Matsushika et al., 2009b). Shahsavaran et al. (2012) discovered that superior temperature resistance could be achieved in *S. cerevisiae* thermostolerant strain C3723 and its derivatives by the overexpression of RSP5 allele of the RSP5 gene that encodes E3 ubiquitin ligase. This simple technique can be applied in industrial fermentation especially in biofuels production from lignocellulosic materials. Yeast, particularly *Saccharomyces cerevisiae* have been shown to be the most common fermenting microorganism used for bioethanol production due to its excellent fermenting capacity, to grow rapidly under anaerobic conditions and high tolerance to ethanol (Shahsavaran et al., 2012). Toivari et al. (2001) investigated the overexpression of the endogenous gene for the enzyme (XKS1) in *S. cerevisiae* that also expresses the *P. stipitis* genes for XR and XDH and under microaerobic conditions, 5.4 g L⁻¹ ethanol was produced from 47 g L⁻¹ xylose during 100 h (Toivari et al., 2001).

**Metabolic pathways of engineered *Saccharomyces cerevisiae***

Enhancement of bioethanol production process for industrial scale with reduction in capital and operation cost, necessities some integrated unit operations using robust microorganisms for better product yields (Zhang, 2008). Ethanol, the most advanced liquid fuel, have been continuously developed using different technologies in order to enhance its productivity. Therefore, the combinatorial metabolic engineering for industrial yeast strains have been expected to further improve xylose utilization, ethanol yield and production rate. Such technology under development is the use of yeasts for the commercial fermentation of xylose to ethanol. *Saccharomyces cerevisiae* which is the yeast mostly used for bioethanol production does not naturally use xylose as a substrate, however must be engineered to both transport and ferment xylose. Recently, designing the metabolic pathway of *Saccharomyces cerevisiae* to expand the substrate of ethanol fermentation has been taken into consideration (Jeffries, 2006; Kristensen et al., 2009; Kim et al., 2013). It was reported that mutagenesis in combination with long term evolutionary engineering was successfully applied to introduce a greater level of tolerance in *S. cerevisiae* D5A+H, together with improved xylose utilization or enhancing yeast tolerance for fermentation (Smith et al., 2014). Results from comparisons between different recombinant *S. cerevisiae* strains suggested that MA-R4 may be a suitable recombinant strain for further study into large-scale ethanol production from mixed sugars present in lignocellulosic hydrolysates (Matsushika et al., 2009a).
In *S. cerevisiae*, xylose catabolism is mediated by a heterologously expressed fungal pathway consisting of xylose reductase (XR) encoded by *XYL1*, xylitol dehydrogenase (XDH) encoded by *XYL2* and xylulose kinase (XKS), producing a pentose phosphate pathway (PPP) intermediate, xylulose-5-phosphate, which can be further metabolized to ethanol (Fig. 3). This pathway is coupled with the endogenous metabolic network by the requirement for NAD(P)H for XR, NAD+ for XDH and ATP for XKS (Fig. 3). Alternatively, heterologous expression of the bacterial xylose isomerase (XI) can be used to convert xylose into xylulose (Kim, 2013; Cao *et al.*, 2014). Jennifer *et al.* (2008) provided that deletion of the PHO13 gene recombinant *Saccharomyces cerevisiae* is one such method to overcome growth inhibition due to overexpression of xylulokinase so that to improve the bioethanol production from lignocellulosic feed stocks (Vleet *et al.*, 2008). It has been verified that strains with very high XYLA copy number have very high xylose assimilation rate (Zhou *et al.*, 2012). Recently, Cao *et al.* (2014) developed a new strategy called two-stage transcriptional reprogramming (TSTR), where key gene expression at both glucose and xylose fermentation phases is optimized in engineered *S. cerevisiae*. This method improved an ethanol yield of 0.48 g/g total sugar and 94% of the theoretical yield was achieved but not appear to improve the specific ethanol productivity (Cao *et al.*, 2014). It was showed that effort in molecular transporter engineering is able to improve the xylose transport characteristics of yeast strains in order to influence industrial fermentations of lignocellulosic biomass (Young *et al.*, 2012; Tanino *et al.*, 2012; Wang *et al.*, 2013; Moon *et al.*, 2013).

Research showed approaches to engineer *S. cerevisiae* HXT transporters to improve second generation bioethanol production showed that the HXT1 transporter seems more suitable for hydrolysates containing xylose/glucose blends, whereas the HXT7 permease would be a better choice for xylose-enriched sugar streams, such as those obtained from the acid, steam explosion and/or hydrothermal biomass pretreatment processes (Goncalves *et al.*, 2014). Different researches on metabolic flux analysis suggested that the expression of the Gxf1 transporter had shifted the control of xylose catabolism from transport to the NAD+ dependent oxidation of xylitol to xylulose (Fonseca *et al.*, 2011).

**Downstream processing**

Membrane pervaporation (PV) or pervaporative separation has been considered as one of the most effective and energy-saving process for separation of azeotropic mixtures. In this process, the component of interest is separated from a mixture of liquids by partial vaporization through a non-porous or porous membrane. The membrane acts as a separator between two phases: the liquid-phase feed and the vapor-phase permeate. Separation of components is based on a difference in transport rate of individual components through the membrane where the target component of liquid feed passes through it by vaporization. This process have been promised to be used as an extractive unit to be coupled to the fermentation unit for ethanol recovery (Jaimes *et al.*, 2014). The pervaporation of bioethanol produced by the fermentation of waste newspaper by *Saccharomyces cerevisiae* decreased process performance. Recently, hydrophobic polymeric polydimethylsiloxane (PDMS) membrane was used to reinforce the pervaporative separation of bioethanol produced from fermentation of lignocellulosic biomass (waste newspaper) and glucose where no significant decreasing process performance was occurred (Trinh *et al.*, 2013). Carbon molecular sieve membranes (CMSMs) was demonstrated to have high performance and potential to separate water from bioethanol through pervaporation or vapor permeation than organic membranes (Liao *et al.*, 2012).

Membranes can be either hydrophilic or hydrophobic. Hydrophilic membrane for removal of water can be divided into different kinds such as: inorganic membrane, polymeric membrane and composite or mixed membrane. Also, membrane pervaporation-bioreactor hybrid for removal of the inhibitors from the fermentation broth and Vacuum Membrane Distillation (VMD)—bioreactor hybrid classified into direct contact membrane distillation (DCMD), air gap membrane distillation (AGMD), sweeping gas membrane distillation (SGMD) and vacuum membrane distillation (VMD) all for separation of ethanol and the other inhibitory compounds from fermentation broths were suggested (Huang *et al.*, 2008). The production of bioethanol of purity up to 99.8% (w/w) has been reported when a novel separation technique by integrating pervaporation and ultrafiltration system was used Neil *et al.* (2014). Among several entrainers like ethylene glycol, glycerol and ionic liquids often used in extractive batch distillation, high purity bioethanol using glycerol as entrainer was reported and has achieved a higher compositions in the distillate in contrast to those reported using ethylene glycol and ionic liquids (Navarrete-Contreras *et al.*, 2014).
In few last years, distillation has showed several critical disadvantages including high cost and limited separation capacity. Different alternatives have been proposed to replace distillation such as non-heating fractional distillation by ultrasonic irradiation, oxidation of impurities by ozone, and adsorption of impurities by activated carbon or zeolite (Onuki et al., 2008).

Conclusion
In the present study, the novel ethanol production by engineered microorganism (S. cerevisiae), accurate fermentation process and favorable ethanol purification processes have been discussed. Recently, lignocellulosic materials often include wood, short-rotation woody crops, agricultural wastes, short-rotation herbaceous crops, animal wastes and a host of other materials (Demirbas et al., 2011) are mostly used due to their availability and cheap source of fermentable sugars for bioethanol production. Pretreatment followed by enzymatic hydrolysis has been reported as one more expensive step for conversion of biomass to fermentable sugars (Sridevi et al., 2011; Balat, 2011; Kim and Kim, 2014). Different inhibitor tolerant strains of S. cerevisiae capable to ferment arabinose, galactose, glucose and mannose and recombinant strains for xylose metabolism are currently in use. As reviewed by many researchers, conversion of fermentable sugars to ethanol have been well performed through simultaneous saccharification and fermentation (SSF) process which improves the ethanol yields by reducing the product inhibition excreted by saccharification products and also eliminates the need for separate reactors for saccharification and fermentation, which results in cost reduction. There are two key separation steps in the biofuels production: First is the separation of fermentation inhibitors after the pre-extraction of hemicelluloses from lignocellulosic biomass. The second which is critically reviewed in this review is the azeotropic nature of ethanol-water mixture posing challenges to remove the last amounts of water producing fuel grade ethanol.

References


Production of bioethanol from wheat straw: An overview on materials for ethanol production.


