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Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation

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ABSTRACT

Wheat straw is an abundant agricultural residue with low commercial value. An attractive alternative is utilization of wheat straw for bioethanol production. However, production costs based on the current technology are still too high, preventing commercialization of the process. In recent years, progress has been made in developing more effective pretreatment and hydrolysis processes leading to higher yield of sugars. The focus of this paper is to review the most recent advances in pretreatment, hydrolysis and fermentation of wheat straw. Based on the type of pretreatment method applied, a sugar yield of 74–99.6% of maximum theoretical was achieved after enzymatic hydrolysis of wheat straw. Various bacteria, yeasts and fungi have been investigated with the ethanol yield ranging from 65% to 99% of theoretical value. So far, the best results with respect to ethanol yield, final ethanol concentration and productivity were obtained with the native non-adapted *Saccharomyses cerevisiae.* Some recombinant bacteria and yeasts have shown promising results and are being considered for commercial scale-up. Wheat straw biorefinery could be the near-term solution for clean, efficient and economically-feasible production of bioethanol as well as high value-added products.

1. Introduction

Global demand for energy continues to grow due to rapidly expanding human population and increase of the industrial prosperity in developing countries. The major energy demand is still supplied from conventional fossil fuels such as oil, coal and natural gas. Utilization of fossil fuels over the last century and following years has drastically increased the level of greenhouse gasses in the earth's atmosphere (Ballesteros et al., 2006). These facts along with inevitable depletion of the world's energy supply, and unstable oil market have renewed the interest of society in searching for alternative fuels. Ethanol has long been 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%. Today, bioethanol is the most dominant biofuel and its global production showed an upward trend over the last 25 years with a sharp increase from 2000. Worldwide production capacity in 2005 and 2006 were about 45 and 49 billion liters per year, respectively and total output in 2015 is forecast to reach over 115 billion liters (Licht, 2006).

Sugar and starch based materials such as sugarcane and grains are two groups of raw materials currently used as the main resources for ethanol production. The third group is lignocellulosic materials representing the most viable option for production of ethanol. Growing demand for human food, as it is for energy, and considering the priority for starving human society could make the first two groups of raw materials potentially less competitive and perhaps expensive feedstocks in the near future compared to lignocellulosic materials (Taherzadeh and Karimi, 2007).

Lignocellulosic waste materials obtained from energy crops, wood and agricultural residues, represent the most abundant global source of renewable biomass (Lin and Tanaka, 2006). Among the agricultural residues, wheat straw is the largest biomass feedstock in Europe and the second largest in the world after rice straw (Kim and Dale, 2004). About 21% of the world's food depends on the wheat crop and its global production needs to be increased to satisfy the growing demand of human consumption (Ortiz et al., 2008) therefore, wheat straw would serve as a great potential feedstock for production of ethanol in 21st century.

The objective of current paper is to review the published investigations on wheat straw conversion to bioethanol and to present the recent advances of the technology, future perspective and challenges encountered throughout the entire process *i.e.* pretreatment, hydrolysis, and fermentation stages.

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2. Wheat straw: production and fate

Wheat (*Triticum aestivum* L.) is the world's most widely grown crop, cultivated in over 115 nations under a wide range of environmental conditions. Over the past 100 years, the yields of wheat have been increased and annual global production of dry wheat in 2008 was estimated to be over 650 Tg (Atwell, 2001). Assuming residue/crop ratio of 1.3, about 850 Tg of wheat residues are annually produced. The straw produced might be left on the field, plowed back into the soil, burned or even removed from the land depending on the decision made by landowner. Disposal of wheat straw by burning has been practiced for a long time. In recent years however, this practice has been challenged due to increased concern over the health effects of smoke from burning fields (Kerstetter and Lyons, 2001). Burning of wheat straw results in large amounts of air pollutants including particulate matter (PM_{10}), CO and NO_2 (Li et al., 2008). Thus, finding an alternative way for disposal of surplus wheat straw is of high interest and an immediate necessity.

Full removal of wheat residue may decrease soil organic matters and lead to soil erosion. The fraction of wheat straw that must be left on the field depends on the weather, crop rotation, existing soil fertility, slop of the land, and tillage practices (Kerstetter and Lyons, 2001). According to Kim and Dale (2004), in order to ensure prevention of soil erosion, 60% ground cover could be considered that roughly requires 1.7 Mg wheat residue per hectare. The global average yield of wheat is around 2.4 Mg ha^{-1} and as a result, approximately 430 Tg of wheat straw is annually available for production of about 120 GL bioethanol. This volume of ethanol can replace about 93(±3) GL of gasoline when differences in their volumetric energy content and octane number are taken into account (Otero et al., 2007). Several reports have suggested that under proper fine tuning of engine parameters, ethanol–gasoline blends will lead to enhanced engine performance and reduced emission of CO (Bayraktar, 2005; Wu et al., 2004). Thus, turning from burning of wheat straw to production of ethanol from surplus wheat straw seems to be a promising approach to gain energy from waste biomass and partly reduce dependence on fossil fuels while contributing to the greenhouse gas effect and improving urban air quality.

3. Wheat straw as a potential feedstock for 2nd generation bioethanol

Wheat straw like any other biomass of lignocellulosic composition is a complex mixture of cellulose, hemicellulose and lignin, as three main components, and a small amount of soluble substrates (also known as extractives) and ash. The overall chemical composition of wheat straws could slightly differ depending on wheat species, soil, and climate conditions. Cellulose, hemicellulose and lignin content of wheat straw are in the range of 33–40, 20–25, and 15–20 (%w/w), respectively (Prasad et al., 2007). The cellulose strains are bundled together and tightly packed in such a way that neither water nor enzyme can penetrate through the structure (Laureano-Perez et al., 2005; Sun et al., 2004). Hemicellulose serves as a connection between lignin and cellulose fibers and it is readily hydrolyzed by dilute acid or base, as well as hemicellulase enzyme. Lignin is covalently linked to cellulose and xylan (predominant hemicellulose carbohydrate polymer in wheat straw) such that lignin–cellulose–xylan interactions exert a great influence on digestibility of lignocellulosic materials (Laureano-Perez et al., 2005). Due to this structural complexity of the lignocellulosic matrix, ethanol production from wheat straw requires at least four major unit operations including pretreatment, hydrolysis, fermentation and distillation. Unlike sucrose or starch, lignocellulosic biomasses such as wheat straw need to be pretreated to make cellulose accessible for efficient enzymatic depolymerization.

4. Pretreatment of wheat straw

The pretreatment aims to improves the rate of production as well as the total yield of liberated sugars in hydrolysis step (Hendriks and Zeeman, 2009). A number of pretreatment methods have been developed and applied for wheat straw biomass. The overall efficiency of the pretreatment process is correlated to a good balance between low inhibitors formation and high substrate digestibility. The pretreatments are roughly classified into physical, physico-chemical, chemical and biological processes (Fig. 1). The applied methods usually use combination of different principles, such as mechanical together with thermal and chemical effects in order to achieve high sugar release efficiencies, low toxicants production, and low energy consumption. In the following section, the major pretreatment methods applied for wheat straw are particularly reviewed.

4.1. Physical

The first step in using wheat straw for ethanol production is size reduction through milling, grinding or chipping (Fig. 1) which can improve the efficiency of downstream processing. However, use of very small particles may not be desirable due to higher energy consumption in milling stage as well as imposing negative effect on the subsequent pretreatment method. Initial and ultimate particle size, moisture content and material properties are among variables that influence both energy consumption and the effectiveness of subsequent processing. Smaller particle size and higher moisture content of straw will lead to higher specific energy consumption. The specific energy consumptions for grinding wheat straw with the hammer mill screen sizes of 0.8 and 3.2 mm were 51.6 and 11.4 kW h t^{-1}, respectively which were higher than corresponding values for corn stover at similar moisture content (Sudhagar et al., 2004). Koullas et al. (1992) reported significant improvement of enzymatic hydrolysis of ball-milled wheat straw. After 2 h ball milling, the maximum degree of saccharification in the following enzymatic hydrolysis step increased to 61.1(%) compared to 17.7(%) of untreated sample. Pedersen and Meyer (2009) estimated the effects of substrate particle sizes (in the range of 53 μm upto 4 cm) on the enzymatic hydrolysis of the wet oxidized and untreated wheat straw. Size reduction enhanced the susceptibility of untreated substrate to enzymatic hydrolysis. Release of glucose and xylose from the smallest straw particles (53–149 μm) increased with 39% and 20% of the theoretical maximal values after 24 h of hydrolysis when compared to the reference sample (2–4 cm). Wet oxidation showed more pronounced effect on larger particles (707–1000 μm) than smaller particles (53–149 μm).

4.2. Physico-chemical

The solubilization of lignocellulose components depend on temperature, pH and moisture content. In lignocellulosic materials such as wheat straw, hemicellulose is the most thermal-chemically sensitive fraction. Hemicellulose compounds start to solubilize into the water at temperature higher than 150 °C and among various components, xylan can be extracted the most easily (Hendriks and Zeeman, 2009). Liquid hot water (LHW), steam explosion (SE) and ammonia fiber explosion (AFEX) are among physico-chemical methods investigated for pretreatment of wheat straw (Fig. 1).

Liquid hot water (LHW) is a hydrothermal pretreatment, where pressure is applied to maintain water in the liquid state at elevated

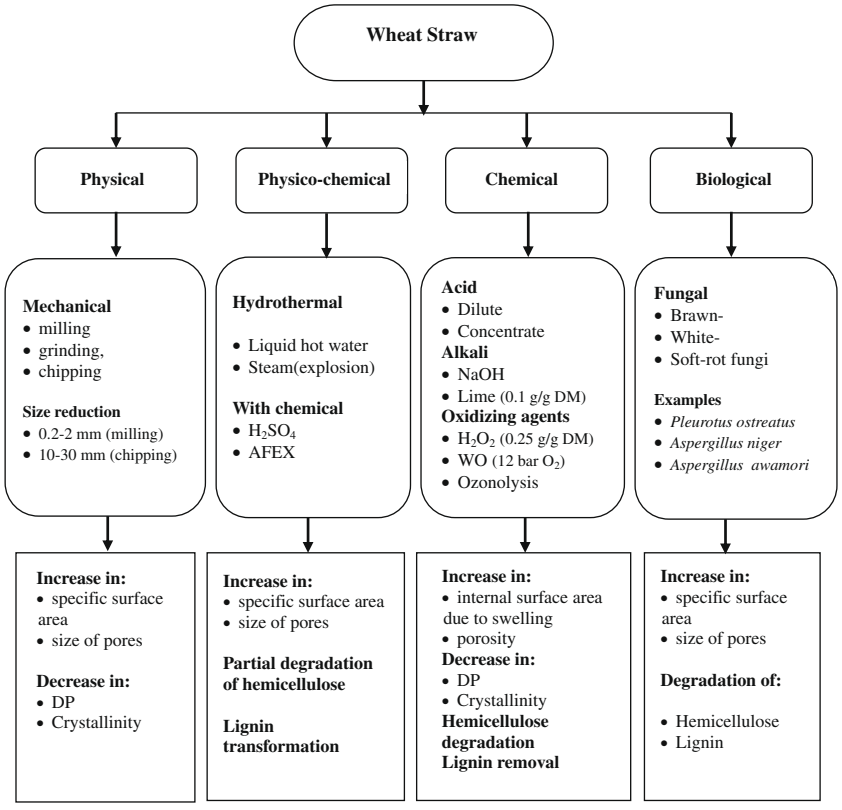

Fig. 1. The most common pretreatment methods used on wheat straw and their possible effects (DP, degree of polymerization; WO, wet oxidation).

temperature. Temperature in the range of 170–230 °C and $P > 5$ MPa are commonly used (Sánchez and Cardona, 2008). It has potential to release high fraction of hemicellulosic sugars mostly in the form of oligomers contributing to reduction of undesired degrading products (Hendriks and Zeeman, 2009; Mosier et al., 2005a,b). Temperature and time showed the most significant effect on the recovery of hemicellulosic sugars and the yield of subsequent enzymatic hydrolysis of pretreated wheat straw (Perez et al., 2007). Perez et al. (2008) reported maximum recovery of hemicellulose-derived sugars (HDS) and the highest yield of enzymatic hydrolysis was achieved under various conditions of time and temperature. Simultaneous optimization of both response variables occurred at 188 °C and 40 min suggesting a two-step pretreatment would be the most adequate process configuration. A pilot scale (up to 100 kg h^{-1}) plant, using two-step hydrothermal pretreatment in continuous operation has been developed for wheat straw (Petersen et al., 2009). The first step is soaking of straw at 80 °C for 5–10 min followed by the second stage treatment at higher temperature. In the second stage, pretreatment at 195 °C and 6–12 min resulted in 70% and 93–94% recovery of hemicellulose and cellulose, respectively.

Steam explosion (autohydrolysis) is one of the most cost-effective and widely used pretreatment methods for wheat straw (Alfani et al., 2000; Ballesteros et al., 2006). In this method, size-reduced biomass is rapidly heated by high pressure steam for a period of time and then the pressure is suddenly reduced which makes the materials undergo an explosive decompression. Temperatures in the range of 160–230 °C for a time period of several seconds to a few minutes are generally applied in SE of wheat straw. The efficiency of SE is affected by several factors including temperature, residence time, particle size and moisture content. Addition of chemicals such as H_2SO_4, or SO_2 in SE can improve the rate and extent of hemicellulose removal and lead to enhanced yield of enzymatic hydrolysis at lower temperatures (Jurado et al., 2009). Beltrame et al. (1992) studied the effect of SE on the fractionation of wheat straw. The maximum delignification occurred with pretreatment at 210 °C and 1–2 min whereas the maximum solubilization of cellulose-rich solid fraction (83.7%) and the highest glucose production (93.5%) during enzymatic hydrolysis attained at 230 °C and 1 min. These values could be compared with maximum glucose production of untreated sample which was only 11.8%, indicating a great improvement of wheat straw digestibility after SE. Ballesteros et al. (2006) investigated the effect of SE on the diluted acid (0.9%) or water-impregnated wheat straw in various temperatures and residence times. The best results obtained from SE of acid-impregnated wheat straw at 180 °C and 10 min where almost complete dissolution of hemicellulose components and the highest yields of glucose and ethanol based on the initial raw material were achieved. Although, SE at higher temperatures increased enzymatic hydrolysis yield of cellulose-rich solid fraction, however the overall yield of glucose was lower due to partial loss of cellulose. In a similar study by Linde et al. (2008) less severe condition in impregnation step was investigated. Interestingly, the highest overall yield of the sum of glucose and xylose was obtained with treatment at 190 °C and 10 min for the wheat straw. It is uncommon for other lignocellulosic materials to reach the maximum yield of hemicellulosic and cellulosic sugars at the same pretreatment conditions.

Ammonia fiber explosion (AFEX) is an alkaline thermal pretreatment during which the lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a period of time followed by a rapid pressure release. Herbaceous and agricultural residues are well suited for AFEX pretreatment. This method does not produce inhibitors for the downstream processes and small particle size is not required for efficacy (Mosier et al., 2005a,b; Sun and Cheng, 2002). The major parameters influencing AFEX process are ammonia loading, temperature, blowdown pressure, moisture content of biomass, and residence time (Holtzapple

et al., 1991; Teymouri et al., 2004). This pretreatment has drawbacks of being less efficient for biomass containing higher lignin content (*e.g.* softwood newspaper) as well as solubilization of very small fraction of solid material particularly hemicellulose (Belkacemi et al., 1998; Sun and Cheng, 2002). Mes-Hartree et al. (1988) made a comparison between steam and ammonia pretreatment of wheat straw. The authors reported that enzymatic hydrolysis was improved by several folds and more or less in the same order of magnitude for both pretreatments. However, the highest glucose concentration (0.38 g/g dry mass) was achieved with ammonia treatment. The AFEX pretreatment of wheat straw unlike other lignocellulosic materials has been rarely reported. While near theoretical sugar yields after AFEX treatment under optimum conditions have been reported for various agricultural residues (Alizadeh et al., 2005; Teymouri et al., 2004), more experimental works are necessary to address the feasibility and efficiency of this pretreatment method for wheat straw.

4.3. Chemical pretreatment

Chemical pretreatment for wheat straw employ different chemicals such as acids, alkalis, and oxidizing agents *e.g.* peroxide and ozone (Fig. 1). Among these methods, dilute acid pretreatment using H_2SO_4 is the most-widely used method. Depending on the type of chemical used, pretreatment could have different effects on lignocellulose structural components. Alkaline pretreatment, ozonolysis, peroxide and wet oxidation pretreatments are more effective in lignin removal whereas dilute acid pretreatment is more efficient in hemicellulose solubilization (Galbe and Zacchi, 2002; Sánchez and Cardona, 2008; Tomas-Pejo et al., 2008).

4.3.1. Acid hydrolysis

Inorganic acids such as H_2SO_4 have been used for pretreatment of wheat straw to improve downstream enzymatic hydrolysis. Based on the dose of acid used in the process, it could be identified as concentrated- and/or dilute-acid hydrolysis (Fig. 1). In the first case, the biomass is treated with high concentration of acids at ambient temperatures, which results in high yield of sugars. Concentrated acid treatment offers advantage of not using any enzymes for saccharification, however, this process has drawbacks including high acid and energy consumption, equipment corrosion and longer reaction time as well as obligation for acid recovery after treatment that largely limit its application (Galbe and Zacchi, 2002; Sun and Cheng, 2002). In the second approach, low-concentration acids *e.g.* 0.5–1% H_2SO_4 and high temperatures are exploited. High temperature is favorable to attain acceptable rates of cellulose conversion to glucose. Despite low acid concentration and short reaction time, application of high temperatures in dilute-acid hydrolysis accelerates the rate of hemicellulose sugar decomposition and increases equipment corrosion (Galbe and Zacchi, 2002; Taherzadeh and Karimi, 2007). The main drawbacks of this method are formation of many inhibiting by-products and pH neutralization requirement for downstream processes (Sun and Cheng, 2002; Talebnia, 2008). In order to decrease sugars degradation, a two-stage process has been developed where hemicellulose sugars are released in the first stage under milder conditions followed by the second-stage hydrolysis of cellulose-rich solid residue performed under harsher conditions. Depending on the nature of lignocellulosic feedstock, a range of temperatures between 140 and 190 °C in the first stage and 190–230 °C in the second stage is commonly used (Galbe and Zacchi, 2002; Saha et al., 2005).

Delgenes et al. (1990) treated wheat straw with 72% (w/v) H_2SO_4 for 30 min at 30 °C and obtained 11.1 g monomeric sugars in total from 18.8 g dry raw material accounting for 59% of maximal theoretical value. In a more comprehensive work, the effects of both concentrated- and dilute-sulfuric acid pretreatment on wheat straw was evaluated by Saha et al. (2005). Concentrate and dilute acid treatments yielded 49% and 63% of the total sugars content. Optimum acid dose for maximum yield of carbohydrates in dilute acid treatment was 0.75% (v/v). The effect of temperature was also studied and formation of furfural was reported only at the highest temperature (180 °C). Two-stage dilute acid was also tried in order to improve the yield of total sugars and to avoid formation of furfural; however, the result was not satisfactory. An investigation on dilute mineral and organic acid pretreatment of wheat straw was made by Kootstra et al. (2009) where the efficiency of sulfuric acid was compared with fumaric and maleic acids. Pretreatments were performed at 130, 150 and 170 °C for 30 min. At the highest temperature, the yield of glucose after enzymatic hydrolysis reached to 98% and 96% for sulfuric acid and maleic acid, respectively. Fumaric acid was less effective than maleic acid. The highest yield of xylose (ca. 80%) was obtained with H_2SO_4 pretreatment at 150 °C, however, the yield decreased at 170 °C due to partial degradation of xylose to furfural. The maximum xylose yield at 170 °C was obtained with treatment using maleic acid. The authors concluded that maleic acid pretreatment of wheat straw is almost as effective as sulfuric acid in respect with enzymatic digestibility where more xylose and less furfural is produced.

4.3.2. Alkaline

Alkaline process is based on utilization of dilute bases in pretreatment of lignocellulosic feedstocks. Sodium, potassium, calcium and ammonium hydroxides are suitable alkaline agents for pretreatment, among which sodium hydroxide has been studied the most (Kumar et al., 2009a,b). Alkaline pretreatment processes utilize lower temperatures and pressures than other pretreatment technologies (Mosier et al., 2005a,b). The effectiveness of this method depends on the lignin content of biomass and therefore, it is well suited for agricultural residues such as wheat straw (Sánchez and Cardona, 2008; Sun and Cheng, 2002). Alkaline pretreatment can largely improve the cellulose digestibility and sugars degradation is less than acid treatment, however, the application is hindered by high cost of alkalis. Utilization of calcium hydroxide (lime) as a cheap alkaline agent and its recovery and regeneration or ammonia which is recyclable due to its volatility could be a solution to this problem (Kim and Holtzapple, 2006; Wyman et al., 2005). Lime pretreatment of wheat straw was explored in a work presented by Chang et al. (1998). They found that for short pretreatment times (1–3 h), high temperatures (85–135 °C) were required to reach high sugar yields, whereas for long treatment times (*e.g.* 24 h), lower temperatures (50–65 °C) were more effective. The optimal lime loading was 0.1 (g/g dry mass). Under all recommended conditions, the yield of reducing sugars was increased by a factor of 10 compared with untreated wheat straw.

4.3.3. Oxidizing agents

4.3.3.1. Alkaline/oxidative pretreatment. In this pretreatment, an oxidizing compound such as hydrogen peroxide (H_2O_2) or peracetic acid ($C_2H_4O_3$) is used in combination with an alkaline (*e.g.* NaOH) and it is usually carried out under mild temperature. This treatment is more effective in improving of crop residue digestibility compared with NaOH treatment alone. Gould (1984) demonstrated the use of H_2O_2 for delignification of agricultural residues including wheat straw. Treatment was performed with 1% H_2O_2 and pH 11.5 at 25 °C for 18–24 h. Under this condition, more than half of the lignin and most of hemicellulose were solubilized. These values were higher than those of NaOH treatment (without H_2O_2 addition) by several folds. Enzymatic hydrolysis of treated wheat straw in presence of H_2O_2 also showed a clear enhancement where nearly 100% conversion was attained at pH 11.5. Treatment with NaOH alone under identical condition exhibited a significant increase in cellulose digestibility only at pH > 12, however, the max-

imum efficiency did not exceed 65%. Alkaline peroxide delignification of agricultural residues showed to be strongly pH-dependent, with an optimum pH of 11.5–11.6. The author also reported that at pH lower than 10, delignification was negligible and at a pH 12.5 and higher, alkaline peroxide treatment showed no real effect on the enzymatic digestibility. The variables of alkaline peroxide pretreatment of wheat straw was optimized by Saha and Cotta (2006). Treatment with NaOH (0% peroxide) yielded about 250 (mg/g) total sugars. This yield was almost doubled with addition of peroxide. The optimum dose of peroxide was 2.15% (v/v) and sugar recovery after enzymatic hydrolysis was slightly higher at 35 °C compared to 25 °C. Increase of treatment time from 3 to 24 h showed a minor effect on enhancement of total sugars yield. The results are in agreement with previous reports suggesting a minimum peroxide/biomass weight ratio of 0.25 is necessary for a good delignification (Gould, 1984).

4.3.4. Wet oxidation (WO)

In wet oxidation, the lignocellulosic biomass is treated with water and high pressure oxygen or air at elevated temperatures (above 120 °C). Typical oxygen pressure range is 120–480 Psi (Schmidt and Thomsen, 1998). WO is an effective pretreatment method for the fractionation of wheat straw into a solubilized hemicellulose fraction and a cellulose-rich solid fraction with high susceptibility to enzymatic hydrolysis. Combination of alkali and WO not only improves the rate of lignin oxidation (and in turn enzymatic hydrolysis) but also prevents formation of furfural and HMF (Bjerre et al., 1996). Acids formed during initial reaction in WO due to solubilization of hemicellulose components catalyze the subsequent hydrolytic reactions through which hemicelluloses are broken down into lower molecular weight fragments that are soluble in water. Lignin degradation is also significant especially at the higher temperatures because phenol-like compounds and carbon–carbon linkage are very reactive under wet oxidation conditions. Lignin is decomposed to CO_2, H_2O and carboxylic acids (Klinke et al., 2002).

Bjerre et al. (1996) investigated the alkaline wet oxidation of wheat straw at 10 bar of oxygen pressure and various temperatures. A maximum delignification of about 65% and hemicellulose solubilization of about 50% occurred at 170 °C within 10 min. The left over solid residue showed the highest enzymatic convertibility where 85 (%w/w) of cellulose was converted to glucose. The effects of oxygen pressure and alkaline (Na_2CO_3) addition on WO of wheat straw were studied and the results indicated slight effects of these two parameters on solubilization of hemicellulose. Almost all hemicellulose (96%) was solubilized by pretreatment with oxygen pressure and without carbonate. However, alkaline addition, regardless to presence or absence of oxygen, decreased formation of furfural by more than 10-fold. The lignin removal, on the other hand, was significantly affected by presence of oxygen (60% compared to 11% without oxygen pressure) (Ahring et al., 1996). Schmidt and Thomsen (1998) optimized the WO of wheat straw with respect to oxygen pressure, reaction temperature and time as the main process variables. The optimum condition was 185 °C, 15 min and 12 bar oxygen pressure. In overall, temperature was found to be more important process parameter than time and oxygen pressure. Formation of some inhibitors in WO of wheat straw has been reported including aliphatic carboxylic acids (mainly formic acid and acetic acid), phenols and 2-furoic acid (Klinke et al., 2001).

4.3.5. Ozonolysis

In this process, ozone is used to solubilize lignin and a small fraction of hemicellulose from wheat straw. Ozonolysis is carried out at room temperature and can effectively remove the lignin without producing any toxic residues. The main drawback of this process is a large amount of ozone utilization that makes the process expensive (Sun and Cheng, 2002). Binder et al. (1980) investigated the delignification of wheat straw by ozone treatment and biodegradability of resultant solid residue. It was demonstrated that a 50% reduction of the original lignin content is optimal for enzymatic hydrolysis. After treatment, 75% of the cellulose was digested within 24 h as compared to 20% in untreated straw. The improved digestibility was attributed to lignin removal as well as reduced degree of polymerization of the treated cellulose. The ozonolysis pretreatment of wheat and rye straw was investigated in a fixed bed reactor under room conditions (Garcia-Cubero et al., 2009). Among the studied variables, moisture content found to be the most significant variable and a reaction controlling parameter for values below 30%. Enzymatic hydrolysis yield of up to 88.6% was attained compared to 29% in non-ozonated wheat straw.

4.4. Biological pretreatment

Biological pretreatment comprises of using microorganisms such as brown-, white-, and soft-rot fungi for selective degradation of lignin and hemicellulose among which white-rot fungi seems to be the most effective microorganism. Lignin degradation occurs through the action of lignin-degrading enzymes such as peroxidases and laccase (Fig. 1) (Okano et al., 2005). The suitable fungi for biological pretreatment should have higher affinity for lignin and degrade it faster than carbohydrate components. Biological pretreatments are safe, environmentally friendly and less energy intensive compared to other pretreatment methods. However, the rate of hydrolysis reaction is very low and needs a great improvement to be commercially applicable. Hatakka (1983) investigated the pretreatment of wheat straw using 19 white-rot fungi and found that 35% of the wheat straw was converted to reducing sugars after five weeks pretreatment with *Pleurotus ostreatus* compared to only 12% conversion of the untreated straw. Five different fungi obtained from screening were evaluated for pretreatment of wheat straw in a study performed by Patel et al. (2007). Pretreatment with *Aspergillus niger* and *Aspergillus awamori* showed the best results regarding to yields of total sugars and ethanol after fermentation.

4.5. Summary of pretreatment methods

Pretreatment plays a significant role in ethanol production from lignocellulosic materials such as wheat straw. The objectives are to increase the surface area and porosity of the substrate, reduce the crystallinity of cellulose and disrupt the heterogeneous structure of cellulosic materials. Thus far, no single pretreatment method was found to meet all these requirements; instead a combination of different methods might be applied. Table 1 describes some the most promising methods and corresponding conditions, recently used for pretreatment of wheat straw. Diverse advantages and drawbacks are associated with each pretreatment method. The shorter reaction times (desired) are generally accompanied with higher temperature (undesired) (Table 1). The choice of appropriate pretreatment method for wheat straw relies on some technological factors including energy balance, higher solid loading, and minimum use of chemicals as well as some environmental factors such as wastewater treatment, catalyst recovery and solvent recycling. Steam explosion is probably the most suitable method for pretreatment of wheat straw in terms of lower reaction time, higher solid loading and minimum use of chemicals (Table 1). However, since the pretreatment has an enormous influence on the efficiency and economy of the subsequent stages, the final decision must me made in the framework of the entire process.

Table 1
Promising methods and corresponding conditions for pretreatment of wheat straw.

Pretreatment technology	Procedure/chemicals	Temp. (°C)	Reaction times	Solid loading (wt.%)	References
Dilute acid	0.5–5.0% H_2SO_4	120–180	5–60 min	5–30	Kootstra et al. (2009) and Saha et al. (2005)
Steam explosion	Saturated steam	160–230	5–30 min	<30	Ballesteros et al. (2006) and Palmarola-Adrados et al. (2004)
Alkaline peroxide	>0.25 g H_2O_2/g biomass, pH 11.5	25–35	3–24 h	<10	Gould (1984) and Saha and Cotta (2006)
Wet oxidation (alkaline)	6–12 bar oxygen pressure (+0.11 g Na_2CO_3/g biomass)	185–195	10–15 min	6	Klinke et al. (2002) and Schmidt and Thomsen (1998)
Lime	0.05–0.15 g $Ca(OH)_2$/g biomass	85–135 50–65	1–3 h 24 h	5–20	Chang et al. (1998) and Saha and Cotta (2007)

5. Hydrolysis

Hydrolysis using appropriate enzymes represents the most effective method to liberate simple sugars from cellulosic materials. Cellulose hydrolysis is catalyzed by a class of enzymes known as cellulases. These enzymes can be produced by fungi such as *Trichoderma reesei* and *A. niger* (Table 2) and/or bacteria such as *Clostridium cellulovorans* (Arai et al., 2006). Most research for commercial cellulase production has focused on fungi since majority of relevant bacteria are anaerobes with a very low growth rates. At least three major groups of enzymes namely, endo-glucanase, exo-glucanase and β-glucosidase are involved in hydrolysis of cellulose to glucose and their action is synergistic. Endo-glucanase attacks regions of low crystallinity in the cellulose fiber and creates free chain-ends. Exo-glucanase degrades the molecule further by removing cellobiose units from the free chain-ends which is then cleaved to glucose by the action of β-glucosidase. The enzymatic hydrolysis can be influenced by substrate and end-product concentrations, enzyme activity and reaction conditions. β-Glucosidase plays a significant role in the hydrolysis process, since cellobiose is an end-product inhibitor of many cellulases including both exo- and endo-glucanases (Galbe and Zacchi, 2002; Rabinovich et al., 2002). β-Glucosidase, in turn, is inhibited by glucose and, enzymatic hydrolysis is thus sensitive to the substrate concentration. Additionally, pretreatment of cellulosic materials and hydrolyzing conditions such as temperature and pH are among factors influencing the efficiency of enzymatic hydrolysis. Most cellulase enzymes show an optimum activity at temperatures and pH in the range of 45–55 °C and 4–5, respectively (Galbe and Zacchi, 2002). Cellulase dosage of 10–30 (FPU/g cellulose) is often used in laboratory studies because it results in an efficient hydrolysis with high glucose yield in a reasonable time (48–72 h) and enzyme cost. However, enzymes loading may vary depending on the pretreatment, type and concentration of raw materials.

The action of cellulolytic enzymes occur through three steps of adsorption, biodegradation and desorption. Cellulase activity decreases during the hydrolysis and it is believed that the irreversible adsorption of enzyme on cellulose is partially responsible for this deactivation. Addition of surfactants may improve the enzymatic cellulose conversion into monomeric sugars (Eriksson et al., 2002). Various mechanisms have been proposed for positive effect of surfactant addition on enzymatic hydrolysis. The surfactant could change or modify the nature of cellulose surface properties, reduce irreversible binding of cellulase on cellulose, prevent enzyme denaturation as well as unproductive binding of enzymes to lignin residues. Non-ionic surfactants such as Tween 20 were shown to be the most effective for enhancing of enzymatic hydrolysis (Kristensen et al., 2007; Tabka et al., 2006). Kristensen et al. (2007) investigated the effects of several non-ionic surfactants on enzymatic hydrolysis of five different types of pretreated wheat straw. All pretreated samples showed increased cellulose conversion with addition of different surfactants. The highest increase in cellulose conversion during enzymatic hydrolysis was 70% obtained with sulfuric acid treated straw when Berol 08 was used as surfactant. The optimum surfactant concentration was approximately 0.05 (g/g dry mass) and was found to be similar, irrespective of pretreatment type. The addition of Tween 20 increased the enzymatic saccharification yield of H_2SO_4 pretreated wheat straw from 488 to 520 (mg/g) (Saha et al., 2005). Similar results was also obtained with lime pretreated wheat straw, while no improvement was observed with alkaline peroxide pretreated wheat straw (Saha and Cotta, 2006, 2007; Saha et al., 2005).

Use of cellulase enzyme supplemented with other enzymes can raise the rate of enzymatic hydrolysis. It is well known that conjugated action of cellulases and hemicellulase results in a higher ultimate sugar production. The dominant hemicellulose polymer in wheat straw is xylan consisting of D-xylose backbone with different side groups including L-arabinose, D-galactose, acetyl, feruloyl, *p*-coumaroyl and glucuronic acid residues (Mazeau et al., 2005). Tabka et al. (2006) explored the effect of adding accessory enzymes including xylanases, feruloyl esterase (FAE) and laccase on the enzymatic hydrolysis of pretreated wheat straw. Feruloyl esterase could act in synergy with xylanases by cleaving diferulic bridges between xylan chains, opening the structures and releasing lignin.

Table 2
Sugar yield in the enzymatic hydrolysis of wheat straw after various pretreatments.

Pretreatment	Enzymes mixture	Source of enzyme	Hydrolysis conditions	Sugar yield (mg/g DM)	% max. theoretical	References
Dilute H_2SO_4 impregnation + SE	Cellulase β-Glucosidase	*T. reesei* *A. niger*	40 °C pH 5.0 96 h	612	99.6	Linde et al. (2008)
0.75% (v/v) H_2SO_4, 121 °C	Cellulase β-Glucosidase Xylanase	*T. reesei* *A. niger* *T. longibrachiatum*	45 °C pH 5.0 72 h	565	74	Saha et al. (2005)
2.15% (v/v) H_2O_2, 35 °C	Cellulase β-Glucosidase xylanase	*T. reesei* *A. niger* *T. longibrachiatum*	45 °C pH 5.0 120 h	672	96.7	Saha and Cotta (2006)
Fine grinding + wet oxidation	Cellulase β-Glucosidase	*T. reesei* *A. niger*	50 °C pH 5.0 24 h	638	92	Pedersen and Meyer (2009)

The best results regarding to glucose yield were obtained with cocktails of cellulose, xylanase and FAE at 50 °C accounting for 81% of the maximum amount of glucose recovery. Some of the most promising results regarding to enzymatic hydrolysis of wheat straw are summarized in Table 2. Enzymatic saccharifications of pretreated wheat straw using different methods (Table 2) were satisfactory with respect to yield of sugars. A blend of fungal cellulase and β-glucosidase enzymes seems to be enough for efficient saccharification of wheat straw after pretreatment at low pH. Addition of hemicellulase enzyme (xylanase activity) could improve the final yields of sugars, where substantial amount of hemicellulose remains in solid residue or in the form of soluble oligomers after pretreatment at high pH *e.g.* alkaline H_2O_2 pretreatment (Table 2).

6. Fermentation

An important factor preventing industrial utilization of lignocelluloses for bioethanol production is the lack of microorganisms able to efficiently ferment (with high yield and high rate) all sugars (both pentoses and hexoses) released during pretreatment and hydrolysis. Regarding to commercial ethanol production, the ideal microorganism should have broad substrate utilization, high ethanol yield and productivity, tolerance to inhibitors present in the hydrolyzates and to high concentrations of ethanol, cellulolytic activity and ability for sugar fermentation at high temperatures (Hahn-Hagerdal et al., 2007). The best known microorganisms for ethanol production from hexoses are the yeast *Sacchamyces cerevisiae* and the bacterium *Zymomonas mobilis* (Claassen et al., 1999) offering high ethanol yields (90–97% of the theoretical) and high ethanol tolerance up to ca. 10% (w/v) in fermentation medium. Ethanol yield of 99% based on initial glucose concentration, was obtained recently with native *S. cerevisiae* strain (Jorgensen, 2009). Main disadvantage of the native strains of *S. cerevisiae* and *Z. mobilis* is their inability to utilize xylose, the main C5 sugar derived from hemicellulose (Rogers et al., 2007; Talebnia and Taherzadeh, 2006). Other microorganisms known to ferment xylose to ethanol, such as enteric bacteria and the yeasts *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* (Chandel et al., 2007; Lin and Tanaka, 2006) are characterized by low ethanol yields and their tendency to re-assimilate the produced ethanol (Karakashev et al., 2007). In order to overcome this drawback, genetically modified strains of *S. cerevisiae* capable of fermenting both hexoses and pentoses have been developed (Karhumaa et al., 2007). However, those strains showed a low productivity with respect to the conversion of xylose to ethanol (Watanabe et al., 2007). Furthermore, there is a practical drawback for the wide implementation of recombinant ethanologens, since the plasmids carrying xylose conversion genes are often rejected by the host (Krishnan et al., 2000).

Taking into account the benefits of fermentation process at elevated temperature including high production rates, facilitated product recovery, utilization of a wide range of substrates and low risk of contamination (Torry-smith, 2002), ethanol fermentation with various strict anaerobic thermophilic bacteria such as *Clostridium* sp. (Claassen et al., 1999) and *Thermoanaerobacter* sp. (Larsen et al., 1997) has been proposed. The main drawback of the thermophilic ethanologens is their low tolerance to ethanol which is less than 30 g/L (Claassen et al., 1999). Additionally, many of these ethanologens produce other products such as volatile fatty acids and lactate besides ethanol. Recently, two novel ethanol-tolerant facultative anaerobic thermophilic strains of *Geobacillus thermoglucosidasius* were isolated from compost (Fong et al., 2006), indicating possibility for isolation of new species/strains from relevant sources. A mixed bacterial culture dominated by *Thermoanaerobacter*, *Thermoanaerobacterium* and *Caldanaerobacter* was recently found to produce simultaneously ethanol and hydrogen from glucose under extreme thermophilic conditions (Zhao et al., 2009).

Ethanol fermentation of wheat straw hydrolyzates as feedstock has been widely studied with different microorganisms including yeasts, bacteria and fungi, usually grown as pure cultures (Table 3). *P. stipitis* (Nigam, 2001), *Kluyveromyces marxianus* (Tomas-Pejo et al., 2009) native (Jorgensen, 2009) and recombinant strains of *S. cerevisiae* (Panagiotou and Olsson, 2007), were the most widely studied yeasts for ethanol fermentation based on wheat straw hydrolyzates as feedstock. So far the best results with respect to ethanol yield, final ethanol concentration and volumetric ethanol productivity were obtained with the native non-adapted *S. cerevisiae* (Table 3). Ethanol yields with *Pichia* sp. have reached up to 0.42 (g/g), however the average volumetric ethanol productivity was approximately half of that registered with *Kluyveromyces* sp. (Table 3). High effective single-batch fermentation approach (86% ethanol yield) employing two phylogenetically different microorganisms were demonstrated by Zayed and Meyer (1996). In this study the hydrolysis of delignified wheat straw to simple sugars was performed by fungus *Trichoderma viride* followed by aerotolerant fermentation of the resulting xylose and glucose to ethanol by yeast *Pachysolen tannophylus*. With respect to thermophiles, ethanol productivity obtained with the *Thermoanaerobacter* sp. grown on pretreated wheat straw (Georgieva et al., 2008) were much lower compared to those obtained with yeasts and fungi (Table 3). We have recently isolated an ethanologen belonging to *Thermoanaerobacter*, producing mainly ethanol from pentoses with an ethanol yield of 70%. The only additional products beside ethanol were acetate and hydrogen (unpublished results).

Recombinant bacteria with deletions of genes responsible for production of by-products have been also considered for ethanol production. A recombinant bacterium (*E. coli* strain FBR5) was

Table 3
Properties of some ethanol producing microorganisms involved in ethanol fermentation of wheat straw-based hydrolyzates.

Microorganism	Phylogeny	Y_E[a] (%)	r_E (g/L h)	C_E (g/L)	Growth conditions	References
S. cerevisiae[b]	Yeast	99	1.16	31.2	Mesophilic, facultative anaerobic	Jorgensen (2009)
Thermoanaero-bacter BG1L1	Bacteria	76	0.2	14.4	Extreme thermophilic (70 °C), strict anaerobic	Georgieva et al. (2008)
Kluyveromyces marxianus CECT 10875	Yeast	65	0.5	36.2	Thermotolerant (42 °C), facultative anaerobic	Tomas-Pejo et al. (2009)
S. cerevisiae strains CPB.CB4	Yeast (recombinant)	84	0.27	12.8	Mesophilic, facultative anaerobic	Otero et al. (2007)
Pichia stipitis	Yeast	82	0.27	19	Mesophilic, facultative anaerobic	Nigam (2001)
Trichoderma viride + *Pachysolen tannophylus*	Fungus				Mesophilic, aerobic fungus	Zayed and Meyer (1996)
	Yeast	86	0.25	11.8	Facultative anaerobic yeast	
E. coli strain FBR5	Bacteria (recombinant)	90	0.4	18.9	Mesophilic, semianaerobic	Saha and Cotta (2006)

Note: r_E, volumetric ethanol production rate; Y_E, ethanol yield; C_E, ethanol concentration reached in the medium.
[a] % of maximum theoretical yield.
[b] r_E and C_E were reported as g kg^{-1} h^{-1} and g kg^{-1}, respectively.

tested for ethanol production from pretreated wheat straw (Saha and Cotta, 2006) and showed the highest ethanol yield among the bacteria studied for bioconversion of wheat straw derived feedstocks (Table 3). Ethanol concentration and volumetric productivity reported were comparable with ethanol concentration obtained with yeast *P. stipitis* and productivity with yeast *K. marxianus* (Table 3). Research efforts regarding ethanol fermentation are still in progress. Finding new wild-type ethanologens or construction of new promising genetic manipulated organism (GMO) with higher ethanol tolerance, productivity and yield will create a basis for future development of commercial lignocellulose-based bioethanol production from wheat straw.

6.1. Different approaches for enzymatic hydrolysis and fermentation

The enzymatic hydrolysis and fermentation process can be accomplished using different strategies: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and direct microbial conversion (DMC). In SHF, hydrolysis and fermentation are carried out in separate vessels under their own optimal conditions; however, end-product inhibition of enzymes' activity and contamination problems are associated with this process. In order to eliminate drawbacks of the SHF process, SSF that combines hydrolysis and fermentation in one vessel has been developed. Sugars produced during hydrolysis are immediately fermented into ethanol and thus, problems associated with sugar accumulation and enzyme inhibition as well as contamination can be avoided (Galbe and Zacchi, 2002; Ohgren et al., 2007). Another advantage of SSF over SHF is the cost reduction resulting from use of only one reactor. The main drawback of SSF is the different optimum temperatures of the hydrolysis and fermentation processes. Most fermenting yeasts have an optimal temperature around 30–35 °C while hydrolyzing enzymes show optimal activities around 50 °C (Kadar et al., 2004).

Saha et al. (2005) evaluated the performance of both SSF and SHF using dilute acid pretreated wheat straw by the recombinant *E. coli* (FBR5). SHF approach worked better than SSF and yielded higher ethanol at shorter fermentation time. Detoxification with overliming method dramatically reduced fermentation time of SHF and enhanced the yield of ethanol in SSF. Better performance of SHF approach than SSF was also reported with the same strain (*E. coli*) and using alkaline peroxide pretreated wheat straw (Saha and Cotta, 2006). However, time required for separate enzymatic hydrolysis in SHF approach was excluded in this aspect. Considering total time (separate hydrolysis + fermentation) in SHF suggests that SSF approach is much more advantageous and works better than SHF. Alfani et al. (2000) estimated SHF and SSF processes for the bioconversion of steam-exploded wheat straw. The authors reported that time needed for completion of SSF and SHF were 30 and 96 h (72 h hydrolysis + 24 h fermentation), respectively. In contrary to substantially faster ethanol productivity in SSF, the final ethanol yield was higher in SHF process (81% of the theoretical compared to 68% in SSF). In DMC, no external enzyme is added and the hydrolytic process is mediated by the enzymes produced from the microorganisms (Demirbas, 2005). To our knowledge, DMC of alkali-pretreated wheat straw to ethanol was investigated only with mesophilic fungus *Fusarium oxysporum* F3 however, yield and volumetric productivity reported, were relatively low (Christakopoulos et al., 1991) .

7. Future perspective and conclusions

As the price of current ethanol feedstocks (*e.g.* Corn) is estimated to increase, lignocellulosic materials remain the only viable candidate to serve as renewable feedstock for ethanol production. There are huge amounts of wheat straw that are currently burned on the field or wasted otherwise which can be used as low value row material for ethanol production. Despite extensive technological advances in ethanol production from lignocellulose feedstocks over last few decades the price of the second generation ethanol is still high and remains around $2.65 per gallon (Kumar et al., 2009a,b). This high price is because of some technological impediments encountered in all different steps of the process.

Pretreatment is estimated to account for 33% of the total cost (Tomas-Pejo et al., 2008). The current leading pretreatment methods for lignocellulosic materials are capital intensive. Economical comparison showed that there is little differentiation between studied pretreatment methods as for instance; low cost pretreatment reactors are counterbalanced by higher cost of catalyst and/or ethanol recovery (Eggeman and Elander, 2005). Development of less energy intensive and more effective pretreatment methods allowing lower amount of enzymes loading can substantially decrease the total cost of cellulosic ethanol.

The next significant technical barrier is cost of enzymes. Joint collaboration and investment has been made with the aim of increasing the effectiveness of enzymes, developing of novel technology for high solid handling and reducing the enzyme cost by several folds. Novozymes AS unveiled its second generation of lignocellulytic enzymes known as Cellic CTec and Cellic HTec which is claimed to be a key step toward delivering commercially viable enzymes for cellulosic ethanol production. These enzymes require one-third the dose of its first generation enzyme, Celluclast, to achieve 80% conversion, and work with a wide range of feedstocks and pretreatments (Bevill, 2009). High solid concentration can significantly decrease the cost of cellulosic ethanol (Galbe and Zacchi, 2002). Due to the current limitation of solid loading in enzymatic hydrolysis and fermentation stages, the resultant ethanol concentration is commonly low which dramatically increases the cost of distillation. Final ethanol concentration higher than 4% (w/w) is necessary to substantially reduce the energy demand in the distillation step (Hahn-Hagerdal et al., 2006). Consequently, a minimum wheat straw loading of ~20% (w/w) is required in order to reach the desired final ethanol concentration.

Additionally, the economy of lignocellulosic ethanol could be improved by simultaneous fermentation of hexose and pentose sugars in fermentation step. The most commonly-used industrial microorganism, *S. cerevisiae*, is not able to uptake pentose sugars. Only a limited number of bacteria, yeasts, and fungi can convert hemicellulose-derived sugars into ethanol with a satisfactory yield and productivity. The ethanol yield of anaerobic bacteria capable of fermenting xylose, is low and they are inhibited at low sugar and ethanol concentrations. Natural xylose-fermenting yeast such as *Pichia* species cannot efficiently tolerate the toxic compounds generated during pretreatment and fungi are commonly too slow for a competitive industrial process (Hahn-Hagerdal et al., 2006). Metabolic engineering concept has been used for efficient fermentation of mixed sugars. Some recombinant bacteria and yeasts such as *E. coli*, *Klebsiella oxytoca*, *Z. mobilis* and *S. cerevisiae* have shown promising results and are being considered for commercial scale-up. However, the willingness of ethanol producers to consider using these strains instead of conventional yeast will depend on demonstrating of several technical concerns including capability of producing ethanol reliably in larger bioreactors, no need for fully aseptic condition to avoid contamination, and excellent tolerance capacity against the inhibitors (Dien et al., 2003).

In recent year, several biorefinery concepts have been introduced as a solution for clean, efficient and economically-feasible utilization of lignocellulosic materials. The modern biorefinery parallels the petroleum refinery and is a factory that processes crops to produce a wide range of product including high value components, transportation fuels and direct energy (Ragauskas et al.,

2006). Wheat straw could be converted into a variety of high value wax products and a number of energy and chemical products. Natural waxes have a wide range of industrial uses in cosmetics, personal care products, polishes and coating with a world market of thousands of ton (Deswarte et al., 2006, 2007). By further decrease in the cost of enzymes for hydrolysis, and modern technology such as process integration for new ethanol plants, the second generation of ethanol, will gain the potential to compete on a large scale with gasoline without subsidies in near future.

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