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Bioethanol Production from Steam Explosion Pretreated Straw

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1. Introduction

1.1 Motivation and environmental aspects

The combustion of fossil fuels is responsible for 73% of carbon dioxide emissions into the atmosphere and therefore contributes significantly to global warming. Interest in the development of methods to reduce greenhouse gases has increased enormously. In order to control such emissions, many advanced technologies have been developed, which help in reducing energy consumption, increasing the efficiency of energy conversion or utilization, switching to lower carbon-content fuels, enhancing natural sinks for carbon dioxide, capture and storage of carbon dioxide, reducing the use of fossil fuels in order to decrease the amount of carbon dioxide and minimizing the levels of pollutants. In the last few years, research on renewable energy sources that reduce carbon dioxide emissions has become very important. Since the 1980s, bioethanol has been recognized as a potential alternative to petroleum-derived transport fuels in many countries. Today, bioethanol accounts for more than 94% of global biofuel production, with North America (mainly the US) and Brazil as the overall leading producers in the world (about 88% of the world bioethanol production in 2009).

Generally, biofuel production can be classified into three main types, depending on the converted feedstocks used: biofuel production of first, second and third generation. Bioethanol production of the first generation is either from starchy feedstocks, e.g. seeds or grains such as wheat, barley and corn (North America, Europe) or from sucrose-containing feedstocks (mainly Brazil). The feedstocks used for bioethanol production of the second generation are lignocellulose-containing raw materials like straw or wood as a carbon source. Biofuel production of the third generation is understood as the production of lipolytic compounds mainly from algae.

The feedstocks of bioethanol production of the first generation could also enter the animal or human food chain. Therefore, bioethanol production of the first generation is regarded critically by the global population, worrying about food shortages and price rises. Other reasons which lead to research and developments in bioethanol production of the second generation are: a shortage of world oil reserves, increasing fuel prices and reduction of the greenhouse effect. In addition to this, the renewable energy directive (EC 2009/28 RED) demands a reduction for Europe of 6% in the greenhouse gases for the production and use of fuels. This reduction is only possible if biofuels are added to diesel fuel or gasoline by the year 2020. It also seems that the target for greenhouse gas reduction for Europe can only be

achieved if the biofuels are mainly from bioethanol of the second generation. Outside Europe (Brasil, USA) the targets can be achieved using first generation biofuels. Hence, research and development on the production of bioethanol of the second generation needs to be intensively promoted, particularly in the European countries.

1.2 State of science and technology

Bioethanol production of the first generation from sugar cane and from wheat or corn is well established in Brazil as well as in the US and Europe. The world's ethanol production in more than 75 countries amounted in 2008 to more than 77 billion litres of ethanol (Sucrogen bioethanol, 2011).

Bioethanol production of the second generation can use lignocelluloses from non-food crops (not counted in the animal or the human food chain), including waste and remnant biomass e.g. wheat straw, corn stover, wood, and grass. These feedstocks are composed mainly of lignocellulose (cellulose, hemicelluloses and lignin).

The process of bioethanol production of the first generation is well established and shown in Fig. 1.

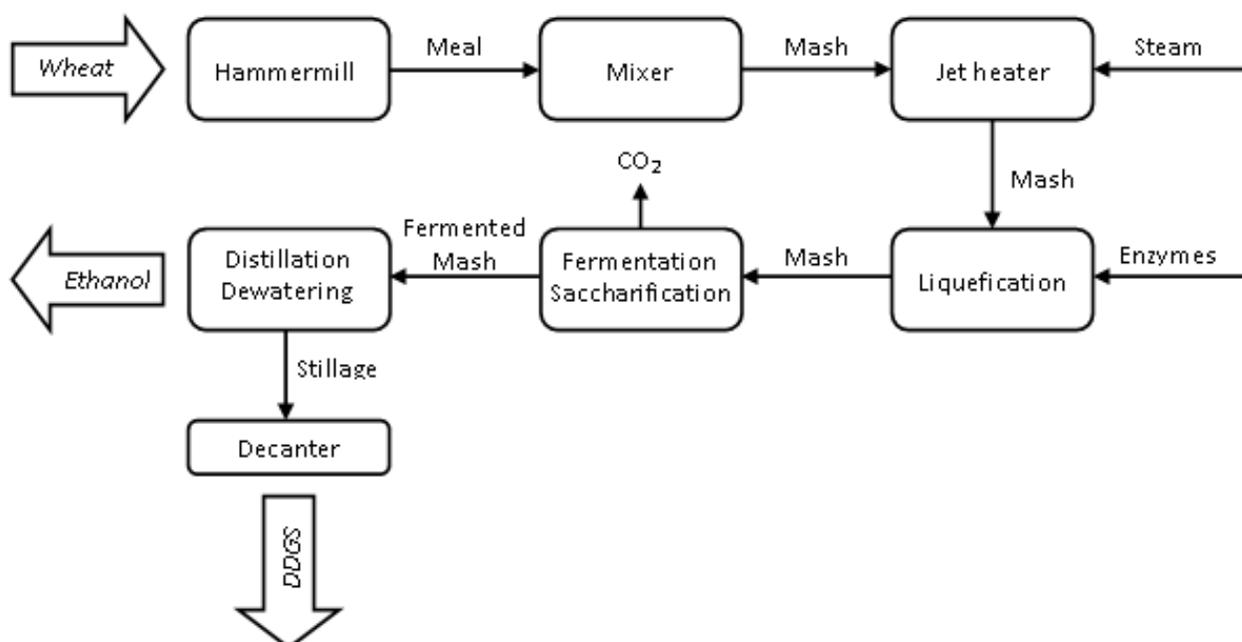


Fig. 1. Flow chart showing bioethanol production from starchy raw materials

The process of bioethanol production from wheat normally consists of five major process steps:

1. Milling of the grain
2. Liquefaction at high temperatures
3. Saccharification (enzymatic degradation of starch)
4. Fermentation with yeast
5. Distillation (rectification) of ethanol

The production of bioethanol from lignocelluloses follows more or less the same principle and is composed of the following sub-steps: milling, thermophysical pretreatment hydrolysis, fermentation, distillation and product separation/processing (Fig. 2).

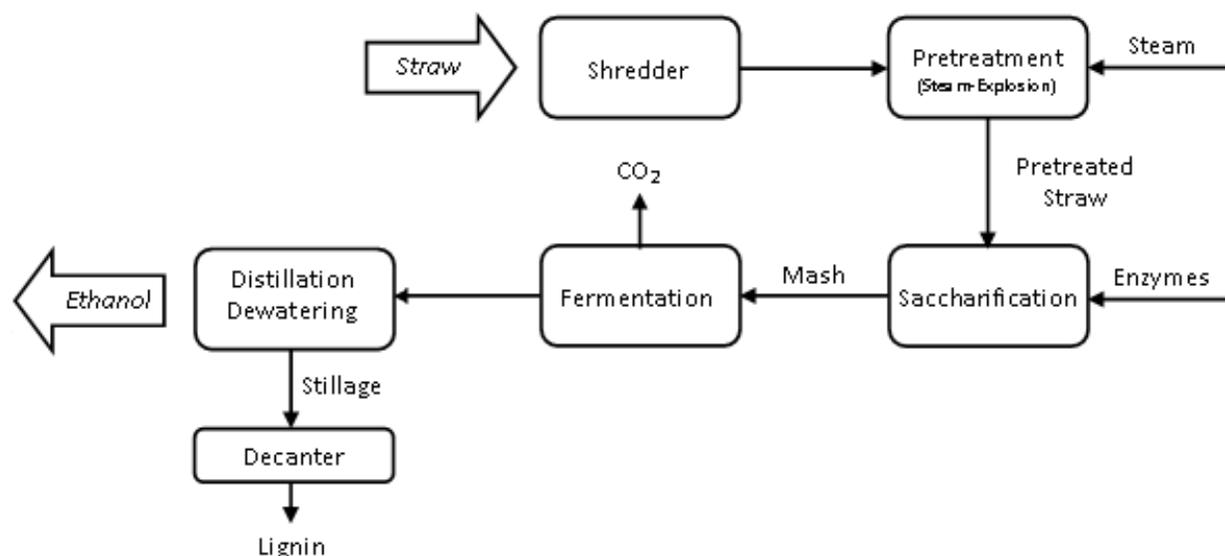


Fig. 2. Flow chart showing bioethanol production from lignocelluloses

The cellulose in the lignocellulose is not accessible to enzymes. Therefore, lignin and/or hemicelluloses have to be removed in order to make the enzymatic degradation of the cellulose possible. Ideal pretreatment should lead to better performance during bioethanol production from lignocelluloses.

The pretreatment should cause the hydrolysis of hemicelluloses, high recovery of all carbohydrates, and high digestibility of the cellulose in enzymatic hydrolysis. No sugars should either be degraded or converted into inhibitory compounds. A high solid matter content and high concentration of sugars should be possible. The process should have low energy demands and require low capital and operational cost.

The pretreatment methods can be classified roughly into three types: thermophysical methods, acid-based methods and alkaline methods. Thermophysical methods like steam pretreatment, steam explosion or hydrothermolysis solubilise most of the cellulose and hemicelluloses. There is only a low level of sugar conversion. Cellulose and hemicelluloses have to be converted enzymatically into C6 sugars (mainly glucose) and to C5 sugars (mainly xylose). Acid-based methods use mineral acids like sulphuric acid and phosphoric acid. Hemicelluloses are degraded to sugar monomers, cellulose has to be converted to glucose enzymatically. Alkaline methods like ammonia fibre explosion leave some of the hydrocarbons in the solid fraction. Hemicellulases acting both on solid and dissolved hemicelluloses are required as well as the cellulolytic enzymes.

Lignocellulose containing substrates are mainly composed of cellulose (40-50%), hemicellulose (25-35%) and lignin (15-20%). Cellulose is a glucose polymer, hemicellulose is a heteropolymer of mainly xylose and arabinose, and lignin is a complex poly-aromatic compound. The different pretreatment methods are necessary to loosen the close bonding between cellulose, hemicellulose and lignin. Wheat (*Triticum aestivum* L.) straw is composed of 45% cellulose, 26% hemicellulose and 19% lignin. Maize (*Zea mays*) straw is composed of 39% cellulose, 30% hemicellulose and 17% lignin.

The high percentage of hemicelluloses and the resulting pentoses, e.g. xylose from the hydrolysis of the polymer, are a further challenge to a cost-competitive bioethanol process with lignocelluloses as carbon source.

Yeasts used for the conversion of sugars into ethanol (mostly *Saccharomyces spec.*) usually only convert glucose into ethanol. C5 sugars like xylose are only converted into ethanol at

low rates by very few yeast (*Pichia spec.*) strains. Research programs are underway either to adapt yeasts for the use of both C5 and C6 sugars or to modify *Saccharomyces* genetically to obtain yeast that produces ethanol simultaneously from C5 and C6 sugars.

Nevertheless, because of its ready availability and low costs, lignocellulosic biomass is the most promising feedstock for the production of fuel bioethanol. Large-scale commercial production of bioethanol from lignocellulose containing materials has still not been implemented.

2. Potential of second generation bioethanol

The world-wide availability of feedstock has to be taken into account if bioethanol from lignocelluloses is to contribute significantly to the world fuel market. A report by Bentsen & Felby (2010) shows existing agricultural residue of 1.6 Gt/year cellulose and 0.8 Gt/year hemicelluloses (figures do not include Africa and Australia). This gives a theoretical quantity of 1.24 Gm³ bioethanol from cellulose (690 l/t using *Saccharomyces spec.*) and 0.480 Gm³ (600 l/t using *Zymomonas spec.*) from hemicelluloses. For comparison: the worldwide production of crude oil is estimated to reach not more than 4.8 Gm³ pa (83 million bbl/day) and is supposed/predicted to decline to under 2.4 Gm³ pa (41.5 million bbl/day) by 2040 (Zittel, 2010). The potential of bioethanol from agricultural residues seems to be high. But not all residues will be available and the conversion rates will not be 100%. Therefore, it is thought that lignocellulose-containing materials have to be produced on agricultural land possibly in combination with the production of feedstocks like wheat, corn or sugar cane. These crops would serve as feedstocks for bioethanol production of the first generation.

The yield per hectare is conservatively estimated at 3000 l and 1500 l per hectare of agricultural land for bioethanol of the first and second generation, respectively. A replacement of 41.5 million bbl/day of crude oil would require an area of land of around 5 million km².

Using DDGS (distillers dried grain solubles) as protein-rich animal feed, taking into account an increase in productivity in agriculture and using intermediate crops as feedstock, the required area could be reduced to under 2.5 million km². This represents approximately 3% of the world's land (Bentsen & Felby, 2010).

3. The production of bioethanol from lignocelluloses

3.1 Pretreatment

Lignocellulose containing biomass has to be pretreated prior to hydrolysis to improve the accessibility of the biomass. For this pretreatment, several processes are available: mechanical treatment for size reduction (e.g. chopping, milling, grinding), hydrothermal treatment (e.g. uncatalysed steam treatment with or without steam explosion, acid catalysed steam treatment, liquid hot water treatment) and chemical treatment (e.g. dilute acid, concentrated acid, lime, NH₃, H₂O₂). Diverse advantages and drawbacks are associated with each pretreatment method (Mosier et al., 2005; Hendriks & Zeeman, 2009; Chen & Qui, 2010; Talebnia et al., 2010).

Steam explosion is a widely-employed process for this pretreatment. This process combines chemical effects due to hydrolysis (autohydrolysis) in high temperature water and acetic acid formed from acetyl groups, and mechanical forces of the sudden pressure discharge

(explosion). The steam explosion process offers several attractive features when compared to other technologies. These include less hazardous process chemicals and significantly lower environmental impact (Alvira et al., 2010). Typical operation conditions for steam explosion treatment of straw - temperature and duration of treatment - are summarised in Table 1.

Biomass	Temperature in °C	Duration of pretreatment in minutes	Catalyst	Reference
Wheat straw	220	2.5	none	Tomás-Pejó et al., 2009
Wheat straw	190	8	none	Ballesteros et al., 2004
Wheat straw	190	10	H ₂ SO ₄	Jurado et al., 2009
Wheat straw	200	10	none	Sun et al., 2005
Wheat straw	200	4.5	none	Chen et al., 2007
Barley straw	210	5	none	Garcia-Aparicio et al., 2006
Barley straw	210	5	H ₂ SO ₄	Linde et al., 2007
Corn stover	200	10	none	Yang et al., 2010
Corn stover	200	5	H ₂ SO ₄	Varga et al., 2004
Rice straw	220	4	none	Ibrahim et al., 2011

Table 1. Typical operation data for steam explosion of straw

According to Overend and Chornet (1987), the severity of the pretreatment can be quantified by the severity factor R_0 . The severity factor combines the temperature of the pretreatment (T in degree Celsius) and the duration of the pretreatment (t in minutes) thus:

$$R_0 = \int_0^t \exp \frac{T(t) - 100}{14.75} . dt \quad (1)$$

The severity factor is based on the observation that it is possible to trade duration of treatment and the temperature of treatment so that equivalent final effects are obtained. However, it is not intended to give mechanistic insight into the process.

3.2 Hydrolysis

Clearly, the hydrolysis step is affected by the type of pretreatment and the quality of this process - particularly by the accessibility of the lignocellulose.

Lignocelluloses can be solubilised by enzymatic or chemical hydrolysis (mainly with acids). Both the pretreatment and hydrolysis are performed in a single step during acid hydrolysis. Two types of acid hydrolysis are usually applied: concentrated and dilute acid hydrolysis (Wyman et al., 2004, Gray et al., 2006, Hendriks & Zeeman, 2009).

Cellulase enzymes from diverse fungi (e.g. like *Trichoderma*, *Aspergillus*) (Dashtban et al., Sanchez, 2009) and bacteria (e.g *Clostridium*, *Bacillus*) (Sun & Cheng, 2002) can release sugar from lignocellulose at moderate temperatures (45-50°C) with long reaction times (one to several days) (reviewed in Brethauer & Wyman, 2010; Balat, 2011).

Three different enzymes work synergistically - the endo- β -1,4-glucanases (EC 3.1.2.4), exo- β -1,4-glucanases (EC 3.2.1.91) and β -glucosidase (EC 3.2.1.21) - to generate glucose molecules from cellulose (Lynd et al., 2002). In addition, enzymes like hemicellulases and ligninases improve the hydrolysis rate and raise the content of the fermentable sugar (Palonen & Viikari, 2004; Berlin et al., 2005).

Diverse factors inhibit the activity of the cellulase and thereby decrease the rate of hydrolysis and the effectiveness of the hydrolysis step: end-product inhibition, easily degradable ends of molecules are depleted, deactivation of the enzymes, binding of enzymes in small pores of the cellulose and to lignin (Brethauer & Wyman, 2010; Balat, 2011).

Hemicellulose is a highly complex molecule and multi-enzyme systems are needed like endoxylanase, exoxylanase, β -xylanase, α -arabinofuranosidase, α -glucuronidase, acetyl xylan esterase and ferulic acid esterase (all produced by diverse fungi e.g. *Aspergillus* and bacteria e.g. *Bacillus*) for the enzymatic hydrolysis (reviewed in Balat, 2011).

3.3 Fermentation

The microorganisms for the ethanolic fermentation process for lignocellulose-containing hydrolysates should ferment both hexoses and pentoses (if both cellulose and hemicellulose are solubilised) to achieve efficient bioethanol production. Unfortunately, no known natural microorganisms can efficiently ferment both pentoses and hexoses, which are generated during hydrolysis from lignocelluloses (Ragauskas et al., 2006). The perfect microorganism for fermentation should exhibit several properties: sugar tolerance, ethanol and thermotolerance, resistance against diverse inhibitors, fermentation of hexoses and pentoses and stability during industrial application.

Diverse microorganisms like *Saccharomyces cerevisiae*, *Pichia stipitis*, *Escherichia coli* and *Zymomonas mobilis* are typically applied in the bioethanol process from lignocellulose. Both generally used microorganisms, the yeast *Saccharomyces cerevisiae* and the bacterium *Zymomonas mobilis*, can convert hexoses into bioethanol offering high ethanol tolerance and ethanol yields. Genetically modified yeast strains from *Saccharomyces cerevisiae* converting both pentoses and hexoses into bioethanol have been generated (reviewed in Vleet & Jeffries, Bettiga et al., Matsushika et al., 2009). *Zymomonas mobilis* was also genetically altered converting xylose into ethanol (reviewed in Girio et al., 2010, Balat, 2011).

Pentoses (xylose, the main sugar from hemicellulose) can be utilized from the yeast strains *Pichia stipitis*, *Pachysolen tannophilus* and *Candida shehatae*. The main disadvantage of these yeast strains is their low ethanol tolerance and ethanol yield. Bacteria like *Escherichia coli* and *Klebsiella oxytoca* take up hexoses and pentoses but lead to very low ethanol yields. Successful genetic modifications have been performed in these bacteria leading to higher ethanol yields (reviewed in Girio et al., 2010; Balat, 2011).

Enzymatic hydrolysis and fermentation can be carried out simultaneously (SSF). This process has several advantages: lower enzyme concentrations, higher sugar yields (no end product inhibition of cellulase), higher product yields, shorter process times and lower risk of contamination. The main disadvantage is the different optimal conditions for the hydrolysis and fermentation reactions (reviewed in Balat, 2011). Performing enzymatic hydrolysis and fermentation separately is known as SHF. Each step has to be carried out under optimal reaction conditions but the end product inhibition of the cellulase reduces the rate of hydrolysis and this type of process is costlier (reviewed in Balat, 2011).

3.4 Distillation

With conventional distillation at atmospheric pressure, the maximum achievable ethanol concentration is 90-95%, because in the system ethanol-water there is an azeotrope at 95.6% (w/w) ethanol, boiling at a temperature of 78.2°C. For the production of anhydrous ethanol

further dehydration of the concentrated ethanol is required. This can be achieved by employing azeotropic distillation, extractive distillation, liquid-liquid extraction, adsorption, membrane separation or molecular sieves (Hatti-Kaul, 2010; Huang et al., 2008).

Separation of ethanol from water is an energy-intensive process. The energy required for production of concentrated ethanol by distillation also depends very much on the feed concentration (Zacchi & Axelsson, 1989). The search for solutions for the reduction of the energy required is a field of intensive research. Membrane separation processes need much less energy for ethanol separation but are not in operation on an industrial scale. First results from a pilot plant using the Siftek™ membrane technology show a reduction of the energy required for dehydration of about 50% (Côté et al., 2010). Process and heat integration techniques also play an important role in energy saving in the bioethanol process (Alzate & Toro, 2006; Wingren et al., 2008). Maximum energy saving in the distillation of about 40% is possible by applying mechanical vapour recompression (Xiao-Ping et al., 2008). Solar distillation of ethanol is under investigation for distillation of bioethanol in smaller plants (Vorayos et al., 2006). The production of solid biofuel or biogas for thermal energy supply also reduces the net energy requirement of bioethanol production (Eriksson & Kjellström, 2010; Šantek et al., 2010).

3.5 Use of residues for energy supply

The stillage from distillation can be separated in a liquid-solid separation step into two fractions. The solid fraction is usually used for solid fuel production. The liquid fraction is either fed to an anaerobic digestion process, generating biogas with a methane concentration of about 60% (Prakash et al., 1998) or is used for solid fuel production together with the solid fraction after evaporation of most of the water. In this case the concentrated liquid fraction is mixed with the solid fraction before drying and pelletizing.

Biogas is used for heat generation or combined heat and power generation for the bioethanol process, whereas solid biofuels can also be sold on the market.

4. Our results for bioethanol production from steam explosion pretreated straw

4.1 Steam explosion pretreatment

4.1.1 Operation of steam explosion reactor

The bulk density of the straw in the steam explosion reactor depends very much on the condition of the straw and the feeding method. When filling the pilot reactor with chopped straw manually, a bulk density of about 60 kg m⁻³ was achieved. Loading baled straw would lead to a bulk density of approximately 150 kg m⁻³ (bulk density of straw bales according Jenkins (1989): 100 – 200 kg m⁻³). The bulk density of straw pellets is 500 kg m⁻³ and higher (Theerarattananoon et al., 2011). For reliable discharge of the treated straw from the reactor in the explosion step, addition of water to the dry straw is usually required. The thermal energy requirement of the steam explosion treatment is met by steam directly fed into the reactor. In small steam explosion units, steam is also optionally used for jacket heating of the reactor. In adiabatic operation, the thermal energy is required for heating up the biomass and the added water. The steam in the vapour phase of the reactor is lost through a vent during the sudden pressure discharge of the reactor. The steam required for heating up the biomass and the added water $m_{st,1}$ (in kg) can be calculated thus:

$$m_{st,1} = m_S \cdot \left(c_{p,S} \cdot \Delta T_S + \frac{m_W}{m_S} \cdot c_{p,W} \cdot \Delta T_W + \Delta h_R \right) \cdot \frac{1}{\Delta h_V} \quad (2)$$

The mass of straw m_S and the mass of the added water m_W are in kg. The specific heat capacity of straw $c_{p,S}$ and the specific heat capacity of water $c_{p,W}$ are in $\text{kJ kg}^{-1} \text{K}^{-1}$. The temperature difference between pretreatment temperature and feed temperature for straw ΔT_S and water ΔT_W are in K. The enthalpy of vaporization for water Δh_V at pretreatment temperature and the net reaction enthalpy Δh_R of the pretreatment process are in kJ kg^{-1} .

The venting loss of steam $m_{st,2}$ (in kg) can be calculated thus:

$$m_{st,2} = m_S \cdot \left[\frac{1}{\rho_{S,b} \cdot \eta_V} - \frac{1}{\rho_S} - \left(\frac{m_W}{m_S} + \frac{m_{st,1}}{m_S} \right) \cdot \frac{1}{\rho_W} \right] \cdot \rho_{st} \quad (3)$$

The bulk density of the straw in the reactor $\rho_{S,b}$ as well as the density of straw ρ_S , the density of water ρ_W and the density of steam ρ_{st} , all at operation temperature and pressure, are in kg m^{-3} . The factor for the volumetric use of reactor volume is η_V .

An increase in steam consumption of 10% can be estimated because of non-adiabatic operation of the steam explosion system and steam leakages (Sassner et al., 2008). The total steam consumption is therefore calculated thus:

$$m_{st} = 1.1 \cdot (m_{st,1} + m_{st,2}) \quad (4)$$

A reduction in the cost of pretreatment can be achieved by minimisation of the specific steam demand. Ahn et al. (2009) determined the specific heat capacity of wheat straw with a water content of 4.3 g water/g dry sample to be $1.63 \pm 0.07 \text{ kJ kg}^{-1} \text{K}^{-1}$. The specific heat capacities of other types of straw were in the same range. The specific heat capacity of water is about 2.5 times higher than the specific heat capacity of straw. Therefore, the total water content of the input material is a main influencing factor on the thermal energy consumption of steam explosion pretreatment. Minimizing the rate of water addition to the straw is a way to reduce the steam consumption. Preheating of the added water using waste heat e.g. from the condenser of the distillation or increasing the bulk density of the straw in the reactor are also ways to reduce the steam consumption (Fig. 3).

A reduction in steam temperature would reduce the steam demand too, but at the same time reduce the effect of steam explosion treatment.

For the discharge of the treated straw from the reactor in the explosion step a certain fraction of the reactor volume has to remain filled with uncondensed steam. The remaining steam-filled fraction of the reactor volume under various operation conditions is shown in Fig. 4.

The steam explosion pretreatment of straw pellets is restricted by the pore volume available for the addition of water and condensing steam. From this point of view, a type of compacted straw with a density between 150 kg m^{-3} and 500 kg m^{-3} would be preferable.

4.1.2 Steam explosion experiments

The pretreatment of the straw was carried out in a steam explosion pilot unit using a reactor with a reaction volume of $0,015 \text{ m}^3$. Explosion was carried out into a cyclonic separator to separate the treated straw from the vapour phase. The vapour was then condensed in a regenerative cooler. The maximum steam temperature of the steam generator was 200°C

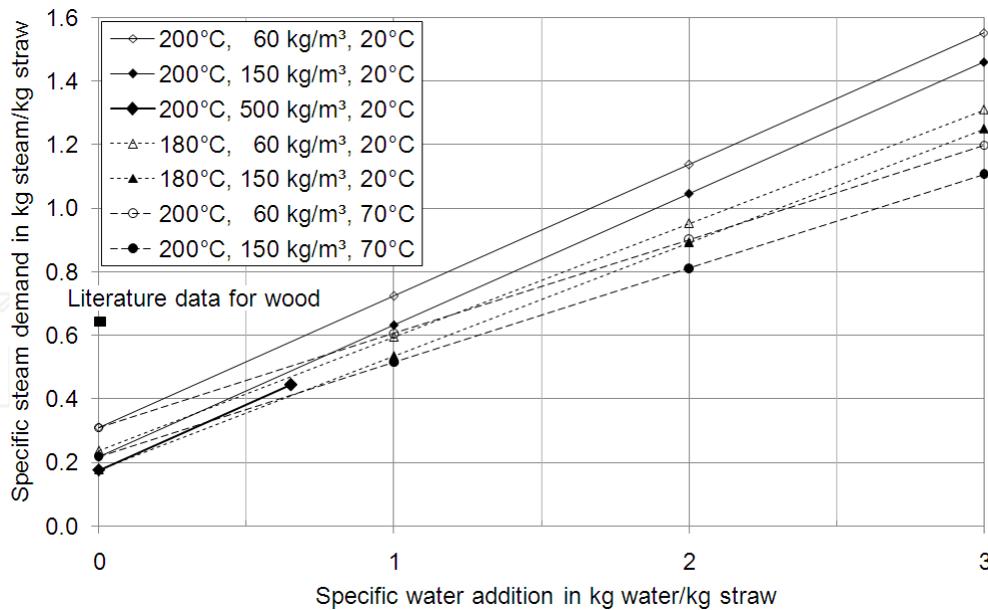


Fig. 3. Specific steam demand in steam explosion pretreatment of straw; general operating data: assumed volumetric use of reactor volume: 0.95; density of straw: 1290 kg m⁻³ (Shaw & Tabil, 2005); net reaction enthalpy neglected; individual operating data (as shown in the legend): temperature of treatment, bulk density of straw, temperature of added water; literature data: thermal energy demand without indication of water content (Zhu & Pan, 2010).

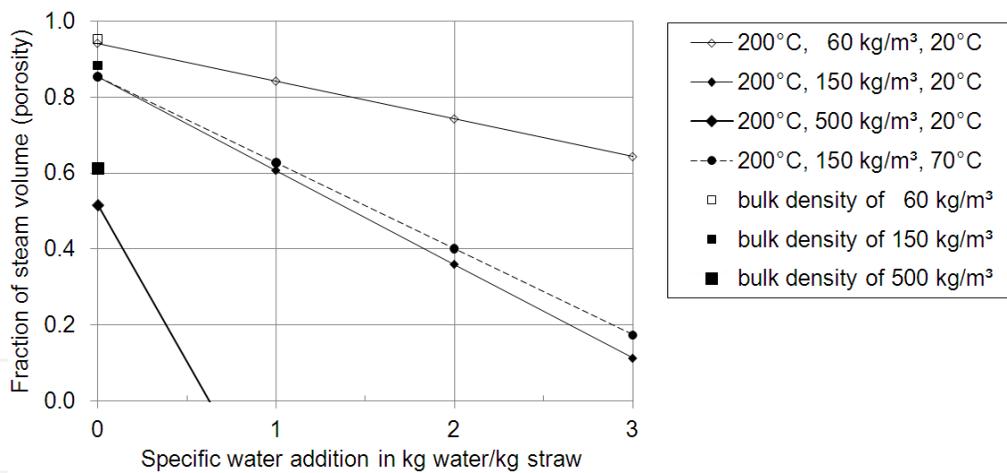


Fig. 4. Remaining steam-filled fraction of the reactor volume under various operating conditions; individual operating data (as shown in the legend): temperature of treatment, bulk density of straw, temperature of added water

(equivalent to a steam vapour pressure of 1.55 MPa). The operation temperature in the reactor is reached via a temperature ramp. In most experiments the mass of added water was 1.0 kg per kg of straw. The operation temperature was generally 200°C and the duration of the treatment was usually 10 minutes. This results in a severity factor of 9500 ($\log(R_0) = 3.98$).

The bulk density of the straw in the reactor was 60 kg m⁻³ for chopped straw. When straw pellets (mixture of Triticale (*Triticosecale Wittmack*) and wheat straw) were pretreated, the

bulk density increased to 520 kg m^{-3} . However, in these cases the volumetric use of reactor volume had to be reduced. Also the ratio of added water was lower.

The steam consumption in the pilot tests was more than two times the calculated value due to only partial thermal insulation of the reactor. In the case of a cold start of the system, steam consumption was even higher.

Figs. 5a and 5b show an example of wheat straw before and after pretreatment. The scanning electron microscope (SEM) images show wheat straw with intact bundles of fibres before pretreatment (Fig. 5a) and the same material after pretreatment (Fig. 5b), where the morphological structure has been broken down. This material is now accessible to the cellulytic enzyme complex.

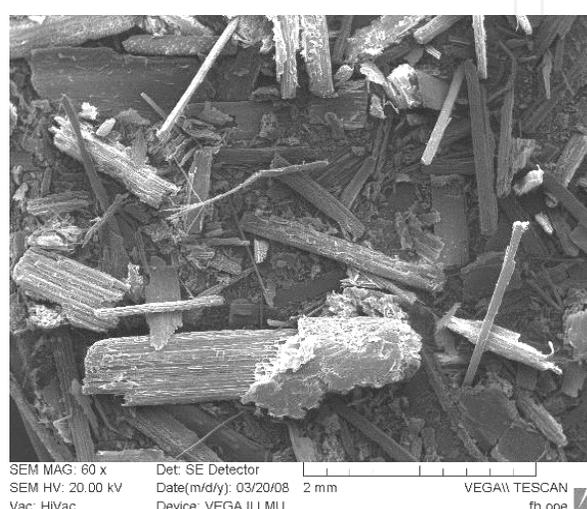


Fig. 5a. Wheat straw untreated (SEM)

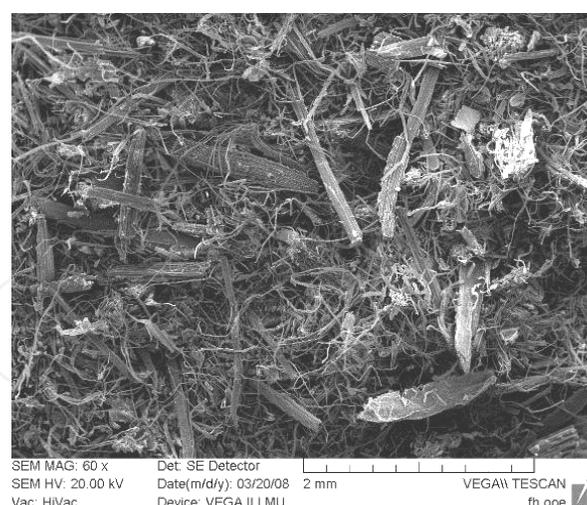


Fig. 5b. Wheat straw treated (SEM)

4.1.3 Recycling of low ethanol concentration solutions into the steam explosion reactor

The outcome of an economic study shows that the most important factor for economic bioethanol production is maximum ethanol output (von Sivers & Zacchi, 1996). A possibility to increase the ethanol output would be the recycling of effluents with low ethanol

concentration, e.g. the stillage from the distillation, which contains about 1% ethanol (Cortella & Da Porto, 2003) or low concentration effluents from membrane separation steps via the steam explosion reactor. In this case, the added water would be replaced by the effluent to be recycled. During the steam treatment, vapour-liquid equilibrium of the ethanol-water system will be reached. Due to the fact that ethanol is more volatile than water, the concentration of ethanol in the vapour phase will be much higher than in the liquid phase. The vapour-liquid equilibrium of the ethanol-water system at 1.5 MPa is shown in Fig. 6.

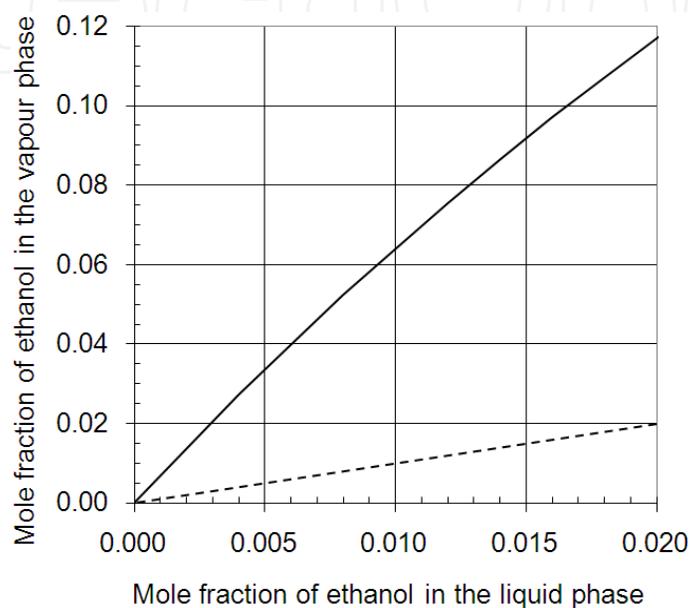


Fig. 6. Vapour-liquid equilibrium of the ethanol-water system at 1.5 MPa, calculated with the Wilson equation (Gmehling & Brehm, 1996)

When the reactor is vented, the exploded biomass is separated from the vapour phase in a cyclonic separator. In the separator secondary vapour is also produced by evaporation cooling of the wet biomass. The vapour phase has to be condensed by cooling at the separator outlet to recapture the ethanol. The collected condensate can be added to the feed of the distillation column.

In a first series of experiments on the recycling of ethanol-containing effluent, the added water in the feed to the steam explosion reactor was replaced by a solution containing 10% (w/w) ethanol. Analyses of the pretreated wet straw are shown in Table 2. The samples were taken from the treated straw heap in the separator immediately after the explosion step and transferred into a gastight bottle. With the exception of ethanol no significant differences were found when 10% ethanol (w/w) solution was used. The ethanol content of 31.6 g/kg feed straw (d.b.) in the treated straw from the experiment with the addition of 10% ethanol solution (w/w) is equivalent to 31.6% of the added ethanol; the remaining 68.4% is expected to be in the condensate. It was not possible to verify this due to limitations in the drainage of such small amounts of condensate from the installed regenerative cooler.

Treated straw samples taken from the separator about five minutes after the explosion step showed a significantly lower ethanol content. The average ethanol content in these samples was 13.5 g/kg of feed straw (d.b.), whereas the concentrations of the other components were

Added water 1 kg/kg wheat straw	Ethanol	Formic acid	Acetic acid	HMF	Furfural
Water	3.7	3.8	16.8	0.3	1.9
10% ethanol (w/w)	31.6	6.1	20.1	0.2	1.0

Table 2. Analyses of steam-exploded wheat straw (pretreatment conditions: 200°C, 10 min); all values in g/kg feed straw (d.b.); averages of two pretreatment experiments; wet straw samples were leached with deionised water, analysis of the filtrate by HPLC

very much the same. This can be explained by the evaporation of ethanol during the cooling of the treated straw. For example, the recycling of a 1% ethanol (w/w) solution would result in a condensate with about 5% ethanol (w/w) considering also the dilution of the liquid phase in the reactor by condensation of steam.

However, recycling of low ethanol concentration effluents could be limited by inhibitors contained in the effluent. Further tests with real effluents are therefore required.

4.2 Hydrolysis and fermentation

4.2.1 Description of the experiments

Bioethanol production from wheat straw was investigated. Several improvements, particularly one washing step and the recirculation strategy, were made. The washed wheat straw was named inhibitor-controlled wheat straw. These improvements increase both the sugar concentration and the bioethanol yield by up to 7% (vol). Also, the lignocellulose-containing substrate corn stover was tested for its potential in bioethanol production. Furthermore, recirculation of bioethanol was performed to ultimately raise the end concentration of bioethanol. Therefore, ethanol was added during the pretreatment process and a possible effect on the hydrolysis and fermentation steps was examined.

The enzyme mixture Accellerase TM1000 from Genencor® was used with enzyme activities of 775 IU cellulase (CMC)/g solids and 138 IU beta-glucosidase/g solids. Suspensions with various dry substances (10-20%) were produced with the pretreated substrate in citrate buffer (50 mM, pH 5.0) and incubated at 50°C for 96 hours in a shaking incubator (100 rpm). The hydrolysis of pretreated substrate was repeated three times in a recirculation process. Sample analysis was performed with HPLC. Diverse salts were added to the straw hydrolysate for fermentation. A wild-type strain of *Saccharomyces cerevisiae* was used exclusively for all experiments. The fermentation process was conducted at 30°C in a shaking incubator for one week (110 rpm).

4.2.2 Results

The glucose concentration obtained after hydrolysis from wheat straw pretreated with different levels/degrees of severity (conditions ranging from 160°C, 10 minutes to 200°C, 20 minutes) is demonstrated in Fig. 7. The pretreatment at 200°C over 20 minutes (severity factor 18000; $\log(R_0)=4.26$) achieved the highest sugar concentration, converting about 100% cellulose during the hydrolysis. Recirculation strategies with wheat straw were developed, where the sugar solution of a first hydrolysis reaction was recycled twice to fresh straw and the subsequent hydrolysis reaction. The glucose concentration was further increased by a recirculation process to fresh washed solids and subsequent hydrolysis from 30 g/l to 143 g/l

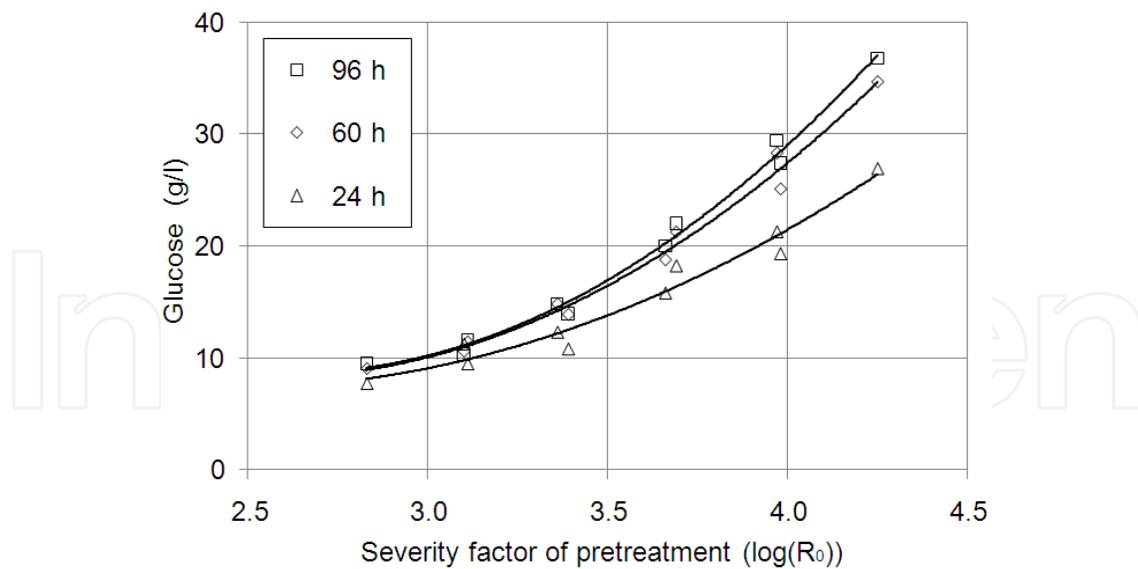


Fig. 7. Glucose concentration after hydrolysis of pretreated wheat straw

(20% solids, third hydrolysis). After fermentation with *Saccharomyces cerevisiae*, an ethanol concentration of 7.5%(vol) was obtained. In Fig. 8, the final glucose concentrations after recirculation processes with inhibitor-controlled wheat straw as well as bioethanol yields after fermentation are shown.

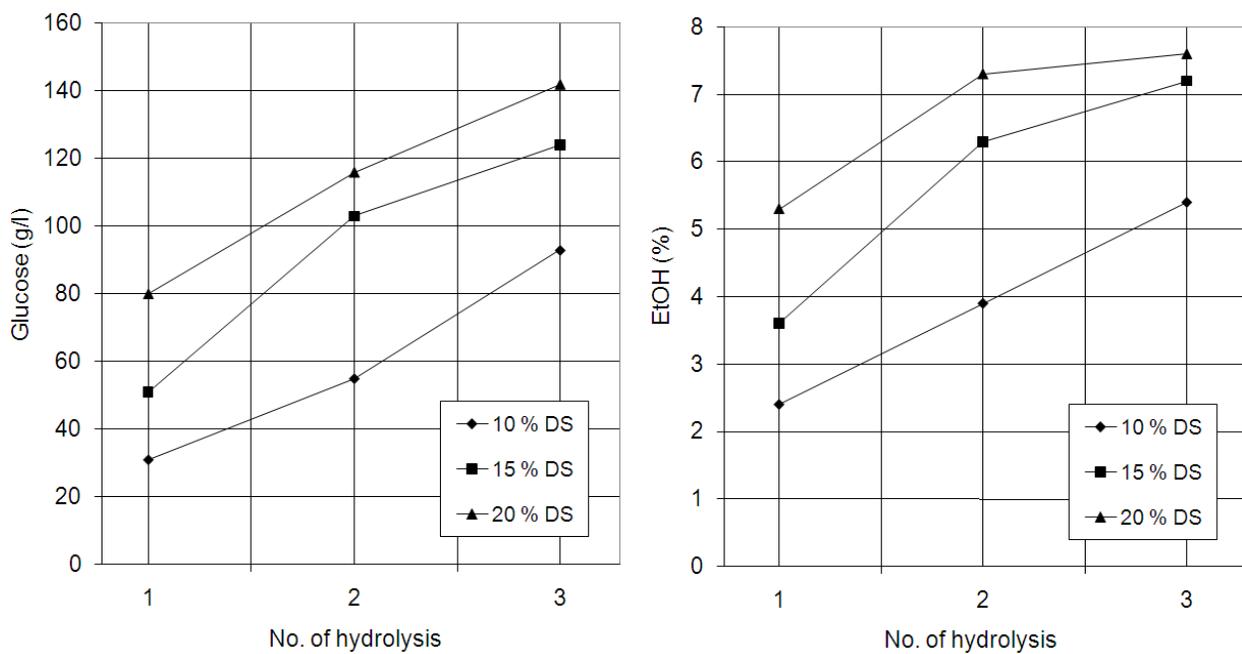


Fig. 8. Produced glucose concentration and bioethanol yields after fermentation of inhibitor controlled wheat straw

Corn stover was pretreated at 190°C for 10 minutes. Initially, 10% of the dry substance corn stover was hydrolyzed and fermented. Here, the sugar concentration was 32 g/l glucose and 10 g/l xylose yielding 1.9% bioethanol (Table 3). The dry substance was increased to 15% and 20%, yielding considerably higher sugar and bioethanol concentrations (Table 3).

	10 % dry substance	15 % dry substance	20 % dry substance
Glucose (g/l)	32	47	58
Xylose (g/l)	10	16	20
EtOH (%(vol))	1.9	2.8	3.9

Table 3. Sugar concentration and ethanol content from corn stover (10, 15 and 20 % solids)

Wheat straw was moistened with water before steam explosion pretreatment. Ethanol was added during pretreatment (10 minutes at 200°C) to test for a possible effect on the hydrolysis and fermentation step. The wet straw was hydrolyzed with the enzymes and fermented with yeast. Additional ethanol during the pretreatment process did not influence the sugar and bioethanol content (Table 4).

	Standard pretreatment	Pretreatment with 10% ethanol (w/w)
Glucose (g/l)	41	40
Xylose (g/l)	20	19
EtOH (%(vol))	2.1	2.2

Table 4. Sugar concentration and ethanol yields after fermentation of standard pretreatment and pretreatment with ethanol (from 10 % dry substance)

Alternatively, pellets from mixed straw were used to increase the dry substance already during the pretreatment step. It was possible to increase the glucose concentration from wet straw pellets to 60 g/l resulting in 2.5%(vol) bioethanol (from 10 % dry substance).

5. Other concepts for the use of lignocellulosic feedstocks

Diverse concepts for the use of lignocellulose-containing plants for bioethanol production are available. In the simplest concept, only the glucose is fermented to bioethanol, with the by-products xylose solution and lignin pellets. The xylose sugars can be used as barrier films, hydrogels, paper additives (Söderqvist et al., 2001; Lima et al., 2003; Grönholm et al., 2004) or in xylitol production (reviewed in Chen et al., 2010). At the moment, the utilization of lignin is unsatisfactory; therefore, the lignin pellets are used as solid biofuel.

The economy of bioethanol production from lignocellulose-containing materials can be improved in a cost-effective concept by simultaneous fermentation of both sugars (glucose and xylose) to bioethanol by diverse microorganisms. In the last twenty years, diverse microorganisms were genetically modified to ferment both glucose and xylose, with good results (reviewed in Hahn-Hägerdal et al., 2007; Matsushika et al., 2009; Jojima et al., Kim et al., Mussatto et al., Weber et al, Young et al., 2010). Furthermore, diverse adaptation programs, mutagenesis and breeding were performed to produce yeasts and other microorganisms with improved xylose fermentation (reviewed in Hahn-Hägerdal et al., 2007; Matsushika et al., 2009; Mussatto et al., 2010). However, in several countries production with GMO is only possible under strict standards and acceptance of GMO in these countries is poor.

In a biorefinery concept, co-production of biofuels, bioenergy and marketable chemicals from renewable biomass sources take place simultaneously. Diverse biorefinery concepts for wheat straw were developed such as: bioethanol from glucose, biohydrogen from xylose and the residual effluents from bioethanol and biohydrogen processes being used for biogas

production (Kaparaju et al., 2009). The biorefinery concept including higher-value chemical by-products and autonomous power supplies will enhance economic competitiveness of second generation plants and, therefore, will make this type of plant economical in the near future.

6. Outlook

Research on bioethanol production from lignocellulose-containing substrates has made great progress over the last decades. As shown by other authors and our own results, the theoretical yield of bioethanol from cellulose (690 l/t cellulose, 283 l/t straw) is almost achievable. The yield of bioethanol from hemicelluloses still has to be increased. Compared to bioethanol production of the first generation, cost-effectiveness also has to be improved. No commercial bioethanol plant using lignocellulose-containing residues as feedstock is in operation in 2011. However, diverse pilot plants are in operation and the first demonstration plants have been completed and running successfully.

The production of biofuels such as bioethanol is often criticized because of the negative impact of the feedstock on biodiversity. The competition of the raw materials for use either as biofuel or for food production is also a major obstacle to increasing bioethanol production capacity. Therefore, lignocellulose-containing residues offer a possibility to satisfy part of the increasing demand for fuel by means of biofuel.

Diverse scenarios are possible - only using first generation fuel, resulting in dramatic increases in world prices for feedstock crops. The stimulation of the second generation results in reduced pressure on world prices for feedstock crop. It is the authors' opinion that the higher demand for biofuels will necessarily lead to the use of lignocelluloses as feedstock to produce biofuels. In order to replace fossil fuels to a larger extent, not only agricultural residue must be used as feedstock. Agriculture has to be geared towards food as well as towards energy production. This will only be possible in the context of a coordinated international effort.

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Recent studies have shown strong evidence of human activity impact on the climate of the planet. Higher temperatures and intensification of extreme weather events such as hurricanes are among the consequences. This scenario opens up several possibilities for what is now called "green" or low carbon economy. We are talking about creating new businesses and industries geared to develop products and services with low consumption of natural resources and reduced greenhouse gases emission. Within this category of business, biofuels is a highlight and the central theme of this book. The first section presents some research results for first generation ethanol production from starch and sugar raw materials. Chapters in the second section present results on some efforts around the world to develop an efficient technology for producing second-generation ethanol from different types of lignocellulosic materials. While these production technologies are being developed, different uses for ethanol could also be studied. The chapter in the third section points to the use of hydrogen in fuel cells, where this hydrogen could be produced from ethanol.

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