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Review

Consolidated briefing of biochemical ethanol production from lignocellulosic biomass



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ABSTRACT

Bioethanol production is one pathway for crude oil reduction and environmental compliance. Bioethanol can be used as fuel with significant characteristics like high octane number, low cetane number and high heat of vaporization. Its main drawbacks are the corrosiveness, low flame luminosity, lower vapor pressure, miscibility with water, and toxicity to ecosystems. One crucial problem with bioethanol fuel is the availability of raw materials. The supply of feedstocks for bioethanol production can vary season to season and depends on geographic locations. Lignocellulosic biomass, such as forest-based woody materials, agricultural residues and municipal waste, is prominent feedstock for bioethanol cause of its high availability and low cost, even though the commercial production has still not been established. In addition, the supply and the attentive use of microbes render the bioethanol production process highly peculiar. Many conversion technologies and techniques for biomass-based ethanol production are under development and expected to be demonstrated. In this work a technological analysis of the biochemical method that can be used to produce bioethanol is carried out and a review of current trends and issues is conducted.

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1. Introduction

Nowadays, the depletion of fossil fuels and the environmental compliance regarding the greenhouse gases has attracted the interest in non-conventional fuel from bioresources [1,2,3,4,5]. For the past few years, the biomass-based ethanol has caught the attention of global industry. According to the Renewable Fuels Association [6], United States (U.S.) and Brazil are the pioneer countries in global bioethanol production with a percentage of approximately 90%. The involvement of several countries has already begun in new pathway development for biogasoline from biomass [7]. Wheals et al. [8] refer that in North America, bioethanol is primarily provided from starch sources (corn starch) while in South America is mostly extracted from sugars (sugarcane juice) and molasses [8,9].

On the other side, the European countries focus on biodiesel and biogasoline production which exceeds 50% of the global production cause of engines development and feedstocks supply costs [10,11,12,13,14]. Despite the fact that most of the countries in the world, China, India and Japan continue to invest in technologies from agricultural residues and appear as future producers [15,16,17,18,19]. Although bioethanol based on corn and sugar is an encouraging replacement to gasoline in transportation sector, the amount produced is insufficient with respect to the annual consuming amount worldwide. There is no black-and-white answer to the question of what constitutes the most suitable feedstock for the bio-based economy. Generally, sugars, oils and proteins can be used in many applications. The concern for the food security has globally increased the interest of researchers to focus on alternative feedstocks [20,21,22].

The nova Institute of Germany claims that lignocellulosic resources are favorable in terms of environmental sustainability and food security as they do not antagonize food crops and animal feed as renewable substrate for bioethanol production [23,24]. Moreover, the availability of lignocellulosic materials in industrial-scale basis is increased cause of the exploitation of industrial wastes and agricultural residues [25,26,27]. Lignocellulosic wastes are a promising feedstock considering its availability and low cost. The utilization of corn stover, rice, wheat and sugarcane bagasse is gaining significant importance worldwide [28,29,30,31].

Nonetheless, the recalcitrant structure of lignocellulose requires high capital cost processing. Therefore, these technologies are not economically achievable [32,33]. During the decomposition of lignocellulosic material, it must be considered that D-xylose is the second important sugar which has to be broken down as is found in high portion in the feedstock [34]. The conversion of biomass to ethanol has 4 main steps: pretreatment, hydrolysis, fermentation and distillation. During the last decades genetic engineering and enzymatic processing have provided significant improvements in all of the four

steps of ethanol production and making capable to ferment different sugars concurrently [35,36,37]. Even though there is a wide range of bacteria, they cannot all be adapted to saccharification process conditions and several bacteria produce low ethanol yields. For this reason, subtle improvements are sometimes required [38].

The microbial contamination is a crucial problem in bioethanol production process. Bacterial infections occur during bioethanol fermentation which consume nutrients necessary for the fermentation itself and it is possible to produce toxic products too. Both of these situations can negatively affect the bioethanol yield [39,40]. The formation of inhibitory by-products during the biofuel production must be taken into account. Pienkos and Zhang [41] refer that pretreatment and conditioning processes release toxic compounds into the hydrolysate which inhibit the bacteria growth and decrease the ethanol yield. The mechanism/methodology applied for biomass pretreatment influences the relevant toxicity rate [41,42]. This review examines recent technologies and trends that are used in lignocellulosic bioethanol production. It also provides a summary of the current problems and barriers concerning the different pathways and analyses potential issues and trends of biotechnological conversion performance.

2. Current status

In 2014, the global production of bioethanol reached 24.5 billion gal, up from 23.4 billion gal in 2013 which shows the international bioethanol market is at a very dynamic stage [43]. More than half (about 60%) of global bioethanol production is based on sugar cane conversion and the rest (40%) comes from other crops [44]. United States and Brazil are the global producers as they produce more than 70% of the global bioethanol production (Table 1).

Even the main source for bioethanol production is considered to be the corn from US and sugar cane from Brazil, any country with agro-industrial economy can be involved in bioethanol fermentation. This is feasible cause of the current progress in bioconversion of non-food crops in large scale production [46] (Fig. 1).

In Europe the biochemical pathways show a crucial potential for research development in conjunction with the progress in biorefineries. It is important to clarify that several technologies are under development such as the SSCF technology which gains space in biotechnology research area. Research requires effort to solve problems concerning process improvement and confront challenges regarding the overall efficiency of a biorefinery [47]. It was also reported in 2009 that notwithstanding the global economic-constraints, bioethanol production continues to increase and to support significantly to the global development [48].

3. Lignocellulosic sources and composition

3.1. Raw materials and characteristics

Sustainable biofuel production in Europe can be met with lignocellulosic biomass usage [49]. There is a wide variety of raw materials that are discerned by their make-up, structure and process-ability. In North America most cultivated land comprises (Table 2).

The land cultivation is mainly based on forestland (around 35%), grazed land (27%) as well as crop lands (19%) which constitute approximately 9.0 million km² [51,52,53]. Forest sources include

Table 1

Top five bioethanol producers (billion gallons) [45]

Country	2008	2010	2012	2014
US	9.31	13.30	13.22	14.34
Brazil	6.47	5.57	5.57	6.19
Europe	0.73	1.21	1.14	1.45
China	0.50	0.54	0.56	0.64
Canada	0.24	0.36	0.45	0.51

Table 2
Total energy potential from different feedstocks (KTOE). [50].

Different biomass sources availability		2004–2010		2020	
		Netherlands	EU-27	Netherlands	EU-27
Biomass from agricultural land and by-products	Woody residues of fruit trees, nuts and berry plantations, olives, citrus and vineyards	16	9362	13	10105
	Straw	39	22936	195	49285
	Manure	3916	56817	4574	46724
	Grassland cutting	38	1097	40	1143
Biomass from forestry	Primary forestry residues	22	20285	71.5	41186
	Round wood	148.4	56735	137.8	56115
	Sawmill by-products (excluding saw-dust)	21	9072	31	10093
	Saw-dust	10	4496	–	4984
	Other industrial wood residue	–	4637	–	5461

woody biomass consisting mainly of residues or by-products from manufacturing processes, biomass plantations, agricultural residues (trees and branches) [54,55]. Cellulose materials can also be collected from municipal and industrial wastes which include food residues and pulping sludge [56,57].

3.1.1. Forest woody sources

According to the taxonomical division of woody materials, there are two species: softwoods and hardwoods. Softwoods are gymnosperms and originate from coniferous trees including pines, spruces and firs. Hardwoods are angiosperms and originate from deciduous trees including oaks, maples and birches [59].

Fig. 2 shows the type of forest biomass that can be supplied globally. Forest biomass represents a valuable feedstock cause of its composition (more lignin and less ash content than agricultural residues). Forestry wastes like wood chips, branches, and sawdusts have also been used as bioethanol feedstocks [60].

3.1.2. Agricultural and municipal solid wastes (MSW)

Agricultural residues are a widespread lignocellulosic biomass source available in many countries. The available amount of agro-residues is estimated to be 1010 Mt. globally, which corresponds to an energy value of 47 EJ [61]. Crops residues consist of an extensive variety of types. They are mostly comprised of agricultural wastes such as corn stover, corn stalks, rice and wheat straws as well as sugarcane bagasse [62]. Crop residues contain more hemicellulosic material than woody biomass (approximately 25–35%) [63]. Besides from environmental point of view, agricultural residues help to avoid non-sustainable cutting trees decreasing the phenomenon of deforestation [30].

Moreover, municipal and industrial solid wastes are also a prospective pathway for biofuels production [64]. Li [65] studied that integrated bioconversion of cellulose-enriched municipal solid waste offers promising alternatives but the processing cost is still high. However, their utilization associated with the disposal of garbage, organic waste and household by-products has to be considered in case of environmental effects [66]. Even though intensifying crop management is applied to improve yields, the high cost of biomass still remains a crucial constraint [67]. According to this study the available amount of biomass for 30% petroleum-based gasoline displacement will almost meet the target of 2030 [68].

3.1.3. Marine algae

Since the 1970s special interest has existed in marine algae as third generation biofuel feedstock but the research was discontinued when funding stopped. Particularly the research has focused on examination of its production efficiency per acre including water consumption and estimation of by-products during ethanol production [62]. Even though exists progress in algae development commercial applications are still limited during the 20th century. Currently, algae conversion is regaining interest as future biofuel feedstock in order to replace energy crops and cover any limitations in supply.

Marine algae are a suitable raw material for several chemical processes especially due to biorefineries expansion that aims at the production of different substances such as biofuels (i.e. bioethanol, biodiesel, biogasoline etc.) and other value-added chemicals [69]. Rodolfi et al. [70] state that algae feedstock can provide 60 times more alcohol than soybeans per acre of land. According to the study of Ferrel and Sarisky-Reed [71] algae can provide ten-fold the amount of ethanol than corn per growing area. Harel [72] refers that algae are

Table 3
Pros-and-cons of potential microorganisms for bioethanol fermentation [140].

Species	Pros	Cons
<i>Saccharomyces cerevisiae</i>	<ul style="list-style-type: none"> –Alcohol yield up to 90% –High tolerance to chemical inhibitors and to ethanol (10% v/v) –Naturally adapted to ethanol fermentation 	<ul style="list-style-type: none"> –Not able to ferment xylose and arabinose sugars –Not able to survive at high temperature of hydrolysis
<i>Z. mobilis</i>	<ul style="list-style-type: none"> –Complaisance to genetic modifications –Bioethanol yield up to 97% –High ethanol tolerance (up to 14% v/v) –Does not require additional oxygen 	<ul style="list-style-type: none"> –Not able to ferment xylose sugars –Low tolerance to inhibitors
<i>Escherichia coli</i>	<ul style="list-style-type: none"> –Complaisance to genetic modification –Ability to use both pentose and hexose sugars –Amenability for genetic modifications 	<ul style="list-style-type: none"> –Low tolerance to inhibitors and ethanol –Narrow pH and temperature growth range –Production of organic acids
Thermophilic species:	<ul style="list-style-type: none"> –Resistance to high temperature of 70°C. –Suitable for consolidated bioprocessing 	<ul style="list-style-type: none"> –Low tolerance to ethanol
> <i>Thermoanaerobacter</i>	<ul style="list-style-type: none"> –Ferment a variety of sugars 	
> <i>Clostridium</i>	<ul style="list-style-type: none"> –Amenability to genetic modification 	

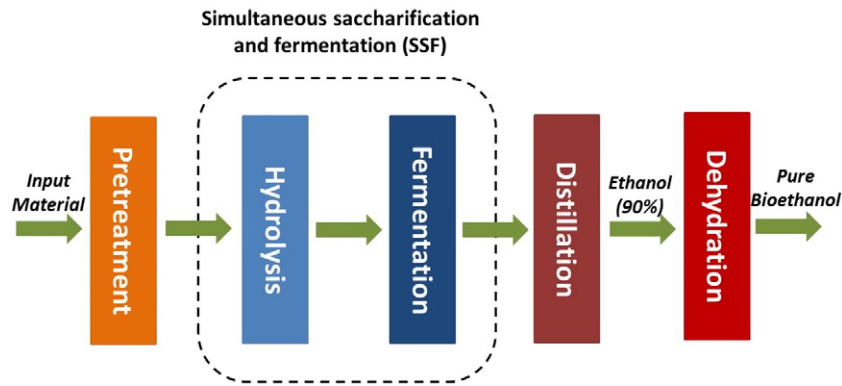


Fig. 1. Stages of bioethanol fuel production.

consuming high amounts of CO₂ during their growth which make them very attractive to use as an environmental friendly feedstock.

3.2. Lignocellulosic molecular components

The main components of lignocellulosic biomass are cellulose (30–35%), hemicellulose (25–30%) and lignin (10–20%) In addition, lignocellulose contains protein, lipids, water and other items [73,74,75,76]. Cellulosic and hemicellulosic polymers constitute approximately 70% of the entire biomass and are connected to the lignin component through a variety of covalent bonds that give the lignocellulosic biomass significant robustness and resistance to (bio-)chemical or physical treatment [77,78].

3.2.1. Hemicellulose

Hemicellulose has a vague and changeable structure of heteropolymers including hexoses (glucose, galactose, mannose), pentoses (xylose, arabinose) as well as sugar/uronic acids (glucuronic, galacturonic, methylgalacturonic) [79]. The hemicellulosic chain consists of xylose (90%) and arabinose (10%). Xylan is the primary component of hemicellulose and its composition varies in each feedstock. For this reason, hemicellulose stands in need of wide variety of enzymes to be completely hydrolyzed into free monomers [80,81,82].

3.2.2. Cellulose

Cellulose is a linear polymer which contains several thousand of 1,4-b-glucosidic bonds connecting thousands of glucose units. The structure is crystalline because of the hydrogen bridges between the polymers. This large amount of hydrogen linkages provides toughness and compactness to the cellulose molecule. Deguchi et al. [83] refer that for the conversion of cellulosic crystalline to an amorphous structure, a temperature of 320°C and a pressure of 25 MPa is required. Cellulose is the richest organic polymer on earth and make up 30% of plant biomass. However, cotton consists of almost 100% cellulose [84].

3.2.3. Lignin

Lignin is a complex polymer coupled via covalent bonds to xylans rendering massiveness and stability to the plant cell wall. It contains three main monomers, coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol [75]. Lignin is a copious natural polymer and a dominant constituent of wood (30–60% for softwoods and 30–55% for hardwoods), while agricultural residues and grasses contain 3–15% and 10–30% respectively [63]. Contrarily, crop residues like corn stover, rice and wheat straws contain particularly hemicellulose. Heretofore, lignin effects on hydrolysis have partially been investigated, even though in recent studies it is reported that lignin characteristics, such as structure and composition, can positively contribute to the whole hydrolysis

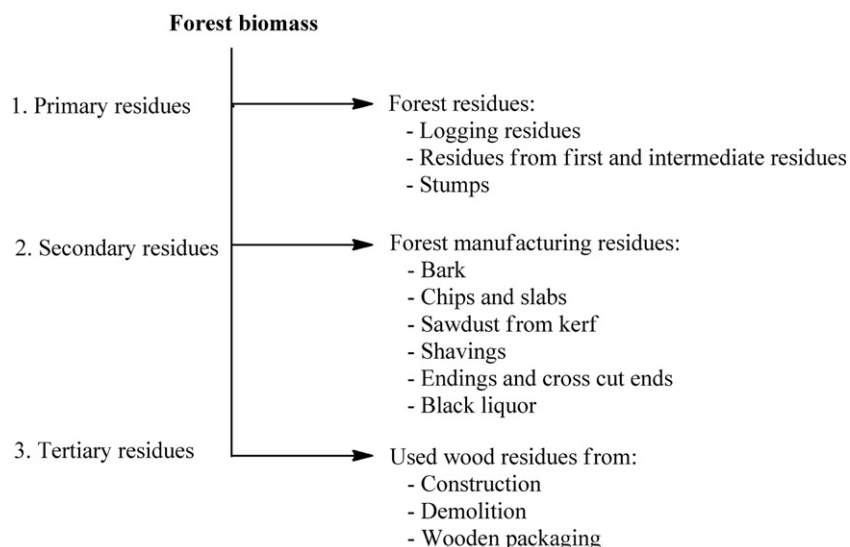


Fig. 2. Different types of forest biomass. Adopted from the source [58].

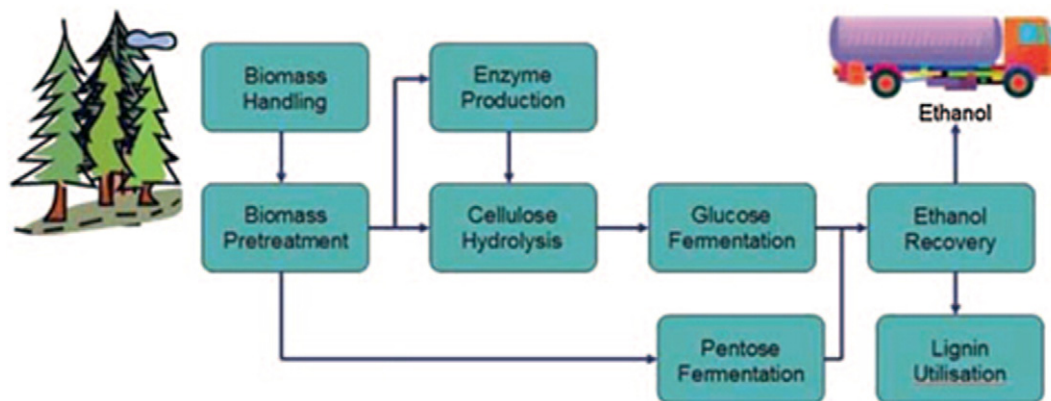


Fig. 3. Schematic of a biochemical cellulosic ethanol production process. Adopted from the source [95].

process [85]. Chen et al. [86] pointed out that lignin modification via genetic engineering techniques could increase the bioethanol yield and furthermore to be a potential source to give biorefineries financial solvency [86].

4. Processing routes to bioethanol

There are two different approaches (i.e. biochemical and thermochemical conversion) for bioethanol production from biomass [87]. Both pathways conclude into fragments of lignin, hemicellulose and cellulose via degradation of lignocellulose. Polysaccharides are hydrolyzed into sugars and subsequently are converted into bioethanol [88,89]. However, these conversion technologies are not similar techniques. Mu et al. [90] state that the thermochemical route includes feedstock gasification at 800°C with a catalytic reaction to ensue. This technology requires high level of heat and results into a synthesis gas (syngas) such as CO, H₂ and CO₂. Syngas can be chemically converted into a mixture of alcohols at 300°C using MoS₂ as the catalyst. Ethanol is separated from the mixture via distillation [91]. Alternatively, syngas can also be further processed into ethanol using the microorganism *Clostridium ljungdahlii*, *Saccharomyces cerevisiae* or *Zymomonas mobilis* [92,93,94].

In contrast to the thermochemical pathway towards syngas, the biochemical route includes mild physical and/or thermochemical pre-treatment, and biological pretreatment using hydrolytic enzymes to degrade cellulose and hemicellulose. The physical and/or thermochemical pretreatment is mainly used to overwhelm contumacious substances and boost cellulose availability/accessibility

to cellulases and hemicellulases in the biological pretreatment to produce the monomeric sugars. [96,98] (Fig. 3).

The upstream process includes hydrolysis of cellulose and breakdown of hemicellulose into soluble sugars. Afterwards the sugars are converted into bioethanol via fermentation and pure ethanol is produced via distillation [88,97]. Contemporaneously, the recalcitrant by-product, lignin, can be combusted and converted into power and heat [89]. In general, biochemical conversion consists of four unit operations i.e. pretreatment, hydrolysis, fermentation and distillation [99,100]. Nowadays, the biochemical approach is the most commonly used process [101].

4.1. Pretreatment

Hydrolysis and downstream processing can be optimized by effective pretreatment. The basic treatment methods include physical and thermochemical processes which disrupt the recalcitrant materials and enable the cellulose to undergo hydrolysis with higher efficiency and lower energy consumption [102]. The pretreatment process required for each feedstock was chosen according to its characteristics. Zhu and Pan [103] reported that agricultural biomass treatment differs from woody biomass because of its physical properties and chemical composition. Unlike agricultural biomass, woody biomass requires high content of energy to reach size reduction for further enzymatic saccharification.

Toxic compounds have also to be considered for evaluating the pretreatment cost. Different substances may act as inhibitors of microorganisms that are used in the ethanol fermentation. These inhibitors include phenolic compounds, furans (furfurals and 5-HMF),

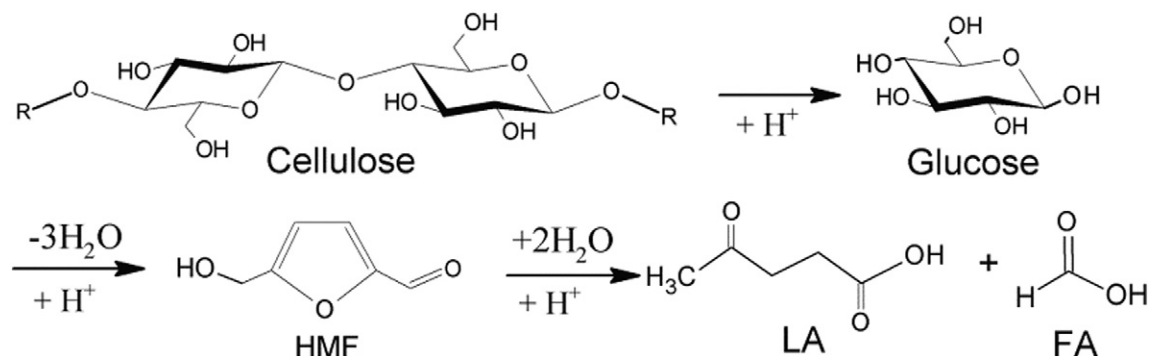


Fig. 4. Mechanism of acid-catalyzed cellulose hydrolysis to glucose. HMF = hydroxymethylfurfural, LA = levulinic acid, FA = formic acid [113,114]

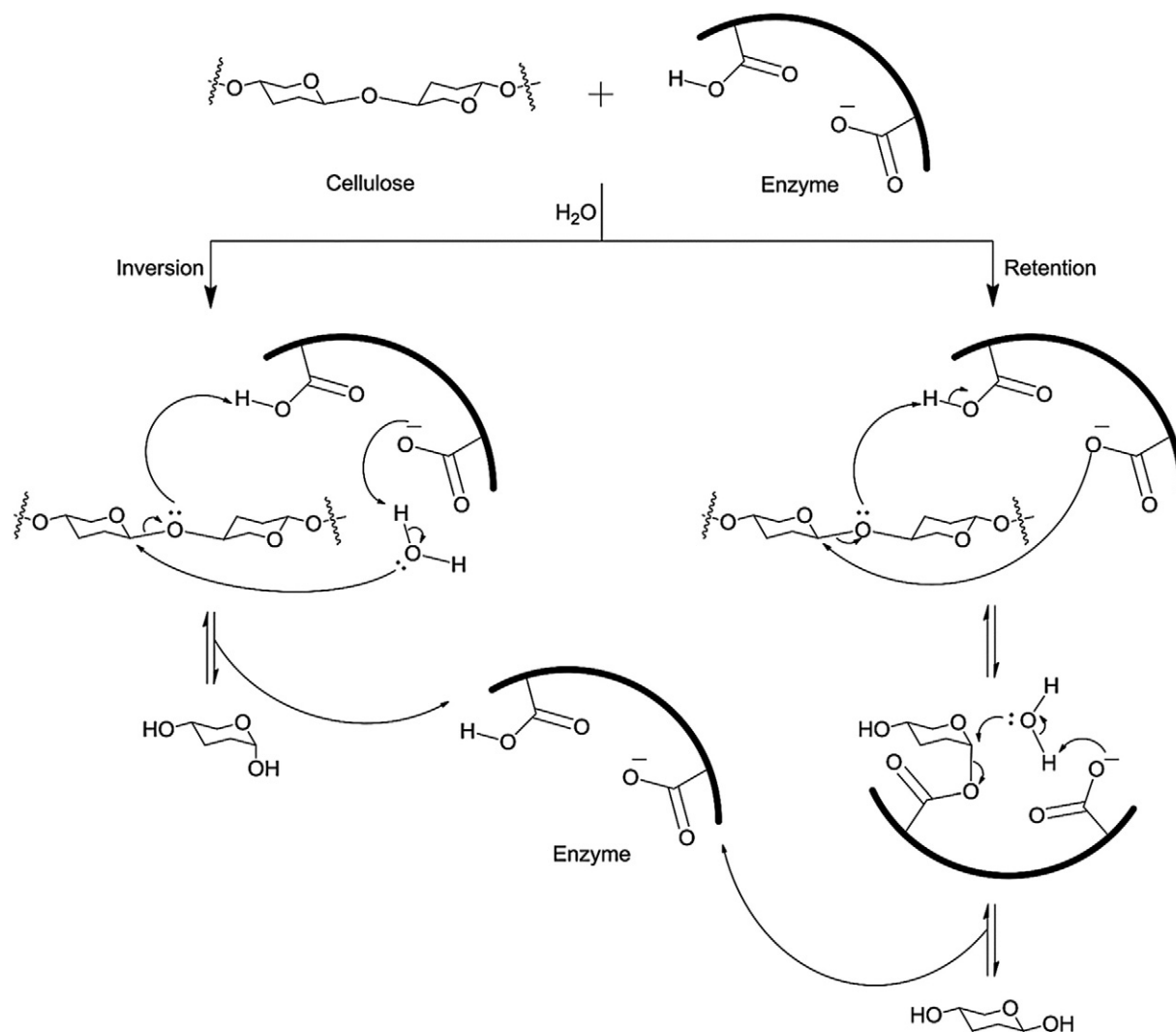


Fig. 5. Mechanism of the enzyme catalyzed hydrolysis of cellulose into glucose [116]

aliphatic acids and inorganic compounds (iron, chromium or nickel). Several alternative measures can be taken to avoid problems caused by inhibitors [104]. The detoxification process is an important step which can affect the pretreatment performance [103,105,106]. General feedstock versatility and toxic inhibitors produced have to be considered on the pretreatment efficiency in order to reach optimal conditions [107].

4.2. Hydrolysis

The performance of the hydrolysis is highly associated to the pretreatment process [80]. During this reaction, cellulose and hemicellulose are hydrolysed into simplistic and soluble compounds available for further conversion (fermentation) to ethanol [88]. There are two different types of hydrolysis processes that involve either acidic (sulfuric acid) or enzymatic reactions. The acidic reaction can be divided into dilute or concentrated acid hydrolysis. Dilute hydrolysis requires a high temperature of 200–240°C to disrupt cellulose crystals [108]. On the other side, concentrated acid hydrolysis is a more effective method as it produces higher amount of free sugars (80%) and lower concentrations of inhibitors. However, this process requires high quantity of acid which makes it usage less attractive [109,110].

When acids are used in the hydrolysis, the phenomenon of chemical dehydration occurs on monosaccharides resulting in the appearance of other compounds like aldehydes [20]. This specific issue has driven the

researcher to focus on enzymatic hydrolysis. Compelling pretreatment is fundamental to an efficient enzymatic hydrolysis [111]. Eggeman and Elander [112] have demonstrated that *Trichoderma reesei* is a very efficient fungus to produce industrial grade cellulolytic enzymes. Recent studies proved that lignin is a source of sustainable energy and added-value compounds. The application of metal components like Ca(II) and Mg(II) could intensify the enzymatic hydrolysis [112,115] (Figs. 4 and 5).

Sewalt et al. [117] have reported that the unfavorable influence of lignin on cellulases activities can be surpassed by ammonium and N-based components. Spindler et al. [118] report that the enzymatic pretreatment can be attained in simultaneous way with the co-fermentation (known as simultaneous saccharification and fermentation (SSF)) process in order to produce ethanol from woody biomass. In SSF process the concentration of saccharides is kept low and cellulose inhibition is deterred. In a separate hydrolysis and fermentation (SHF) process cellulases (hydrolytic enzymes) are inhibited by glucose and cellobiose (saccharide products) resulting in a slower process and a lower yield of fermentable sugars [119].

4.3. Fermentation

Fermentation is the following step and requires the presence of microorganisms to degrade sugars into alcohols and other end products. The previously described processes are fundamental for the

fermentation process [88,89]. Typically *S. cerevisiae* converts the sugars into ethanol under anaerobic conditions at a temperature of 30°C. In this pathway other by-products are also generated in the form of CO₂ and N-based compounds. *S. cerevisiae* is a prevalent microorganism and provides a high yield of ethanol (12.0–17.0% w/v; 90% of the theoretical yield) from sugars [119,120].

The SHF is the traditional method for bioethanol production. Several studies have reported the weakness of *S. cerevisiae* to ferment only hexose sugars and the interest for versatile-acting microorganisms increased [121]. To date, extensive research has been conducted to develop microorganisms which enable to i) ferment pentose and hexose sugars synchronously available from the hemicellulose fraction and ii) endure under inhibitory conditions. Recently, research attention focuses on efficient techniques like SSF in order to establish a consolidated bioprocessing so that hydrolysis and fermentation occur in a single reactor. This leads to a reduction in costs and avoidance of high amount of inhibitory compounds. While there is a wide variety of microorganisms which are able to convert sugars to ethanol as well as the use of one microorganism seems promising for efficient fermentation, their limitation from the standpoint of ethanol yield, tolerance to chemical inhibitors and temperature is still obvious in many demonstrated projects [85] (Table 3).

The end-product from fermentation process is a mixture of ethanol–water and requires further separation through a distillation process. Fractional distillation is a very common process to separate ethanol from water based on their different volatilities. The distillation column is heated and on the top of the column the distillate (bioethanol) is collected as it has lower boiling point (78.3°C) whereas water's boiling point is (100°C). However, the concentration of the ethanol distillate is about 92% and further dehydration is required to obtain 99% ethanol [25].

5. Recent issues in bioethanol production

5.1. Is recalcitrance of biomass a barrier?

Although lignocellulosic biomass is a promising feedstock for biorefineries, its recalcitrant structure and complexity make up an economic and technical constraint to lignocellulosic-based biofuel production. The three constituents of biomass (cellulose, hemicellulose and lignin) enhance its compactness and strength. There are strong linkages between molecules resulting in a complex structure of lignocellulosic material. As a consequence, it is necessary to use specific enzymes as a pre-treatment for fermentation [122].

Moreover, there are other materials which are inhibitory, such as xylose, and must be removed in order to prevent any negative influence to enzymatic hydrolysis [123,124]. Recent studies have indicated that bioconversion efficiency is related to the pretreatment performance [103]. For instance, the recent SPORL treatment technology is of great interest for its broad on acting in different types of woody materials [126,127]. Zhu et al. [128] reported that SPORL technology is effective for softwoods (e.g., spruce and red pine) and capable to solve problems concerning their poor digestibility in enzymatic saccharification. The SPORL was effective even when it was applied to directly pretreat wood chips without chip impregnation. Generally, each feedstock has different characteristics and for this reason the pretreatment process has to be chosen carefully [125].

The recalcitrance issue still remains a technical constraint that has to be eliminated. This problem is not current but is concerned to the evolution mechanism of natural plants which have developed those mechanisms to resist and avoid the attack of insects on their sugars. In general this 'natural' recalcitrance of plants makes up an impediment for the transformation of lignocellulosic biomass into fermentable sugars. For this reason, research development has been focused on sugars capture by re-engineering (genetic techniques applied in cell wall structure) in order to increase the sugar yields

following by enzymatic hydrolysis. The use of such approaches may promote and accelerate the future use of lignocellulosic feedstocks for the bioethanol industry [129].

5.2. Sustainable balance of water-biofuels

Water consumption in sustainable biorefineries is a crucial issue considering the industrial and agricultural practices implemented to date [130]. Although water resources are not constraint for countries such United States, Canada and Brazil, for other countries like China and India water availability is a crucial issue which project investments have to be encountered [131,132]. In United States, the production of energy feedstocks and fuels requires substantial water input. So far, bioethanol from lignocellulosic resources is produced in laboratory and pilot scale.

The Argonne National Laboratory refers that the water requirements for lignocellulosic ethanol production vary with technology and invokes that nearly 35 l of water required to produce biochemically 3.5 l of cellulosic ethanol [133,134]. The U.S. National Academy of Science (NAS) has reported that the overuse of water via the expansion of energy crops makes up serious problem. Even the biorefineries consume a specific amount of water, the main problem is concerned with the water used for cultivation [135,136]. Huffaker [137] states that significant steps are required and must include best available techniques (BATs) (for instance recycling) for sustainable use of water.

5.3. Gap between biotech research and commercialization

Bioethanol production from lignocellulosic biomass at large scale has not yet been demonstrated as an economically feasible option. Research efforts have to focus on second generation (cellulose-based) bioethanol because it has potential to be improved. A wide variety of technical problems occur in the different steps of bioethanol processing from pretreatment to the final separation of the ethanol–water mixture. Further development has to be carried out in order to mature and consequently to industrialize the second-generation-based production technologies. However, the comprehension of the interconnection between science and applied technology is crucial to identify the voids and rifts of research-industry system, so that through an overall analysis the socio-economical, technical and environmental aspects can be determined [138].

However, in order to reduce the cost of bioethanol production, it is necessary to clarify the important technological steps (i.e. enzyme development: activity, stability and production costs). Many companies are developing enzymes to increase the range of applications and the performance of the enzymatic hydrolysis of cellulose and hemicellulose. The hydrolysis may involve the application of micro-organisms (fungi, yeast, bacteria) and/or enzymes. The choice of micro-organisms and/or catalysts has to be made in terms of type and quantity as this has an impact on conversion rates and process stability. However, the use of enzymes and microorganisms increases the production cost of lignocellulosic ethanol. Further research has to be conducted in the area of microorganisms and enzymes to increase the conversion efficiencies, decrease the cost of microorganisms and enzymes to positively contribute to profitable lignocellulosic-based ethanol production plants [139].

5.4. Bioethanol-based economy

Bioethanol economy is based on different factors like feedstock availability, bioprocessing technology efficiency, and end-products characteristics. There is a wide variety of sources (corn starch, sugar cane lignocellulosic biomass, etc.) with low cost and high availability which can be used for bioethanol. Research & Development communities have to focus on the development of cheap and efficient bioconversion technology of solid cellulosic materials into bioethanol

as a feasible industrialized technology in order to be considered economically attractive.

Furthermore, significant initiatives like the registration of cellulosic bioethanol for sale and use under the RFS eliminate the gap between research and commercialization. Blenders and refiners of transportation fuels are obligated under the RFS to include certain percentages of renewable fuels in their total fuel sales. Industry ensures that since cellulosic bioethanol technology is ready for commercialization. The production of bioethanol could reach the required levels to be economically viable from the demand caused by the RFS. Both lawmakers and industry expect that the creation of a guaranteed market as federal programs such as grants, loans, and tax incentives boost the market introduction of this fuel [140,141].

However, the lignocellulosic-based ethanol is not yet widely demonstrated because of its high costs [142]. In addition, efforts have to be continued and studies to be carried out to optimize the efficiency of the existing process technology from the pretreatment to the dehydration [143]. There are margins for further development and combination (i.e. consolidated bioprocessing) of these pilot technologies in order to achieve higher bioethanol yields. Especially processes based on enzyme technology have high cost and for this reason have to be improved [144]. Bioethanol production plays a key role on bio-based economy as there are strategic perspectives for global producers, mainly US and Europe, especially when the price of oil is reduced.

6. Conclusion

In the next decades, biomass will be the most meaningful renewable energy source as an alternative to fossil fuels. Lignocellulosic bioethanol is a potential pathway for the global producers which provide renewable fuels. Bioethanol production will be probably the most successful biofuel because it has plenty of usable forms (heat, power, electricity or vehicle fuel). Different feedstocks can be used in bioethanol production and studies have focused on their characteristics. The benefits anticipated from mandated use of cellulosic biofuels include energy security through domestic production of transportation fuel and environmental improvement through the reduction of greenhouse gas and other particulate emissions associated with fossil fuel combustion. Additional benefits include creating new markets for agricultural products, keeping productive farmland in use, and improving trade balances.

The main steps leading to the end-user product (bioethanol) are pretreatment, hydrolysis, fermentation and separation/distillation. High attention has to be given for all four major steps so that the bioconversion will be optimized and the ethanol yield increased. In the USA and Europe, previous and planned research initiatives and efforts are still funded by federal sources. Also significant research funding exists through various companies which are making investments in applied research that addresses topics concerning the genetics of energy crops, the production of stable and active hydrolytic enzymes, the further development of yeast and bacterial ethanol fermentation systems. Even though technological advances and research efforts are still progressing, multiple configurations of systems and techniques are developed in order to design efficient, sustainable and economically feasible bioethanol production technologies and confront issues concerning the feedstocks and operations costs.

Conflict of interest

There is no conflict of interest.

References

- [1] Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, et al. The path forward for biofuels and biomaterials. *Science* 2006;311:484–9. <http://dx.doi.org/10.1126/science.1114736>.
- [2] Demain AL, Newcomb M, Wu JHD. Cellulase, clostridia and ethanol. *Microbiol Mol Biol Rev* 2005;69:124–54. <http://dx.doi.org/10.1128/MMBR.69.1.124-154.2005>.
- [3] Hill J, Nelson E, Tilman D, Polasky S, Tiffany D. Environmental, economic and energetic costs and benefits of biodiesel and ethanol biofuels. *Proc Natl Acad Sci U S A* 2006;103:11206–10. <http://dx.doi.org/10.1073/pnas.0604600103>.
- [4] Lin Y, Tanaka S. Ethanol fermentation from biomass resources: Current state and prospects. *Appl Microbiol Biotechnol* 2006;69:627–42. <http://dx.doi.org/10.1007/s00253-005-0229-x>.
- [5] Caledria K, Jain AK, Hoffert MI. Climate sensitivity uncertainty and the need for energy without CO₂ emission. *Science* 2003;299:2052–4. <http://dx.doi.org/10.1126/science.1078938>.
- [6] RFA. US fuel ethanol industry biorefineries and capacity. Washington, DC: Renewable Fuels Association; 2014[<http://www.ethanolrfa.org>].
- [7] Goldemberg J. Ethanol for a sustainable energy future. *Science* 2007;315:808–10. <http://dx.doi.org/10.1126/science.1137013>.
- [8] Wheals AE, Basso LC, Alves DMG, Amorim HV. Fuel ethanol after 25 years. *Trends Biotechnol* 1999;17:482–7. [http://dx.doi.org/10.1016/S0167-7799\(99\)01384-0](http://dx.doi.org/10.1016/S0167-7799(99)01384-0).
- [9] Basso LC, Basso TO, Rocha SN. Ethanol production in Brazil: The industrial process and its impact on yeast fermentation. In: Dos Santos Bernardes MA, editor. *Biofuel production – recent developments and prospects*. 1st ed. Rijeka or Croatia: InTech Pr; 2011. p. 85–100.
- [10] European Union. Directive on the promotion of the use of energy from renewable sources. *Off J Eur Union* June, 2009.
- [11] Flach B, Bendz K, Lieberz S. EU 28 – Biofuels annual. Final report. Washington (DC): U.S. Department of Agriculture, global agriculture information network (GAIN), GAIN report no. NL4025; 2014.
- [12] Shikida PFA, Finco A, Cardoso BF, Galante VA, Rahmeier D, Bentivoglio D, et al. A comparison between ethanol and biodiesel production: The Brazilian and European experiences. In: Domingos Padula A, Silveira dos Santos M, Benedetti Santos OI, Borenstein D, editors. *Liquid biofuels: Emergence, development and prospects*, lecture notes in energy 27. 1st ed. London: Springer- Verlag Pr; 2014. p. 25–53. <http://dx.doi.org/10.1007/978-1-4471-6482-1>.
- [13] Rösch C, Skarcka J. The European biofuels policy and sustainability. *Int Assoc Energy Econ* 3rd Q 2009;31–5.
- [14] European Academies Science Advisory Council (EASAC). The current status of biofuels in the European Union, their environmental impacts and future prospects. Report. Halle (Saale): German National Academy of Sciences Leopoldina; 2012[Policy Report No. 19].
- [15] Swart JAA, Jiang J, Ho P. Risk perceptions and GM crops: The case of China. *Tailoring Biotechnol* 2008;33:11–28.
- [16] Li J, Liu Y, Cheng JJ, Mos M, Daroch M. Biological potential of microalgae in China for bio refinery-based production of biofuels and high value compounds. *N Biotechnol* 2015;32:588–96. <http://dx.doi.org/10.1016/j.nbt.2015.02.001>.
- [17] Gunatilake H, Roland-Holst D, Sugiyarto G. Energy security for India: Biofuels, energy efficiency and food productivity. *Energy Policy* 2014;65:761–7. <http://dx.doi.org/10.1016/j.enpol.2013.10.050>.
- [18] Qiu H, Sun L, Huang J, Rozelle S. Liquid biofuels in China: Current status, government policies, and future opportunities and challenges. *Renewable Sustainable Energy Rev* 2012;16:3095–104. <http://dx.doi.org/10.1016/j.rser.2012.02.036>.
- [19] Koizumi T. Biofuel and food security in China and Japan. *Renewable Sustainable Energy Rev* 2013;21:102–9. <http://dx.doi.org/10.1016/j.rser.2012.12.047>.
- [20] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresour Technol* 2002;83:1–11. [http://dx.doi.org/10.1016/S0960-8524\(01\)00212-7](http://dx.doi.org/10.1016/S0960-8524(01)00212-7).
- [21] Hazell PBR, Evans M. Environmental, economic and policy aspects of biofuels. In: Galarraga I, González-Eguino M, Markandya A, editors. *Handbook of sustainable energy*. 1st ed. Cheltenham- Northampton: Edward Elgar Pr; 2011. p. 375–92.
- [22] German L, Schoneveld GC, Pacheco P. The social and environmental impacts of biofuel feedstock cultivation: Evidence from multi-site research in the forest frontier. *Ecol Soc* 2011;16:24. <http://dx.doi.org/10.5751/ES-04309-160324>.
- [23] Thompson W, Meyer S. Second generation biofuels and food crops: Co-products or competitors. *Global Food Secur* 2013;2:89–96. <http://dx.doi.org/10.1016/j.gfs.2013.03.001>.
- [24] Carus M, Dammer L. Food or non-food: Which agricultural feedstocks are best for industrial uses? Paper. Hürth: nova-Institut GmbH; nova paper #2 on bio-based economy; 2013.
- [25] Cardona CA, Sánchez OJ. Fuel ethanol production: Process design trends and integration opportunities. *Bioresour Technol* 2007;98:2415–57. <http://dx.doi.org/10.1016/j.biortech.2007.01.002>.
- [26] Metzger JO, Hüttermann A. Sustainable global energy supply based on lignocellulosic biomass from afforestation of degraded areas. *Naturwissenschaften* 2009;96:279–88. <http://dx.doi.org/10.1007/s00114-008-0479-4>.
- [27] Anwar Z, Gulfranz M, Irshad M. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. *J Radiat Res Appl Sci* 2014;7:163–73. <http://dx.doi.org/10.1016/j.jrras.2014.02.003>.
- [28] Kadam KL, McMillan JD. Availability of corn stover as a sustainable feedstock for bioethanol production. *Bioresour Technol* 2003;88:17–25. [http://dx.doi.org/10.1016/S0960-8524\(02\)00269-9](http://dx.doi.org/10.1016/S0960-8524(02)00269-9).
- [29] Knauf M, Moniruzzaman M. Lignocellulosic biomass processing: A perspective. *Int Sugar J* 2004;106:147–50.
- [30] Kim S, Dale BE. Global potential bioethanol production from wasted crops and crops residues. *Biomass Bioenergy* 2005;26:361–75. <http://dx.doi.org/10.1016/j.biombioe.2003.08.002>.
- [31] Cheng KK, Cai BY, Zhang JA, Ling HZ, Zhou YJ, Ge JP, et al. Sugarcane bagasse hemicelluloses hydrolysate for ethanol production by acid recovery process. *Biochem Eng J* 2008;38:105–9. <http://dx.doi.org/10.1016/j.bej.2007.07.012>.
- [32] Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, et al. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. *Science* 2007;315:804–7. <http://dx.doi.org/10.1126/science.1137016>.
- [33] Michelin M, Ruiz HA, Silva DP, Ruzene DS, Teixeira JA, Polizeli MLTM. Cellulose from lignocellulosic waste. *Polysaccharides* 2015;475–511. http://dx.doi.org/10.1007/978-3-319-16298-0_52.

- [34] Lachke A. Biofuel from D-xylose – The second most abundant sugar. *Resonance* 2002;5:50–6. <http://dx.doi.org/10.1007/BF02836736>.
- [35] Torney F, Moeller L, Scarpa A, Wang K. Genetic engineering approaches to improve bioethanol production from maize. *Curr Opin Biotechnol* 2007;18:193–9. <http://dx.doi.org/10.1016/j.copbio.2007.03.006>.
- [36] Senthilkumar V, Gunasekaran P. Bioethanol production from cellulosic substrates: Engineered bacteria and process integration challenges. *J Sci Ind Res* 2005;64:845–53.
- [37] Yang B, Dai Z, Ding SY, Wyman CE. Enzymatic hydrolysis of cellulosic biomass. *Biofuels* 2011;2:421–50.
- [38] Weber C, Boles E. Sugar-hungry yeast to boost biofuel production. *Sci Daily* 2010; 92:881–2.
- [39] Beckner M, Ivey ML, Phister TG. Microbial contamination of fuel ethanol fermentations. *Lett Appl Microbiol* 2011;53:387–94. <http://dx.doi.org/10.1111/j.1472-765X.2011.03124.x>.
- [40] Muthaiyan A, Limayem A, Ricke SC. Antimicrobial strategies for limiting bacterial contaminants in fuel bioethanol fermentation. *Prog Energy Combust Sci* 2011;37: 351–70. <http://dx.doi.org/10.1016/j.pecs.2010.06.005>.
- [41] Pienkos PT, Zhang M. Role of pretreatment and conditioning processes on toxicity of lignocellulosic biomass hydrolysates. *Cellulose* 2009;16:743–62. <http://dx.doi.org/10.1007/s10570-009-9309-x>.
- [42] Jönsson LJ, Martín C. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour Technol* 2016;199: 103–12. <http://dx.doi.org/10.1016/j.biortech.2015.10.009>.
- [43] Demirbas MF, Balat M. Recent advances on the production and utilization trends of bio-fuels: A global perspective. *Energy Convers Manage* 2006;47:2371–81. <http://dx.doi.org/10.1016/j.enconman.2005.11.014>.
- [44] Dufey A. Biofuels production, trade and sustainable development: Emerging issues. Environmental economics programme, sustainable markets discussion paper no. 2. London: International Institute for Environment and Development (IIED); September, 2006.
- [45] RFA-Renewable Fuels Association (RFA). Ethanol industry statistics, Washington, DC, USA; 2007[cited; available from: www.ethanolrfa.org].
- [46] Lin Y, Tanaka S. Ethanol fermentation from biomass resources: Current state and prospects. *Appl Microbiol Biotechnol* 2006;69:627–42. <http://dx.doi.org/10.1007/s00253-005-0229-x>.
- [47] Gnansounou E. Production and use of lignocellulosic bioethanol in Europe: Current situation and perspectives. *Bioresour Technol* 2010;101:4842–50. <http://dx.doi.org/10.1016/j.biortech.2010.02.002>.
- [48] Balat M, Balat H. Recent trends in global production and utilization of bio-ethanol fuel. *Appl Energy* 2009;86:2273–82. <http://dx.doi.org/10.1016/j.apenergy.2009.03.015>.
- [49] Lucia LA. Lignocellulosic biomass: A potential feedstock to replace petroleum. *Bioresources* 2008;3:981–2.
- [50] Alterra & IASA. Atlas of EU biomass potentials deliverable 3.3: Spatially detailed and quantified overview of EU biomass potential taking into account the main criteria determining biomass availability from different sources, atlas of EU biomass potentials, IEE 08 653 S12, 529 241; 2012.
- [51] Hu G, Heitmann JA, Rojas OJ. Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues. *BioResources* 2008;3:270–94.
- [52] Miao Z, Grift TE, Hansen AC, Ting KC. An overview of lignocellulosic biomass feedstock harvest, processing and supply for biofuel production. *Biofuels* 2013;4: 5–8.
- [53] Office of Biological and Environmental Research. Lignocellulosic biomass for advanced biofuels and bioproducts: workshop report. U.S. Department of Energy Office of Science; 2015[Report DOE/SC-0170].
- [54] Návár J. In: Momba M, Bux F, editors. Measurement and assessment methods of forest aboveground biomass: A literature review and the challenges ahead. *Biomass*, 1st ed[Rijeka: Sciyo; 2010. p. 27–64.
- [55] Köhl M, Lasco R, Cifuentes M, Jönsson Ö, Korhonen KT, Mundhenk P, et al. Changes in forest production, biomass and carbon: Results from the 2015 UN FAO Global Forest Resource Assessment. *For Ecol Manage* 2015;352:21–34. <http://dx.doi.org/10.1016/j.foreco.2015.05.036>.
- [56] Nielsen L, Hazel D, Frederick D, Nicoles E. Using municipal waste sites for cellulosic biomass production in North Carolina. Final report. North Carolina: NC State University, Department of Environment and Natural Resources; 2013[Report No. WB-0016].
- [57] Chester M, Martin E. Cellulosic ethanol from municipal solid waste: A case study of the economic, energy, and greenhouse gas impacts in California. *Environ Sci Technol* 2009;43:5183–9. <http://dx.doi.org/10.1021/es802788z>.
- [58] Elumalai S, Pan XJ. Chemistry and reactions of forest biomass in biorefining. In: Zhu JY, Zhang X, Pan XJ, editors. Sustainable production of fuels, chemicals, and fibers from forest biomass. Washington DC: American Chemical Society; 2011. p. 109–44. <http://dx.doi.org/10.1021/bk-2011-1067.ch005>.
- [59] Bond BH. Wood identification for hardwood and softwood species native to Tennessee. Agricultural Extension Service The University of Tennessee. [Report No. PB1692].
- [60] Amarasekara AS. Feedstocks for cellulosic ethanol production. In: Amarasekara AS, editor. Handbook of cellulosic ethanol. US: John Wiley & Sons & Scrivener; 2014. p. 43–129. <http://dx.doi.org/10.1002/9781118878750.ch3>.
- [61] Gabrielle B, Gagnaire N. Life-cycle assessment of straw use in bio-ethanol production: A case study based on biophysical modelling. *Biomass Bioenergy* 2008;32: 431–41. <http://dx.doi.org/10.1016/j.biombioe.2007.10.017>.
- [62] U.S. Department of Energy Biomass Program. http://www.eere.energy.gov/biomass/pdfs/biomass_deep_dive_pir.pdf; 2009.
- [63] Demirbas A. Bioethanol from cellulosic materials: A renewable motor fuel from biomass. *Energy Source* 2005;27:327–37. <http://dx.doi.org/10.1080/00908310390266643>.
- [64] Shi AZ, Koh LP, Tan HTW. The biofuel potential of municipal solid waste. *GCB Bioenergy* 2009;1:317–20. <http://dx.doi.org/10.1111/j.1757-1707.2009.01024.x>.
- [65] Li A. Bioconversion of biodegradable municipal solid waste (BMSW) to glucose for bio-ethanol production. London: University College of London; 2008[Doctoral thesis].
- [66] Khanna M. A billion tons of biomass a viable goal but at high price. Illinois: News Bureau; 2011[<https://news.illinois.edu/blog/view/6367/205414>].
- [67] Aoun WB, Gabrielle N, Gagnepain B. The importance of land use change in the environmental balance of biofuels. *Oilseeds Fats Crops Lipids* 2013;20:1–12. <http://dx.doi.org/10.1051/ocl/2013027>.
- [68] Khanna M, Chen X, Huang H, Önal H. Supply of cellulosic biofuel feedstocks and regional production pattern. *Am J Agr Econ* 2011;93:473–80. <http://dx.doi.org/10.1093/ajae/aaq119>.
- [69] Bonvicini G. Algae bioenergy siting, commercial deployment and development analysis. Final report. Brussels: European Commission DG ENERGY; 2015[Report no. 12–920–H3].
- [70] Rodolff L, Zitelli GC, Bassi N, Padovani G, Biondi N, Bionini G, et al. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photo-bioreactor. *Biotechnol Bioeng* 2009;102:100–12. <http://dx.doi.org/10.1002/bit.22033>.
- [71] Ferrel J, Sarisky-Reed V. National algal biofuels technology roadmap. US Department of Energy (DOE). Office of Energy and Renewable Energy; 2010.
- [72] Harel A. Noritech seaweed biotechnology Inc. Rotterdam, NL: Algae World Conference; 2009.
- [73] Yang SH. Chemistry of cellulosic plant. 3rd ed. Beijing: China Light Industry Press; 2001.
- Chen H, Chen H. Brief introduction to the biotechnology of lignocellulose. *Biotechnology of lignocellulose: Theory and practice*. Beijing: Chemical Industry Press Pr; 2014. p. 1–24.
- [74] Badger PC. In: Jannick J, Whipseyke A, editors. Trends in new crops and new uses. Alexandria, VA: ASHS Press; 2000. p. 17–21.
- [75] Mielenz JR. Ethanol production from biomass: Technology and commercialization status. *Curr Opin Microbiol* 2001;4:324–5. [http://dx.doi.org/10.1016/S1369-5274\(00\)00211-3](http://dx.doi.org/10.1016/S1369-5274(00)00211-3).
- [76] Girio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasic R. Hemicelluloses for fuel ethanol: A review. *Bioresour Technol* 2010;101:4775–800. <http://dx.doi.org/10.1016/j.biortech.2010.01.088>.
- [77] Edey LA, Doherty WOS. Fractionation of a lignocellulosic material. Patent US 20100196967 A1; 2008.
- [78] Balat M, Balat H, Öz C. Progress in bioethanol processing. *Prog Energy Combust Sci* 2008;34:551–73. <http://dx.doi.org/10.1016/j.pecs.2007.11.001>.
- [79] Saha BD. Hemicellulose bioconversion. *J Ind Microbiol Biotechnol* 2003;30:279–91. <http://dx.doi.org/10.1007/s10295-003-0049-x>.
- [80] Girio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasic R. Hemicelluloses for fuel ethanol: A review. *Bioresour Technol* 2010;101:4775–800. <http://dx.doi.org/10.1016/j.biortech.2010.01.088>.
- [81] Sun R, Sun XF, Tomkinson J. Hemicelluloses and their derivatives. In: Gatenholm P, Tenkanen M, editors. Hemicelluloses: Science and technology. Washington DC: American Chemical Society Pr; 2004. p. 2–22. <http://dx.doi.org/10.1021/bk-2004-0864.ch001>.
- [82] Ebringerova A, Hromadkova Z, Heinze T. Hemicellulose. *Adv Polym Sci* 2005;186: 1–67. <http://dx.doi.org/10.1007/b136816>.
- [83] Deguchi S, Mukai SA, Tsudome M, Horikoshi K. Facile generation of fullerene nanoparticles by hand-grinding. *Adv Mater* 2006;18:729–32. <http://dx.doi.org/10.1002/adma.200502487>.
- [84] Klemm D, Schmauder HP, Heinze T. Cellulose. *Biopolymers Online* 2005;6: 277–312. <http://dx.doi.org/10.1002/3527600035.bp016010>.
- [85] Ladisch MR, Mosier NS, Kim Y, Ximenes E, Hogsett D. Converting cellulose to biofuels. *SBE special supplement biofuels*. CEP 2010;106:56–63.
- [86] Chen F, Srinivasa RMS, Temple S, Jackson L, Shadle G, Dixon RA. Multi-site genetic modulation of monolignol biosynthesis suggests new routes for formation of syringyl lignin and wall-bound ferulic acid in alfalfa (*Medicago sativa* L.). *Plant J* 2006;48:113–24. <http://dx.doi.org/10.1111/j.1365-313X.2006.02857.x>.
- [87] Demirbas A. Progress and recent trends in biofuels. *Prog Energy Combust Sci* 2007; 33:1–18. <http://dx.doi.org/10.1016/j.pecs.2006.06.001>.
- [88] Chandel AK, Chan E, Rudravaram R, Narasu ML, Rao LV, Ravindra P. Economics and environmental impact of bioethanol production technologies: an appraisal. *Biotechnol Mol Biol Rev* 2007;2:14–32.
- [89] Gamage J, Howard L, Zisheng Z. Bioethanol production from lignocellulosic biomass. *J Biobased Mater Bioenergy* 2010;4:3–11. <http://dx.doi.org/10.1166/jbmb.2010.1071>.
- [90] Mu D, Seager T, Suresh Rao P, Zhao F. Comparative life cycle assessment of lignocellulosic ethanol production: Biochemical versus thermochemical conversion. *Environ Manage* 2010;46:565–78. <http://dx.doi.org/10.1007/s00267-010-9494-2>.
- [91] National Renewable Energy Laboratory (NREL). Thermochemical ethanol via indirect gasification and mixed alcohol synthesis of lignocellulosic biomass. Technical report NREL/TP-510-41168. Colorado: U.S. Department of Energy, Office of Energy Efficiency & Renewable Energy; 2007[Contract No. DE-AC36-99-GO10337].
- [92] Younesi H, Najafpour G, Mohamed AR. Ethanol and acetate production from synthesis gas via fermentation processes using anaerobic bacterium, *Clostridium ljungdahlii*. *Biochem Eng J* 2005;27:110–9. <http://dx.doi.org/10.1016/j.bej.2005.08.015>.
- [93] Liu K, Atiyeh HK, Stevenson BS, Tanner RS, Wilkins MR, Huhnke RL. Continuous syngas fermentation for the production of ethanol, n-propanol and n-butanol. *Bioresour Technol* 2014;151:69–77. <http://dx.doi.org/10.1016/j.biortech.2013.10.059>.
- [94] Liu K, Atiyeh HK, Stevenson BS, Tanner RS, Wilkins MR, Huhnke RL. Mixed culture syngas fermentation and conversion of carboxylic acids into alcohols. *Bioresour Technol* 2014;152:337–46. <http://dx.doi.org/10.1016/j.biortech.2013.11.015>.
- [95] Johnson T, Johnson B, Scott-Kerr C, Kiviaho J. Bioethanol-status report on bioethanol production from wood and other lignocellulosic feedstocks. 63rd Appita annual conference and exhibition. Melbourne; 2010.
- [96] Yang B, Wyman CE. Pretreatment: The key to unlocking low-cost cellulosic ethanol. *Biofuels Bioprod Biorefin* 2008;2:26–40. <http://dx.doi.org/10.1002/bbb.49>.
- [97] Zhu JY, Wang GS, Pan XJ, Gleisner R. Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. *Chem Eng Sci* 2009;64:474–85. <http://dx.doi.org/10.1016/j.ces.2008.09.026>.

- [98] National Renewable Energy Laboratory (NREL). Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: Dilute-acid pretreatment and enzymatic hydrolysis of corn stover. Technical report NREL/TP-5100-47764. Colorado: U.S. Department of Energy, Office of Energy Efficiency & Renewable Energy; 2011[Contract No. DE-AC36-08GO28308].
- [99] Sánchez OJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour Technol* 2008;99:5270–95. <http://dx.doi.org/10.1016/j.biortech.2007.11.013>.
- [100] Spataro S, Bagley DM, MacLean HL. Life cycle evaluation of emerging lignocellulosic ethanol conversion technologies. *Bioresour Technol* 2010;101:654–67. <http://dx.doi.org/10.1016/j.biortech.2009.08.067>.
- [101] Fehrenbacher K. Logen suspends U.S. cellulosic ethanol plant plans. [accessed October 22] <http://earth2tech.com/2008/06/04/iogen-suspends-us-cellulosic-ethanol-plant-plans-2009>.
- [102] Zheng Y, Pan Z, Zhang R. Overview of biomass pretreatment for cellulosic ethanol production. *Int J Agric Biol Eng* 2009;2:51–68. <http://dx.doi.org/10.3965/j.issn.1934-6344.2009.03.051-068>.
- [103] Zhu JY, Pan HJ. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation. *Bioresour Technol* 2010;101:4992–5002. <http://dx.doi.org/10.1016/j.biortech.2009.11.007>.
- [104] Jönsson LJ, Alriksson B, Nilvebrant NO. Bioconversion of lignocellulose: Inhibitors and detoxification. *Biotechnol Biofuels* 2015;6:16. <http://dx.doi.org/10.1186/1754-6834-6-16>.
- [105] Zhu JY, Xuejun P, Zalesny RS. Pretreatment of woody biomass for biofuel production: Energy efficiency, technologies, and recalcitrance. *Appl Microbiol Biotechnol* 2010;87:847–57. <http://dx.doi.org/10.1007/s00253-010-2654-8>.
- [106] Laser M, Schulman D, Allen SG, Lichwa J, Antal MJ, Lynd LR. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. *Bioresour Technol* 2002;81:33–44. [http://dx.doi.org/10.1016/S0960-8524\(01\)00103-1](http://dx.doi.org/10.1016/S0960-8524(01)00103-1).
- [107] Wyman CE, Dale BE, Elander RT, Holtzapfle M, Ladisch MR, Lee YY. Coordinated development of leading biomass pretreatment technologies. *Bioresour Technol* 2005;96:1959–66. <http://dx.doi.org/10.1016/j.biortech.2005.01.010>.
- [108] Xiang Q, Lee YY, Pettersson PO, Torget RW. Heterogeneous aspects of acid hydrolysis of α -cellulose. *Appl Biochem Biotechnol* 2003;107:505–14. <http://dx.doi.org/10.1385/ABAB:107:1-3:505>.
- [109] Torget R, Werdene P, Himmel M, Grohmann K. Dilute acid pretreatment of short rotation woody and herbaceous crops. *Appl Biochem Biotechnol* 1991;24:115–26. <http://dx.doi.org/10.1007/BF02920238>.
- [110] Hamelinck CN, Van Hooijdonk G, Faaij APC. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass Bioenergy* 2005;28:384–410. <http://dx.doi.org/10.1016/j.biombioe.2004.09.002>.
- [111] Hendriks ATWM, Zeeman G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour Technol* 2009;100:10–8. <http://dx.doi.org/10.1016/j.biortech.2008.05.027>.
- [112] Eggeman T, Elander RT. Process and economic analysis of pretreatments technologies. *Bioresour Technol* 2005;96:2019–25. <http://dx.doi.org/10.1016/j.biortech.2005.01.017>.
- [113] Kupiainen L, Ahola J, Tanskanen J. Kinetics of formic acid-catalyzed cellulose hydrolysis. *BioResources* 2014;9:2645–58. <http://dx.doi.org/10.15376/biores.9.2.2645-2658>.
- [114] Xiang Q, Lee YY, Pettersson PO, Torget RW. Heterogeneous aspects of acid hydrolysis of α -cellulose. *Appl Biochem Biotechnol* 2003;107:505–14. <http://dx.doi.org/10.1385/ABAB:107:1-3:505>.
- [115] Liu H, Zhu JY, Fu S. Effects of lignin-metal complexation on enzymatic hydrolysis of cellulose. *J Agric Food Chem* 2010;58:7233–8. <http://dx.doi.org/10.1021/jf1001588>.
- [116] Bhaumik P, Dhepe PL. Conversion of biomass into sugars. (Chapter 1), In: Murzin D, Simakova O, editors. *Biomass sugars for non-fuel applications*RSC green chemistry no. 44, the Royal Society of Chemistry; 2016. <http://dx.doi.org/10.1039/9781782622079-00001>.
- [117] Sewalt VJH, Glasser WG, Beauchemin KA. Lignin impact on fiber degradation. 3. Reversal of inhibition of enzymatic hydrolysis by chemical modification of lignin and by additives. *J Agric Food Chem* 1997;45:1823–8. <http://dx.doi.org/10.1021/jf9608074>.
- [118] Spindler DD, Wyman CE, Grohmann K. The simultaneous saccharification and fermentation of pretreated woody crops to ethanol. *Appl Biochem Biotechnol* 1991;28/29:773–86. <http://dx.doi.org/10.1007/BF02922648>.
- [119] Kumar S, Singh SP, Mishra IM, Adhikari DK. Recent advances in production of bioethanol from lignocellulosic biomass. *Chem Eng Technol* 2009;32:517–26. <http://dx.doi.org/10.1002/ceat.200800442>.
- [120] Claassen PAM, Van Lier JB, Lopez Contreras AM, Van Niel EWJ, Sijtsma L, Stams AJM, et al. Utilization of biomass for the supply of energy carriers. *Appl Microbiol Biotechnol* 1999;52:741–55. <http://dx.doi.org/10.1007/s002530051586>.
- [121] Martín C, Galbe M, Wahlbom CF, Hahn-Hagerdal B, Jönsson LJ. Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilizing *Saccharomyces cerevisiae*. *Enzyme Microb Technol* 2002;31:274–82. [http://dx.doi.org/10.1016/S0141-0229\(02\)00112-6](http://dx.doi.org/10.1016/S0141-0229(02)00112-6).
- [122] Yang B, Wyman CE. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. *Biotechnol Bioeng* 2004;86:88–95. <http://dx.doi.org/10.1002/bit.20043>.
- [123] Qing Q, Yang B, Wyman CE. Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. *Bioresour Technol* 2010;101:9624–30. <http://dx.doi.org/10.1016/j.biortech.2010.06.137>.
- [124] Ximenes E, Kim Y, Felix S, Mosier NS, Ladisch MR. Inhibition of cellulolytic enzymes due to products of hemicelluloses hydrolysis. A special conference on the Society for Industrial Microbiology: 32nd symposium on biotechnology for fuels and chemicals. FL: Clear Water Beach; 2010.
- [125] Olofsson K, Bertilsson M, Lidén G. A short review on SSF – An interesting process option from lignocellulosic feedstocks. *Biotechnol Biofuels* 2008;1:1–7. <http://dx.doi.org/10.1186/1754-6834-1-7>.
- [126] Wang GS, Pan XJ, Zhu JY, Gleisner R, Rockwood D. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. *Biotechnol Prog* 2009;25:1086–93. <http://dx.doi.org/10.1002/btpr.206>.
- [127] Shuai L, Yang Q, Zhu J, Lu FC, Weimer PJ, Ralph J, et al. Comparative study of SPORL and dilute-acid pretreatments of spruce cellulosic ethanol production. *Bioresour Technol* 2010;101:3106–14. <http://dx.doi.org/10.1016/j.biortech.2009.12.044>.
- [128] Zhu JY, Pan XJ, Wang GS, Gleisner R. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour Technol* 2009;100:2411–8. <http://dx.doi.org/10.1016/j.biortech.2008.10.057>.
- [129] Abramson M, Shoseyov O, Hirsch S, Shani Z. Genetic modifications of plant cell walls to increase biomass and bioethanol production. In: Lee JW, editor. *Advanced biofuels and bioproducts*. New York: Springer; 2013. <http://dx.doi.org/10.1007/978-1-4614-3348-4>.
- [130] Keeny D, Muller M. Waste use by ethanol plants potential challenges. Minneapolis, MN: Institute for Agriculture and Trade Policy; 2006 7.
- [131] Yang H, Zhou Y, Liu J. Land and water requirements of biofuel and implications for food supply and the environment in China. *Energy Policy* 2009;37:1876–85. <http://dx.doi.org/10.1016/j.enpol.2009.01.035>.
- [132] Huang J, Yang J, Msangi S, Rozelle S, Weersink A. Global biofuel production and poverty in China. *Appl Energy* 2012;98:246–55. <http://dx.doi.org/10.1016/j.apenergy.2012.03.031>.
- [133] Berndes G. Bioenergy and water: the implications of large-scale bioenergy production for water use and supply. *Glob Environ Change* 2002;12:253–71. [http://dx.doi.org/10.1016/S0959-3780\(02\)00040-7](http://dx.doi.org/10.1016/S0959-3780(02)00040-7).
- [134] M W, Mintz M, Wang M, Arora S. Consumptive water use in the production of bioethanol and petroleum gasoline. Final report. Lemont: Argonne National Laboratory; 2008[Contract No. DE-AC02-06CH11357].
- [135] National Research Council (NRC). Water implication of biofuels production in the United States. Washington DC. USA: National Academy of Science Press; 2007.
- [136] Jackson H. U.S. corn boom has downside for gulf. Associated Press; 2007.
- [137] Huffaker R. Protecting water resources in biofuels production. *Water Policy* 2010;12:129–34. <http://dx.doi.org/10.2166/wp.2009.113>.
- [138] Russo L, Ladisch M. Gaps in the research of 2nd generation transportation biofuels. Final report of task 41, project 2. IEA bioenergy, T41 (2):01; 2008.
- [139] European Biofuels Technology Platform. Development of enzymes and processes for cellulosic ethanol production. Ethanol fact sheet. <http://www.biofuelstp.eu/factsheets/ethanol-fact-sheet.html>.
- [140] Garver MP, Liu S, Gupta VK, et al. Development of thermochemical and biochemical technologies for biorefineries. *Bioenergy research: Advances and applications* Elsevier; 2014. p. 457–88. <http://dx.doi.org/10.1016/B978-0-444-59561-4.00027-9>.
- [141] AVL MTC, Motortestcenter AB. Blending of ethanol in gasoline for spark ignition engines – Problem inventory and evaporative measurements. Rapport nr MTC 5407, Sweden; 2005.
- [142] Lynd LR, Van Zyl WH, McBride JE, Laser M. Consolidated bioprocessing of cellulosic biomass: An update. *Curr Opin Biotechnol* 2005;16:577–83. <http://dx.doi.org/10.1016/j.copbio.2005.08.009>.
- [143] Talebnia F, Karakashev D, Angelidaki I. Production of bioethanol from wheat straw. An overview on pretreatment, hydrolysis and fermentation. *Bioresour Technol* 2010;101:4744–53. <http://dx.doi.org/10.1016/j.biortech.2009.11.080>.
- [144] Weber C, Farwick A, Benisch F, Brat D, Dietz H, Subtil T, et al. Trends and challenges in the microbial production of lignocellulosic bioalcohol fuels. *Appl Microbiol Biotechnol* 2010;87:1303–15. <http://dx.doi.org/10.1007/s00253-010-2707-z>.