

State of the Art in Bioethanol Production

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Abstract. The objective of the present study is to provide an overview of available literature on problems and potential solutions in bioethanol production. The preparation of an overview of bioethanol as motor fuel requires knowledge of its chemical-physical properties and different production methods. The study points out the most popular opinions and test results to characterise the production of bioethanol. This overview considers potential methods for producing ethanol and production technologies suitable for ethanol as motor fuel, especially most recent achievements in converting carbohydrates into ethanol.

Key words: Biofuel, bioethanol, production methods, lignocellulosic biomass, bacteria

INTRODUCTION

There are two global biorenewable transportation fuels that might replace oil-derived gasoline and diesel fuel. These are bioethanol and biodiesel. Owing to its widespread availability, biorenewable fuel technology will potentially result in the employment of more people than fossil-fuel-based technology (Demirbas, 2006).

Biofuels obtained from renewable sources can be classified on the basis of their production technologies, biofuels of first and second generation and biofuels of third and fourth generation. The first-generation fuels refer to biofuels made from plants rich in oil or sugar. The feedstock for such biofuels consists in oil plants (plant seeds) which are pressed to yield oil that can be processed into diesel fuels by esterification; sugar-containing feedstock is processed to yield ethyl alcohol, which is then used as gasoline additive or individual fuel. However, the production of first-generation biofuel is economically unreasonable, because of discarding cellulose and hemicellulose – which constitute the majority of the carbon resource of the plants – in the course of the process. Furthermore, the biofuels of this generation also compete with food products intended for human consumption.

The second-generation biofuels (*Biomass to Liquid*) are made from organic materials, such as straw, wood residues, agricultural residues, reclaimed wood, sawdust, and low-value timber. Feedstock also includes short rotation plants and trees (perennial grasses, short-rotation coppice) and quickly growing algae. Although the second-generation biofuels allow improving CO₂ balance, they do not yield major benefit in comparison with the first-generation fuels, considering the high amount of fossil fuels used for their production.

Biofuels of the third and fourth generation are produced from algae by using modern gene and nanotechnologies.

Bioethanol as motor fuel for internal combustion engines

Ethanol or ethyl alcohol ($\text{CH}_3\text{CH}_2\text{OH}$) with molecular weight $M = 46.7$ is also known as alcoholic spirit, grain spirit, absolute alcohol and ethyl hydrate. Depending on its water content, production method and final use, there are several ethanol products available on the market. 99% alcohol (mostly referred to as absolute alcohol) is used for preparing tinctures and pharmaceutical preparations, solvents and preservatives, antiseptics and perfumes. Ethanol represents a crucial functional component in the composition of alcoholic drinks produced by carbohydrate fermentation. If alcohol is used for purposes other than drink, it is denatured with such additives as methanol, pyridine, formaldehyde, etc. Tables 1 and 2 provide an overview of biofuels by generations, including respective feedstock and production processes.

Table 1. First and second generation biofuels, their feedstock and technological processes (Sims et al, 2008)

First-generation (conventional) biofuel			
Type of biofuel	Name	Biomass feedstock	Production process
Bioethanol	Conventional bioethanol	Sugar beet, sugar cane, sugar sorghum	Hydrolysis & fermentation
Pure plant oil	Pure plant oil (PPO)	Oil plants (e.g. rape seed)	Cold-pressing/extraction.
Biodiesel fuel (plant energy)	Rape methyl-/ethyl ester) RME/REE Fatty acids methyl/ethyl ester (FAME/FAEE)	Oil plants (e.g. rape/turnip rape seeds, sunflower seeds, soy beans, etc.)	Cold-pressing/extraction/transesterification.
Biodiesel fuel (waste grease)	Fatty acids methyl/ ethyl ester (FAME/FAEE)	Biodiesel cooking and deep-fry grease	Transesterification
Biogas	Upgraded biogas	(Wet) biomass	Anaerobic digestion
Bio-ETBE		Bioethanol	Chemical syntheses
Second-generation biofuel			
Type of biofuel	Name	Biomass feedstock	Production process
Bioethanol	Cellulose ethanol	Lignocelluloses	Upgraded hydrolysis & fermentation
Synthetic biofuels	Mixed higher alcohols Bio-dimethyl ether	Lignocelluloses	Gasification + syntheses
Biodiesel (hybrid biodiesel from the first and second generation)	NExBTL	Plant oils and animal fats	Hydrogenation (Refining/enrichment)
Biogas	SNG (Synthetic Natural Gas)	Lignocelluloses	Gasification & syntheses
Bio-hydrogen		Lignocelluloses	Gasification & syntheses or biological process

Table 2. Third and fourth generation biofuels, their feedstock and technological processes (Demirbas, 2009)

Third-generation biofuel			
Type of biofuel	Name	Biomass feedstock	Production process
Biodiesel	<i>Oilgae</i> Algae diesel	<i>Algae</i>	Gene and nanotechnology & esterification
Fourth-generation biofuel			
Type of biofuel	Name	Biomass feedstock	Production process
Bio gasoline	Synthetic oil	Vegetable oil	Hydrolytic
Bio jet fuel		(CENTIA TM oil	conversion/deoxygenating
Biodiesel		from algae)	

ANALYSIS OF PRODUCTION TECHNOLOGIES OF ETHANOL

Ethanol production is commonly classified into chemical and microbiological. Chemically synthesized ethanol is produced by hydration of ethylene, a by-product of oil-manufacturing. This is industrial alcohol with wide range of applications. Fig. 1 lists the sources for industrial ethanol.

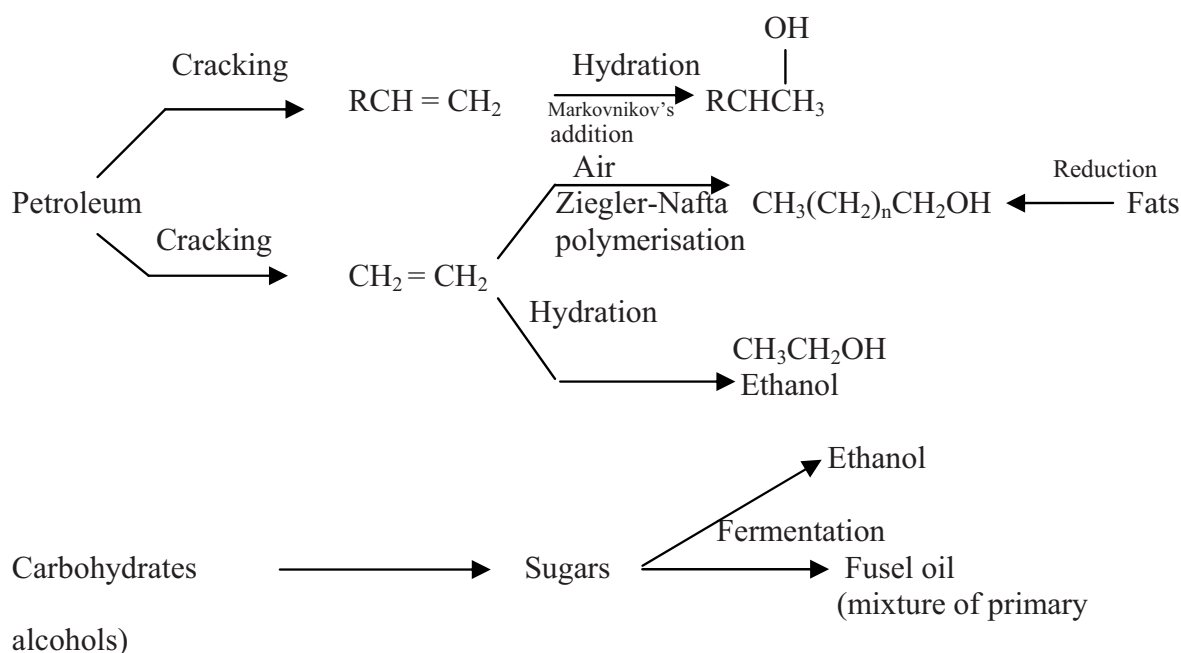


Fig. 1. Sources of industrial production of ethanol (Ullmann, 1990a).

Physical properties

In its plain form, ethanol is a colourless liquid. It is soluble in water as well as in ether, acetone, benzene, and other organic solvents. Anhydrous alcohol is hygroscopic; it achieves certain stability after absorption of water to the extent of 0.3-0.4%. The main parameters of anhydrous ethanol are the following (Ullmann, 1990a),

Boiling point	78.39°C
Liquefaction temperature	-114.15°C
Refractive index n at 20°C	1.36048
Densities, d_4^{20} ; d_4^{15} ;	0.79356; 0.78942
Flash point (in closed cup)	13° C
Dynamic viscosity η	1.19 mPa·s ⁻¹
Calorific value,	
• lower	29,895 kJ kg ⁻¹
• upper	29,964 kJ kg ⁻¹

Azeotropic mixture consists of 95.57% ethanol and 4.43% water by volume. Therefore, normal distillation allows yield of 95.57% ethanol by volume. Further removal of water from azeotropic mixture can be done either by using tertiary solvent, molecular sieves, membrane method or some other method.

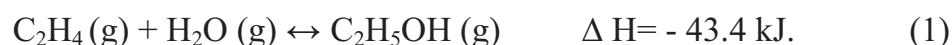
Chemical properties

Chemical properties of ethanol are dominant due to the presence of a functional group – OH in the compound, which enables industrially important chemical reactions, such as dehydration, halogenization, recovery of esters, and oxidation (Ullmann, 1990b).

Synthetic ethanol

There are two main methods for industrial production of synthetic ethanol (Ullmann, 1990a),

1) Direct catalytic ethylene hydration reaction,



Reaction kinetics in the presence of phosphoric acid and multiple catalysts has been studied by several authors (Ullmann, 1990a). For example H_3PO_4 – in the presence of silica gel, and blue lead oxide – in the presence of silica gel catalyst. A nomogram has been prepared depending on the variation of process parameters, pressure, temperature, molar water-ethylene ratio. Under normal reaction conditions, the molar ethylene water ratio is 1.1; at temperatures between 250-300°C, at the pressure of 5-8 MPa, ethanol conversion reaches 7-22%.

Conversion is more efficient at lower temperatures (reaction 1), but this also involves formation of diethyl ether by secondary reaction,



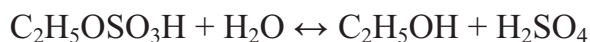
At higher pressure ethylene is polymerized, yielding butylenes and higher olefins.

Technical and patented literature describes several catalysts for ethylene hydration (Ullmann, 1990a). Diatomic loams (diatomite, zeolite) are used.

2) Indirect ethylene hydration, esterification-hydrolysis or H₂SO₄ process, based on absorption of high volume ethylene in concentrated sulphuric acid (Ullmann, 1990d). Using diluted H₂SO₄ produces diethyl ether in addition to ethanol.



Hydrolysis is performed in three steps,



Products include ethanol and 5-10% diethyl ether. Ether yield is verified by varying reaction conditions, especially by changing ethylene and sulphuric acid ratio. Industrial production makes use of additional methods as well (methanol homologization, methanol and methyl acetate carbonylation, syngas heterogeneous and homogenous catalysis). Chemically synthesized methods will not be further considered by the authors of the present article, because we focus on the use of feedstock of biological origin for producing motor fuel.

Ethanol recovery from fermentation of carbohydrates

Biochemically, ethanol (first-generation) is produced from plant feedstock containing high quantity of carbohydrates. Fermentation involves yeasts. The commonest yeast species include highly productive species, such as *Saccharomyces cerevisiae*, *S. uvarum* (formerly *S. carlsbergensis*), and *Candida utilis*. The species *Saccharomyces anamnesis* and *Schizosaccharomyces pombe* have also been used. The species *Kluyveromyces* together with the ferment lactose are good for producing ethanol from whey (Ullmann, 1990d).

Ethanol production by yeast is characterized by high selectivity, low accumulation of by-products, high ethanol yield, high fermentation rate, good tolerance toward both increased ethanol and substrate concentrations, and lower pH value. Viability and genetic stability of yeast cells under process condition and at high temperature are also desirable.

At present, bioethanol as a fuel is mostly produced by fermenting plant carbohydrates with yeast. Carbohydrates are grouped as soluble carbohydrates – sugars (e.g. sucrose from sugar cane, sugar sorghum or sugar beet), storage carbohydrates – such as starch from grains and tubers, structural carbohydrates – such as cellulose, hemicellulose and pectin. The principal carbohydrate is starch contained in grain crops – corn, wheat, barley and oats, but also in potato, Jerusalem artichoke, etc. Two major forms of starch exist – amylose and amylopectine (BeMiller, 1996). Amylose is a straight chain polymer of glucose molecules joined by α (1-4) glycosidic bonds (Fig. 2). This primary structure results in long polymers coiling into a helical conformation (BeMiller, 1996).

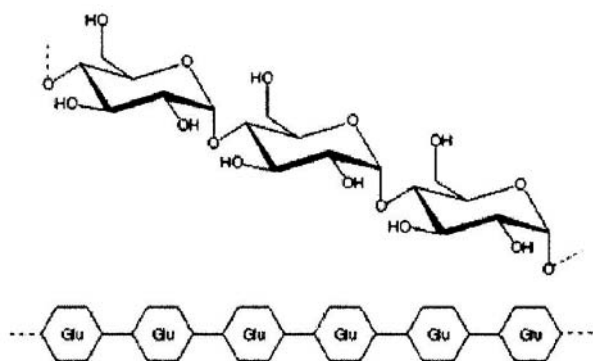


Fig. 2. Structure of amylose (BeMiller, 1996).

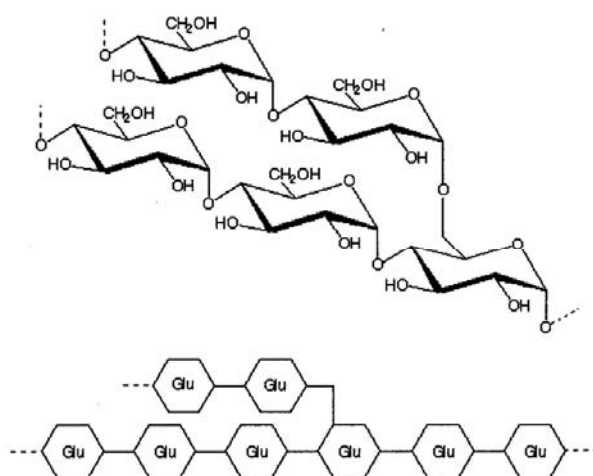
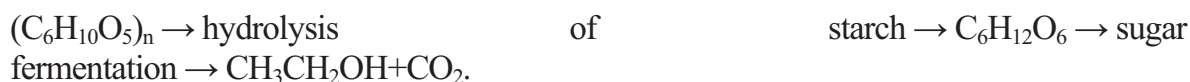


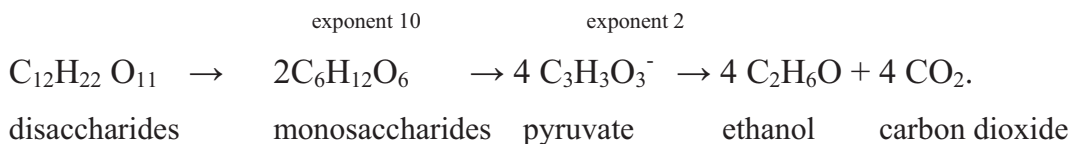
Fig. 3. Structure of amylopectin (BeMiller, 1996).

Amylopectin (Fig. 3) is also primarily a straight chain of glucose molecules joined by α (1-4) glycosidic bonds, but it also includes branches occurring at every 24 to 30 glucose units consisting of α (1-6) bonds. Starch is semi-crystalline and transitions to an amorphous state (a gel) at 60-70°C, through a gelatinization process where water molecules disrupt the hydrogen bonds within and between starch molecules. Starch, especially gelatinized starch, can be easily hydrolyzed to yield individual glucose molecules.

Hydrolysis of starch (polysaccharides) into sugars by enzymatic activity and fermentation into ethanol by yeast enzymes takes place according to the following scheme,



Summary reaction,



Production of ethanol as motor fuel

There are two types of industrially manufactured ethanol fuels for engines, anhydrous and hydrous ethanol fuel (Ullmann, 1990). Anhydrous ethanol is used in the production of Ed75-Ed 85 Automotive Spark-Ignition Engines. These fuels are specified by Standard ASTM D 5798 and E85 in Europe by Swedish SVENSK STANDARD SS 155480,2006 and European Standard CWA 15293,2005.

Anhydrous bioethanol as motor fuel

For producing fuel ethanol from grains, modern industry uses dry-grind process which involves 4 major steps, preparation of the grain (grinding, liquefaction, and saccharification), fermentation of the sugars, recovery of the ethanol, and drying of the ethanol (Fig. 4).

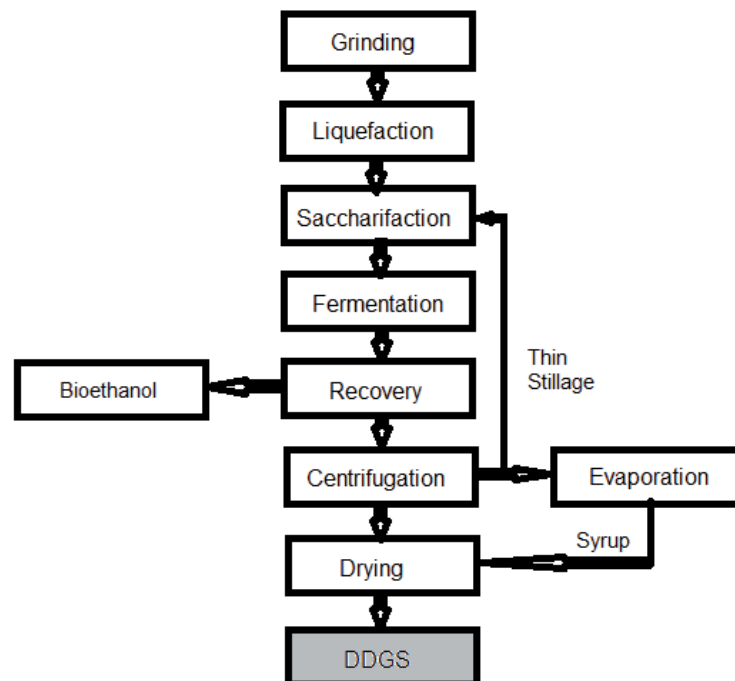


Fig. 4. Technological overview of dry grind bioethanol process (McAloon, 2000).

Preparation of the grain takes 8-12 days (Kwiatkowski, 2006). Broken kernels and foreign materials such as metal, dirt, cobs, etc. are removed. The cleaned grain is then ground in hammer mills fitted with screens, which provide grain particles of a more uniform size so that more than 90% of the ground grain has a diameter of 0.5 to 2mm (Rausch, 2005).

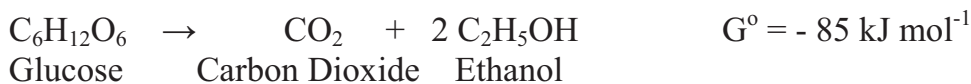
Liquefaction involves combining the ground grain with process water to form slurry which is approximately 30% solids by weight (Kwiatkowski, 2006). Ammonia and lime are added at this step to adjust the pH of the slurry to 6.5. The ammonia, which contains nitrogen, also serves as a nutrient for the yeast. The slurry is heated to 88°C by direct steam injection. Then a thermostable enzyme (α -amylase) is added to cleave the starch molecules at random points along the middle of the polymer chain and to break the starch into smaller water soluble fragments called dextrins. After approximately one hour, the output from the first step of

liquefaction is combined with recycled water from the end of the ethanol distillation process, so that water accounts for approximately 15% of the final volume of the mash (McAloon, 2000). As the liquefied slurry is cooled to 60°C, it is channelled to the jet-cooker (Kwiatkowski, 2006). Using a new enzyme technology developed by *Genencor* allows for the rapid hydrolysis of granular starch and eliminates the need for gelatination of the starch slurry by jet-cooking, thus significantly lowering the energy consumption (Shetty, 2005). After that, during the second stage of liquefaction, H₂SO₄ is added to the slurry to lower the pH to 4.5. An additional enzyme, glucoamylase (also called β -amylase) is added to break the starch and dextrins into glucose via a stepwise hydrolysis of glucose. The slurry is held at 60°C for 5-6 hours as the glucoamylase hydrolyzes the dextrins to fermentable glucose (Schenk, 2002). After saccharification, the slurry (which is now called mash) is cooled to 32°C. The cooled mash then enters the fermentation tanks.

A popular alternative to mash-presaccharification is to add glucoamylase during the filling of the fermentor. In that case the saccharification and fermentation of the starch takes place simultaneously (Power, 2003).

Sugar fermentation

Fermentation under anaerobic conditions uses microorganisms *Saccharomyces cerevisiae* to convert sugars to ethanol. Ethanol production process results in the production of ethanol and CO₂ and heat. One molecule of glucose yields 2 molecules of ethanol and 2 molecules of CO₂. One kilogram of glucose will theoretically produce 0.51 kilogram of ethanol and 0.49 kilogram of CO₂.



In the fermentation step, yeast grown in seed tanks is added to the mash to ferment the simple sugars to ethanol. The other components of the grain kernel (protein, oil, etc.) remain largely unchanged during the fermentation process. E.g. corn oil helps to prevent foaming during the fermentation. Fermentation is a downstream process that occurs continuously.

Fermentation is a continuous process also in case of dry-grind facilities. While continuous fermentation has greater reactor productivity (one fermenting for approximately 46-48 hours) because it is continuously operating with high yeast loads, much more care needs to be exercised to prevent contamination with bacteria, especially species of *Lactobacillus*. These bacteria allow production of organic acids that lower ethanol yields (Graves, 2006), (Bayrock, 2001), (NL514253).

Ethanol production by yeast has high selectivity, low accumulation of by-products, high ethanol yield, and high fermentation rate. Yeasts have to be viable and genetically stable, but also tolerant to high temperatures during the process.

In addition to ethanol, carbon dioxide (CO₂) is also produced during fermentation. Usually, the carbon dioxide is not recovered as a sellable product. If recovered, this carbon dioxide can be cleaned, compressed and sold for carbonation of soft drinks or frozen into dry-ice for cold product storage, for sandblasting in car service and metal industry, etc. If the carbon dioxide is not recovered, it is cleaned and vented to the atmosphere.

Production of hydrated bioethanol as motor fuel

A system has been developed for the production of 85-95% bioethanol by volume with low energetic value as motor fuel. Such a fuel is used in engines that require pure alcohol and not gasoline or gasoline blended fuels. This distillation process is mostly used in Brazil. Hydrated ethanol to be used as motor fuel is called AEHC 'hydrated Ethanol Fuel' (E100) (Orlando Volpato Filho, 2009), relevant specification has been filed (Fuel Ethanol Specifications Brazil, 2008-2010).

Ethanol pre-distillation and post-distillation (fusel oil) fractions are removed similar to traditional technology used in production of dehydrated ethanol. Process is complete when fractional distillation does not reduce water content any more (azeotropic mixture, ca 95.6% by volume). Maximum final water content is 4.9% by volume.

Ethanol dehydration

Industrial dehydration of ethanol is performed by distillation or non-distillation methods. Dehydrating ethanol-H₂O mixture (94.75% by vol.) by using distillation methods is possible when using azeotropic distillation, i.e. by using triple azeotropes. The third component in ethanol water mixture is benzene or toluene (Ullmann, 1990a).

Non-distillation methods allow significant reduction in the need for energy required for removal of water. Most commonly used non-distillation methods in ethanol industry include solvent extraction, carbon dioxide extraction, adsorption method using molecular sieves, and membrane technology (Ullmann, 1990a).

In case of solvent extraction ethanol is dissolved in certain liquids that are practically non-soluble in water. This difference in solubility can be used for recovery of ethanol from water solution by solvent extraction. For instance, solvent extraction is used by Energol Corporation (USA), which has low total energy consumption (3,500-3,700) per kJ kg⁻¹ ethanol.

The University of Pennsylvania and General Electric has developed a process that uses di-butyl phthalate as a water-immiscible solvent for purifying ethanol. This solvent has much higher boiling point than ethanol, and ethanol can therefore be separated in a single distillation step with low losses of solvent.

Extraction with carbon dioxide uses so-called critical liquids, i.e. gases that are compressed to a point where they acquire equilibrium between gas and liquid. Extraction is performed selectively from grain mash with CO₂ at its critical point, 7.3 MPa and 31°C. Ethanol jet is brought to the pressure of ca 4.8 MPa to remove CO₂. CO₂ is removed and ethanol remains in a liquid state. This method requires only 1/3 to 1/2 of the energy of normal distillation, but the expenses of entire process are 20% higher. When using low-cost CO₂ as a by-product of fermentation process, the price drops significantly. This is important because some of the solvents will inevitably leak during ethanol distillation and have to be replaced.

Dehydration by adsorption method uses molecular sieves with pores that allow water to pass through, but retain ethanol. Molecular sieves may be synthetically produced or naturally occurring zeolites (e.g. clinoptilolites) or proprietary resins. The 95 vol. ethanol is dehydrated in molecular sieve columns; 75% of adsorbed material is water, 25% is ethanol. When the column is saturated, the stream is directed to a fresh column and the saturated column is regenerated. The

regeneration stream containing 25 vol. ethanol is fed back to the ethanol distillation system.

Dehydration by membrane technology is based on vaporization of water through membrane module. This is a new method where evaporator consists of several semi-permeable membrane modules made of poly(vinyl alcohol) resins. The 94 vol. ethanol is preheated to 60°C and pumped to the semi-permeable membrane modules of the pervaporator. Water permeates the membrane down its concentration gradient; a phase change occurs from the liquid phase at the membrane inlet to the vapour phase in the permeate. Water is thus separated without azeotrope formation. The driving force for permeate flow is provided by a vacuum of less than 1 kPa at the permeate inlet. The total energy consumption is the sum of the evaporation and the condensation enthalpies.

The condensed permeate contains a small amount of ethanol and can be recycled to a rectifying or distillation tower for recovery of ethanol. A pressure of (4-7) MPa is usually applied to remove the water by forcing it across the membrane. The ethanol retention of new noncellulosic membranes is much higher than that of the cellulose acetate membranes used earlier (80% compared to 50%). Reverse osmosis may prove useful for savings in energy costs by concentrating ethanol to about 10% prior to distillation.

The researchers of Latvian universities (Bremers et al, 2009; LV13691) have experimented with removing water from ethanol by using new methods, as this process is the most energy-intensive stage in ethanol recovery. They have suggested dehydration of bioethanol already during the rectification process as well as performing simultaneous adsorption by supplementing the rectification column with a substance adsorbing water molecules (either laboratory zeolite or Zeolite Sylobead MS564). Combination of rectification and water adsorption allows reducing the number of rectification column beds and the energy spent on removing water by ca 70%.

Production of bioethanol from cellulose biomass

Lignocellulose biomass, including wood waste, agricultural waste, household waste, etc. represents a renewable resource which has stored solar energy in its chemical bonds (McKendry, 2002). It has great potential for bioethanol production, when compared to ethanol produced from grain, tubers and sugar plants, because it is a widely available cheap feedstock which does not compete with human food products. General scheme of producing ethanol from biomass is shown in Fig. 5.

It is known that the main difficulty in converting lignocellulose biomass into second-generation ethanol consists in breaking down structural and chemical biomass complex (Fig. 6). In the course of breakdown process cellulose feedstock is affected by enzymes which allow further recovery of ethanol. Biomass consists of polysaccharides – cellulose and hemicellulose, which are hydrolysed into single sugar components, followed by further recovery of ethanol by well-known and elaborated fermentation technologies. Enzymatic activity in lignocellulose hydrolysis gives a good yield and minimum amount of by-products; it has lower energy consumption, milder operating conditions and represents an environmentally friendly processing method (Saha, 2000; Wingren et al., 2005).

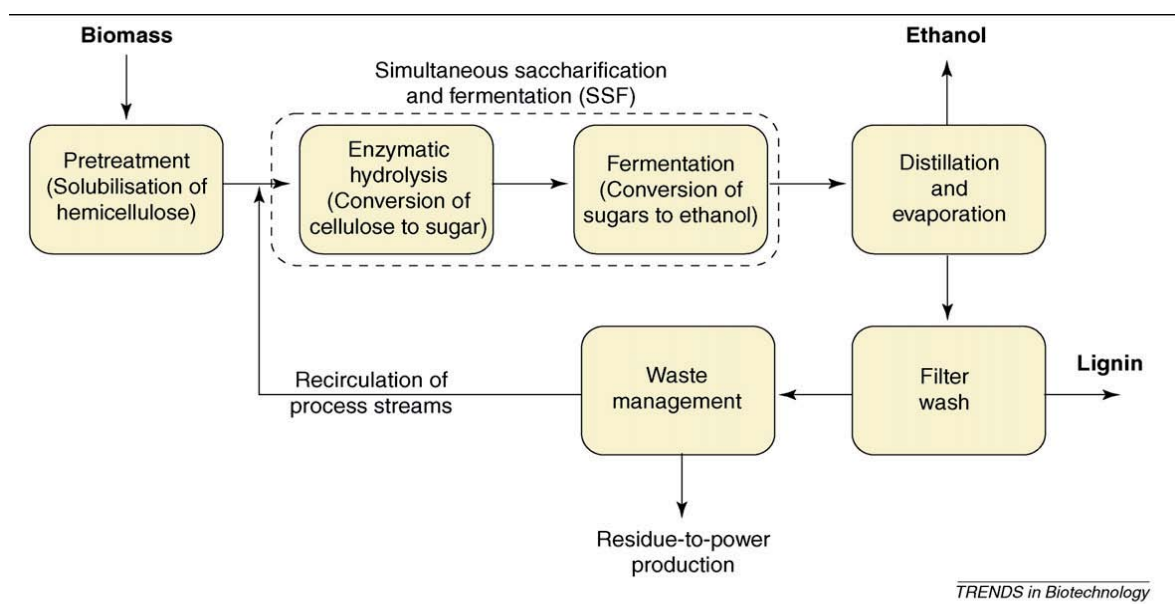


Fig. 5. Scheme for production of ethanol from biomass (Hahn-Hägerdal & Pamment, 2004).

Considering that the sugars required for fermentation are bound to the lignocellulose structure, pre-treatment of biomass is required in order to remove and/or modify lignin and hemicellulose matrix before enzymatic hydrolysis of polysaccharides. Unlike starch which is a crucial source of energy in plants, cellulose has mostly structural role as it provides plant cells with mechanical durability with hemicellulose and lignin. Natural cellulose materials do not have high reactivity; therefore, fermentable saccharification requires large cellulose surface and broken cellulose microfilm structure. Reactivity of natural substrates is also reduced by lignin.

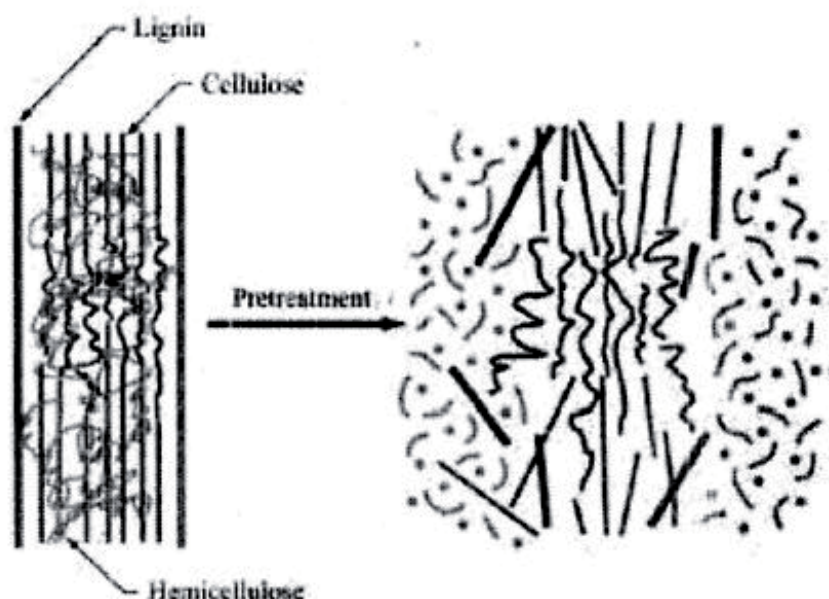


Fig. 6. The structure of lignocellulose material and changes induced by pre-processing (Yi Zheng et al., 2009).

The goals of pre-treatment of lignocellulose feedstock are to preserve hemicellulose, reduce generation of inhibitors and energy cost (National Research Council, 1999). Pre-treatment techniques have generally been divided into three distinct categories, including physical, chemical and biological pre-treatment (Yi Zheng et al., 2009). Physical method consists in steam explosion without catalysts, hot water liquid pre-treatment, mechanical grinding and high energy radiation.

Steam explosion is the most widely used method due to its low cost (McMillian, 1994; Hsu, 1996; Chandra, 2007). Here hemicellulose hydrolysis is performed by using steam and organic acids (Weil et al, 1997). Crucial factors with regard to steam explosion include time, temperature, particle size and humidity (Ballesteros et al., 2008; Negro et al. 2003). Usually the temperature is between 160 and 270°C, processing time ranges from seconds to a couple of minutes. About 90% of the pre-treated mass is subject to further enzymatic activity, whereas relevant percentage in case of non-treated mass is only 15% (Grous et al., 1986). This method provides low hemicellulose yield (Wright, 1988; Excoffien et al., 1991; Heitz et al., 1991).

Hot water pre-treatment is performed with water in liquid state at elevated temperatures (Brandon et al., 2008; Dien et al., 2006; Negro et al., 2003; Rogalinski et al., 2008). This method increases cellulose processing output, extraction of sugars and recovery of pentose, but resulting pre-hydrolysate may contain sugar fermentation inhibitor (Van Walsum et al., 2008). Conversion of lignocellulose material depends on its origin. Pre-hydrolysates rich in sugar can be fermented into ethanol directly. Ca 80% of hemicellulose produced from corn fibre (Allen et al., 2001) and sugar cane bagasse (Laser et al., 2002) can be subject to enzymatic process. Wheat straw sugar yields 53% and enzymatic hydrolysis yields 96% (Perez et al., 2007).

The most efficient but also the most expensive and energy-intensive method is mechanical comminution of lignocellulose feedstock. Biomass material can be comminuted by various chipping, grinding and milling. The milling can be further detailed into hammer and ball-milling (wet, dry, and vibratory rod/ball milling) (Rivers et al., 1987; Yoshida et al., 2008), compression milling (Ryu et al., 1982), (Tassinari et al., 1977), pan milling (Zhang et al, 2007), etc. Mechanical processing disrupts cellulose structure, reduces polymerization level, increases special surface of cellulose biomass when biomass is broken down to smaller particles.

Chemical alkali-based methods are used in order to reduce the price of pre-treatment (Abdi, et al., 2000, Carrillo et al., 2005, Pinto et al., 1996; Silverstein et al., 2007); physical methods include γ -radiation (Yang et al., 2008) and (Youssef & Aziz, 1999) dilution with sulphuric acid (Ballesteros et al., 2008; Martin et al., 2007; Marzioletty et al., 2008; Sun et al., 2005; Zheng et al., 2007; Zheng et al., 2008), and other diluted acids such as diluted HNO_3 (US5221357, US5366558), HCl (Mehlberg, 1979, Herrera et al., 2003), H_3PO_4 (Israilides et al., 1978), (Um et al., 2003; Vazquez et al., 2007). But basic chemical pre-treatments include concentrated HCl and H_2SO_4 (Goldstein et al., 1983; Vedernikov et al., 1991), in order to produce fermentable sugars.

Biological pre-treatment uses microorganisms for decomposing wood, the effect of white and brown soft-rot fungi and bacteria in order to modify the

chemical composition and/or structure of lignocellulose biomass so that it could be subjected to processing with enzymes (Kurakake, 2007; Lee, 2007, (Singh, 2007). Brown soft-rot fungi have greater impact on cellulose and less on lignin, whereas white soft-rot fungi have greater impact on the lignin component (Schurz, 1978). Zheng et al. have studied (Zheng, 2009) organisms that are more efficient in decomposing lignin.

Research on biomass pre-treatment for the purpose of breaking down lignocellulose feedstock has continued for years, but none of the results have been introduced in industrial application of ethanol production due to low economical profitability. Only a few pre-treatment methods such as diluted acid and steam explosion have been tested with success. Until this day the best-known practice in the world is sample production of ethanol from cellulose in relevant Iogen Corporation facilities in Canada. Iogen Corporation uses modified steam explosion pre-treatment of the feedstock in order to enhance enzymatic cellulose ethanol production at the yield of 340 l/t per fibre (Iogen Corporation, 2010).

These days the factory cost of ethanol production from cellulose still exceeds the production cost of grain ethanol by 2.5-4 times. In June 2006 the price of bioethanol made of lignocellulose was 0.59 USD/l in the United States of America. The United States have set a goal of producing bioethanol from lignocellulose at the price of 0.28 USD/l by 2012 (Solomon, 2007).

Already in 2008 Japanese researchers reported on the successful completion of the programme for producing ethanol from cellulose by using only microorganisms. Until then chemicals, such as H_2SO_4 , etc. had been used for cellulose decomposition to glucose. They have prepared large quantities of bacteria for that purpose. In that case there is no need for expensive special lignocellulose biomass pre-treatment. This is the most prospective modern tendency in industrial production of ethanol, as it would be the cheapest production method in the future (as it does not require chemicals or large amounts of water, it is less polluting and provides maximum yield when producing ethanol from carbohydrates. Bacteria prevent a problematic situation that might occur when using fungi. Several authors have analysed the problems arising from soft-rot fungi, because the use of fungi leads to generation of inhibitors that hinder decomposition of carbohydrates during the pre-treatment process. Generation of inhibitors is impossible when using bacteria.

The bacterium *Escherichia coli* KO11 has been used in hydrolysis of sugar cane bagasse (Hahn-Hägerdal, 2004), hemicellulose corn fibres (O'Brien, 2004) and *E. coli* FBR5 corn cobs (Hahn-Hägerdal, 2004), and rice kernels (Saha, 2005). Patent No. WO2009008206 registered by Japanese researchers Nanba Hiromi and Tanaka Hidehiko allows conversion of cellulose material by using *koji mold* bacteria from rice malt.

Bioethanol from algae

Production of motor fuel from algae has been subject to research for decades. Now there is an opportunity to produce bioethanol simultaneous to the third-generation biofuel – algae diesel (*Oilgae*) (WO2010006228, WO2009154437, US2009298159, CN191580857, etc). The production scheme is shown in Fig. 7. Carbohydrates in algae oil can still be converted to starch.

For years Japanese researchers have studied the saccharification of sea algae by marine bacteria (Mitsufumi Matsumoto, 2003), (Tadashi Matsunaga, 2009). There are several new patents on recovery of starch from algae, US2009075353, US2010041926, JP55011317, JP1023001, WO2009125037, etc.

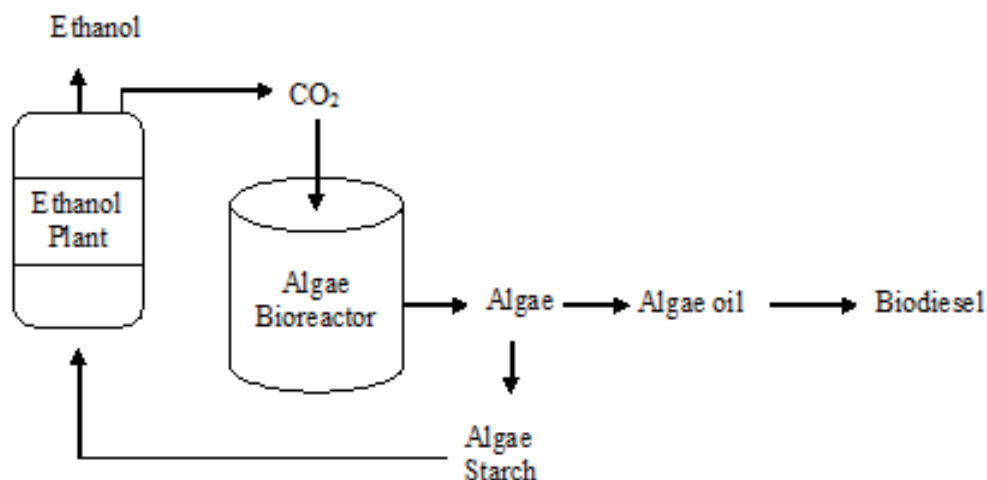


Fig. 7. Technological scheme for producing biodiesel and ethanol from algae <http://www.oilgae.com/algae/pro/eth/eth.html>.

CONCLUSIONS

This present study provides an overview of potential methods for industrial ethanol production. The main attention is paid to carbohydrates as technologies for producing bioethanol are based on renewable resources. It comprises both the potential recovery of dehydrated bioethanol and hydrated bioethanol for the purpose of using them as motor fuels. In addition to sugar and starch based and lignocellulose feedstock used for producing ethanol, the authors have also pointed out the most recent tendencies of the last decade.

These include,

- 1) Production of ethanol from cellulose by using bacteria; this method allows exclusion of energy-intensive pre-treatment of feedstock and combine hydrolysis and sugar fermentation process,
- 2) Algae oil and ethanol recovery by using gene modification and nanotechnology.

Institute of Technology of Estonian University of Life Sciences continues activities for gaining knowhow for producing bioethanol in farm environment at optimum price as well as for its use in internal combustion engine.

REFERENCES

- Abdi, N., Hamadache, F., Belhocine, D., *et al.* 2000. Enzymatic saccarification of solid residue of olive mill in a batch reactor. *Biochem. Eng. J.*, **6**, 77-183.
- Aden, A., *et al.* 2002. Lignocellulosic biomass to ethanol processing design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. National Renewable Energy Laboratory Technical Report NREL/TP-510-32438 (<http://www.nrel.gov/docs/fy02osti/32438.pdf>).

- Agarwal, A. K. 2007. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. *Progress in Energy and Combustion Science*, **33**(3), 233-271.
- Albrecht, A., Grondin, O., Le Berr, F., Le Sollic, G. 2007. Towards a stronger simulation support for engine control design, a methodological point of view, *Oil & Gas Science and Technology – Rev. IFB*, **62**(4), 437-456.
- Al-Hasan, M. I. & Al-Momany, M. 2008. The effect of iso-butanol-diesel blends on engine performance. *Transport*, **23**(4), 306-310.
- Allen, S. G., Schulman, D., Lichwa, J., *et al.* 2001. A comparison between hot liquid water and steam fractionation of corn fiber. *Ind. Eng. Chem. Res.*, **40**, 2934-2941.
- Arsie, I., Pianese, C., Sorrentino, M. 2007. Effects of control parameters on performance and emissions of HSDI diesel engines, investigation via two zone model *Oil & Gas Science and Technology – Rev. IFB*, **62**(4), 457-469.
- Ballesteros, I., Ballesteros, M., Manzanares, P., *et al.* 2008. Dilute sulfuric acid pretreatment of cardoon for ethanol production. *Biochem. Eng. J.*, **42**, 84-91.
- Bayrok, D. & Ingledew, W.M. 2001. Changes in steady state on introduction of Lactobacillus contaminant to a continuous culture ethanol fermentation. *Journal of Industrial Microbiology and Biotechnology*, **27** (1), 39-45.
- BeMiller, J. N. & Whistler, R. L. 1996. Carbohydrates. In Food Chemistry (3rd ed., 157-223). New York; New York, Marcel Deker.
(http://www.bioenergybaltic.ee/bw_client_files/bioenergybaltic/public/img/File/Lep7_028WFinalB.pdf).
- Boychenko, S., Shkilnuk, I., Turchak, V. 2008. The problems of biopollution with jet fuels and the way of achieving solution. *Transport*, **23**(3), 253-257.
- Brandon, S. K., Eiteman, M. A., Patel, K., *at al.* 2008. Hydrolysis of Tifton 85 bermudagrass in a pressurized batch hot water reactor. *J. Technol. Biotechnol.*, **83**, 505-512.
- Bremers, G., Birzietis, G., Blija, A., Škele, A., Danilevics, A. 2009. Bioethanol congruent dehydration. In, *Proceedings of the 8th International Scientific Conference 'Engineering for Rural Development'*. Latvia University of Agriculture, Jelgava, **376**, 148-155.
- Butkus, A., Pukalskas, S., Bogdanovicius, Z. 2007. The influence of turpentine additive on the ecological parameters of diesel engines, *Transport*, **22**(2), 80-82.
- Carillo, F., Lis, M. J., Colom, X., *et al.* 2005. Effect of alkali pretreatment on cellulose hydrolysis of wheat straw, Kinetic study., **40**, 3360-3364.
- Chandra, R. P., Bura, R., Mabey, W. E., *et al.* 2007. Substrate pretreatment, the key to effective enzymatic hydrolysis of lignocellulosics. *Adv. Biochem. Engin/ Biotechnol.*, **108**, 67-93.
- Demirbas, A. 2006. Global biofuel strategies. *Energy Edu. Sci. Technol.*, **17**, 27-63.
- Demirbas, A. 2009. Biohydrogen, For Future Engine Fuel Demands. In Chapter 3. *Biofuels*, pp. 61-84. Springer Verlag.
- Dien, B. S., Li, X. L., Iken, L. B., *at al.* 2006. Enzymatic saccharification of hot-water pretreated corn fiber for production of monosaccharides. *Enzyme Microb. Technol.*, **39**, 1137-1144.
- Dukulis, I., Pirs, V., Jesko, Z., Birkavs, A., Birzietis, G. 2009. Development of Methodics for Testing Automobiles Operating on Biofuels. In, *Proceedings of the 8th International Scientific Conference 'Engineering for Rural Development'*. Latvia University of Agriculture, Jelgava, **376**, 148-155.
- European Standard CWA 15293, 2009. *Automotive fuels - Ethanol E85 - Requirements and test methods*.
- Excoffier, G., Toussaint, B., Vignon, M. R. 1991. *Biotech. Bioeng.*, **28**, 792-801.

- Fuel Ethanol Specifications Brazil. 2008-2010. National Department of Fuels Technical Regulation DNC - 01/91. Specifications for Anhydrous Fuel Ethanol ("AEAC") and Hydrous Fuel Ethanol "AEHC"). http://www.fuel4life-biofuels.com/specifications_fuel_ethanol_hydrous_and_anhydrous.pdf
- Goldstein, I. R., Pereira, H., Pittman, L. J., *et al.* 1983. The hydrolysis of cellulose with superconcentrated hydrochloric acid. *Biotech. Bioeng. Symp.*, **13**, 17-25.
- Gardner, K. H. & Blackwell, J. 1974. The structure of Native cellulose. *Biopolymers*, **13**, 1975-2001.
- Gorianoff, W., A. Butlerov, A. 1876. *Justus Liebigs Ann. Chem.*, **180**, 245.
- Graves, T., Narendranath, N. V., Dawson, K., Power, R. 2006. Effect of pH and lactic or acetic acid on ethanol productivity by *Saccharomyces cerevisiae* in corn mash. *Journal of Industrial Microbiology and Biotechnology*, **33**(6), 469-474.
- Grous, W. R., Converse, A. O., Grethlein, H. E. 1986. Effect on steam explosion pretreatment on pore size and enzymatic hydrolysis of poplar. *Enzyme Microb. Technol.*, **8**, 274-280.
- Hahn-Hägerdal, B. & Pamment, N. 2004. Microbial pentose metabolism. *Appl. Biochem. Biotechnol.*, **113-116**, 1207-1209.
- Harris, H. G. & Himmelblau, D. M. 1964. Kinetics of the Reaction of ethylene with Sulfuric Acid. *J. Chem. Eng. Data*, **9**, 61-65.
- Heitz, M., Capek-Menard, E., Koeberle, P. G., *et al.* 1991. Fractionation of *Populus tremuloides* in the pilot plant scale, optimization of steam pretreatment conditions using STAKE II technology. *Bioresour. Technol.*, **35**, 23-32.
- Herrera, A., Tellez-Luiz, S. J., Ramirez, J. A., *et al.* 2003. Production of xylose from sorghum straw using hydrochloric acid. *J. Cereal Sci.*, **37**, 267-274.
- Hsu, T. A. 1996. Pretreatment of biomass. In, Wyman, C. E. (Ed.), editor. Handbook on Bioethanol, production and utilization. Washington, DC, Taylor & Francis, 179-212.
- Iogen Corporation. Iogen's Cellulosic Ethanol Demonstration Plant. http://www.iogenenzymes.com/company/demo_plant/index.html
- Israilides, C. J., Grant, G. A., Han, Y. W. 1978. Sugar level, fermentability, and acceptability of straw treated with different acids. *Appl. Environ. Microbiol.*, **36**, 43-46.
- Jeuland, N., Montage, X., Gautrot, X. 2004. Potential of ethanol as a fuel for dedicated engine, *Oil & Gas Science and Technology – Rev. IFB*, **62**(4), 559-570.
- Kurakake, M., Ide, N., Komaki, T. 2007. Biological pretreatment with two bacterial strains for enzymatic hydrolysis of Office paper. *Curr. Microbiol.*, **54**, 424-428.
- Kwiatkowski, J. R., McAloon, A. J., Taylor, F., Johnston, D. B. 2006. Modeling the Process and Costs of Fuel Ethanol Production by the Corn Dry-Grind Process. *Industrial Crops and Products*, **23**, 288-296.
- Laser, M., Schulman, D., Lichwa, J., *et al.* 2002. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for conversion to ethanol. *Bioresour. Technol.*, **81**, 33-44.
- Lebedevas, S. & Lebedeva, G. 2009. The problems of using alcohol biofuel mixtures in the Lithuanian transport system. *Transport*, **24**(1), 58-65.
- Lebedevas, S., Vaicekauskas, A., Suškov, P. 2007a. Presumptions of effective operation of diesel engines running on RME biodiesel. Research on kinetics of combustion of RME biodiesel. *Transport*, **22**(2), 126-133.
- Lebedevas, S., Vaicekauskas, A., Lebedeva, G., Makareviciene, V., Janulis, P. 2007b. Change in operational characteristics of diesel engines running on RME biodiesel fuel. *Energy and Fuels*, **21**(5), 3010-3016.
- Lee, J. W., Gwack, K. S., Park, J. Y., *et al.* 2007. Biological pretreatment of softwood *Pinus densiflora* by three white rot fungi. *J. Microbiol.*, **45**, 485-491.

- Li, D.-G., Zhen, H., Xingcai, L., Wu-Gao, Z., Jian-Guang, Y. 2005. Physico-chemical properties of ethanol-diesel blend fuel and its effect on performance and emissions of diesel engines. *Renewable Energy*, **30**(6), 967-976.
- Martin, C., Alriksson, B., Sjode, A. *et al.*, 2007. Dilute sulfuric acid pretreatment of agricultural and agro- industrial residues for ethanol production. *Appl. Biochem. Biotechnol*, **137**, 339-352.
- Marzaletti, T., Olarte, M. B. V., Sievers, C., *et al.* 2008. Dilute acid hydrolysis of Loblolly pine, A comprehensive approach. *Ind. Eng. Chem. Res.*, **47**, 7131-7140.
- McAloon, A., Taylor, F., Yee, W., Ibsen, K., Woolay, R. 2000. Determining the cost of Producing ethanol from corn strach and lignocellulosic feedstocks. NREL/TP-580-288893.
- McKendry, P. 2002. Overview of Biomass. Energy Production from Biomass (Part 1) . *Bioresour. Technol.*, **83**(1), 37-46.
- McMillian, J. D. 1994. Pretreatment of lignocellulosic biomass. In, Himmel, M.E.; Baker, J. Q.;
- Mitsufumi Matsumoto, Hideaki Miyashita, Tadashi Matsunaga. 2003. Saccharification of marine microalgal using marine bacteria for ethanol production. *Appl. Biochem. Biotechnology*, **105**, 247-254.
- Mehlberg, R. L., Tsao, G. T. 1979. Low liquid hemicellulose hydrolysis of hydrochloric acid. Presented at the 178th ACS National Meeting Washington, DC.
- National Research Council. 1999. Committee on Biobased Industrial Products-Priorities for Research and Commercialization. National, Academy Press, 1999.
- Negro, M. J., Manzanares, P., Oliva, J. M., *at al.* 2003. Changes in various physical/chemicals parameters of *Pinus pinaster* wood after steam explosion pretreatment. *Biomass Bioenergy*, **25**, 301-308.
- Negro, M. J., Manzanares, P., Ballesteros, I., *at al.* 2003. Hydrothermal pretreatment conditions to enhance ethanol production from popular biomass. *Appl. Biochem. Biotechnol.*, **105**, 87-100.
- Offeman, R. D., Stephenson, S. K., Robertson, G. H., Orts, W. J. 2005. Solvent Extraction of Ethanol from Aqueous Solutions. I. Screening Methodology for Solvents. *Ind. Eng. Chem. Res.* **44** (17), 6789–6796.
- O'Brien, D. J. *et al.* 2004. Ethanol recovery from corn fiber hydrolysate fermentations by pervaporation. *Bioresour. Technol.* **92**, 15–19.
- Oilgae. Com., 2009. <http://www.oilgae.com/algae/pro/eth/eth.html>
- Olt, J., Mikita, V., Södro, T., Tamm, R., Raidla, E., Küüt, A., Ritslaid, K. 2009. Mixtures of bioethanol and gasoline as otto motor fuel. Rouse, 01-03.2009. Energy efficiency and agricultural engineering., **920**, 591-600.
- Orlando Volpato Filho. 2009. Gasoline C made with hydrous ethanol. Delphi South America Technical Center – Brazil.
http://www.epiphergy.com/uploads/Brazil_Hydrous_Ethanol.pdf
- Overend, R. P. (Ed.), editors. Enzymatic Conversion of Biomass for Fuels Production. Washington, DC, ACS Symp. Ser. ACS, 1994, **566**, 292-324.
- Perez, J. A., Gonzalez, A., Olivia, J. M., *et al.* 2007. Effect of process variables on liquid hot water pretreatment of wheat straw for bioconversion to fuel-ethanol in a batch reactor. *J. Chem. Technol. Biotechnol.*, **82**, 929-938.
- Petrović, J., Ivković, Vujačić I., Žeželj, S. 2009. Possabilities of buses on alternative fuel in public urban transport in Belgrade, *Technological and Economic Development of Economy*, **15**(1), 78-89.
- Pinto, J. H. & Kamden, D. P. 1996. Comparison of pretreatment methods on the enzymatic saccarification of aspen wood. *Appl. Biochem. Biotechnol.*, **61**, 289-297.

- Power, R. F. 2003. Enzymatic conversion of starch to fermentable sugars. In Jacques, K. A., Lyons, T. P., Kelsall, D. R. (Eds.), *The Alcohol Textbook*, 4th Edition (pp. 23-32). Bath, England, Nottingham University Press.
- Raslavičius, L. & Markšaitis, D. 2007. Research into three-component biodiesel fuel combustion process using a single droplet technique, *Transport*, **24**(4), 312-315.
- Raslavičius, L. & Bazaras, Ž. 2009. The analysis of the motor characteristics of D-RME-E fuel blend during on-field test. *Transport*, **24**(3), 187-191.
- Rausch, K. D., Belyea, R. L., Ellersieck, M. R., Singh, V., Johnston, D. B., Tumbleson, M. E. 2005. Particle size distributions of ground corn and DDGS from dry grind processing. *Transactions of the Asae*, **48**(1), 273-277.
- Rivers, D. B. & Emert, G. H. 1987. Lignocellulose pretreatment, a comparison of wet and dry ball attrition. *Biotech. Lett.*, **9**, 365-368.
- Rogalinski, T., Ingram, T., Brunner, G. J. 2008. Hydrolysis of lignocellulosic biomass in water under elevated temperatures and pressures. *J. Supercrit. Fluids*, **47**, 54-63.
- Ryu, D. D., Lee, S. B., Tassinari, T., *et al.* 1982. Effect of compression milling on cellulose structure and enzymatic hydrolysis kinetics. *Biotech. Bioeng.*, **24**, 1047-1067.
- Saha, B. C. 2000. Alpha-L-Arabinofuranosidases, biochemistry, molecular biology and application in biotechnology. *Biotechnol. Adv.*, **18**(5), 403-423.
- Saha, B. B. *et al.* 2005. Dilute acid pretreatment, enzymatic saccharification, and fermentation of rice hulls to ethanol. *Biotechnol. Prog.*, **21**, 816-822.
- Schurz, J. 1978. Bioconversion of Cellulosic Substances into Energy. Chemicals and Microbial Protein Symp. Proc., Ghose, T. K., Ed. New Delhi, IIT, Delhi, pp.37.
- Shetty, J. K., Lantero, O. J., Dunn-Coleman, N. 2005. Technological advances in ethanol production. *International Sugar Journal*, **107**(1283), 605.
- Silverstein, R. A., Chen, Y., Sharma-Shivappa, R. R., *et al.* 2007. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresour. Technol.*, **98**, 3000-3011.
- Sims, R., Taylor, M., Saddler, J., Mabee, W. 2008. From 1st- to 2nd- generation biofuel Technologies- Full report- An overview of current industry and RD& Activities. International Energy Agency, Nov. 2008.
<http://www.economicsclimatechange.com/2009/01/from-1st-to-2nd-generation-biofuel.html>
- Singh, P., Suman, A., Tiwari, P , *et al.* 2008. Biological pretreatment of sugarcane trash for its conversion to fermentable sugars. *Word J. Microbiol. Biotechnol.*, **24**, 667-673.
- Solomon, B. D., Barnes, J. R., Halvorsen, K. E. 2007. Grain and cellulosic ethanol, History, economics, and energy policy. *Biomass and Bioenergy*, **31**(6), 416-425.
- Sun, Y., Cheng, J. J., *et al.* 2005. Dilute acid pretreatment of rye straw and bermudagrass for ethanol production. *Bioresour. Technol.*, **96**, 1599-1606.
- Specifications Brazilian fuel ethanol. National Department of Fuels. Technical Regulation DNC - 01/91 Specifications for Anhydrous Fuel Ethanol ("AEAC") and Hydrous Fuel Ethanol ("AEHC"). http://www.fuel4life-biofuels.com/Specifications_Brazilian_fuel_grade_ethanol_hydrous_and_anhydrous_2010_Fuel4Life.pdf
- Tadashi Matsunaga. 2009. Marine microalgae. *Biotechnol. Bioeng.*, **95**, 22-28.
- Tassinari, T. & Macy, C. 1977. Differential speed two roll mill pretreatment of cellulosic materials for enzymatic hydrolysis. *Biotech. Bioeng.*, **19**, 1321-1330.
- Teeri, T. T. 1997. Crystalline cellulose degradation, new insight into the function of cellobiohydrolases. *Tibtech*. **15**, 160-167.
- Thirouard, B. & Cherel. 2006. Nature of CAI combustion and air/fuel ratio stratification effects. *Oil & Gas Science and Technology- Rev. IFP*, **61**(1), 95-119.

- Török, A. 2009. Theoretical estimation of the environmental impact of biofuel mixtures. *Transport*, **24**(1), 26-29.
- Ullmann's Encyclopedia of Industrial Chemistry. Ethanol. 1990a, **A9**.
- Ullmann's Encyclopedia of Industrial Chemistry. Ethanol. 1990b, **A1**.
- Um, B. H., Karim, M. N., Henk, L. L. 2003. Effect of sulfuric and phosphoric acid pretreatments on enzymatic hydrolysis of corn stover. *Appl. Biochem. Biotechnol.*, **105**, 115-125.
- Van Walsum, G. P., Allen, S. G., Spenser, M. J., *et al.* 1996. Conversion of lignocellulosics pretreated with liquid hot water to ethanol. *Appl. Biochem. Biotechnol.*, **57/58**, 157-170.
- Vazquez, M., Oliva, M., Tellez-Luiz, S. J., *et al.* 2007. Hydrolysis of sorghum straw using phosphoric acid, evaluation of furfural production. *Bioresour. Technol.*, **98**, 3053-3060.
- Vedernikov, N., Karlivans, V., Roze, I., *et al.* 1991. Mechanochemical destruction of plant materials-polysaccharides in presence of small amounts of concentrated sulfuric-acid. *Sibirskii Khimicheskii Zhurnal*, **5**, 67-72.
- Weil, J. R., Sarikaya, A., Rau, S-L., *et al.* 1997. Pretreatment of yellow poplar sadust by pressure cooking in water. *Appl. Biochem. Biotechnol.*, **68**, 21-40.
- Wingren, A., Galbe, M. Roslander, C., *at al.* 2005. Effect of reduction in yeast and enzyme concentrations in a simultaneous- saccharification-and-fermentation-based bioethanol process. *Appl Biochem Biotechnol.*, **122**, 485-499.
- Wright, J. D. Ethanol from biomass by enzymatic hydrolysis. *Chem. Eng. Prog.*, **84**, 62-74.
- Yoshida, M., Liu, Y., Uchida, S., *et al.* 2008. Effects of cellulose crystallinity, hemicellulose, and lignin on the enzymatic hydrolysis of *Miscanthus sinensis* to monosaccharides. *Biosci. Biotechnol. Biochem.*, **72**, 805,810.
- Zhang, W., Liang, M., Lu, C. 2007. Morphological and structural development of hardwood cellulose during mechanochemical pretreatment in solid state through pan milling. *Cellulose*, **14**, 447-456.
- Zheng, Y., Zhang, R. H., Pan, Z., *et al.* 2007. Evaluation of different biomass materials as feedstock for fermentable sugar production. *Appl. Biochem. Biotechnol.*, **136/140**, 423-435.
- Zheng, Y., Pan, Z., Zhang, R. H., *et al.* 2008. Non-ionic surfacants and non-catalytic protein treatment on enzymatic hydrolysis of pretreated Creeping Wild Ryegrass. *Appl. Biochem. Biotechnol.*, **146**, 231-248.
- Yang, C. P., Shen, Z. Q., Yu, G. C., *et al.* 2008. Effect and aftereffect of gamma radiation pretreatment on enzymatic hydrolysis of wheat straw. *Bioresour Tehnol.*, **99**, 6240-6245.
- Yi Zheng, Zhongli Pan, Ruihong Zhang. 2009. Overview of biomass pretreatment for cellulosic ethanol production. *Int. J. Agric.Biol. Eng.*, **3**(2), 51-68.
- Youssef, B. M., Aziz, N. H. 1999. Influence of gamma-irradiationon the bioconversion of rice srtaw by *Trichoderma viride* into single cell proteiin. *Cytobios*, **97**, 171-183.