BIOETHANOL AS A FUEL TO REDUCE THE GREENHOUSE EFFECT

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Abstract. In the review with 208 references the greenhouse effect and the pollution problems from the exhaust gases of petrol (gasoline) engines are discussed. It is shown that ethanol is currently the only alternative renewable biofuel that has achieved noticeable market success, particularly in Brazil and to an extent as petrol blends in the USA. Investigations on gene engineering, process and apparatus developing, sources base enlargement, etc., carried out in many countries, especially in the USA, to produce bioethanol as a fuel economically are reported. It is shown that the production of bioethanol fuel is important for every country, because its main source will be agricultural, forestry, and municipal cellulosic wastes, which should be processed on the spot to avoid large transport costs.

Key words: ethanol, biofuel, biomass, greenhouse effect (GHE), air pollutants.

INTRODUCTION

Global warming from the increase in the amount of "greenhouse gases", mainly carbon dioxide, has become a major scientific and political issue during the past twenty years. It has been established that the atmospheric carbon dioxide level has increased by about 25% since 1850. Consequently, infrared radiation is being trapped in the atmosphere, producing a global warming or so-called greenhouse effect (GHE). Climatic models suggest that global average surface temperatures will increase by 2–6 °C during the first half of the next century and the sea level will rise by 0.5 to 1.5 m [1], which could lead to unpredictable environmental and economic effects.

The main cause of the GHE is the carbon dioxide formed in the process of burning non-renewable fossil fuels (coal, petrol, natural gas). Thus, it would be possible to reduce the GHE by using alternative fuels, such as liquid fuels

produced from plant biomass. The photosynthetic process utilizes carbon dioxide from air to synthesize polysaccharides (starch, cellulose, hemicellulose, sugars, etc.) for the plant biomass. The main pollutant in the process of burning liquid fuels produced from biomass (such as ethanol) is also carbon dioxide. Thus, by using biofuels, a part of carbon dioxide is constantly kept in the circulation, reducing its excess formation and accumulation in the atmosphere.

USE OF BIOETHANOL AS AN ALTERNATIVE FUEL IN DIFFERENT COUNTRIES

Bioethanol as a fuel became important only in the 1970s in connection with the world energy (petrol) crisis. Today, in spite of the relief of the crisis, bioethanol as a fuel continues to be actual, particularly in connection with the danger of the GHE. The biofuel programmes have nowadays a world-wide distribution extending from Europe [2], including Finland [3–6], France [7], Italy [8], Sweden [9–11], Germany [12, 13], Romania [14], Poland [15–18], Hungary [19], Denmark [20, 21] etc., to Canada [22–25], Australia [26–28], New Zealand [29], South Africa [30, 31], Japan [32–34], China [35], South Korea [36], Indonesia [37], Jordan [38], India [39–42], Turkey [43], Malaysia [44, 45], etc.

In 1975, Brazil was the first country where a governmental programmes was established to make ethanol a primary transportation fuel for otto-motor vehicles – the ProAlcool Program [46]. This programme has achieved a great technical success. During the 1980s, most of the new vehicles (around 90%) were ethanol fuelled and the alcohol fleet reached almost 5 million vehicles [47, 48]. Currently, Brazil is going towards the ProAlcool Program II [49].

In 1980, the Japanese Government promoted the establishment of the Research Association for Petroleum Alternatives Development, which included a group for biomass conversion and utilization. The main cause of this governmental programmes was the oil crisis of the 1970s, but also the environmental pollution problems, mainly the GHE [33]. The construction of an integrated bench-plant for ethanol fuel production from cellulosic biomass was started in 1983. This technology included the pretreatment of biomass, saccharification, enzyme recovery, sugar concentration, fermentation by continuous process with immobilized yeast cells, and ethanol concentration and dehydration by supercritical fluid extraction with carbon dioxide. Other dehydration units such as pervaporation and azeotropic distillation were also constructed for comparison.

In the USA, the National Renewable Energy Laboratory [50, 51], the National Fuels Laboratory [52], and many universities work hard on the problems of alternative fuels, mainly on ethanol-based fuels. The petrol–ethanol blend marketed as gasohol (90% gasoline + 10% alcohol) was worked out and its production began. Already in 1971, a review on the use of ethanol as a blending material to extend motor petrol was published [53]. Since then, many reviews have been published on the new technologies in the field of alcohol fuels,

including the different blends synthesis, the processes used, properties, engine-performance evaluations, economics, safety measures, pollution effects, combustion studies, and studies of sources for alcohol fuels [54–72]. Numerous scientific investigations are currently carried out to cut the production costs of ethanol from agricultural products and waste [73, 74]. In 1994, a historical, technological, and economical review on renewable transportation fuels in the United States was published by Sheehan [75]. It has been prognosticated that commercial use of ethanol will start after the year 2000. The potential sources for bioethanol production in the United States are considered to be corn, wood, and cellulosic agricultural, wood, and municipal wastes [75]. The corn ethanol industry in the USA can at best supply roughly half of the current gasohol market. As a net result, this would represent less than 5% of the total petrol market. Based on the calculations for the year 2010, the resource of cellulosic waste (agricultural, forestry, municipal solid wastes) could cover the total light duty vehicle energy demand in the USA by over 110% [75].

Calculations have shown that if fuel ethanol production from biomass was organized in all countries it would be possible to solve the problem of carbon dioxide pollution and reduce the GHE significantly.

USE OF ETHANOL–PETROL BLENDS AS FUEL

A blend of 10% ethanol with 90% petrol, marketed as gasohol, can actually be burned without the modification of current engines [76].

Several investigations have shown that using ethanol or ethanol–petrol blends as fuels in otto-motors increases the octane number and thermal efficiency of the fuel, but decreases its calorific value. With the increase of ethanol concentration in the petrol, the concentration of carbon oxide (CO), NO_x, and hydrocarbons in the exhaust gases decreases [77]. Thus, an exhaust gas from a $85:15$ petrol:ethanol mixture has been shown to contain 70 ppm hydrocarbons and 0.6% CO. In comparison, the fuel containing no additive contained 150 ppm of hydrocarbons and 1.8% CO. Also, the nitric oxide content in the exhaust gases was 40% of that observed in the absence of the ethanol additive [78]. A high ethanol content in the petrol mixture leads to a decrease in CO, hydrocarbons, and NO_x content in the motor exhaust gases. For example, Suzuki Motor Co Ltd has patented an alcohol and petrol mixture with a high ethanol content (80%). This mixture was tested in a motorcycle, and the emissions were: CO ~8, hydrocarbons 4.2, and N-oxides 0.01 g/km. In comparison, the numbers for a petrol without ethanol additive were ~10, 7.2, and 0.025, respectively [79].

The importance of ethanol as a new petrol component has been demonstrated in Poland [16]. In the carburettor engines, CO emission decreased by 25–35% when they were fuelled with petrol containing 4–8 volume % of ethanol, and N-oxides and hydrocarbon emissions dropped by 5–10%. For cars with fuel-injected engines, CO emission decreased on an average by 20% [15]. In Spain it

was found that when using blends of petrol with 15% ethanol, carbon dioxide emission decreased by 23.6% [80]. In the field study in Albuquerque, New Mexico, in the winters of 1994 and 1995, gasohol was used to reduce the pollutants content in the air. However, the peroxyacetal nitrate content in the air for the winters of 1994 and 1995 was reported to be 0.4 and 0.2 ppb, respectively, while in the summer of 1993, when gasohol was not used, it was 0.1 ppb [81]. From the discussion by Whitten [82] and Gaffney et al. [83], it seems clear that the content of peroxyacetal nitrate was likely not due to the acetaldehyde formation from ethanol. Nevertheless, the content of aldehydes in the exhaust gases when using ethanol as a motor fuel was a little higher than that from the petrol without ethanol. The use of oxygenated petrol began in the winter of 1992 in California to reduce the unhealthy carbon monoxide concentration in many urban areas. California's modified oxygenated fuels programme resulted in an approximately 5–10% reduction in mean ambient CO concentrations depending on the statistical approach used [84].

Today, all the high octane number petrols contain methyl-*tert*-butyl ether (MTBE) as an additive. The addition of MTBE reduces the CO content in the engine exhaust gases and increases the octane number value of the petrol. Both MTBE and ethanol have the potential to provide anti-knocks and have a low cost for increasing the octane number value [85]. However, MTBE is produced from 2-methylpropene and methanol, both of which are produced from petroleum, which is not renewable. Besides, ethanol is more effective in reducing the content of CO than MTBE. Thus, the use of petrol with 20% ethanol content allowed reduction of the CO content by 60%, while the use of petrol containing 27% of MTBE reduced CO only by 40% [86]. Moreover, sulphur oxide emission for ethanol is 60–80% lower than that for reformulated petrol. Also, volatile organics content in the exhaust fumes of ethanol-containing fuel is 13–15% less than in reformulated petrol exhaust fumes [75].

Additives for ethanol–petrol blends to reduce the content of pollutants in the engine exhaust gases have also been elaborated. For instance, adding 0.1–0.2% of a mixture of 30% benzyl alcohol and 70% phenol decreased the CO content of the exhaust gases by 64–75% [87]. Addition of 0.1% naphthalene gave the same effect [88]. In recent years, only one publication on an additive for reducing the content of pollutants in the exhaust gases was published. This additive contains higher alcohols, ketones, and ethers with tertiary alkyl groups and aliphatic and silicone compounds, it increases engine power and reduces the emission of pollutants [89].

Many authors have tried to determine the octane number value for alcohol–petrol blends [90–94]. Thus, Scheller [92] showed that the octane number for gasohol was by 4.5 octane number units higher than that of the unleaded petrol alone. Allsup and Eccleston [93] showed that the octane number of the blend was approximately 3.6 units higher than that of the petrol without ethanol. In field studies, the optimum octane number value for alcohol–petrol blends has been estimated to be 86.5 for Pb-free fuel and 88 for low-Pb fuel. The theoretical

estimation of the octane number values has yielded 90–100 and 100–101, respectively [91].

Many petrol and ethanol blends containing water have also been patented [95–98]. These mixtures contain usually a surfactant.

CORROSION AND STABILITY PROBLEMS

Scheller showed that after 1.5×10^6 miles of road testing, gasohol use was not associated with any unusual engine wear [92]. Also, Finnish investigators Nylund et al. established in field tests with different ethanol fuelled engines that none of the test engines showed signs of accelerated wear of the kind that could have considerably shortened their life-time [5]. However, a methanol–petrol blend is considered to be a more difficult fuel both with regard to corrosion and fuel stability [5, 92, 99].

Even though it has been shown that fuels containing ethanol do not cause corrosion of the engine, many additives have been patented to avoid the problem of corrosion. Such additives are, for instance, the following amine derivatives: tetramethyltrimethylene-diamine 0.005–0.03% [100] and amine synthesized from N-monotallow-1,3-propane diamine, 5-amino-1,4-tetrazol and HCHO, 0.004% [101]. Also, analogous compounds in a concentration of 38 ppm [102], and also compounds prepared from aminotriazole and polyisobutenyl succinic anhydride in a concentration of 7.68 ppm [103], aminophosphate [104], 3-(tridecyloxy)-propylamine, 0.02% [105], alkoxyamines, 1% [106], prepared from itaconic and alkyl trimethylene diamine, 0.1% [107], N-oleyl-3-aminopropionic acid in oil, 0.1% [108], prepared from aminotetrazole and sarcosine [109], amine and dilauryl phosphate [110], reaction products of isatoic anhydride and tert-alkyl primary amine, 382 ppm [111], prepared from secondary amine and isatoic anhydride [112], reaction products of isatoic anhydride with secondary fatty alkylamines [113], aminated polyisopropoxylated polyethoxylated alkylphenols have been used as petrol additives [114].

In the USA, patents for gasohol-induced corrosion inhibition by means of using low water content fuels have been obtained. In Brazil, corrosion inhibitors of the neat ethanol fuel (which contains 0.2–5.0% of water) have been patented, the additives used are dicyclohexylamine nitrite [115], mixture of phenol and amines [116], and polymerized organic acids, e.g. trimer of linolic acid 0.0005–0.5% [117], and polyisobutylenyl succinic acid [118]. The corrosion effect of the Brazil fuel ethanol is mainly due to the impurities, sulphates and chlorides [119].

Many substances for the inhibition of phase separation have also been patented. Ethanol can absorb moisture from air and thus, two phases could form. Many additives for maintaining the homogeneity of ethanol–petrol blends have been worked out. They contain tert-butylalcohol and dodecylglycerol [120], nonionic surfactants [121–126], higher alcohols, fusel oil and eucalyptus oil [127], saponified fatty material [128], n-butanol [129], neutralized soybean oil

[130], methyl and ethyl carbonate [131], hexanol, butylamine and pentanone mixture [132]. Diethylamine prevents corrosion of the steel as well as separation of water [133]. Mixtures of nonionic surfactants and higher alcohols [134] also prevent separation of wet ethanol from petrol.

The elaboration of the ingredients for petrol and ethanol blends against corrosion and separation are all from the period of 1979–86. In the later years the corrosion problem has usually been prevented with using ethanol of high purity and separating wet ethanol by adding surfactants or higher alcohols or their mixtures.

TECHNOLOGICAL PROBLEMS RELATED TO ETHANOL PRODUCTION

The technology for producing ethanol from biomass has been dynamically developing [67–69]. With the improved technology, the cost of ethanol in the USA fell from $ 0.95 per litre in 1980 to $ 0.32 per litre in 1992 [75].

Different resources are available for bioethanol production, depending on the climatic and agricultural conditions in any given country. In Brazil, the main source of fuel ethanol is sugarcane. For the northern countries, it could be corn or cellulosic biomass (hard- and softwood, agricultural and forestry residues, and cellulosic municipal waste), but also algae.

The current technology for ethanol production from corn is based on the conversion of starch to ethanol. Starch is treated with starch-degrading enzymes to produce simple sugars, which can then be converted to ethanol by yeast fermentation. Ethanol is removed by rectification, and the final step in order to produce anhydrous ethanol is extractive rectification [135, 136].

The technology of producing ethanol from cellulosic biomass is based on chemical hydrolysis of cellulose to sugars and then fermentation of the sugars to ethanol. The main components for producing ethanol from biomass are two families of sugar polymers – cellulose and hemicellulose. Cellulose fibres in plants are embedded in a matrix of hemicellulose and lignins, which cannot be used for ethanol production. The first attempts to produce ethanol from cellulosic biomass were made in the late 1890s by Simonsen. The yields were 7.6 litres of pure ethanol per 100 kg of dry wood [cited in 75]. Nowadays, pretreatment of biomass is carried out with dilute acids. The soluble pentosis solution obtained from hemicellulose is separated and fermented to ethanol. The residual mass is subjected to simultaneous saccharification and fermentation (SSF) process. From the reaction mixture, ethanol is distilled and rectified. Anhydrous ethanol is obtained by extractive rectification using molecular sieves or by membrane technology. Membrane technology can be used also in the fermentation process. The SSF process is available after application of the strains of *Trichoderma reesei* [137], which are the best available sources of commercial cellulase, an enzyme that converts cellulose to hexoses. Also, bacteria have been discovered

that produce more potent forms of celluloses which have enhanced temperature tolerance and stability of the product [138]. Ultimately, genetic engineering will have an important role in developing organisms with this type of combined enzyme production [75]. Genetic engineering techniques have been also used to get organisms that can ferment pentoses from hemicellulose to ethanol more efficiently and that enable to reduce the cost of production. Thus, the yield of ethanol from the biomass with the use of modern technologies has increased during the last hundred years from 7.6 litres per 100 kg dry wood to almost 40 litres per 100 kg dry biomass [75].

Although the production costs for bioethanol are remarkably low, an important consideration in bioethanol production is the transportation cost. The fuel ethanol production must be organized near to the production of biomass, to make it economical.

TECHNOLOGIES USED IN THE PRETREATMENT PROCESS

Several techniques have been developed for the pretreatment process to facilitate the enzymatic conversion of cellulosic biomass to sugars. In steaming the cellulosic raw material at 200°C for 20 min, the lignin depolymerizes and dissolves in the hemicellulose solution in hot water. The lignin becomes readily into solution in dilute NaOH, from which it can be recovered as an active chemical by acidification. Glucose yields are 70–80% of cellulose [139]. Pretreatment of the biomass with diluted sulphuric acid hydrolyses all hemicelluloses, but solubilizes very small amounts of lignin and cellulose. Hydrolyzed hemicellulose solution is easily fermented to ethanol. Cellulose in pretreated hardwoods becomes highly digestible by cellulase from *Trichoderma reesei*, the same happens to grasses [140]. The lignin from the wood sources can also be used as a fuel component after the relevant treatment. It is possible to convert lignin to methyl or ethyl aryl ethers, which are antiknock and pollutant-decreasing additives for petrol [141].

The combination of the wet oxidation process (water, oxygen pressure, elevated temperature) and alkali (sodium carbonate) hydrolysis has been investigated as an efficient method for pretreating wheat straw for solubilization of hemicellulose without generating inhibitors, produced during the breakdown of carbohydrates. Fermentation of the hydrolyzates was carried out by a thermo-anaerobacterium. The highest ethanol yields were obtained in hydrolyzates treated at oxygen pressures of 3 atm with the exception of the hydrolyzates without addition of carbonate [142].

For hardwood, a combination with dilute acid and steam has been worked out. The wood chips were soaked in 0.4% sulphuric acid solution and then pretreated with steam at 200–230°C for 1–5 min. After pretreatment, 90–95% of the hemi-cellulose and as much as 20% of the cellulose were solubilized in water, and 80% of the remaining cellulose could be hydrolyzed to glucose by cellulose enzyme.

The prehydrolyzates could be readily fermented by the unadapted yeast *Saccharomyces cerevisiae* D_5A [143]. Also, alkali (NaOH, NH_4OH, and lime) were used for the pretreatment before enzymatic hydrolysis of lignocellulosic biomass, which gave a higher yield of ethanol compared to the untreated material [144].

For softwood impregnation with SO_2 was found to be preferable, since approximately the same sugar yields resulted when compared to the dilute sulphuric acid and steam, but better fermentability was achieved, thus increasing ethanol yield [145].

Recently, a new pretreatment process of the biomass was developed by Belkacemi et al. [146]. Lignocellulosic materials such as alfalfa, red canary grass, and agricultural residues were pretreated, using the Ammonia Fiber Explosion process. The pretreated materials were directly saccharified by cellulolytic enzymes. The ultrasonic treatment reduces cellulose requirements by one third or even by half. When mixed waste office paper was degraded by the combination of ultrasonic treatment and fermentation with *Klebsiella oxytoca* P2, a 15% increase in ethanol yields was achieved [147].

FERMENTATION AND ENZYMATIC PROCESS AND THE USE OF GENE TECHNOLOGY

A problem in fermenting the lignocellulosic material is the requirement of separate fermentation of the hemicellulose and cellulose hydrolysates. The yeast *Saccharomyces cerevisiae* is effective only for fermenting hexoses to ethanol. However, technologies are now available which allow the conversion of lignocellulose into fuel ethanol using genetically engineered bacteria. The use of gene engineering to obtain more effective microorganisms for the production of ethanol has developed very fast in recent years. Thus, using gene engineering with a URA 1 gene from *S. cerevisiae*, *Pichia stipitis* is able to ferment xylose and glucose together [148]. The genetically engineered recombinant yeast *Saccharomyces* 1400 (pLNH 33) can also utilize glucose and xylose simultaneously to produce ethanol [149]. Also, the possibility of using cellolytic fungus *Aspergillus terreus* for ethanol fermentation from glucose, hexoses, pentoses, and disaccharides has been shown [150].

Recombinant *Zymomonas* containing *Escherichia coli* enzyme genes are useful for fermenting pentoses and glucose produced by hydrolysis of hemicellulose and cellulose to ethanol [151, 152]. Genes encoding *Zymomonas mobilis* pyrvuate decarboxylase and alcohol dehydrogenase have been integrated into the chromosome of *Escherida coli* B to produce strain KO11. This organism can efficiently ferment all sugars present in the polymers of hemicellulose. *Klebsiella oxytoca* M5A1 has been genetically engineered in a similar manner to produce strain P2 for ethanol production from cellulose [153]. *Escherichia coli* KO11 containing the *Klebsiella oxytoca* casAB operon was used to ferment

mixed waste office paper to ethanol. This process is rapid and the yield is higher than 90% [154, 155]. Also the genetically modified "old soldier" *Saccharomyces cerevisiae* improves essentially the fermentation process. Thus, it was shown that the strain YPB-G can produce ethanol in media with starch concentration above 100 g/L [156]. Two respiratory-deficient nuclear petites, FY23Δpet191 and FY23Δcox5A of *S. cerevisiae*, have high ethanol tolerance and increase the productivity of ethanol 30–40% [157]. Recombinant of *S. cerevisiae* and *Zymomonas mobilis* has a good strain stability, ethanol tolerance and produces ethanol efficiently from lignocellulosic wastes, ethanol obtained this way can economically compete with petrol more efficiently [158].

A comparative evaluation indicates that recombinant strains of *Z. mobilis* are very competitive with other recombinant bacteria and yeasts which are able to convert xylose to ethanol. Also, studies have shown that the strains of *Z. mobilis* are relatively tolerant to some of the inhibitory compounds (e.g. acetic acid, furfurol), found in lignocellulosic hydrolysates [159]. Recombinant cyanobacteria with *Zymomonas* pdc and adh genes are also used for the production of ethanol [160].

New cloned microorganisms are used in practice. For instance, the gene encoding pyruvate decarboxylase from *Chlamydomonas reinhardii* improves the efficiency of ethanol production from polysaccharides during fermentation [161].

In the USA, 13 enzymatic preparations are commercially available, which have been used in the food, detergent, and textile industries and have been examined for processing biomass from feedstocks. Monoclonal antibodies specific for *Trichoderma reesei* CBH1 and *Aspergillus niger* β-D-glucosidase have been developed [162]. Also, mutations of cellulytic fungus *Aspergillus nidulas* can be used for the conversion of cellulosic materials [163].

Enzymes produced from other strains, for instance from *Candida peltata* strain NRRLY-21603, are effective for the treatment of cellulosic and lignocellulosic materials to convert cellulose to glucose [164]. A new, very interesting, extremely thermophilic anaerobic bacterium *Thermoanaerobacter mathranii* A3M1 was used for the production of ethanol with high productivity at a very high temperature, so that ethanol could be removed from the stream by *in situ* distillation [19, 165]. This may prove to be a new way for developing the production of ethanol from biomass.

Recently, a method of introducing new genes into cyanobacteria has been reported. The coding sequences of pyruvate decarboxylase (pdc) and alcohol dehydrogenase II (adh) from the bacterium *Zymomonas mobilis* were cloned into the shuttle vector pCB4 and then used to transform the cyanobacterium *Synechoccus* sp. strain PCC 7942. As cyanobacteria have simple growth requirements and use light, CO_2, and inorganic elements efficiently, production of ethanol by cyanobacteria is a potential system for bioconversion of solar energy and CO_2 into a valuable resource [166]. The SSF process of pretreated poplar with *Saccharomyces cerevisiae* D_5A was studied by Kadam & Newman [167], and it was shown that a low-cost medium containing 0.3% corn steep and

2.5 mM MgSO₄ 7H₂O could be used. All the components are available on a commercial basis making the use of this medium industrially relevant.

Mixed cultures can also be used for the production of ethanol from a mixture of cellulosic glucose and hemicellulosic xylose. For ethanol production, the process was studied in continuous aerated conditions with a respiratory deficient mutant *S. cerevisiae* CBS 1200, associated with *Pichia stipitis* NRRL 11545 using a microfiltration membrane bioreactor. Under these conditions ethanol was produced with a yield of 0.43 g/g. The glucose and xylose conversion yields were 100% and 60%, respectively, giving overall substrate conversion yield of 80% [168].

The immobilization of the yeast plays an important role in continuous fermentation of saccharides to ethanol. In Brazil, it has been shown that the sugar cane stake itself can be a suitable base [169]. The immobilized cells of *Zymomonas mobilis* are more efficient than *S. cerevisiae* for ethanol production on Ca-alginate matrix which is better than κ-canageenan [170]. Immobilized yeast makes possible the continuous ethanol fermentation process, which increases the efficiency through recycling of biomass into the fermentor. Thus, the thermotolerant yeast strain *Kluyveromyces marxianus* IMB3 immobilized in calcium alginate and the porous volcanic mineral, kissiric, at 45°C with 90–94% efficiency [171, 172]. Immobilization decreased the fermentation time markedly and the productivity of ethanol increased [173]. With the continuous alcoholic fermentation by co-immobilized *S. cerevisiae* and *Candida shehatae,* it is possible to carry out simultaneous cellulose and hemicellulose hydrolyzates fermentation from glucose and xylose mixtures [174].

The recycling of the enzyme in the enzymatic process decreases the ethanol cost by 11%. Despite a significant reduction, the enzyme cost still represents about 18.5–22.7% of the total cost of ethanol production from hardwood and softwood [175]. A novel bioreactor design with plastic composite supports, which stimulate biofilm formation and supply nutrients to the attached micro-organisms, has been developed. Using this novel bioreactor design, increased productivity in low cost medium can be achieved beyond conventional fermentations. Thus, *S. cerevisiae* yielded two to ten times higher ethanol production in this novel bioreactor [176].

NEW DEVELOPMENTS IN TECHNOLOGY

The technology for the production of ethanol from biomass is constantly developing, and new processes [177, 178] and apparatus [179] are being patented.

The hydrolysis of cellulose and the fermentation of hexoses and pentoses can be carried out simultaneously [177]. Chemical mutagens for enhancing the efficaciousness of microorganisms to increase the production of ethanol [180] and also electric field [181] have been used.

The biochemical conversion of cellulosic biomass to fuel-ethanol can be carried out efficiently and economically using the SSF process. This process integrates the enzymatic hydrolysis of cellulose to glucose, catalysed by the synergistic action of cellulase and β-glucosidase, with the fermentative synthesis of ethanol. The SSF process is in continuous development [182–184], new microorganisms are used [185] and reactor construction is being modified [186]. The enzymatic reaction in the SSF process is operated at temperatures much lower than the optimum level. To alleviate this problem, a nonisothermal simultaneous saccharification and fermentation (NSSF) process was proposed. Thus, the saccharification and fermentation occur simultaneously, yet in two separation reactors that are maintained at different temperatures. Lignocellulosic biomass is retained inside a column reactor and hydrolyzed at the optimum temperature for the enzymatic reaction, 50 °C. The effluent from the column reactor is recirculated through fermentor, which runs at its optimum temperature, 20–30 °C. Both ethanol yield and productivity in the NSSF process are substantially higher than those in the SSF [187]. Formally, it is more correct to call the NSSF process a two-step process rather than a simultaneous process.

Many investigations have been made in order to broaden the sources for the production of bioethanol, for instance from industrial waste [188] and from algae [189]. Wang et al. [190] showed that the efficacy of ethanol production from rye is similar to that of wheat, reaching 91–93%, thus ethanol obtained from rye is cheaper than that obtained from wheat as rye is cheaper than wheat. Czarnecki et al. obtained ethanol from rye with high efficiency with *Zymomonas mobilis* [191]. For the production of bioethanol, triticale showed the highest potential in Denmark, with a biomass production of 15.8 t dry matter/ha, closely followed by rye varieties and then winter wheat [21]. In Greece, Nigeria, and China bioconversion of agricultural crops to ethanol by the SSF process has been carried out. The most promising results were achieved with sweet sorghum, converted into ethanol by mixed cultures of *Fusarium oxysporum* and yeast [192]. Thus, the plant disease causer *F. oxysporum* was usefully employed.

It is possible to produce ethanol also from oilseed residue, one of such methods has been worked out by Henkel Corp, USA. The oilseeds were deoiled and the residue, comprising complex polymeric carbohydrates, was saccharified to form saccharides. The saccharides were fermented to ethanol and other lower alcohols and then the extracted oil was transesterified with the obtained ethanol. Thus, it is possible to produce fatty acid lower alkyl esters (which can be used as bio-diesel fuel) and glycerol [193]. This ethanol can also be used directly as otto-motor fuel.

The preextraction in the ethanol production process from corn is also effective. Thus, the Sequential Extraction Processing is a new process for ethanol production, which has a potential to produce more valuable co-products than alternative processes. The oil and protein are extracted and residual corn fibre is subjected to fermenting process. The ethanol yield was increased by 10% [194]. Kumar et al. [195] elaborated an interesting process to produce ethanol from

solid food wastes and by-products. The solid food processing wastes and by-products are co-fermented with cheese whey. Cheese whey is used as a wetting agent that provides macro- and micronutrients for the microorganisms. This procedure induces a 33–36% increase in alcohol yield and also significantly reduces the fermentation time (approximately 5 times).

In the petrol–ethanol blends, absolute ethanol is used. The main technologies for producing absolute ethanol are extractive rectification, drying with molecular sieves, pervaporation–membrane technology, and supercritical CO_2 extraction. From the fermentation liquid, ethanol is usually removed by distillation.

In recent years the separation process of ethanol from the fermentation broths with supercritical CO_2 extraction has been paid increasingly more attention. The process is usually carried out at 40 °C and under 100 atm pressure [34, 196]. The supercritical extraction process is energy efficient like the corresponding conventional separation process. It involves the separation and drying of ethanol [197]. The production of gasohol is also possible by the extraction of azeotropic ethanol with petrol [198, 199].

In the USA, the economical production of ethanol from waste feedstocks is already carried out [200]. Over 1.3 billion gallons of potential fuel ethanol is produced annually in the United States. Approximately 95% was derived from corn starch in 1996. With the increased attention to clean air and oxygenates for fuels, an opportunity exists for an expanded ethanol fuel industry. Corn fibre represents a renewable resource that is available in sufficient quantities from corn wet milling industry to serve as a low cost feedstock for production of fuel ethanol [201]. In 1996 about 10% of all petrol in the USA was blended with 10% ethanol [50]. Abundant biomass available in the country could provide the feedstock for an ethanol industry. The use of thermophilic bacteria and upflow solids retaining bioreactors are in the focus. Continuous hydrolysis and fermentation of first pretreated hardwood and later paper sludge have been examined [202]. In Sweden annually 30 million SEK is allocated for the continuation of developing ethanol as a motor fuel for the period 1998–2004.

On a recent ACS symposium, a very comprehensive review on biofuel cells was given and the process of using bioethanol oxidation for obtaining electrical power was discussed [203]. The biological fuel cells can have practical meaning through genetic engineering of the enzymes as catalysts maximizing the cell voltage and minimizing the costs. It is of interest to mention that Daimler-Benz has already constructed a fuel-cell-powered experimental car [204].

ARGUMENTS AGAINST THE USE OF BIOETHANOL FUEL

The two main arguments against the production of bioethanol as a fuel are the large amount of water used in the enzymatic and fermentation processes and the high energy requirement.

In order to reduce the water consumption and to increase the productivity of ethanol in the fermentation process, a new method has been worked out in Japan. A continuous ethanol production without effluence of wastewater is carried out using a closed circulation system which integrates a cell retention culture system and distillation system to separate ethanol. The stirred ceramic membrane reactor, a jar fermentor fitted with asymmetric porous alumino ceramic membrane rods, was used for retaining a high density of cells and for extraction of the culture supernatant that was continuously fed to the distiller to evaporate ethanol [205]. Water economy is also possible by simple recycling. In ethanol production from lignocellulose by enzymatic hydrolysis and fermentation, it is possible to minimize the addition of fresh water and wastewater streams. The streams can be reduced to a large extent by recycling without decreasing the productivity of ethanol [206].

The second argument against bioethanol as a fuel is the cost of energy in the production process. Easterly [207] showed that a plant producing 57.9 million gallons of ethanol fuel per year using wood as the feedstock can produce also 36.1 MW power generated with a steam turbine using various organic waste streams from the ethanol process as fuel for producing electrical power and process heat. The total plant demand for electricity is estimated to be 22.7 MW, which results in a 13.4 MW power surplus. The excess of electricity can be sold.

Elliott et al. [208] mention that although ethanol may be classified as a renewable energy source associated with solar energy, it uses agricultural resources that will become increasingly needed for food as the population grows, especially in Asia. However, most probably in the future the main sources for the production of fuel ethanol will be cellulosic wastes, not agricultural resources [75].

SUMMARY

The arguments presented above should show without doubt that fuel ethanol has great prospects as a motor fuel. The main sources for fuel ethanol are agricultural, forestry, and municipal wastes. The production is relatively cheap and economically profitable. However, the transportation costs of the wastes are relatively high, therefore the production of fuel ethanol must be organized in close vicinity to where the wastes are formed.

Many possibilities remain for further improvements in ethanol production from biomass. These include the development of enzyme-based systems which eliminate the need for dilute acid hydrolysis or other pretreatments, improvements in existing pretreatments for enzymatic hydrolysis, process improvements to increase the effective use of cellulose and hemicellulose enzymes, improvements in rates of ethanol production at decreased nutrient costs, increases in ethanol concentrations achieved in biomass, increased resistance of the biocatalysts to lignocellulosic derived toxins, etc. To be useful, each of these

improvements and also to use the SSF process must result in a decrease in the cost of ethanol [149].

Production of bioethanol fuel should be considered in every country in order to combat the global GHE. As to Estonia, there are two perspective sources available for the production of fuel ethanol from biomass: the cellulosic wastes and grain. Considering that approximately 200 thousand hectares of possible grain fields are out of agricultural use today and grow weeds, grain can be considered an important source for bioethanol production in Estonia. The first steps would be to start immediately more detailed investigations in this field, taking into account Estonian industrial, agricultural, and economic conditions, and to begin with technological research.

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BIOETANOOL KUI KASVUHOONEEFEKTI VÄHENDAV KÜTUS

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Ülevaates on käsitletud bensiinimootorite heitgaaside põhjustatud õhusaaste-probleeme ja kasvuhooneefekti. On näidatud, et etanool on alternatiivne bio-kütus, mida kasutatakse laialdaselt Brasiilias ja segus bensiiniga ka USA-s. Mitmetes riikides, eriti USA-s tehtud teaduslike uurimistööde (geenitehnoloogia, protsesside ja seadmete arendamine, toormebaasi laiendamine ja muud vald-konnad) alusel on osutunud võimalikuks toota bioetanooli kui mootorikütust ökonoomselt. Bioetanoolkütuse tootmine on oluline iga riigi jaoks, sest perspek-tiivseks toormeks on põllumajanduse, metsatööstuse ja olme tselluloossed heit-med, mida on ökonoomne töödelda kohapeal vältimaks liigseid transpordi-kulusid.