Ecological Engineering and Environment Protection, No 3, 2012, pp. 46-57

THE CONTRIBUTION OF SECOND GENERATION BIOETHANOL TO SUSTAINABLE ENERGY INDEPENDENCE

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ЗНАЧЕНИЕТО НА БИОЕТАНОЛА ОТ ВТОРО ПОКОЛЕНИЕ ЗА СТАБИЛНА ЕНЕРГИЙНА НЕЗАВИСИМОСТ

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Abstract. In this review the basic approaches for development and improvement of bioethanol industry, its perspectives and problems encounter are summarized. Bioethanol produced from plant biomass by biochemical method (second generation bioethanol) is object of special attention. The perspectives and quality of this biofuel are compared with those of bioethanol produced from starch feedstock (first generation bioethanol). The advantages as well as problems concerning manufacturing and commercialization of second generation bioethanol are discussed also. It is pointed on the necessity of global approach to develop and stimulate such industry. The initial steps of commercialization of second generation bioethanol as well as future impacts of such industry on sustainability and environmental are presented also.

Key words: bioethanol, commercialization, sustainability, environment

INTRODUCTION

The major challenges of 21 century are depletion of fossil energy resources and continuously growing necessity of energy supply. Especially the transport sector is currently heavily dependent from petrol oil and such problems are source of economical and political instability and crisis. These and other factors constrain industrially developed countries to make serious efforts for their energetic independence. One possible way to achieve such goal is to develop sustainable manufacturing of biofuels, mainly biodiesel and bioethanol. Biofuels should ideally retain the advantages of petroleum oil with regard to being relatively cheap and rich in energy and should in addition provide a net energy gain, have environmental benefits and be producible in large quantities without impacting on food supplies [1]. As a result of such increased global demand biofuels production and consumption has more then quadrupled between 2000 and 2008 (Fig.1). Currently, biofuels provide for over 1.5% of the energy used for transport [2]. Future targets and investment plans suggests growth of biofuel ethanol will continue in the near future, rising to around 100 000 Ml in 2014, 45% more than produced in 2008. Total plant capacity could exceed 120 000 Ml/year by that time, given an analysis of the number of plants presently under construction and those planned [3].

Bioethanol is more popular, distributed and consumed product then biodisel and dominate in current transportation systems and corporate plans over the next decade. Bioethanol is considered as a viable alternative to fossil fuels and provide a potential route to avoiding the global political instability and environmental issues.

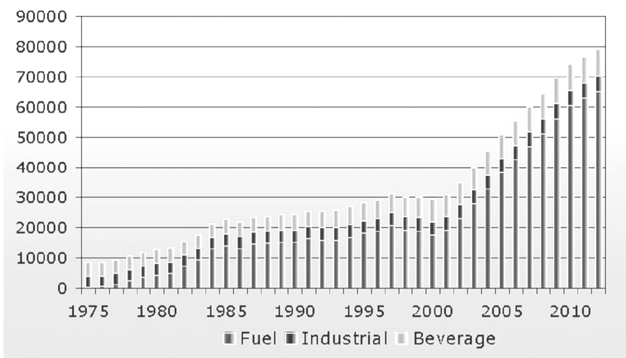

Fig.1. World production of ethanol (million liters)

Ethanol has an energy density of 24.0 MJ/liter, which is only around 68-75% the density of gasoline at 32-35MJ/liter [4]. Ethanol is currently used as gasoline additive in many of world countries. It has already replaced methyl tertiary butyl ether as gasoline additive and oxygenate to reduce air pollution. Although the energy density of ethanol is lower than gasoline, its octane rating is 35-40% higher and the fuel can improve thermal efficiency when compared with pure gasoline. Depending from sources used for its manufacturing bioethanol is regarded as belonging to first and second generations. From chemical point of view both are the same organic molecule called as ethanol (or alcohol) that is widely used for drinking and other purposes, but additionally is subjected to dehydratation.

First generation bioethanol (1G-bioethanol) includes that is produced by alcohol fermentation from plant-derived sugars, derived from 'food' crops, such as sugar cane, corn, wheat, and sugar beet. Its manufacturing is simple and well developed, and can be and is produced widely in the world. The most popular1G-bioethanol is corn ethanol in USA and sugarcane ethanol in Brazil. However, sustainable and economic production of 1G-bioethanol come under close scrutiny and several important limitations constrain its global expansion due to: i.) relatively high price, ii.) limited amounts of agricultural products and, iii.) competition with markets of food and feed products. Furthermore, the amounts of crops available are not enough to satisfy its requirements as transport fuel. For example, some calculations suggest that conversion of all corn in USA to ethanol would satisfy only 12 % of its need in gasoline [1]. Additionally, it had engendered increasing disputes over its participation in greenhouse gas reduction. When take into account emissions from production, transport and life cycle assessment from 1G- bioethanol frequently approach those of traditional fossil fuels. The other disadvantage of 1G- bioethanol is its low energy balance - 1,25 [4].

Second generation bioethanol (2G-bioethanol, also called as cellulosic ethanol) is also typically ethanol but is produced from non-food biomass. There is opinion that 2G-bioethanol production is more sustainable and has a lower impact on food production. The goal of its manufacturing is to extend the amount of biofuels that can be produced sustainably by using biomass consisting of the residual non-food parts of current crops, such as stems, leaves and husks that are left behind once the food crop has been extracted, as well as other crops that are not used for food purposes. Compare to 1G-biethanol and biodisel its energy balance is more then >4 and this is serious advantage over biofuels compared [1]. The cumulative impact of these various concerns have stimulated the interest in developing 2G-bioethanol, a research topic that has continued over three decades.

PLANT BIOMASS AS SOURCE OF RENEWABLE ENERGY

Humanity has unlimited source of renewable material that can be used as energy source in the form of plant biomass. Every year, photosynthesis produces near 120 billion ton of dry matter in the Earth which corresponds to more than 40 billion ton of oil. Nevertheless such biomass is used mainly by direct burning and gasification or production of biogas from municipal and agricultural wastes.

Plant biomass is regarded as the only foreseeable renewable feedstock for sustainable production of renewable transport fuels. The term biomass for energy is often used to mean plant based material derived from living, or recently living organisms. As an energy source, biomass can either be used directly, or converted into other energy products such as biofuel. Within this definition, biomass for energy can include five basic categories of material: (i) Virgin wood, from forestry, arboricultural activities or from wood processing, (ii) Energy crops, high yield crops grown specifically for energy applications, (iii) Agricultural residues: residues from agriculture harvesting or processing, (iv) Food waste, from food and drink manufacture, preparation and processing, and post-consumer waste, and (v) Industrial waste and co-products from manufacturing and industrial processes. Agriculture and wood industry residues could provide a huge income of lignocellulose is a significant feedstock and can be used as optimal feedstock for cellulosic ethanol. Enormous amounts of sawdust, waste products of agriculture, wood industry and various municipal wastes could be used for such purposes also. Plant biomass is largely accessibilitable, comparatively cheep and in big access in nature. It is not compatible to food crops and is neutral toward CO_2 emissions. For such reasons industrial production of 2G-ethanol could be regarded as alternative of sugarcane and starch ethanol (1G-ethanol). Till now more attention was attracted to utilization of waste biomass, originated from wood and cellulose-paper industry as well as agriculture waste. Industrial biomass can be grown from numerous types of plants, including miscanthus, switchgrass, hemp, corn, poplar, willow, sorghum, sugarcane, bamboo, and a variety of tree species, ranging from eucalyptus to oil palm.

Plant biomass is composed from three major components: cellulose, hemicelluloses and lignin. In this review these components are discussed according to their importance for bioethanol manufacturing only. The average percents of each component in plant biomass and corresponding dry contents are listed in Table 1. The data listed are approximately because they varied for each plant species and various parts of plants also.

Table 1. Average percents of cellulose, hemicelluloses and lignin in plant biomass.

Plant biomass	Component
Cellulose	33 - 51
Hemicelluloses	19 - 34
Lignin	21 - 32
Others	1 - 5

The lignin content in plant biomass is quite high (21-32% of the dry matter), but this polymer cannot be is not object of this review. Cellulose is most distributed polymer in the nature. It is homo-polymer composed from β 1→4 linked glucose monomers and possess stable structure that make it very resistant to hydrolysis. However, if hydrolysis is performed, the resulted glucose molecules are object of easily conversion into ethanol. Hemicellulose is the second major component of plant biomass. The term hemicellulose is used to designate heterogeneous group of highly branched hetero-polymers composed from various pentose and hexose monomers. Main hexoses (C6) of hemicelluloses are glucose, mannose, galactose and rhamnose. Hemicellulose have a high content of pentose sugars (C5), preliminary D-xylose (up to 20% of the dry matter) and L-arabinose [5]. Xylose is the second most abundant monosaccharide after glucose, and the most prevalent pentose sugar found in lignocelluloses. The relative proportion of the individual sugars depends on the raw material; the hemicellulose fraction of hardwoods and agricultural raw materials is rich in pentoses sugars, while softwood hemicellulose only contains minor fraction of pentose sugars D-xylose and L-arabinose.

Lignocellulose represents the most widespread and abundant source of carbon in nature and is the only source that could provide a sufficient amount of feedstock to satisfy the world's energy and chemicals needs in a renewable manner [6,7]. The main technological impediment to more widespread utilization of lignocellulose for production of fuels and chemicals is the lack of low-cost technologies to overcome the recalcitrance of its structure [8]. Producing biofuels such as ethanol from cellulosic plant material has the potential to meet capacity requirements without impacting directly on food production [9].

INDUSTRIAL MANUFACTURING OF 2G-BIOETHANOL

There are two basic methods for manufacturing of ethanol from lignocelluloses: the first method (called thermo-chemical) includes transformation of lignocelluloses to gaseous CO and H_2, flowed by catalytic conversion of these gases into ethanol [10].

The second method (called also as biochemical or cellulolitic) is based on hydrolysis of pretreated biomass, followed by fermentation. Both approaches have the final step distillation of resulted product. The object of this review is cellulolitic method. It consists of five steps: (1) initial fragmentation and pretreatment, (2) hydrolysis, (3) separation of sugars from other materials, (4) fermentation, and (5) distillation for recovery of pure ethanol.

Fragmentation and pretreatment

The aim of fragmentation is to diminish size of biomass by grinding and usually more intensive mechanical disintegration is necessary. Physical pretreatment is often called size reduction to reduce biomass physical size. Fragmentation process is followed by pretreatment with diluted acids, alkali, organic solvents, steam explosion, etc. Pretreatment make biomass more accessible to the next operations and contribute for removing of undesired lignin. Every kind of biomass requires its own pretreatment and correspondingly this is the most expensive step of cellulosic ethanol manufacturing. Besides effective cellulose liberation, an ideal pretreatment has to minimize the formation of degradation products because of their inhibitory effects on subsequent hydrolysis and fermentation processes. The presence of such inhibitors seriously hampers ethanol production and increase the cost of final product, because additional procedures for detoxification are necessary. Inhibitors complicate the ethanol production and increase the cost of production due to entailed detoxification steps.

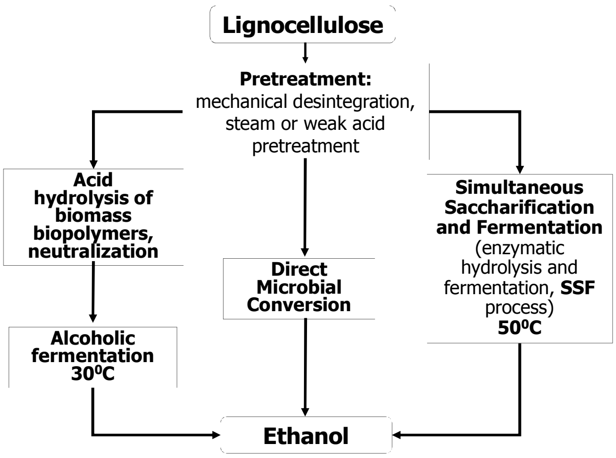

Fig.2. Scheme of basic methods for manufacturing of 2G-bioethanol.

Chemical hydrolysis

The next step is hydrolysis of lignocelluloses into sugar monomers. At least two main technologies (chemical and enzymatic) are used for such purposes (Fig.2). Acid hydrolysis is more rapidly and cheep, but is environmentally dangerous. According to the concentrations of acids employed such hydrolysis is divided by method that use diluted acids and required high temperatures and pressures and method that utilize concentrated acid at relatively moderate temperatures and pressures. The aim of hydrolysis is to break hydrogen bonds in lignocellulose and to liberate corresponding hexoses and pentoses. However, as a result of chemical hydrolysis many harmful side products are usually formed. The most dangerous of them are very strong cellular inhibitors furfural and hydroxymethyl furfural. Correspondingly, one of the most serious problems, which are necessary to be solved toward development of cellulose bioethanol technology is to overcome toxic action of such inhibitors. Several ways could be used including neutralization by additional chemical treatment or construction of microorganisms that are resistant to inhibitors. The action of these toxic interfere by synergistic way with the harmful influence of ethanol liberated during fermentation process.

Enzymatic hydrolysis

This method is based on the action of various enzymes called cellulases that are manufactured by genetically engineered microbial strains. It is regarded as very perspective but encounter two obstacles: enzyme hydrolysis is slow and the price of enzymes is still high. However, enzyme treatment has several important advantages. It occur at a relatively mild condition (50 °C and pH5), and gives possibility for economy of energy and chemicals. Enzymatic process enable effective cellulose breakdown without the formation of byproducts that would otherwise inhibit enzyme activity. Importantly enzyme hydrolysis is environmental safe technology. Enzyme treatment also gives possibility for simultaneous performing of two independent processes (hydrolysis and fermentation) into one vessel. Such process is called simultaneous saccharification and fermentation (SSF). During SSF solid cellulose fraction is degraded by the action of cellulases and liberated glucose together with sugars from liquid hemicelluloses fraction are subjected to fermentation.

Three types of enzymes: cellulases, hemicellulases and ligninases posses the ability to degrade plant cell wall [11]. Various enzyme companies have also contributed significant technological breakthroughs in the mass production of such enzymes at competitive prices. The group of cellulases include endoglucanase, exoglucanase and β-glucosidase, while the most widespread hemicellulases are xylanases with endo- and exo-actiivities. Cellulases produced by various microorganisms, mainly from bacteria and fungi usually are used for industrial purposes. Among them very popular are celluolitic enzymes systems produced from filamentous fungi *Trichoderma reesei*. Continuous efforts for changing of chemical hydrolysis with enzyme treatment are performed from many groups but in spite of serious success, high price of such enzymes hamper their large scale application [12,13]. Various strategies are tested in attempts to decrease the price of cellulases and to enhance their activity. One way is to search for novel microorganisms with better biosynthetic capabilities. The second include works on improvement of cellulases synthesis in organisms widely used as producers. For such purposes all achievements of genetic and metabolic engineering like as, super-expression under controls of very strong promoters, increased secretion and thermostability are used. The other groups of strategies are concentrated on improvement of plants that are perspective for bioethanol industry. Methods of plant genetic engineering give possibility to construct plants with elevated solubility of cellulose. Especially attractive is idea to construct plants in which cellulolitic enzymes are synthesized by heterologous expression of various bacterial or fungal genes and stored inside the plant into specialized cellular organelles - lysosomes [14].

Ethanol producing microorganisms

The lack of industrially suitable microorganisms for converting biomass into fuel ethanol has traditionally been cited as a major technical roadblock to developing a bioethanol industry. Production of 2G-bioethanol requires a fermenting microorganism(s) that convert all types of sugars in the raw material to ethanol in high yield and with a high rate. The fermentation performance of such industrial microorganisms depends from its ability to realize process water economy, to posses inhibitor tolerance, to give high ethanol yield, and specific ethanol productivity. Complete substrate utilization is one of the prerequisites to render 2G-ethanol process economically competitive. This means that all types of sugars in cellulose and hemicellulose must be converted to ethanol by microorganism(s). The other important characteristics are the ability to resist against harmful action of ethanol synthesized (ethanol tolerance) as well as temperature changes (thermotolerance). Many bacterial, yeast and mold species are able to ferment sugars to ethanol, but only limited number of naturally occurring microorganisms are preferred for large scale fermentations. They include two bacterial species - *Escherichia coli* and *Zymomonas mobilis* and three yeasts: *Saccharomyces cerevisiae, Pichia stipitis* and *Hansenula polymorpha.* The most important characteristics of these microorganisms are summarized in Table 2.

Table 2. Main fermentation properties of natural ethanol producing microorganisms

Organism	Glucose fermentation	Xylose fermentation	Resistance to ethanol	Thermo-tolerance (^{0}C)
Saccharomyces cerevisiae	++++	-	++++	37
Escherichia coli	+++	++	++	42
Zymomonas mobilis	++++	-	++++	30
Pichia stipitis	+++	+++	++	37
Hansenula polymorpha	+++	++	+++	48 - 50

Legend: (-) absence of property; (+) week performance; (++) normal performance; (+++) good performance; (++++) very good performance

E. coli is one of the most commonly used host organisms for metabolic engineering and industrial applications, because it is easy to manipulate genetically and can produce wide variety of anaerobic fermentation products. Some strains are ethanologenic and are able to produce ethanol from various carbohydrates by using simple fermentation conditions for producing high concentrations of ethanol. Ethanologenic *E. coli* also ferment hexose sugars, but xylose fermentation is slow or incomplete. Several ethanologenic *E. coli* strains have been developed to improve ethanol production through numerous rounds of modification and are used for pilot and industrial scales for manufacturing or 2G-bioethanol [15].

Z. mobilis produces ethanol with stoichiometric yields using the Entner-Doudoroff pathway and displays high specific ethanol productivity. This bacterium posses several advantages with respect to producing bioethanol like as: higher sugar uptake and ethanol yield (up to 2.5 times higher), lower biomass production, high ethanol tolerance up to 16% (v/v). Additionally, it does not require controlled addition of oxygen during the fermentation, and its cells are amenable to genetic manipulations. However, in spite of these attractive advantages, several factors prevent the commercial usage of *Z. mobilis* in 2G-ethanol production. Resulted biomass has unpleasant odors and can not be used by animals. The other problem is that its substrate range is limited to glucose, fructose and sucrose only. Wild-type *Z. mobilis* cannot ferment C5 sugars like xylose and arabinose which are important components of lignocellulosic hydrolysates. Unlike *E. coli* and yeast, *Z. mobilis* cannot tolerate toxic inhibitors present in lignocellulosic hydrolysates such as acetic acid and various phenolic compounds. Despite intensive efforts, the industrial exploitation of *Z. mobilis* has so far not materialized and works on this organism has concentrated on introducing pathways for the fermentation of arabinose and xylose [16].

Baker's yeast *Saccharomyces cerevisiae* are the best microorganism used for ethanol production. In spite of intensive search for its competitors, *S. cerevisiae* still remains as the best ethanol producer. Important advantage of *S. cerevisiae* is that after finishing of fermentation process resulted biomass can be used as feed additive for animals foraging. The other advantages are that is ethanol tolerant, knowledge of its genetics and physiology is highly developed, and it is generally regarded as being safe [17]. *S. cerevisiae* utilize glycolytic pathway (Embden- Meyerhof-Parnas pathway) in which one molecule glucose is converted into two molecules pyruvate. Resulted pyruvate is converted by pyruvate decarboxylase to acetaldehyde. The final step is conversion of acetaldehyde to ethanol by the action of alcohol dehydrogenase. Ethanol production process results in the production of ethanol, CO_2 and heat. One molecule of glucose yields 2 molecules of ethanol and 2 molecules of CO_2. One kilogram of glucose will theoretically produce 0.51 kilogram of ethanol and 0.49 kilogram of CO_2 [18]. Specially *S. cerevisiae* strains for ethanol production usually are named as distillery yeasts and can be categorized as strains selected for continuous and strains for butch fermentations. Some strains ferment faster or are able to convert substrate to ethanol with increased yields. From the genetic point distillery strains are predominantly homothallic, but some heterothallic strains are reported also. As a rule distillery strains have ploidy higher that haploid and strains with elevated ploidy are usually observed. Most of them were spontaneously selected during production process from"wild" distillery strains [19]. While ethanolic fermentation of hexoses using *S. cerevisiae* is well established on large scale, the conversion of pentose sugars (C5) xylose and arabinose to ehanol encounters serious problems because native *S. cerevisiae* strains are unable to grow on D-xylose and L-arabinose.

The other promising yeast organisms are those with native ability to ferment xylose, like non-conventional yeast *Hansenula polymorpha*. This is thermotolerant yeast able for growth at 48-50 ºC. It ferments glucose, cellobiose, xylose and some strains grow on L-arabinose. Importantly, *H. polymorpha* possesses very strong constitutive and regulatable promoters from genes involved in methanol utilization pathway. It is widely used in industry for production of recombinant proteins (phytase, hepatitis B surface antigen etc). Additionally, *H. polymorpha* belongs to the best studied yeasts with very well developed tools for molecular research. Due to such and other abilities ***H.*** *polymorpha* is regarded as a cell factory for production of various chemicals, heterologous proteins, etc [20]. *H. polymorpha* produce ethanol at elevated temperatures 48-50 ºC but the yield is very low and could not satisfy requirements for industrial manufacturing.

From the literature is evident that there are no known natural organism with the ability to convert both C6 and C5 sugars to produce high ethanol yields. For such reasons during the last two decades, numerous microorganisms have been genetically engineered to selectively produce ethanol. As a result now we have four main groups of engineered organisms able for simultaneous fermentation of C5 and C5 sugars. They include recombinant strains of *E. coli, Z. mobilis, S. cerevisiae,* as well as native xylose-fermenting yeasts (*P. stipitis* and *H. polymorpha*). The presently most efficient microorganisms for fermentation of detoxified lignocellulose hydrolysates are recombinant strains of *E. coli* in which genes encoding for alcohol gehydrogenase and pyruvat decarboxylase from *Z. mobilis* are heterologously expressed [21]. Significant research efforts have focused on the metabolic engineering of *S. cerevisiae* for fast and efficient pentose utilization. The most serious task for this organism is conversion of xylose in xylulose. If such reaction is performed, resulted xyloluse can be easily phosphorilated to xylulose-5-phosphate and converted to ethanol via xylulose-phosphate cycle. Attempts to develop a strain of *S. cerevisiae* capable of using xylose have focused on adapting the xylose metabolic pathway from xylose-utilizing yeasts such as *Pichia stipitis*. Xylose utilization by *S. cerevisiae* has been achieved by expression of the *Pichia stipitis* genes encoding the NAD(P)H-dependent xylose reductase and the NAD^+-dependent xylitol dehydrogenase [22], or by expression of genes encoding xylose isomerase [23].

As a result recombinant S. *cerevisiae* strain, expressing all these genes was constructed, followed by a directed evolution strategy to improve xylose utilization rates. There are also reports for construction of *S. cerevisiae* strain that consumes L-arabinose and produces ethanol [24]. Methylotrophic yeast *H. polymorpha* is attractive as candidate for SSF because give possibility for ethanol fermentation at elevated temperatures that are suitable for the action of cellulases enzymes. However, the ethanol yield at such conditions is low and intensive works for its improvement are in progress [25, 26]. It is necessary to point that most of genetically engineered organisms revealed their abilities in laboratory conditions by using pure sugar substrates. In many laboratories intensive works on construction and selection of strains with increased resistance to various inhibitors in culture media are performed. There are reports for using such organisms in pilot and semi-industrial conditions, but their genetic characteristics are still unknown because of the company secrets.

2G-bioethanol commercialization

Historically the concept for 2G-bioethanol started as abstract scientific idea and for a long time was developed in laboratory scales only. Cellulosic ethanol concept became more realistic by means of numerous academic and applied studies generously supported by government and private investments. As a result of such efforts and advertisement in the first decade of the 21st century, a lot of companies announced plans to build commercial 2G-ethanol plants, but most of those plans eventually fell apart, and many of the small companies went bankrupt. Nevertheless the process of industrial 2G-ethanol production was not fully blocked and now we can talk about initial steps of its commercialization. Cellulosic ethanol commercialization is the process of building an industry and corresponding logistics for 2G-ethanol manufacturing together with its deployment and large scale use as transportation fuel. Such process is in its initial stage and 2G-ethanol production currently exists at "pilot" and "commercial demonstration" scale. By today (2012), there are many demonstration plants throughout the world, and handful of commercial-scale plants which are in operation or close to it. Very active in the field of 2G-ethanol are Canada and United States in North America. Canadian companies such as Iogen, POET, and Abengoa, are building refineries producing 2G-bioethanol in overall of 6 million liters per year. Other companies, such as Diversa, Novozymes, and Dyadic, are specialized in producing enzymes that could enable a cellulosic ethanol future. Commercialization in the United States resulted in building of plants that produce totall 12 million liters per year, and an additional 26 new plants able to produce 80 million liters per year are under construction. Such activity is a consequence of US Energy Independence and Security Act. According to this document during the period of 2007 to 2022 year, 137 billion liters of liquid fuel must originate from renewable sources including sets a ceiling of 57 billion liters for the amount that can be produced from corn starch and 80 billion liters of biofuels from other conventional feedstock such as sugar as well as nonconventional cellulosic feedstock. Such ambition programs are supported by serious investments in scientific and RD&D activities also [27]. European Community (EC) also provides policy for encouraging and stimulating biofuel production and consumption and especially bioethanol. There is demand that EC countries have to increase biofuel consumption to 5.75% of all fuels [28]. There are regulations concerning fuels quality as well as tax (duty) discounts encouraging the use of petrol-bioethanol mix according European standard EN228:1999 [29]. In Europe, several plants are operational in Denmark, Germany, Spain, and Sweden, and capacity of 10 million liters per year is under construction [30]. The most ambition program for cellulosic ethanol commercialization functions in Italy. Italy-based Mossi & Ghisolfi Group broke ground for its 13 million US gallons per year cellulosic ethanol facility in Crescentino in northwestern Italy. The project will be the largest 2G-ethanol project in the world, 10 times larger than any of the currently operating demonstration-scale facilities. The plant is "expected to become operational in 2012 and will use a variety of locally sourced feedstocks, beginning with wheat straw and Arundo donax, a perennial giant cane"[31]. In United Kingdom a $400 million investment program to cover the construction of a world scale ethanol plant and a high technology demonstration plant to advance development work on the next generation of biofuels has been announced by BP, Associated British Foods and DuPont. The 2G-bioethanol plant will be built on BP's existing chemicals site at Saltend, Hull [32]. The other countries that actively work on development of its cellulosic bioethanol industries are Australia, China and Japan.

Described progress toward 2G-commercialization is obvious and impressing but the amounts of bioethanol produced are still very low compared with those of 1G-alternative. This is because 2G-bioethanol production will continue to face major constraints to full commercial deployment [3]. The processing technologies are relatively immature with pilot plant supplying less then 0.1% of world biofuel production [33]. In general terms to achieve sustainable solution of biofuels problem it is necessary to make transition from 1G- to 2G-bioethanol. Such transition and integration between both bioethanol productions is most likely to encompass the next one to two decades. When 2G-bioethanol became fully commercialized it is likely it will be favored over 1G alternatives. Some authors proposed that if 2G-bioethanol commercialization succeeds in the 2013-2015 time frame, the rapid deployment of this biofuel will occurs world-wide beyond 2020 [34].

Success in the commercial development and deployment of 2G-bioethanol will require significant progress in numerous of areas if the technological and cost barriers they currently face are overcome. For the solution of such problems is necessary actively to promote further development of 2G-ethanol as an alternative to conventional petroleum transportation fuels through a variety of mechanisms. To achieve such goal is necessary to increase government's investments for RD&D programs. Such investigations include research to develop better cellulose hydrolysis enzymes and ethanol-fermenting organisms, to engineering studies of potential processes, to co-funding initial 2G-ethanol plants from cellulosic biomass demonstration and production facilities. This research is conducted by various laboratories, as well as by universities and private industry. Engineering and construction companies and operating companies are generally conducting the engineering work. The other ways for 2G-ethanol commercialization are its geographical expansion and efforts to make this product cost effective. For future 2G-bioethanol industry is important to work on the geographic distribution of residues and wastes that can be used as feedstocks. For such reasons it is necessary poor countries to be encouraged to develop 2G-bioethanol industry. 2G-bioethanol can substitute for fossil fuels only if large-scale production is feasible, environmentally friendly and compatible with socio-economic structure of society. The most serious problems with decreasing of its costs are concern with logistics of providing a competitive, all-year-round supply of biomass feedstock. Harvesting, treating, transporting, storing, and delivering large volumes of biomass feedstocks, at a desired quality, all-year-round, to a biofuel processing plants requires careful logistical analysis prior to plant investment and construction. Supplies need to be contracted and guaranteed by the growers in advance over a prolonged period in order to reduce the project investment risk. For such reasons it is necessary to guarantee feasibility of a large scale biomass and reliable transportation between biomass sources and end use points. Decreasing of transportation costs is very important pre-requisition for 2G-ethanol commercialization also. The other profits can be obtained from selling of additional valuable co-products isolated during production of 2G-bioethanol. Such products are wastes obtained after distillation of fermentation media that can be used for foraging of animals as well as several chemicals produced in small volumes but with very high price. Such possibility offers the potential to increase overall revenue from the process and improve the economics of the process.

There is no doubt that impressive progress with 2G-ethanol production has been made during the past three decades. However, full commercialization of 2G-bioethanol appear to remain some years away. Future successful progresses include development of improved micro-organisms and the evaluation of innovative conversion technologies with improved performance and efficiencies. There is also better understanding that unless obvious breakthrough in several areas followed by lower of production costs and accelerating investments and deployment, it is expected that successful commercialization of 2G-ethanol will take another decade or so. During this period, demonstration and 2G-ethanol plants will be continuously improved in order that the products become competitive with petroleum fuels as well as with 1G-ethanol. Therefore a long term view for the potential of 2G-bioethanol should be taken. Even with generous government subsidies, the commercial risks remain high, especially with recent widely fluctuating oil prices and global financial turmoil adding to the investment uncertainty. Possible solution of this problem is further international co-operation and collaboration. Those are necessary to continue and became more effective and productive. Therefore, the problems described are basis for further cost reductions and increased production efficiency.

2G-bioethanol and sustainability

The term sustainability was introduced in the late 1980s as a part of the concept "sustainable development" that means "development which meets the needs of the present without compromising the ability of future generations to meet their own needs" [35]. In ecology, sustainability describes how biological systems remain diverse, robust, and productive over time, a necessary precondition for the well-being of humans and other organisms. Long-lived and healthy wetlands and forests are examples of sustainable biological systems. From the data reported in previous section is obvious that development of biofuels industry will impact by various ways local and global ecosystems. Therefore, it is necessary to evaluate and understand the sustainability of biofuels, especially because of the significant increases in production mandated by many countries. Here we will try to estimate the effect of such rapidly increasing industry on sustainability in the context of bioethanol because sustainability will be a strong factor in the regulatory environment and investments. In such respect sustainable development and sustainability science are combination of economic development, social development and environmental/resource sustainability. The expansion of 2G-ethanol manufacturing may offer opportunities that enhance energetic sustainability when compared to other transportation fuel alternatives. Sustainable 2G-bioethanol industry require several pre-requisitions: economic and social development as well as environmental/resource sustainability. If the process of its commercialization proceeds with the same dynamic we can expect that such new industry will impact various different area including society, ecology, biodiversity, climate, etc. The extent and nature of these impacts may vary from positive to negative through the entire process from feedstocks production to conversion, distribution, and end use. The potential effects of feedstock production are likely to have a significant influence on the sustainability of biofuels [36, 37].

Methods used for bioethanol sustainability assessment are diverse and even controversial. One of them is dealing with the existing environmental certification systems and for their applicability to the growing bioenergy trade [38]. The other authors propose system approach, incorporating the latest scientific knowledge and social values [39]. They include next principles: (1) Legality, (2) Planning, monitoring and continuous improvement, (3) Greenhous gas emissions, (4) Human and labor rights, (5) Rural and social development, (6) Local food security, (7) Conservation, (8) Soil, (9) Water, (10) Air, (11) Use of technology, inputs, and management of waste, and (12) Land rights [40]. The other authors include also the role of bioethanol on environmental protection and biodiversity as well as quality of life and the dialog on ethic of sustainable development [41].

The land used for feedstock production is a key factor in determining biofuels sustainability. Ultimately, the long-term sustainability of 2G-bioethanol throughout the world depends on land-use practices and landscape dynamics. Land-management decisions often invoke trade-offs among potential environmental effects and social and economic factors as well as future opportunities for resource use. The other factors include agricultural subsidies, influence the cost of food commodities and their impact on the poor. Food-biofuel competition could be avoided by focusing biofuel production on less productive lands. Possible solution of such problems is utilizing of marginal agricultural land that has been abandoned or set aside for conservation purposes to grow biomass. However, some authors pointed that the potential to use marginal land for bioethanol feedstock production is limited and is necessary carefully to make decision what kind of plants would be cultivated [42].

Solomon [40] analyzed various dimensions for biofuels, like as: optimal scale (resource assessment, land availability, and land use practice), economic and energy efficiency, equitable distribution of biofuels, socio-economic issues, as well as environmental emissions and effects. He concluded unequivocally that G1-bioethanol is unsustainable and has significant environmental costs [40]. He proclaimed that "... only cellulosic ethanol has the potential to be produced and consumed in a sustainable basis, based on all possible socio-economic and environmental criteria, including the meeting of soil residue maintenance requirements" [40]. The basis for such conclusions are several reasons: a larger resource and land base for the feedstock, higher energy return on investment, potentially greater economically efficiency, equitable resource distribution, little or no conflict with food resources, and much lower greenhouse gas emissions and other environmental effects [40]. Solomon's analysis indicate for the brilliant future for 2G-bioethanol if its commercialization proceeds rapidly and all problems that constrain large scale production will be solved. Finally, if bioethanol want to be part of solution of sustainable energy problem it is necessary to accept a degree of scrutiny unprecedented in the development of a new industry. That is because sustainability deals explicitly with the role of biofuels in ensuring the well-being of our planet, our economy, and our society both today and in the future.

Environmental impact of 2G-bioethanol

Large scale commercialization of 2G-bioethanol throughout the world will have various long standing effects on environmental. Some consequences of such impacts are predictable and positive, but the other are difficult for assessment and provoke uncertainty and protests from environmentalists. Obviously the global development of bioethanol industry and utilization of bioethanol as liquid fuels will have global impact on the planet and the climate. The positive and negative effects of biofuels on land, water and air have to be carefully investigated and discussed. Some authors predict that development of bioethanol industry can create conflicts with environmental protection in many areas of the world. The first and very serious conflict is about land, its use, conservation and preservation. It is predicted that further increasing of 1G-bioethanol based on crops leads to land exhaustion and soil erosion. Expansion of G1- bioethanol may increase environmental pressures due to the higher levels of fertilizer use and from here to increased amounts of contaminated with nitrates ground waters. Such employment has negative effects on biodiversity also [43]. In contrast to such dark expectations the same authors suggests that development of 2G-ethanol will reflect in positive manner on land utilization. The production of 2-G bioethanol is expected to fare better than 1G-bioethanol production on most of these factors, although the magnitude of these differences may vary significantly among feedstocks. Commercialization of 2G-ethanol will have benefits on the quality of air and water also, and from here on many ecosystems. 2G-ethanol gives possibility for restoration of contaminated water resources, which are evaluated as sources of nutrients and water to improve feedstock productivity. 2G-ethanol is expected to result in fewer greenhouse gas and air pollutant emissions. According to US Department of Energy studies conducted by the Argonne Laboratories of the University of Chicago, cellulosic ethanol reduces greenhouse gas emissions by 85% over reformulated gasoline. By contrast, 1G-ethanol, which usually uses natural gas to provide energy for the process, reduces greenhouse gas emissions by 18% to 29% over gasoline.

Described advantages of 2G-bioethanol explain why several government programs for creation of industrial production of plant biomass were proclaimed. They include cultivation and utilization of grasses and shrubs that can grow on soils that are not suitable for feedstock, as well as in countries with adverse climate were claimed recently. It is possible to develop industrial production of wood by cultivation of rapidly growing woods as, poplars, willow, etc. There are recommendations for cultivation of plants that are not popular, but posses high carbohydrate content and could be easily converted into ethanol. Such plants are regarded as perspective for bioethanol industry, for example plants that grow on nonproductive soils or in harsh conditions in deserts, semi-deserts, soils with elevated salts, contaminated area, etc. Additionally plant biomass can be cultivate by growing of certain plants. Among them great expectations are assign of species like Switchgrass (*Panicum virgatum*) and Miscanthus (*Miscanthus giganteus*) due to their high productivity per acre. Use of non-food and non-feed renewable feedstock for fuel ethanol production has energetic, environmental and economic advantages as compared to use of food and feed stocks. It is proposed that only ethanol, obtained from plant biomass (lignocellulose), cultivated at low-fertility soils with minimal use of fertilizers and pesticides, or residues of agriculture and wood industry could satisfy needs of the world in fuels [3]. It is important that utilization of plant biomass is technologically possible and is based on existing industry. In the case that planed programs are realized in large scales such industry will be competitive and profitable.

CONCLUSIONS

This paper describes progress and problems toward development of technology for production of bioethanol from lignocellulose, called also as second generation bioethanol (2G-bioethanol). From the data presented is evident that impressing achievements have been obtained during the past three decades in such field. However, full commercialization of 2G-bioethanol appear to remain some years away and the amounts of 2G-bioethanol produced are still very low compared with those of 1G-alternative. 2G-bioethanol is not a panacea for solving fuel problems and its main advantage is that it will offer a domestic source of a significant fraction of our fuels needs. 2G-bioethanol can substitute for fossil fuels only if large-scale production is feasible, environmentally friendly and compatible with socio-economic structure of society.

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