

## PRODUCTION OF ALCOHOL FOR FUEL AND ORGANIC SOLVENTS

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### Summary

The presented article describes the production of fuel ethanol and organic solvent

(acetone and butanol) using biotechnological methods. Its history and production volumes, together with environmental and economical aspects, are discussed in what follows. As ethanol producers, the yeasts of the genus *Saccharomyces* and bacterial strains—especially *Zymomonas mobilis*—are considered on laboratory and industrial scales, whereas *Clostridium acetobutylicum* presents the solvent producer. As main raw material the various grains are used, although sugarcane and sugarbeet juices are also suitable for ethanol production including continuous fermentation processes. Lignocellulose is another promising raw material for ethanol production. On an industrial scale, ethanol is predominantly produced from grain in batch fermentation processes. For this process it is recommended to use thermostable  $\alpha$ -amylases for starch liquefaction followed by a simultaneous saccharification and ethanol fermentation for modern ethanol production systems using grain as a raw material. Such a system exhibits a productivity of 100-150g L<sup>-1</sup>h<sup>-1</sup> on a laboratory scale using immobilized or flocculating cell cultures, recycling, vacuum, or extractive fermentation processes. Distillation, rectification and molecular sieve dehydration are recommended for obtaining a fuel grade ethanol with 99.6% ethanol content. The fusel oil fractions can be included in fuel grade ethanol.

Gasoline needs at least a 5% ethanol additive for oxygenating the fuel. An alternative is the production of ETBE replacing MTBE as oxygenate. A regulated tax policy by government is necessary to compete with present oil fuel.

The biotechnological production of organic solvents competes with chemical synthesis. An application of immobilized *C. acetobutylicum* in continuous fermentation achieves a system productivity of up to 2 to 3g L<sup>-1</sup>h<sup>-1</sup> on laboratory scale. The by-products (stillage in dry form) could be used for animal feeding.

Bioindustry and agriculture must be integrated in semiclosed production systems to become more economical.

## **1. Production of Fuel Ethanol**

### **1.1. Introduction**

#### **1.1.1. History and Actuality**

In accordance with the prognoses of the World Energy Council in 1994, fossil fuels will continue to provide the bulk of energy demands for the next few decades. At current consumption rates, coal has some 250 years of reserves, oil over 40 years and natural gas over 65 years. One of the alternative energy resources is renewable energy including biomass (*see also Energy from Biomass and Other Organic Residues*). The use of ethanol produced from biomass as fuel positively influences the CO<sub>2</sub> balance and would be in line with the 1992 Rio Declaration and the 1997 Kyoto protocol. In accordance with the European Union Clean Air Act 1994, 2% of oxygen must be added to transportation fuels, which corresponds to a 5% ethanol addition. Methyl Tertiary Butyl Ether (MTBE) as oxygenate has shown a negative environmental effect, and must be replaced with ETBE (Ethyl Tertiary Butyl Ether). The physical properties of ethanol, which are close to the properties of oil products, are presented in Table 1.

Property	Value
Boiling point at 101,3 kPa	+78.3°C
Melting point at 101,3 kPa	-115°C
Combustion temperature in air	+426°C
Firing temperature	+14°C
Density	0.78927 g/cm <sup>3</sup>
Octane number	106
Minimal combustion heat	26.9 MJ/kg

Table 1. Physical properties of ethanol.

The worldwide bioethanol production volume is about 3 was 42.3x10<sup>9</sup> L per year by the year 2004 with about 30.0 x10<sup>9</sup> L used as biofuel . The average growth rate of ethanol production during the period from 1985 to 1997 was 6 percent per year. The total production is estimated to be 36-37x10<sup>9</sup> L year<sup>-1</sup> by the year 2005 with 60 percent being used as biofuel. The largest markets for fuel ethanol can be found in Brazil [14x10<sup>9</sup> L year<sup>-1</sup> ] and in the USA [5The largest producers of ethanol are Brazil [14.6 x10<sup>9</sup> L year<sup>-1</sup>] and USA [14.3 x10<sup>9</sup> L year<sup>-1</sup>]. EU produced 2.6 x10<sup>9</sup> L year<sup>-1</sup> ]. The President of the United States announced a tripling of the use of biomass technologies ethanol [data by the year 2010 in order to produce fuels and materials thereby increasing the income to farms, lessen oil imports and lessen the risk of global warming. Fuel ethanol is therefore going into direct competition with the petrochemical products.2004].

Fuel ethanol production declined since the early 1888's. Ethanol derived from sugarcane has been used as a fuel in Brazil since 1903, adding 5 percent to gasoline and reached a production level of 650 million litresliters by 1941. Gasoline blended with 30-50 percent ethanol and named Latol has been used also in cars in Latvia before World War II, as well as, ethanol additive to gasoline was used in some other countries at this time. Ethanol from cellulose raw materials has been the main strategy for fuel production in Sweden (*see also Cellulose Bioconversion to ethanol*).

Petroleum products dominated in the post-World War II era up to the energy crisis in 1973. OPEC price fixing has influenced the Brazilian economy to a high degree, with more than 80% of petroleum products imported, accounting for approximately 42% of the nation's energy consumption and about 50% of their foreign exchange outlays. The national Brazilian "PRO-ALCOOL" program was developed during the period from 1975 to 1985, resulting in the establishment of 600 distilleries producing a total of about 16x10<sup>9</sup> L of ethanol. Pure ethanol and blends of gasoline with up to 22% anhydrous ethanol have been used as motor fuel. In order to allow the use of pure ethanol as fuel, Volkswagen and other companies developed a pure ethanol combustion engine and produced 4.5 million cars during this decade.

A decade later, fuel ethanol production from corn was developed in the USA, which recommended a 10% ethanol blend in gasoline and the production of ETBE as octane enhancer. Since 1981, a 10% ethanol-gasoline blend has been used in Canada at

approximately the same price as regular gasoline.

In order to allow the use of pure ethanol as fuel, some companies developed a pure ethanol combustion engine which allows to use as fuel ethanol and gasoline in any proportions.

The production of fuel ethanol in Europe is very limited when compared with that in the USA. The projected demand for fuel ethanol in the EC is about  $3 \times 10^9$  L by the end of the century. The raw materials for ethanol production in the EC are wheat and sugar beet. In France, ETBE production already plays an important role in the protection of agriculture.

### 1.1.2. Environmental Effects of Fuel Ethanol

The following pollutants emitted through the exhaust pipes of internal combustion of gasoline- and diesel-powered vehicles have a negative effect on the environment: carbon monoxide (CO), hydrocarbons, nitrogen oxides (NO<sub>x</sub>), sulfur dioxide (SO<sub>2</sub>), particulates, volatile organic compounds (VOC), and lead. The EC green paper reported that, in 1986, 22.5% of total CO<sub>2</sub> emissions (= 577 million t) were released by the transport sector, of which 79.7 % was from road transport alone. The share of the other pollutants from road transport is 53.6% NO<sub>x</sub>, 27.1% VOC, and 2.9% SO<sub>2</sub>. The three most negative pollutants are CO, hydrocarbon, and NO<sub>x</sub>. The addition of fuel ethanol to gasoline cuts down the content of sulfur, aromatics, and olefines, thereby reducing significantly the emissions of sulfur, toxic and photochemical reactive substances—as well as CO—into the atmosphere. With the use of pure ethanol cars, Brazil was able to reduce the CO emissions tenfold in light vehicles over the period between 1980 and 1995. Lead compounds were completely phased out from Brazilian gasoline in 1991. Brazil's experience shows that the introduction of gasohol, a 20% ethanol blend, had an immediate impact on the air quality of Sao Paulo and other large cities. The only negative effect of fuel ethanol is the increase in acetaldehyde emission. Ethanol and ETBE are efficient oxygenates, can be substituted for lead in gasoline, and actually reduce the amount of CO emission as well as uncombusted hydrocarbons. The oxygenated products in the USA are regulated by the “Clean Air Act Amendments of 1990”. All member states of the EC are required (since 1989) to have lead-free petrol in their own territory.

### 1.1.3. Economic Aspects and Government Policies Affecting Fuel Ethanol Production and Marketing

Grain production costs for large-scale dry milling plants are presented in table 2.

	US cents/ L	%
Net corn	17.0	60.07
Energy	4.2	14.84
Emzymes and yeast	1.6	5.65
Misc. chemicals	1.6	5.65
Maintenance	1.8	6.36

Direct costs	26.2	92.5
Direct operating costs	2.1	7.42
Total	28.3	100

<sup>a</sup> DDGS - Distillers dried grains with solubles.

Table 2. Grain ethanol production costs for large-scale dry milling plants (at US\$ 120 t<sup>-1</sup> corn and US\$ 160 t<sup>-1</sup> DDGS).

Ethanol production not only improves air quality, but also influences positively the development of agriculture and unemployment as well as reducing foreign oil importation. It has been the policy of the US government since 1978 to encourage the development and use of ethanol-blended fuels and oxygenates including ETBE. The Energy Tax Act of 1978 established the first Federal incentive for gasoline containing 10% (v/v) ethanol. The tax on oxygenated fuel was reduced. The Federal Government of Brazil decided in 1975 with the “Decret 76.593/75” to encourage the production of ethanol to replace gasoline, fixing the price of gasoline at double the value of that in the USA. Ethanol production was very attractive under conditions where its selling price was 59% of the price of gasoline. Today, most national ethanol markets are in balance or strictly state controlled. Worldwide over 90% of ethanol is produced for the local market, with world trade being handled in the tariff range of from 30 to 100%.

## 1.2. Background

### 1.2.1. Producers and Regulation of Metabolism of Ethanol Synthesis

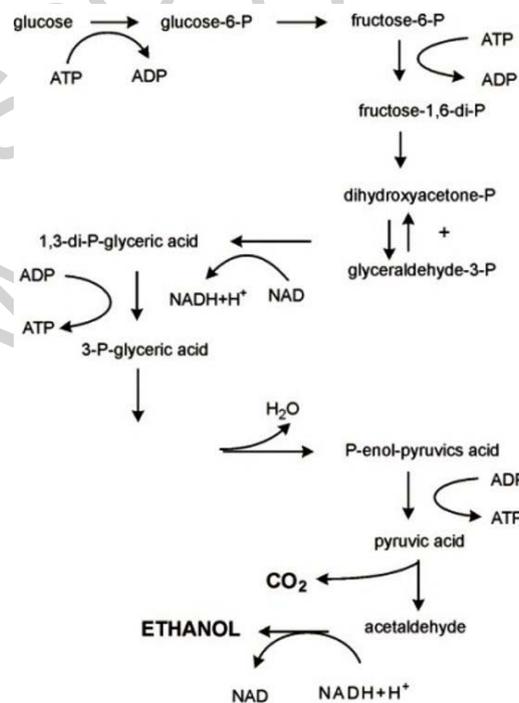


Figure 1. Embden-Meyerhof (glycolytic) pathway.

The most commonly used ethanol producers (*see also Ethanol Fermentation*) are yeasts using species of *Saccharomyces* and *Kluyveromyces*, e.g. *S. cerevisiae*, *S. uvarum*, *Kluyveromyces fragilis*. Under anaerobic conditions, yeast converts sugars to ethanol following the Embden-Meyerhof Pathway (EMP) (*see also – Basic Strategies of Cell Metabolism*) (Figure 1).

Two moles of ethanol, CO<sub>2</sub> and ATP are produced from one mole of glucose:



Some nutrients are utilised for new biomass synthesis, cell maintenance and by-product formation (e.g. glycerol, succinate). The synthesis of ethanol by yeasts is tightly coupled with cell growth, which means that growing yeasts produce ethanol much faster than non-growing yeasts. For a more effective fermentation, yeasts must therefore be supplied with small amounts of oxygen and other medium components (nitrogen, minerals, vitamins) for the biosynthesis of the polyunsaturated fatty acids and lipids of their cell membrane. The provision of yeasts with oxygen or polyunsaturated fatty acids and sterols ensures cell membrane integrity and increases ethanol tolerance.

A number of bacteria can also produce ethanol. However, until around the year 2000 ethanol producing bacteria were only used on laboratory scale. Some of these bacteria produce not only ethanol but also a number of other end products such as higher alcohols, organic acids, polyols, ketones, or various gases (Table 3).

<b>Mesophilic organisms</b>	<b>Thermophilic organisms</b>
<i>Clostridium sporogenes</i>	<i>Bacillus stearothermophilus</i>
<i>Clostridium indolis</i>	<i>Clostridium thermohydrosulfuricum</i>
<i>Clostridium sphenoides</i>	<i>Clostridium thermosaccharolyticum</i>
<i>Clostridium sordelli</i>	<i>Clostridium thermocellum</i> <i>Thermoanaerobium brockii</i>
<i>Erwinia amylovora</i>	<i>Thermoanaerobacter ethanolicus</i>
<i>Leuconostoc mesenteroides</i>	
<i>Sarcina ventriculi</i>	
<i>Spirochaeta aurantia</i>	
<i>Spirochaeta litoralis</i>	
<i>Streptococcus lactis</i>	
<i>Zymomonas mobilis</i>	

Table 3. Ethanol producing bacteria.

The most promising bacterium for fuel ethanol production is without any doubt the gram-negative bacterium *Zymomonas mobilis*. This bacterium converts sugars (glucose, fructose, sucrose) to ethanol using the Entner-Doudoroff Pathway (*see also – Basic Strategies of Cell Metabolism; – Biomass and Organic Waste Conversion to Food, Fuel, Fertiliser, Energy and other Products*). In this case, two moles of ethanol and 1 mol ATP are formed from 1 mol glucose. *Zymomonas mobilis* can tolerate relatively high levels of ethanol. The rate of alcohol production by *Zymomonas mobilis* exceeds that of

yeast. This is caused by the mechanism of “uncoupled growth”, whereby ethanol production is independent of the active growth of the bacterium. This property is favourable for intensive ethanol production. The disadvantage of this bacterium is its relatively narrow range of substrates. Genetic modification of *Z. mobilis* by different genetic engineering techniques aims to obtain strains capable of utilise other substrates (starches, lactose, cellulose, xylose), and is a very promising direction of investigation at the beginning of the century. Recombinant plasmids encoding  $\beta$ -galactosidase, fused proteins, and lactose permease from *Escherichia coli* were introduced into *Z. mobilis*. This strain of *Z. mobilis* can produce ethanol from lactose, but cannot grow on lactose as its sole source of carbon. Another example may be the genetically modified strain *Z. mobilis* IFO 13,756 being capable to assimilate galactose and attempts have also been made to express the  $\alpha$ -amylase gene in *Z. mobilis*.

### 1.2.2. Raw Materials—Renewable Resources

Ethanol production is based on sugarcane, sugar beet, grain, starch, or hydrolysates of lignocellulosic materials as well as on byproducts of certain industries (molasses, wine substrates, whey, waste sulfite liquor). Table 4 shows average yields for ethanol production from different crops.

Crops	Ethanol yield ( l/ha )
Sugar cane	5150
Cassava	4450
Sweet sorghum	2800
Corn grain	2800
Wheat	2081
Sugar beet	4755
Eer. Artichoke	5000

Table 4. Ethanol yields from different crops.

The energy output/input ratio for the production of ethanol is from sugar beet 1.63, from wheat 1.14, and from corn (=maize) –1.25. The most promising raw material for fuel ethanol production would be lignocellulose, since the global photosynthesis yields an annual net production of  $1-1.75 \times 10^{11}$  t of terrestrial dry biodegradable material, 40% of which is cellulose.

### 1.3. Industrial Technologies

Present industrial fuel ethanol production is based on the use of yeast species of the genera *Saccharomyces* and *Kluyveromyces*. Under anaerobic conditions, each gram of glucose can theoretically give 0.51g ethanol. In practice, however, the ethanol yield does not exceed from 90 to 95%, and on industrial scale from 86 to 90%, of the theoretical value. Some parts of the nutrients are required for biomass synthesis and cell maintenance reactions. About 4 or 5% of the total substrate is usually utilised for glycerol and succinate formation. The medium for ethanol production requires small quantities of O<sub>2</sub>, P, S, K, Mg, Mn, Co, Cu, and Zn as well as organic growth factors

such as amino acids, nucleic acids, and vitamins. Yeasts are very susceptible to ethanol inhibition. For example, 1 or 2% (w/v) ethanol decreases cell growth, and 10 % (w/v) stops ethanol synthesis. During the fermentation process, fusel oils are formed from  $\alpha$ -keto acids, derived from deaminations of amino acids. The major fusel oil component is isoamyl alcohol (from 40 to 60%), with optically active amylalcohol and isobutylalcohol making up from 15 to 20% each of the total. In general, the yield of fusel oil is 20 L m<sup>-3</sup> of ethanol produced. Fusel oils can remain in the final product as a component of fuel ethanol.

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## Biographical Sketches

**Martins Bekers**, Latvian, born 1928. *Education:* Graduated from the Faculty of Food Industry at the Latvian Agricultural University (1953), Ph.D (1959), Dr.habil.eng (1972), Dr habil.biol (1992), Professor of biotechnology in Riga Technical University (1972).

*Experience:* Engineering in Riga Yeast factory (1953-1959) and Latvian Food Industry Technology and Reconstruction bureau (1959-1962); Vice-Director and Head of laboratory of Institute of Microbiology, Latvian Academy of Sciences (1962-1993); Director of Institute of Microbiology and Biotechnology (MBI) University of Latvia (1993-1997); Head of laboratory of Industrial Microbiology and Food Biotechnology of MBI (1997-2006).

*Honors and awards:* Full member of Latvian Academy of Science; Dr.h.c of Latvian Agricultural University; Latvian State Prize (1965, 1980). 2003 Latvian State Three-Star Order. *Interests.* Biotechnology of lysine, ethanol, lactic acid production; osmotolerance and anabiosis of microorganisms; biofuel problems. *Publications:* 9 monographs, 580 articles, 95 patents. *Head of research projects:* Dehydration of yeasts; Production of vitamine B<sub>12</sub>; Production of lysine; Transformation of products of photosynthesis; ethanol and levan production by free and immobilized *Zymomonas mobilis*; Biofuel (ethanol, biodiesel and biogas) production under Latvian conditions.

*Courses:* Introduction in biotechnology (Riga Technical University); Food biotechnology (University of Latvia). Detailed information in: <http://www.lza.lv/scientists/bekersm.htm>

**Amands Vigants**, Latvian, born 1967. *Education:* 1992.- University of Latvia, Faculty of Chemistry, chemist Specialized in analytical chemistry. 1999. - obtained the degree of Dr.biol. in University of Latvia. Theme of dissertation: "Growth of bacteria *Lactobacillus casei*, *Lactobacillus plantarum* and *Zymomonas mobilis* and product synthesis under conditions of increasing medium osmolality".

*Experience:* 1990-1993 - Company RECET. Work connected with monitoring and purification of industrial wastewater. 1993-2006 - Institute of Microbiology and Biotechnology University of Latvia, Laboratory of Technical Microbiology and Food Biotechnology. Since 1995 lead the Center of Chromatographic Analysis of institute. 1995.-1996. participate in the project "Osmoinduced metabolism network in bacteria" supported by Danish Council of Science. 1999-2001 – "postdoc" studies in GKSS research center Institute of Chemistry (Teltow, Germany). 2001-2006 project leader from Latvian side in bilateral cooperation project between GKSS research center and LU MBI. Since 2002 senior researcher in LU MBI. 2001-2004 and 2004-2007 head of projects supported by Latvian Council of science: "Investigation of catalytic properties of *Zymomonas mobilis* levansucrase; enzymatic bioconversion of sucrose" and „Biosynthesis of valuable products of *Zymomonas mobilis* by levansucrase and glucose-fructose oxidoreductase – influence of physico-chemical factors on stability and activity of enzymes".

*Research interests:* Chromatographic methods of analysis of biological objects. Physiology of bacteria and influence of different growth medium factors on metabolism. Main object of investigations - *Zymomonas mobilis*. Others research interests deals with purification and immobilization of enzymes. Biotechnology of fermented food.

Detailed information in: <http://www.lu.lv/instituti/mbi/industrial/enzymatic.html> *Publications:* 18 articles, 3 patents, 17 conferences proceedings and thesis