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Biofuels

S D Varfolomeev, E N Efremenko, L P Krylova

Contents

I.	Introduction	491
II.	Efficiency of solar energy conversion through photosynthetic biomass production	493
III.	Biomethane	495
IV.	Bioethanol	496
V.	Biobutanol	498
VI.	Biodiesel	499
VII.	Biohydrogen	502
VIII.	New approaches towards biofuel production	504

Abstract. The main types of biofuels that can be obtained from biorenewable energy sources are analyzed. As the sources, different types of biomass accumulated as the result of photosynthetic conversion of solar energy are considered. The synthesis of biofuels is characterized by the use of combined chemical and biotechnological approaches. The bibliography includes 260 references.

I. Introduction

Energy resources and power consumption are the key problems of stable development of the modern technological society. The Nobel Prize winner academician N N Semenov was among the first to point out the necessity of radical changes in the power production industry and the development of renewable energy sources. Analyzing the dynamics of energy consumption and the environmental characteristics of heat pollution and CO_2 contamination, he wrote:[1] 'Due to the future rapid depletion of common fuel resources and increase in the carbon dioxide content in the atmosphere, the mankind is confronted with the urgent problem of developing a radically new basis for world's energy production. Apparently, little time is left to do this, merely about 100 years.'

The present situation poses the problem of global energy security and the antirecessionary policy in energetics.[2] The substantial and all-increasing role in world's power production was played by renewable energy sources based on solar energy. From the viewpoint of the Nobel Prize winner, academician Zh I Alferov, sticking to solar energy should be considered not only as the safe but also as the only possible long-term future scenario for the mankind.[3] In the past decade, it became obvious that the forms and methods of solar-energy conversion are abundant and diverse. Nowadays, methods of electricity production by wind turbines have become quite common. The solar energy conversion systems based on the use of heat and heat engines are being actively developed. The solar energy luminous flux is highly intense; in deserted mountain areas at low latitudes its density may reach 0.5 – 1.8 kW m^{-2}. Solar thermal power stations using parabolocylindric and parabolic concentrators can convert solar energy to electricity with the efficiency of 25% – 30%. In 2008, at the National Laboratory of Sandia (USA), the efficiency of 31.2% was reached with the use of a parabolic concentrator with the Stirling engine. Solar power stations are becoming quite competitive with conventional power production methods.

Systems of direct solar energy conversion to electricity equipped with semiconductor converters were being developed extremely actively. The power of photoelectrical semiconductor converters installed all over the world increases exponentially with the doubling time of 1.5 – 1.8 year. The leaders in these 'races' are China, Japan and EU countries.[4]

Very dynamic progress was observed in the recent development of photovoltaic converters of solar energy.[5, 6] Figure 1 shows the increase in the overall capacity of semiconductor photoelectric converters as compared with the present values and the predicted growth in the electricity global production by all methods used so far (thermal power stations on coal, oil and gas; hydroelectric power stations, nuclear stations). It is seen that in 2025 – 2035, the amount of electricity produced with photovoltaic solar energy converters can become equal to that produced by

S D Varfolomeev, L P Krylova N M Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, ul. Kosygina 4, 119334 Moscow, Russian Federation. Fax (7-499) 137 41 01, tel. (7-495) 939 35 89, e-mail: sdvarf@sky.chph.ras.ru (S D Varfolomeev), tel. (7-495) 939 72 85, e-mail: lpkrylova@sky.chph.ras.ru (L P Krylova)
E N Efremenko Department of Chemistry, M V Lomonosov Moscow State University, Leninskie Gory, 119991 Moscow, Russian Federation. Fax (7-495) 939 35 89, tel. (7-495) 939 31 70, e-mail: elena_efremenko@list.ru

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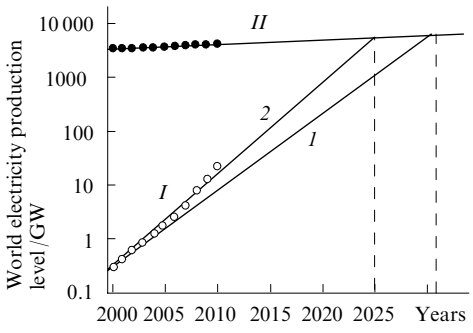


Figure 1. Dynamics of electric power production using semiconductor solar energy converters (*I*) as compared with the conventional power production methods (*II*).[4]
Extrapolated from the data of 2000–2005 (*1*) and 2005–2010 (*2*).

conventional methods. The similar trend becomes evident when comparing the growth dynamics in the production of oil and liquid biofuels (bioethanol and biodiesel) (Fig. 2). Bearing in mind the rapid progress in the wind power production and solar thermal power production, it can be argued with confidence that in the first quarter of the 21st century, the era of traditional fossil fuels will come to end and solar energy will occupy the first place in world's power resources. Current progress confirms the predictions made by academician Semenov.

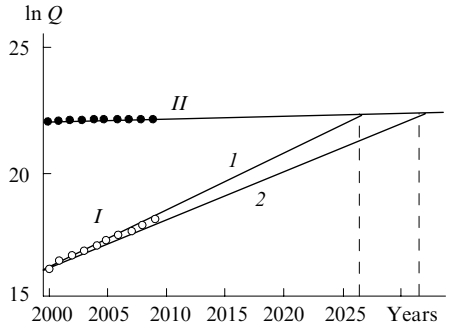


Figure 2. Dynamics of bioethanol and biodiesel production (*I*) as compared with oil production (*II*).[6]
Extrapolated from the data of 2000–2005 (*1*) and 2005–2010 (*2*). *Q* is the production volume/billions of tons.

Energy demands are largely determined by the world population, *i.e.*, the development of world power production correlates with the growth of population.

In the past 30–50 years, the increase in the *per capita* power production failed to keep pace with the population growth. Since the middle 1960s, world's annual power production *per capita* had reached a constant level ($\sim$60 GJ) and tends to decrease. This was determined exclusively by the rapid growth of world population.[7,8]

What will be the future demands of mankind in energy and are the renewable energy sources, particularly, biofuels, able to meet these demands?

It was demonstrated that at present we observe a decrease in the population growth rate.[9] Mathematical simulation of this process made it possible to prognose the growth dynamics as the system passes to the stable state with the limiting population of 9–11 billion in 80–100 years. Assuming that the power consumption *per capita* will increase to reach 100 GJ, the power required for the stable existence of mankind is assessed to be $\sim 10^{21}$ J. Taking into account the development of energy-saving technologies that allow the energy consumption to be substantially reduced, the future Earth population will be comfortably supplied with this power. The energy density in sunny territories is sufficiently high, *i.e.*, 800–1500 W m^{-2}. It was assessed that for the power consumption of 800 W m^{-2} with the efficiency of 30% and 12-h illumination, the required energy can be collected from the area of 2.6×10^5 km^2. This is a negligible part of Earth surface (for comparison, the Sahara desert territory is 8.6×10^6 km^2).

A substantial contribution to the development of power production was made by biofuels, *i.e.*, energy resources obtained by photosynthetic conversion of solar energy followed by the chemical and biotechnological processing into liquid or gaseous fuels. The fundamental problems of bioenergy sources production were repeatedly discussed earlier;[10–13] however, this field is being developed sufficiently quickly and requires permanent analysis. The biofuel production increases exponentially with doubling time of 2–4 years.[13] In 1974, the Brasil ethanol project was launched, which to a certain degree provided the growth in the ethanol fuel production. In 2002–2008, world's ethanol production increased 3.5-fold mainly due to the US bioethanol industry. The current yearly world oil production is 5.5×10^{12} litres. The present-day ethanol production is $\sim 5 \times 10^{10}$ litres per year. It can be easily calculated that with the rate of increase in biofuel production remaining unchanged, the world biofuel industry can reach the present oil production level as regards traditional fuels in 15–25 years (see Fig. 2). Moreover, this does not concern biofuel production from grain but includes only lignocellulose-containing feedstocks, first of all, agricultural wastes and residues of processing industries.

Quite a number of publications showed the analysis of scientific and technological prospects of biofuel production in different countries all over the world,[14–18] including Sweden, the Netherlands,[19] Greece,[20,21] Brasilia,[22] Argentina,[23,24] China,[25] England,[26] Taiwan,[27] Korea.[28] The economic aspects of this production were also discussed.[29]

The fundamental stimulus to the development in biofuel industry were the environmental advantages of their use. Biofuels are the products of photochemical reduction of CO_2 and their large-scale production and use in place of conventional hydrocarbon fuels could stabilize the existing level of carbon dioxide accumulated in atmosphere and prevent its increase.

The wide use of solar energy can qualitatively change the state of the environment. As a result of using fossil fuels, large amounts of heavy metals, sulfur compounds, carcinogenic hydrocarbons are emitted to the atmosphere. All this is untypical of methods utilizing solar radiation. The principal feature of using the energy of Sun is the absence of heat contamination. Any other power sources (fossil fuels, nuclear and thermonuclear energy) are associated with the liberation of excessive heat; with the increase in the given type of power production, this will inevitably lead to heat contamination and global overheat of Earth.[1] The future development in this field undoubtedly depends on the accessibility of materials for fabricating solar converters, *i.e.*, on the level of their production.

II. Efficiency of solar energy conversion through photosynthetic biomass production

Photosynthesis, *i.e.*, a complex physicochemical process to reduce carbon dioxide to carbohydrates and other organic compounds with simultaneous water fission, which is accompanied by molecular oxygen formation, serves the basis of biofuel production. In the present review, the photosynthesis is considered from the viewpoint of efficiency of solar energy conversion. The potentially feasible and truly attainable production rates of different systems with respect to biomass production as the feedstock for secondary processes to afford bioenergy resources were analyzed.

Building qualitatively substantiated models of this process seems to be necessary in view of assessing the possibility to improve the actual characteristics. Recently, the efficiency and production rates of photosynthesizing systems were analyzed within the framework of a kinetic approximation.[30]

The state of a photosynthetic unit within this kinetic scheme can be presented as follows:

$$A_1 \xrightarrow[h\nu]{k(I)} A_2 \xrightarrow{k_e} A_3 \xrightarrow{k_{CO_2}} A_1.$$

The complex physicobiochemical process of CO_2 reduction was conditionally divided into the following stages: the primary process that occurs under the action of light quanta ($A_1 \rightarrow A_2$) and depends on the intensity of light absorption [$k(I)$], the electron-transport process ($A_2 \rightarrow A_3$) that can be characterized by the rate constant k_e and, finally, the transport of electrons to CO_2 to afford carbohydrates. What stage is the limiting one depends on the conditions and the ratio of rate constants of mentioned processes.

The energy conversion factor (η) within a photosynthetic process can be defined as

$$\eta = \eta_1 \eta_2 \eta_3 \frac{J_p}{I},$$

where J_p is the stream of the generated product expressed in electrical units; I is the absorbed light intensity; η_1, η_2, η_3 are the coefficients determined by the features of the photosynthesis mechanism, namely, η_1 is the fraction of photosynthetically active radiation (pigment's absorption spectrum) in the total solar flux equal to ~ 0.45; η_2 reflects the two-quanta mechanism of electron transfer within the framework of functioning of photosystem I and photosystem II, it is equal to 0.41 as regards the potential energy and ~ 0.35 as regards the free energy;[10] and coefficient η_3 characterizes the reflecting ability of the system and is equal to 0.8 – 1.0.

According to the kinetic scheme, the generated product stream is determined by the equation

$$J_p = \frac{k(I)A_0}{\{1 + [k(I)/k_e]\} + [k(I)/k_{CO_2}\{CO_2\}]} - \beta A_0, \qquad (1)$$

where A_0 is the total concentration of photosynthetic units per surface unit, $\{CO_2\}$ is the CO_2 concentration. The additional term βA_0 is associated with the consumption of stored energy in the system functioning.

Equation (1) characterizes the production rate of photosynthesis and reflects an important feature in the system behaviour, namely, the 'saturation' of the product stream for high light intensities and high CO_2 concentrations. In the limit, for the 'saturating' values of both the light intensity and the concentration of the final electron acceptor (CO_2), we obtain

$$J_p = (k_e - \beta)A_0 \text{ for } I \rightarrow \infty, \{CO_2\} \rightarrow \infty.$$

The maximum value of the energy conversion factor realized for $J_p/I = 1$, was $\eta_1 \eta_2 \eta_3 \approx 0.15$.

The dependence of η on the light intensity has two peculiarities.

1) A threshold light intensity (I_0) exists below which the system fails to store energy. The larger β value (dark utilization of stored energy), the higher the threshold light intensity that, being exceeded, marks the onset of energy storage.

2) The dependence of the energy conversion factor on the light intensity is a function with maximum (at $I = I_m$).

It is possible to quantitatively assess the limiting yield reached in systems based on photosynthesis. Assuming that in the limit, one reaction centre corresponds to one molecule of photosynthetic pigment, for the extinction ratio to be 10^4 litre mol^{-1} cm^{-1}, the surface concentration of photosynthetic units should be 10^{-3} mol m^{-2}. The limiting production rate of the system is 0.27 g m^{-2} s^{-1} with respect to absorbed CO_2 and 0.19 g m^{-2} s^{-1} with respect to formed carbohydrates. If one assumes that the time of highly intense illumination of the system is 10 h, then the limiting daily production is 9.9 kg m^{-2} with respect to absorbed CO_2 and 6.75 kg m^{-2} with respect to carbohydrates. It should be stressed that the above estimates are the limiting theoretical values that can be realized with the maximally high concentration of reaction sites (one pigment molecule per reaction site) for saturating light densities and saturating CO_2 concentrations.

The real production rate of photosynthetic systems was substantially lower than the theoretical limit. As a rule, the systems were limited by the light, the low CO_2 concentrations and factors such as limitations on water, mineral salts, *etc*.

Microalgae or microphyte cultivated in different systems including flow-through types exhibited the highest efficiency. Data is known[30] on the photoenergy conversion in a system containing thermophilic microalgae *Chlorella*. A theoretical model describes adequately the dependence of energy conversion factors on the passed light intensity and the carbon dioxide concentration. System parameters were as follows: the limiting efficiency with respect to CO_2 $k_eA_0 = 350 \pm 50$ litre m^{-2} day^{-1} (680 g m^{-2} day^{-1}); a parameter characterizing the consumption of stored energy (respiration), $\beta A_0 = 6$ litre m^{-2} day^{-1} CO_2. Correspondingly, $I_0 > 20$ W m^{-2}, $I_m = 170$ W m^{-2}. Taking into account that $\eta_1 \eta_2 \eta_3 = 0.15$, the solar energy conversion factor is 5.5% for the total light intensity I_m. The limiting carbohydrate production rate of the system k_eA_0 found by extrapolating experimental data to the saturating values of light intensity and CO_2 concentration was ~ 470 g m^{-2} day^{-1}.

This is a very large quantity, however, 15-times lower than the theoretical limit. The analysis makes it possible to outline the pathways towards enhancing the production rates of photosynthesizing systems and increasing the solar energy conversion factors.

1) Decreasing the unproductive use of energy.

2) Passing to thermophilic photosynthesis.

3) Increasing the number of reaction sites with respect to the number of pigment molecules of the 'photosynthetic' system.

Increasing the temperature (path 2), all other conditions being equal, increased the rate constants of the interprotein electron transfer (k_e) and thus enhanced the system efficiency. The theoretical dependences of production rates of photosynthetic systems on the light intensity were reported [30] under an assumption that k_e depends on the temperature according to the Arrhenius law with the activation energy of 12.5 kJ mol^{-1} (experimentally determined activation energy).

It is known that a reaction site involves $\geqslant$100 chlorophyll molecules. Correspondingly, for high degrees of light conversion at high chlorophyll concentrations, relatively low concentrations of reaction sites in the system can be expected. Modernization of the system can involve the use of methods of selection, molecular genetics and genetic engineering (path 3).

The theoretically optimal photosynthesis can be carried out for the solar energy density of 'several Suns' and in principle for very dense solar fluxes of several 'tens' or 'hundreds' Suns. In the latter case, the stability of the photosynthetic process at high temperatures, the photooxidation and other photodestructive processes become the key problems. Probably, these problems can be solved with the transition to artificial systems of organized molecules that simulate photosynthesis.

Real photosynthetic systems differ rather widely in the production rate and the efficiency of solar energy conversion. Table 1 shows the data [31] on the rates of production of different biomass types and the efficiency of solar energy conversion. It is evident that the efficiency of energy conversion lies in the range of 0.2% – 5%; in this case, the production rate of plants could reach 100 – 150 tonnes of biomass from 1 hectare per year.

Table 1. Biomass production rates of different systems and attained efficiencies of solar energy conversion.[31]

Biomass source	Production rate /t hectare^{-1} year^{-1}	Efficiency of conversion (%)
Water hyacinth (USA, Mississippi)	11 – 33	0.31 – 0.94
Sorghum (USA, Texas)	22 – 40	0.55 – 0.94
Maize (USA, Minnesota; Israel)	24	0.79
Wood (England)	34.1	1.79
Rice (Australia, New South Wales)	35.5	1.04
Wood plantations (Congo)	36.1	0.95
Algae of sewage treatment plants (USA, California)	49 – 74	1.3 – 1.9
Sugarcane (Java)	75 – 87	2.2 – 2.6
Algae (Thailand)	164	4.9

High crops are typical of cane plants (sugar cane, miscanthus), namely, $\sim$75 – 100 t hectare^{-1} year^{-1}. It was shown[32] that under optimal cultivation conditions miroalgae, can fix CO_2 and accumulate up to 300 – 400 t hectare^{-1} year^{-1} of biomass.

The theoretical limit of production rates for systems of this kind was found to be 21 600 t hectare^{-1} year^{-1}. Thus, the most efficient real systems can reach only 1.5% – 2% of the theoretical limit in production rates, which is determined by the electron transport rates in protein systems. These calculations have shown that the given methods of solar energy conversion have a considerable potential for development.

For the production rate of 50 – 100 t hectare^{-1} year^{-1} of biomass (sugar cane, miscanthus, microalgae), the energy efficiency corresponds to 28 – 56 t hectare^{-1} year^{-1} of crude oil (calculations take into account the calorific powers of chlorella and oil).[31] Below, heats of combustion of different biomass components and certain fuel types are shown.[31]

Substances	Heat of combustion /MJ kg^{-1}
Monosaccharides	15.6
Polysaccharides	16.7
Proteins	24.0
Lignin	25.1
Lipids	39.8
Terpenes	45.2
Triglycerides	36.5 – 40.0
Timber	
birch	20.0
pine	21.0
Chlorella	27.0
Vegetable (rapeseed) oil	39.8
Amorphous carbon	33.8
Aliphatic hydrocarbons	43.3
Crude oil	48.2

Considerable resources of biomass for the biofuel production were associated with the use of agriculture wastes. In average, the mass of non-productive part of crops is higher as compared with its productive part (*i.e.*, their ratio $Q > 1$):[32]

Crop	Q	Crop	Q
Maize	1.1	Barley	2.5
Rape	1.43	Rice	2.5
Cotton	2.45	Oats	3.0

Correspondingly, for Russia that produces 70 – 80 million tonnes of grain per year, the volumes of biomass wastes amounted to 175 – 200 million tonnes. According to the above data, the energy content of this biomass amount was 89 – 102 millions ton in the aliphatic hydrocarbon equivalent. For a comparison, the annual consumption of gasoline in Russia was $\sim$30 million tonnes. The problem lies in the development of an efficient and economically attractive technology for synthesizing bioenergy sources from biomass of this kind.

In this review, we consider a wide set of products including methane, ethanol, butanol, biodiesel, hydrogen and biohydrocarbons as the different types of biofuel. The development of this research and technology trend is characterized by the combined use of chemical and biotechnological methods and approaches. Moreover, the environmental aspect was among the key criteria in selecting the direction of development. The proper combination of

chemical and biotechnological methods is known to lead to environmentally acceptable and safe processes.

III. Biomethane

A combustible marsh gas, methane, formed as the result of a complex biological process has been known for a long time; however, it is only in recent decades that considerable progress was observed in understanding of the nature and mechanism of methane formation and also in the practical use of this phenomenon, which was determined by the solution of the following complex problems:

— the nature of the microbiological community responsible for this process was studied in detail and is continued to be studied;

— the main chemical reactions responsible for the substance transformations were identified as well as the enzymatic microbial systems that act as catalysts of these processes;

— kinetic models of the action of symbiotrophic methanogenic associations that quantitatively describe the dynamics of this process and reveal the main regulating factors responsible for the stability of this process were developed;

— various reactor designs that allow the efficient conversion of organic compounds to biogas were developed and used, the high-rate diagrams of this process were realized.

Detailed description of microbiology of methanogenesis was beyond the scopes of our review and can be found in other publications.[33–41]

The key feature of methanogenesis is its multistage nature, where many stages are catalyzed by particular groups of microorganisms. A methane-generating microbial association is symbiotrophic, *i.e.*, the reaction products of one microorganism or their group serve as the substrates for functioning of the other microorganisms.

The methane formation proceeds under strictly anaerobic conditions and involves several radically different groups of microrganisms.[42] A large group of bacteria (association) takes part in breaking down biopolymeric molecules to monomeric components, which is followed by their chemical modification. Bacterial associations converts polysaccharides, proteins and lipids to low-molecular compounds to afford acetic acid, hydrogen and CO_2.[31]

A large and diversified group of microorganisms entering this association includes *Bifidobacterium*, *Butyrovibrio*, *Eubacterium*, *Lactobacillus*. Under the action of exogenic enzymes, hydrolysis occurs (proteins to amino acids, polysaccharides to oligo- and monosaccharides, triglycerides to glycerol and fatty acids, nucleic acids to nitrogen-containing heterocycles, ribose and inorganic phosphates). The further hydrocarbon conversion follows the classical glycolytic pathway (the Embden–Meyerhof pathway) to form pyruvic and lactic acids or ethanol.

The next group of microorganisms with the general name of acetogenic bacteria are responsible for the acetic acid formation. Acetogens convert alcohols and higher acids formed upon glycolysis to afford acetic acid, CO_2 and hydrogen. Then, acetic acid is transformed under the action of methanogenic bacteria to methane and carbon dioxide.

Methanogens are characterized by wide diversity as regards their morphology, the cell wall structure, metabolic and physiological properties. These microorganisms were united in a large common group due to the following three important characteristics: accumulation of large amounts of methane, strict anaerobism and belonging to the Archaea domain.[42]

The main materials dominating in biomass are carbohydrates. The reaction of a carbohydrates (glucose) conversion can be written as follows:

$$C_6H_{12}O_6 \longrightarrow 3\,CH_4\,(gas) + 3\,CO_2\,(gas).$$

Catalytic amounts of microorganisms are involved in conversion of substances. The standard free energy and the enthalpy of this reaction at pH 7 and 25 °C were found to be 418 and 131 kJ, respectively. Methane amounted to 27% of the total mass and 95% of the energy content. This reaction is highly attractive with respect to energy. Exothermal losses were relatively small, the process occurred spontaneously with separation of methane from the aqueous phase.

In the course of methanogenesis, quite different compounds, for example, alcohols, amino acids, glycerol, *etc.*, undergo catalytic transformations. It was shown that virtually any organic compound including various xenobiotics can undergo biodegradation. A methanogenic microbial association can transform yperite, sarin, soman and their chemical modification products to carbon dioxide, inorganic phosphate, ammonia, hydrogen sulfide in the course of methane production.[43,44]

Individual reactions that proceed in a symbiotrophic methane-generating associations look rather unusual. Particularly, water can act in these processes as an oxidant to form molecular hydrogen (oxidation of ethanol, butyric and propionic acids with water). The fatty acid transformation to form acetic acid (β-oxidation process) proceeds by the following scheme:

$$C_{n-1}H_{2n-1}COO^- + 2\,H_2O \rightleftharpoons$$
$$\rightleftharpoons C_{n-3}H_{2n-5}COO^- + CH_3COO^- + 2\,H_2 + H^+$$
$$(+48.3\ kJ\ mol^{-1}),$$

$$C_{n-3}H_{2n-5}COO^- + 2\,H_2O \rightleftharpoons$$
$$\rightleftharpoons C_{n-5}H_{2n-9}COO^- + CH_3COO^- + 2\,H_2 + H^+$$
$$(+48.3\ kJ\ mol^{-1}),$$

$$C_2H_5COO^- + 3\,H_2O \rightleftharpoons$$
$$\rightleftharpoons CH_3COO^- + 3\,H_2 + HCO_3^- + H^+$$
$$(+76\ kJ\ mol^{-1}).$$

Under standard conditions, the reaction proceeded in the reverse direction as regards thermodynamics. In really functioning methane-generating associations, this process was possible due to the efficient transformation of products (acetic acid and molecular hydrogen) to methane. The positive values of free energy of β-oxidation were relatively low (48.3 kJ mol^{-1}). It was due to the far negative free energies of hydrogen absorption and acetic acid conversion that the overall process became thermodynamically possible.

In certain modes, where the growth and functioning of methanogenic microorganisms was hindered or blocked (*e.g.*, at low pH), acetic acid and hydrogen were accumulated in the system and the water oxidation reactions proceeded in the opposite direction. This additionally blocked the methanogenesis and led to accumulation of fatty acids (butyric, valeric, *etc.*).

Simulating the dynamics of methane formation by taking into account accumulation and consumption of intermediates and the dynamics of microorganism growth seems

interesting. Such simulations relied on theoretical studies of functioning of symbiotrophic associations.[45,46] These studies were based on experimental investigations of this process with identification of time variations of the maximally large number of chemical compounds.[45,47–50] Individual stages were assumed to be catalyzed by enzymatic systems of individual microorganisms; moreover, the microbe growth depended on the concentration of these substrates. The minimal system of differential equations described the dynamics of changes in concentrations of the starting compound and the catalyzing microorganism and took into account the inhibition of this process by hydrogen and the pH-dependent lysis of the catalytic system; the dynamics of changes in concentrations of hydrogen, ethanol, acetic and butyric acids and also the effect of pH, the inhibition by excessive hydrogen, butyric acid and ethanol; the dynamics of accumulation of CO_2 and methane. Adequate agreement between the theory and the experiment was demonstrated. This simulation allowed the kinetic parameters of the process to be determined and the behaviour of the system to be predicted throughout the studied time intervals. It was important to identify parameters the changes of which were the most noticeable for the system. These were, first of all, the specific growth rates of individual microorganisms and the constants of inhibition by hydrogen ions and hydrogen molecules. The kinetic analysis made it possible to gain dynamic information on the substances involved in this process that cannot be obtained directly, for example, the concentrations of individual microorganisms in the association. The obtained data formed the rational basis for the development of highly efficient reactors for biomethane synthesis.

At present, different reactors of biomass conversion to methane have been developed, tested and are in operation.

A significant stage in identification of methanogenesis processes was the use of immobilized cells of methanogenic associations. Studying the methanogenesis in concentrated cellular suspensions revealed a phenomenon of self-organization of cells into granules to afford a heterogeneous biocatalyst. The use of high biocatalyst concentrations allowed the methane production rate of this process to be increased 50–100-fold for the case of soluble organic substances.[51–54] To date, a considerable part of food industry plants are equipped with biogas sewage treatment units manufactured by Biothane (the Netherlands). The basic unit of the modular type includes a 50 m^3 reactor made of fibreglass plastic and a reactor for biomass conditioning with a pumping facility. The unit size was 5.8 m^2, its efficiency reached 1000 kg of COD (COD is the chemical oxygen demand), the processed sewage volume was 480 $m^3\ day^{-1}$, the yield with respect to gas was 0.04–0.1 litre $s^{-1}\ m^{-3}$ of the reactor.

IV. Bioethanol

At present, ethanol mixed (up to 10%) with petrol is actively used as the fuel for internal combustion engines. To date, only 7% of ethanol was produced by its chemical synthesis and 93% was obtained by the biotechnological methods that employ yeast cells (most often, *Saccharomyces cerevisiae*) as the main catalysts capable of producing and withstanding the ethanol concentrations up to 18 vol.%.

The present volume of the annual ethanol world market chemical is assessed as about 40–50 billion litre[55] of which ~60% is used as gasoline additive, 25% is used in the chemical industry, 15% is used in food industry, with the latter part constantly decreasing. At the same time, according to the opinion of a number of researchers,[56] the development of profitable fuel ethanol production industry is only possible with the further substantial decrease in its prime cost. The main ways for the reduction of its cost could be replacement of grain used now for its production by wastes of various industries and also the modernization of ethanol production technology by applying biotechnological methods, namely, through searching for natural genetic cultures of its producers or creating them, stabilizing them by immobilization, optimizing the organization of the process, which includes carrying out different stages simultaneously.

The ethanol production conventionally used grain (starch-containing feedstocks). By the action of amylolytic enzymes, starch was transformed to glucose and the latter was converted to the target product. At the same time, there are several extra sources for the ethanol synthesis. Thus, lignocellulose subjected first to physicochemical treatment and later to the chemical or enzymatic hydrolysis to afford monosaccharides (mainly glucose and xylose) under the action of cellulolytic enzymes (cellulase and hemicellulase) can the successfully used in ethanol production.

As the starting material in ethanol production, the food industry wastes can be used, particularly, those containing lactose, which can be hydrolyzed to glucose and galactose by the action of β-galactisidase, which is followed by the monosaccharide conversion to ethanol.[57]

Modernization of the ethanol technology by biotechnological methods was first of all associated with the use of heterogeneous biocatalysts (HBC) in the form of immobilized cells of yeast and bacteria.[58–61] The use of HBC considerably simplified technological operations on separation of cells from the fermentation medium, provided high cell concentrations in reactors and made microorganisms resistant to low pH (~3.0) and high concentrations of ethanol, side products and starting chemicals and also allowed the repeated use of HBC in a biotechnological process and provided high degrees of conversion of the original substrate to the target product.[62] Immobilization made it possible to utilize microorganism cultures already used in industry, well studied and adapted to the conditions of the process and to increase their efficiency without resort to search and adaptation of new cultures (Table 2).

Of special interest were the processes that allowed integrating several technological stages necessary for bioethanol production, for example, the stages of enzymatic hydrolysis of substrates to monosaccharides and the stage of monosaccharide conversion to bioethanol.

The integration of the enzymatic hydrolysis of lactose contained in buttermilk and the conversion of glucose to ethanol into a single stage at 36 °C was found to increase the target metabolite concentration by 20% and halve the total time of the process as compared with separate stages. The degree of lactose hydrolysis increased from 70% to 80%.[63,64] Moreover, as compared with the two-stage process, the concentration of immobilized cells and enzyme substrate can also be halved (to 20 and 2.5 g $litre^{-1}$, respectively) as compared with the two-stage process.

The possibility of prolonged use of immobilized cells in the integrated process under optimal conditions in a 50-litre reactor was demonstrated. With the increase in the number of run cycles, their duration changed not too strongly remaining within 22–25 h. The average concentration of

Table 2. Examples of ethanol production from different substrates by immobilized HBC based on microorganism cells.[62]

Microoraganisms	Carrier	Substrate	Reducing sugars /g litre^{-1}	Ethanol /g litre^{-1}
Saccharomyces pastorianuse + *Aspergillus awamori*	cellulose granules	starch	100	44.5
Saccharomyces cerevisiae	PVS cryogel or calcium alginate gel	cheese whey	60	22
Kluyveromyces marxianus	the same	cane molasses	140	52 – 57
Zymomonas mobilis	carraginane gel with K^+ couterion	corn starch	162	70
Saccharomyces cerevisiae	the same	waters of pineapple processing	82.3	37
Aspergillus niger + *Zymomonas mobilis*	calcium alginate gel	potato starch	50	18.9
Saccharomyces cerevisiae	the same	beetroot molasses	109	46.2
Zymomonas mobilis + *Saccharomyces diastaticus*	"	manioc starch	150	46.9
Kefir yeast (*Torulopsis kefir*)	delignified cellulose	buttermilk	52.5	20.3

ethanol accumulated in buttermilk by the end of each cycle was 22 g litre^{-1}, *i.e.*, ~90% of theoretically possible.[63]

Comparing the results obtained with free yeast cells with the data on the use of HBC in the combined processes of lactose hydrolysis and glucose conversion to ethanol demonstrated obvious advantages of the latter. The ethanol production rate of immobilized cells exceeded that of free cells by a factor of 14.

The use of cellulose-containing materials (CCM) as the main source of ethanol production predetermined the necessity of preliminary hydrolysis of hemicellulose and cellulose to monosaccharides. The efficiency of this hydrolysis depended on the conditions of physicochemical pretreatment of starting materials (steam explosion, acid hydrolysis, grinding, delignification with alkaline solutions) and its subsequent enzymatic hydrolytic processing.

It is known that the medium obtained by CCM physicochemical pretreatment contained large amounts of substances (degradation products of cellulose, hemicellulose and lignin) capable of reducing the catalytic action of enzymes and inhibiting the fermenting activity of free yeast cells (furfural, tannins, acetic acid, terpenes, *etc.*),[65] which lowered down the overall ethanol production rate. In connection with this, the interest in the use of highly stable HBC based on immobilized microorganism cells was especially high.[58 – 61, 66]

The conversion of different hydrolyzed substrates employing free yeast cell produced ethanol in the yields of 50% – 80%.[67 – 69] It was found that the concentration of ethanol accumulated in the medium was determined in many respects by the concentration of sugars accessible for cells to be converted to ethanol, *i.e.*, in fact, by the efficiency of chosen enzyme complexes.[70] Moreover, the immobilized cells could efficiently catalyze the ethanol formation in CCM fermentation product both with and without the removal of suspended substrate particles.

Attention was focused on the enzymatic hydrolysis of CCM combined with glucose conversion to ethanol,[71 – 76] where saccharification and fermentation (SSF) occurred simultaneously. In this process, enzyme hydrolyzed polysaccharides to monosaccharides to be immediately converted to ethanol by microorganisms, which made it possible to reduce the sugar concentration in the medium and avoid its inhibiting action on the enzymatic catalysis,[77] to shift the chemical equilibrium of the overall process to the formation of the final product thus increasing the degree of substrate hydrolysis and enhancing the overall efficiency. Although studies associated with the quest for the ways to intensification of this process and reducing its cost are now carried out all over the world, the studies that discuss the possibility of the commercial use of immobilized HBC in SSF were scarce,[78 – 80] which was apparently dictated by the absence of practically usable HBC samples in the world market. Moreover, nowadays the possibility of using HBC based on immobilized microbial cells in the ethanol production was discussed more often in the context of starch-containing substrates[71, 78 – 80] and lactose-containing wastes[81] rather than in the context of CCM. This was due to the fact that the latter source of sugars was considered difficult to process and contain the larger amount of substances capable of exerting negative effect on cells as compared with the first two substrates.

In addition to cell immobilization, in the context of SSF process, keen attention was drawn to the choice and the use of microorganisms capable of producing ethanol under conditions most favourable for the enzymatic hydrolysis of CCM, *i.e.*, at pH 5.0 and 45 °C. In line with this, attention was focused on thermotolerant yeast, which, in addition to providing high ethanol production rates in cultivating media with low pH that prevent their contamination with foreign microflora, proved to be intrinsically capable of catalyzing efficient fermentation at elevated temperatures (45 – 50 °C).[66, 67, 79, 82]

The interest in *Zymomonas mobilis* bacteria as the ethanol producers is virtually lost today,[60] because these bacteria are tolerant only to ethanol concentrations up to 120 g litre^{-1} and do not withstand the low pH of the medium (< 3.5) and the elevated culturing temperatures.

Special attention was drawn to yeast cultures genetically modified to acquire additional enzymatic activity, which allowed cells to combine the metabolic activity with respect to ethanol with the hydrolytic activity with respect to polysaccharide substrates.[69, 76] However, due to the well-known limitations to the active use of genetically modified organisms in large-scale commercial processes, the natural cultures are sill preferred.

Natural yeast cultures traditionally used for converting sugars into ethanol are known to fail not only to maintain

enzymatic hydrolysis of different CCM but also to convert pentoses (xylose and arabinose) often present in high concentrations in conventional media (up to 20 g litre^{-1}) to sugars. It was found that the conversion of pentoses to ethanol can employ mycelial fungi that, on the one hand, are heterotrophic eukaryotic microorganisms and, on the other hand, exhibit extreme diversity of fermentation systems.[83,84] Hence, by selecting the medium and the culturing conditions, it proved possible to exert a desired effect on the metabolic properties of cells of these microbes to obtain biomass with the necessary induced enzymatic characteristics.[85] Mycelial fungi *Aspergillus, Mucor* and *Rhizopus* can be used in ethanol production from a wide spectrum of substrates.[86] For certain substrates, the yield of ethanol reached 76% of the theoretical value for the initial sugar concentration of 50 g litre^{-1}. High sugar concentrations were found to inhibit the fermentation process and reduce ethanol yields. It was also shown that cells of these microorganisms in their free state are very sensitive with respect to ethanol concentrations accumulated in the medium.

It was demonstrated that both free and immobilized cells of mycelial fungi can be used in converting sugars contained in fermentation products of various agricultural wastes. For immobilized cells, the ethanol concentrations were found to be higher (by up to a factor of 22.5, depending on the culture and the substrate) as compared with free cells under the same conditions.[87]

According to the obtained data, cells of mycelial fungus *Fusarium oxysporum* could be used in ethanol production by the cellulose SSF process both in their free[88] and immobilized states;[87] however, the process proved to be rather long ($\sim$200 h) and the ethanol yield did not exceed 60% of the theoretical value. Apparently, the rate of sugar conversion to ethanol was substantially lower for mycelial fungi as compared with yeast. This was explained by the fact that fermentation produced acetic acid as the by-product, which was accumulated in the medium and with respect to which the mycelial fungi cells were very sensitive.[88] At the same time, the obtained ethanol concentrations ($\sim$ 20 g litre^{-1}) were rather high for mycelial fungi cells and, according to the literature data,[83] could considerably inhibit their metabolic activity.

Thus, to date, the best bioethanol producers by the SSF process are immobilized yeast cells that exhibited the higher ethanol production rates as compared with immobilized cells of mycelial fungi, which was due to their higher tolerance to negative factors that accompany this process.

V. Biobutanol

At present, butanol is produced from oil by hydrolysis of haloalkanes or hydration of alkenes. However, in line with the growing interest in biofuels, attention was drawn to the production of biobutanol,[89] *i.e.*, butanol synthesized from biomass.

Biobutanol has the same characteristics as butanol synthesized from oil and is considered as biofuel.[90] Used to fuel the vehicles, it exhibits the following advantages:

— butanol contains 25% more energy as compared with ethanol and liberates 10% more energy in the working cycle as compared with petrol;

— butanol is safe to use, evaporating 6-times slower than ethanol and being 13.5-times less volatile than petrol;

— butanol displays no corrosivity and can be transported by the existing fuel pipes;

— butanol is not only miscible with petrol but also can completely replace petrol in internal combustion engines.

Fermentation of sugars with *Clostridium acetobutylicum* cells to afford a mixture of solvents acetone–butanol–ethanol (ABE) was among the first and the most extensively used methods of biobutanol production. The commercial production of butanol started in 1919 and demonstrated sharp growth in 1927 due to the development of car industry because butanol served the basis for the production of quickly-drying varnish. Moreover, during the World War II, butanol was used in the production of synthetic caoutchouc. In the 1950s, the microbiological method of butanol production was replaced by its synthesis from petroleum by the hydrolysis of haloalkanes or the hydration of alkenes. Nowadays, the opposite trend is observed caused by the use of biomass in place of petroleum in the butanol production. This process represents the anaerobic conversion, *e.g.*, of glucose to butyric, propionic, lactic and acetic acids (the stage of organic acid production), accompanied by a decrease in the medium pH (Fig. 3). For pH 4.5 and lower,[91] the process shifted to accumulation of acetone, butanol and ethanol. This dependence on the pH was discovered for the ABE fermentation by V N Shaposhnikov back in 1939. The maximum yield of butanol for the case of glucose fermentation was 25%. Its production was limited by the fact that in concentrations of 1%–2%, butanol substantially inhibited the cell metabolism. In connection with this, the majority of practical applications did not use butanol concentrations higher than 1.3%.[89,90]

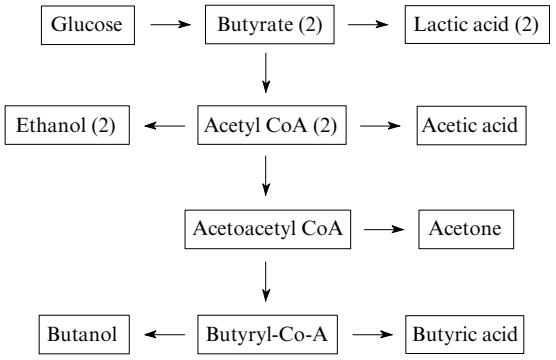


Figure 3. Scheme of glucose conversion in the ABE process under the action of *Clostridium* genus cells.
Numbers in parentheses show the number of molecules.

Studying the effect of temperature on the production of solvents by *Clostridium acetobutylicum* cells has shown that the total yield of neutral products increased with the decrease in temperature mainly due to the decrease in the amount of produced acetone.[92] The butanol production proved to be almost unaffected by temperature variations in the range of 25–40 °C.

In was found that for the 32% conversion of glucose into solvents by *Clostridium acetobutylicum* cells, the solvent ratio in the ABE mixture was 1.9 : 0.6 : 6. With the addition of 2 g litre^{-1} of acetic acid, the amount of acetone increased and the ABE ratio became 3 : 0.5 : 6. The introduction of the same concentration of butyric acid led to a different ABE ratio, 2.4 : 0.8 : 6 (Ref. 93).

With the increase in the hydrogen partial pressure in the ABE fermentation, the yields of butanol and ethanol from

glucose were shown to increase on average by 18% and 13%, respectively, whereas the yields of acetone and hydrogen decreased on average by 40% and 30%.[94]

In the 1980s, attention was drawn to the *Clostridium acetobutylicum* culture as the biobutanol producer. The concentration of cells in the nutrient medium was found to have a considerable effect on the rate of biobutanol accumulation in the basal glucose medium.[95] Thus, it became evident that high concentrations of cells in the medium should be created in order to make the ABE process commercially attractive. Insofar as anaerobic cells of the *Clostridium* family were characterized by sufficiently low specific growth rates, they were proposed to be used in the immobilized form to provide high concentrations in the reactor.[96–98]

For cells immobilized in the calcium alginate gel, it was found that bubbling nitrogen containing 10% hydrogen through the nutrient medium increased the solvent yields almost 5-fold and the addition of 10% of CO_2 to the bubbled gas flow was found to further increase the butanol yield by 15%.[97]

In a number of studies, the simultaneous use of several cultures of the *Clostridium* family in the ABE process was demonstrated to be highly efficient.[99] To date, the use of immobilized *Clostridium tyrobutyricum* and *Clostridium acetobutylicum* cultures allowed the Environmental Energy company to reach the maximum butanol production rate in the ABE process equal to 4.64 g litre^{-1} h^{-1}; moreover, a high (42 mass %) degree of conversion of consumed glucose to solvents was achieved.[89, 90]

The possibility of organizing a continuous ABE process was demonstrated. It was found that free *Clostridium acetobutylicum* cells confined in a flow-through reactor were capable of fermenting mixtures of substrates consisting of, *e.g.*, glucose and glycerol.[100] It should be stressed that immobilized cells under analogous conditions proved to be much more efficient catalysts of this process as compared with free cells. Thus, *Clostridium acetobutylicum* cells immobilized on hollow fibres were shown to provide the incredibly high butanol production rate[101] equal to 4.6 g litre^{-1} h^{-1} for the dilution rate of the reactor medium of 0.9 h^{-1} and the medium pH 4.3.

Genetic engineering of cells in the *Clostridium* family as the biobutanol producers deserves mention.[102–105] The efforts of genetics were aimed at minimizing the side products of butanol synthesis, for example, acetone, that are accumulated in the course of fermentation. The TargeTron process was used in the destruction of the acetoacetate decarboxylase gene.[102] This sharply decreased the acetone concentration in the medium (from 50 mmol litre^{-1} for the original culture to 5 mmol litre^{-1} for the modified culture) with the butanol content above 70% in the overall solvent mixture.

In the USA, a new culture, *Clostridium beijirinckii*, was developed,[103, 104] which at present is of commercial importance in biobutanol production. The high level of glucose conversion to solvents was demonstrated for both free and immobilized cells. Thus, the immobilization of *Clostridium beijirinckii* cells as biofilms in a flow-through reactor[104] afforded butanol in the concentration of 4.3 g litre^{-1} with the glucose utilization ratio of 30.3% for the medium dilution rate of 1.0 h^{-1}.

In addition to attempts to improve butanol producing cultures, the quest for new cultures with metabolism shifted to butanol production was continued. Recently, the cells of a new culture, *Clostridium saccharobutylicum* were isolated and proved capable of fermenting various carbohydrates with the butanol yield reaching 80% (9.2–9.7 g litre^{-1}) of the overall amount of formed solvents.[105]

The studies aimed at making the ABE process more attractive were recently associated with not only the design and search for new cultures of butanol producing cells but also with the widening the circle of substrates convertible to biobutanol. Particularly, it was shown that enzymatic hydrolyzates of active silt, *i.e.*, the main component of systems used in cleaning sewages of the palm oil and sago-palm starch production, can be utilized in the ABE fermentation catalyzed by *Clostridium saccharoperbutylacetonicum* cells.[106] The silt hydrolyzate was used as a substitute for the complete medium for cell growth and metabolism, containing the sources of carbon, nitrogen and trace minerals. In this case, butanol was used in the concentration of 3.5 g litre^{-1}. Bubbling the medium with nitrogen in the absence of pH control increased the yield of butanol. The use of sago-palm starch as the main component of the medium in place of glucose allowed a high butanol concentration (10.4 g litre^{-1}) to be reached.

However, the contemporary production of butanol is still in the very beginning and, unfortunately, faces many accompanying problems. This is determined by the fact that the technology of this fuel production is more complicated as compared with, say, bioethanol. The large energy and water inputs required in the pretreatment of original materials, the staged mechanism of biomass conversion to fuel components, the high sensitivity of cells with respect to butanol and acetone, the high cost of genetically modified cultures of microorganisms and the difficulties of their cultivation, the high costs of separation of target products from side products — all these factors pose serious problems for the technology of biobutanol production.[107]

VI. Biodiesel

The use of vegetable oil and its derivatives as energy resources and fuels has a long history. The diesel engine inventor, Rudolph Diesel demonstrated its operation at the 1900-World Exhibition in Paris by using peanut oil; he noted that with time the use of vegetable oils may acquire importance comparable with the use of petroleum or coal oil. In 1911, he wrote that a diesel engine can run on vegetable oil, which could help the countries employing this engine to develop their agriculture.[31]

Despite several obvious advantages such as their relative accessibility, renewability, the high energy content (nearly 88% of the traditional diesel fuel of the petroleum origin), the absence of sulfur and aromatic compound impurities and high biodegradability,[108–112] the direct use of vegetable oils in engines have not found application. This was first of all associated with their high viscosity and low fluidity, which are responsible for incomplete combustion.[108] Numerous methods were proposed for processing vegetable oils to improve their characteristics, namely, preparation of microemulsions with alcohols (ethanol, methanol), pyrolysis with thermal decomposition to hydrocarbons, catalytic cracking. However, the most widely used methods were the transesterification of fatty acids with ethanol or methanol, because the thus synthesized products proved to be close to conventional diesels in operational characteristics and process flow diagram is very simple and reliable.[108, 113–121] Fatty acids and their derivatives, triglycerides, form a class of

biological molecules with the highest energy content. The majority of plants, animals, fungi and bacteria contain fatty acids and triglycides of fatty acids with even numbers of carbon atoms.[122]

A complex of enzymes can synthesize first of all unbranched C_{16}- and C_{18}-acyl derivatives of coenzyme A. The process starts with acylcoenzyme A the acyl group of which is transferred to the acyl-carrier protein (ACP), a process mediated by acetyltransacylase. This is followed by two enzymatic reduction stages involving NADPH to produce butyryl-ACP. Its further reaction with malonyl-ACP to evolve CO_2 leads to elongation of the carbon chain up to six carbon atoms. The further NADPN-involving reduction of oxo and hydroxy groups is ended with the formation of hexanoyl-ACP. The processes were repeated to produce fatty acids with the number of carbon atoms 16, 18, 20.[123] Fatty acids with odd numbers of carbon atoms were formed as the result of propyonyl-ACP condensation with malonyl-ACP. For higher plants and animals, the saturated fatty acids undergo further transformations under the action of enzymes-desaturases to introduce one or several double bonds in the *cis*-configiration into the aliphatic chain. Many polyunsaturated fatty acids exhibited pronounced physiological activity.[124]

The production rates of different systems on the production of lipids, oils, triglycerides of aliphatic acids were quite diverse. Below, the data on the biodiesel production rates of different systems are compared.[29, 111, 125]

Source of triglycerides, aliphatic acids	Potential production rate /litre hectare^{-1} year^{-1}
Palm	6000
Coconut	2600
Rape	1180
Peanuts	1050
Sunflower seeds	950
Soy	450
Maize	160
Microalgae	20 000 – 80 000

By transesterification with methanol or ethanol, biodiesels with high operational characteristics can be obtained. The composition of different substrates with respect to aliphatic acids was quite diverse;[29, 108] moreover, the outstanding potential of microalgae deserves mention (Table 3).

The operational characteristics of produced biodiesels depended to a certain extent on the composition of fatty acids,[123] particularly, on the cetane number that characterizes the fuel ability to compression ignition (the characteristic of a hydrocarbon cetane, *i.e.*, hexadecane, conditionally taken for 100 points) correlated with the hydrocarbon chain length and the degree of its saturation.[126 – 128] For methyl esters of stearic, oleic, linoleic and linolenic acids, the cetane numbers are 77, 54, 37 and 27, respectively. The increase in the degree of saturation decreased the cetane number of fuels.

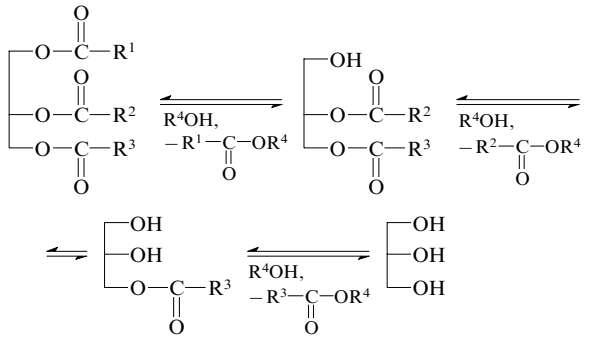
1. Chemical basics of biodiesel synthesis

Transesterification of fatty acid triglycides to methyl and ethyl esters (biodiesels) represents a 'classical' reaction of nucleophilic substitution that proceeds in several stages to afford di- and monoesters of glycerol.

Table 3. Composition of fatty acids from different-origin lipids.[a]

Source	Palmitic (16/0)	Palmitoleic (16/1)	Stearic (18/0)	Oleic (18/1)	Linolic (18/2)	Linolenic (18/3)
Cotton	28.7	0	0.9	13.0	57.4	0
Rapeseed	3.5	0	0.9	64.1	22.3	8.2
Sunflower	6.4	0.1	2.9	17.7	72.9	0
Flax	5.1	0.3	2.5	18.9	18.1	55.1
Wheat	20.6	1.0	1.1	16.9	56.0	2.9
Oil palm	42.6	0.3	4.4	40.5	10.1	0.2
Maize	11.8	0	2.0	24.8	61.3	0
Soy	13.9	0.3	2.1	23.2	56.2	4.3
Peanut	11.4	0	2.4	48.3	32.0	0.9
Olives	5.0	0.3	1.6	74.7	17.6	0
Microalgae	12 – 21	55 – 57	1 – 2	58 – 60	4 – 20	14 – 30
Fungi	7 – 23	1 – 6	2 – 6	19 – 81	8 – 40	4 – 42
Bacteria	8 – 10	10 – 11	11 – 12	25 – 28	14 – 17	–

[a] In parentheses, the numbers of carbon atoms/double bonds are shown.



R^4 = Me, Et.

The final products are esters of fatty acids and glycerol. No radical difference was observed in carrying out these reactions or in the properties of resulting fuels. However, methanol was preferred for economy reasons.[108, 129, 130]

The following methods were developed for converting vegetable oils and fats to biodiesels:

— acid-catalyzed process;

— base-catalyzed process (including alkali-catalyzed);

— carrying out reactions in supercritical methanol or ethanol;

— fermentative process catalyzed with lipases.

2. Acid-catalyzed transesterification

The acidic process was preferentially carried out with substrates containing relatively high amounts of free fatty acids. Sulfuric acid was conventionally used as the catalyst.[131 – 138]

The reaction proceeded at 50 – 65 °C for 0.5 – 5 h with the yield of up to 95% – 99%. Usually, the 6 – 20-fold molar excess of alcohol was taken; however, in certain cases, the alcohol : triglyceride ratio could reach 56.[133] Thus the reaction with methanol was carried out in the presence of 1% sulfuric acid solution for the molar ratio methanol : triglyceride = 30 : 1 at 65 °C for 50 h with the conversion by 99%. The same degree of conversion was achieved for the reaction with ethanol at 78 °C in 18 h and with butanol at 117 °C in 3 h.

A reaction involving sulfonic acid was described.[139, 140] A reaction in which $Fe_2(SO_4)_3$ or cation-exchange resin acted as the heterogeneous catalyst was reported.[141]

3. Base-catalyzed transesterification

A process that employs alkalis (NaOH, KOH) as the catalysts was studied most thoroughly.[129, 132–138, 141–146] The base-catalyzed reaction proceeded much faster than its acid-catalyzed counterpart.[115] Traditionally, 0.5%–1% sodium or potassium hydroxide was used. The reaction was carried out at temperatures below the alcohol boiling point for the alcohol : troglyceride ratio of 5–10. The reaction time was from 5 min to 5 h. A comparison of three homogeneous catalysts, NaOH, KOH and MeONa, was carried out.[147] In this series, the best characteristics were exhibited by sodium methoxide.[147, 148]

From the viewpoint of attaining the final yeilds of the target product, sodium and potassium hydroxides demonstrated virtually the same results;[138] however, from the viewpoint of technology, potassium hydroxide should be preferred. This is associated with the properties of fatty acids formed in small amounts as side-products. Potassium salts, in contrast to sodium salts, are liquid and can be easily removed from reaction vessels.[138, 147]

The TiO_2-ZrO_2 and $Al_2O_3-ZrO_2$ systems at high temperatures (175–200 °C) were also used as the catalysts. Good results were demonstrated by zeolites, MgO, CaO.[149–155]

Data on the kinetic behaviour of this process and the simulation of reaction dynamics can be found in several studies.[140, 156–158] A sufficiently adequate description of experimental data was obtained and rate constants for elementary stages were determined (at 50 °C).

4. Transesterification in supercritical fluids

The highly intense process of triglycide conversion to biodiesel was realized by carrying out reactions in supercritical alcohols. Critical values of temperature and pressure allowed the reaction to be carried out under relatively mild conditions at high rates.[108, 114, 129, 155, 156, 159–162] The alcohol was usually taken in excess and the process duration was 5–40 min.

Alcohol	T_{crit} /°C	p_{crit} /MPa
Methanol	239.2	8.1
Ethanol	243.2	6.4
n-Propanol	264.2	5.1
n-Butanol	281.2	4.9

The advantage of this process is that it requires no catalyst, which substantially simplifies the product isolation.

A kinetic study of transesterification in supercritical methanol was carried out.[160]

5. Fermentative transesterification involving lipases

The process of triglycide fermentative modification involving lipases to afford biodiesel attracted keen attention. Its advantages included the environmental cleanness of this method, the suitable reaction time and the ease of product isolation.[156, 163–166] Lipases were conventionally used in their immobilized form.[167–169]

The main problem of using lipases was associated with the low stability of enzymes and their sensitivity to high methanol concentrations.

Comparing different methods of triglycide transesterification showed that each method has its own advantages and drawbacks.

Processes catalyzed by acids and bases proceeded at relatively low temperatures (55–80 °C) at industrially acceptable rates. The acid process was insensitive with respect to the presence of fatty acids in the substrate, whereas for the alkaline process this led to accumulation of undesirable salts, *i.e.*, soaps. The formation of glycerol posed in both cases certain technological problems. The reaction was sensitive with respect to the presence of water in the substrate.

Methods based on the use of immobilized enzymes and supercritical fluids lacked these drawbacks. At the same time, the disadvantages of these methods involved the high cost and instability of enzymatic catalysts, the high temperatures and pressures when the process is carried out in supercritical alcohols, the relative complexity of the technological equipment used.

At present, a technology that employs the alkaline process of triglycide transesterification is the most commonly used process.[1, 157]

Table 4 compares certain properties of diesels of the petroleum origin and biodiesels.[31] It is evident that these biofuels have lower energy content and higher viscosity. At the same time, biodiesels are characterized by the higher cetane number and the higher pour point temperatures, *i.e.*, better operational characteristics.

Biodiesel proved to be much more 'friendly' to the environment.[129, 170] The use of biodiesel decreased the CO emission by 10%, hydrocarbon emission by 20% and particulate exhausts by 10%. The only negative factor was a certain increase in emitted nitrogen oxides (2%). Of fundamental importance was the biodegradability of biodiesel,[171–173] namely, biodiesel virtually totally disappeared in a year even in Arctic regions.[174]

Table 4. Comparative properties of diesel fuels of the oil origin and biodiesel fuels.[31]

Properties	Diesel of oil	Biodiesel origin			
		soy oil		rapeseed oil	
		methyl ester	ethyl ester	methyl ester	ethyl ester
Specific gravity	0.849	0.886	0.881	0.880	0.876
Kinematic viscosity (40 °C) /mm s^{-1}	2.98	3.89	4.49	5.65	6.17
Boiling point /°C	~190	339	357	347	373
Ignition point /°C	74	188	171	179	124
Pour point /°C	~ −20	−3	−3	−15	−10
Cetane number	49	55	53	62	65
Energy content (MJ kg^{-1}/MJ $litre^{-1}$)	45/38.6	39.7/52.2	39.9/35.2	40.54/35.7	40.51/38

6. Ideal biodiesel 'SuperCetane'

In addition to transesterification, other reactions can be used for producing diesels from biomass. Particularly, the process of catalytic reduction of triglycides with hydrogen to corresponding hydrocarbons deserves mention.[175, 176]

$$\left.\begin{matrix}OR^1\\OR^2\\OR^3\end{matrix}\right\} + 6\,H_2 \longrightarrow Pr^nH + R^1H + R^2H + R^3H + 3\,H_2O.$$

The process occurred at temperature of 300–400 °C, pressure of 2–10 atm, with cobalt-molybdenum catalysts. The obtained products represented the ideal diesel fuel with the cetane number of 75–100 (the conventionally used diesel of the oil origin had the cetane number of 40–50) and could be used as the conventional fuel additive increasing their cetane number. The catalytic deoxygenation of stearic acid was observed to afford n-heptadecane and n-pentadecane.[177]

The possibility is considered of using hydrocarbons derived from triglycerides of the plant or microbiological origin as aircraft fuel.

7. Biodiesel from microalgae

Recently, attention was focused on the synthesis of biodiesel fuel by using microalgae. The idea of employing these algae as primary solar energy converters followed by processing the biomass or cell chemical components to afford various energy resources is not new.[10, 11, 178–180] Microalgae represent relatively efficient solar energy converters with well organized stages of carbon dioxide reduction to afford the whole complex of energetic biomolecules including carbohydrates, proteins, lipids, triglycerides.[181–193]

The algae biomass allowed different types of biofuel including methane to be produced;[10, 186, 187, 190, 194–196] however, interest was drawn to the synthesis of biodiesel.[111, 180, 197–204]

Advantages of using microalgae in the production of biodiesel are as follows:

1. Many types of microalgae contain large amounts of lipids and triglycerides of fatty acids. Sometimes their content reaches record breaking values (up to 70% in mass).

2. Under optimal growth conditions, microalgae are reproduced sufficiently quickly (in a full day, up to three cell duplications could occur), which allowed very high biomass 'crops' to be obtained.

3. The growth of microalgae can be intensified by adding carbon dioxide; moreover, the absorption of industrial carbon dioxide produced by power stations can be organized (algae absorb 1.8 kg CO_2 per kg of biomass.

4. Microlgae are a potential convenient object for genetic engineering aimed at improving their photosynthetic and triglyceride-producing activities.

5. Microalgae can be cultivated on territories useless from the viewpoint of growing agricultural plants, in deserts and semi-deserts, on open water areas by utilizing seawater and industrial or domestic sewages.

From the viewpoint of biodiesel production, microalgae proved to be 20–50-times more efficient than traditional vegetable sources of triglycerides.[125, 204] It is interesting that the use of microalgae can cover 50% of the USA needs in diesel fuel by using only 1.1%–2.5% of its land devoted to agriculture (to afford 58 700–136 900 litre of fuel per hectare).[204] Taking into account the prospects of using water areas and deserts, the potential resources of biodiesel derived from microalgae is virtually limitless.

In addition to using microalgae, the use of heterotrophic microbes[205, 206] that produce large amounts of triglycerides is also of interest.[207]

There are over 100 thousand different microalgae of which only a small fraction were examined for the content of lipids and triglycides. The microalgae of almost all taxonomic groups can be used in the biodiesel production.[204] Triglycerides of microalgae are characterized by the relatively high oleic acid content.

The fundamentally important technological feature of microalgae-based biodiesel production is the stage of their cultivation for which quite diverse reactors were designed. The technologically significant and acceptable solutions were the open flow-through reactor[208] and the tubular photobioreactor.[209–211] The efficiencies of these two systems were compared.[204] The open flow-through reactor (open pond cultivation) represents a trench 0.25–0.40 m deep with variable flow direction (978 m^2) that covers a certain area. The photobioreactor is a tubular system. The latter reactor is substantially more efficient (by a factor of 10–15) per illuminated surface unit. Biomass was separated from the aqueous phase by centrifugation and triglycerides were synthesized by their extraction with organic solvents.

A very important development in the microbiological method of biodiesel production was the use of biomass left after the triglyceride isolation. It was shown that anaerobic conversion of wastes to methane that utilized residues in the form of nitrogen and phosphate fertilizers makes this process economically attractive.[212, 213]

A certain difference of microbiological biodiesel from vegetable biodiesel was the its comparatively higher contents of polyunsaturated fatty acids including eicosopentaenoic and docosohexaenoic acids.[14] This led to higher instability of this fuel due to the higher trend to oxidation.[215] The European Standard on biodiesel restricts the content of methyl linolate in it (not higher than 12%). The content of unsaturated acids could be substantially reduced by catalytic hydrogenation.[216, 217]

VII. Biohydrogen

Hydrogen as a fuel exhibits several exclusive characteristics, surpassing all compounds used as fuels in the energy content per mass unit; moreover, water is the only product of its combustion.

In modern industry, the largest part of hydrogen was synthesized by the methane steam reforming or the catalytic conversion of hydrocarbons. However, a serious drawback of these methods was the emission of considerable amounts of CO_2 to the atmosphere. Hydrogen can also be synthesized by water electrolysis but due to the high price of electricity, the share of this method in the world production does not exceed 5%.

As a consequence, attention is drawn to the hydrogen syntheses involving biocatalysts, that can be classified as follows:[218]

— water biophotolysis employing green and blue-green algae (cyanobacteria);

— photo-decomposition of organic compounds by photosynthetic bacteria;

— dark fermentation of organic substances;

— hybrid systems using photosynthetic organisms and hydrogen-producing bacteria.

1. Biophotolysis of water involving microalgae

Photosynthesis in plants and microalgae involves the water oxidation (oxygen formation) and the transfer of electrons to carriers (ferredoxins, NADPH) that have redox potentials close to the hydrogen electrode potential. In the presence of catalysts of electron transfer to hydrogen ions (hydrogenase enzymes), the formation of molecular hydrogen was observed in the system.[10] Thus, the photosynthesizing systems are capable of water fission to hydrogen and oxygen and performing water biophotolysis.

Green and blue-green algae break water molecules to hydrogen and oxygen through direct and indirect photolysis. The direct photolysis that directly involves the electron-transport chain represents water fission to hydrogen by green algae according to the following general reaction:

$$2\,H_2O \xrightarrow{h\nu} 2\,H_2 + O_2.$$

This process could be generated by cells of the well studied *Chlamydomonas reinhardtii* algae that contain two ferredoxin-dependent hydrogenases. These hydrogenases exhibit high activity but can be stably synthesized only under anaerobic conditions. Oxygen formed inactivates hydrogenase. As the result, the duration of hydrogen evolution turned out to be much limited.[219] A large number of studies were devoted to hydrogenase properties and mechanism of its operation.[218, 220–228]

Immobilization of *Chlamyodomonas reinhardtii* on glass was conducted under conditions of sulfur deficiency and the high density of cells.[221] The maximum hydrogen volume produced by this system was 380 ml in 23 days and the highest rate of hydrogen accumulation was 45 ml day^{-1}. For *Chlamyodomonas reinhardtii* cells incorporated into calcium alginate,[222] the hydrogen production rate under anaerobic dark conditions in the presence of excessive nitrogen was 0.032 μmol h^{-1} mg^{-1} of dry biomass.

The indirect biophotolysis represented a combination of two reactions realized by green or blue-green algae, where hydrogen was formed due to the presence of hydrogenase or nitrogenase.

$$6\,CO_2 + 6\,H_2O \xrightarrow{h\nu} C_6H_{12}O_6 + 6\,O_2,$$
$$C_6H_{12}O_6 + 6\,H_2O \xrightarrow{h\nu} 12\,H_2 + 6\,CO_2.$$

The process could be carried out in two different reactors that provided separate production of hydrogen and oxygen.

Nitrogenase in cyanobacteria is preferentially localized in heterocysts that lack photosystem II, *i.e.*, are incapable of evolving oxygen, while oxygen forms in vegetative cells.[219]

Hydrogen-producing cyanobacteria can be nitrogen-fixing and nitrogen-nonfixing. The first type includes freshwater *Anabaena* sp., seawater *Calothrix* sp., *Oscillatoria* sp.; the second type can be exemplified by *Synechococcus* sp., *Gloebacter* sp. and *Anabaena* sp. All of them exhibited high hydrogen production rates as compared with other types of cyanobacteria.[218]

The hydrogen production rate of *Spirulina platensis* cells was shown to fall down with lowering the medium pH; however, by maintaining pH ~ 7, high hydrogen yields could be reached.[223]

In 24 h after the beginning of the process, the hydrogen production rate of *Anabaena* sp. was 85 and 9 μmol h^{-1} mg^{-1} of chlorophyll under argon and nitrogen, respectively, which substantially exceeded the values obtained in air. Moreover, as a whole, the oxygen yield also increased.[224]

The effect of the dark/light conditions on hydrogen production by *Nostoc muscorum* cyanobacterial cells was studied.[225] According to the obtained data, the higher the hydrogen yields increased with elongation of the illumination period; moreover, the amount of evolved oxygen was insignificant in all these cases.

However, as a whole, the use of cyanobacteria was complicated by the low values of efficiency and process rate and also by the simultaneous oxygen evolution.[219]

2. Photodecomposition of organic substances by photosynthetic bacteria

Photodecomposition of organic substances was performed by purple non-sulfur bacteria due to the presence of nitrogenase under the conditions of deficiency of all nitrogen forms by using the energy of light and for a wide spectrum of organic acids, for example, acetic acid [229]

$$MeCOOH + 2\,H_2O \xrightarrow{h\nu} 4\,H_2 + 2\,CO_2.$$

Photosynthetic bacteria were the research object for a long time due to sufficiently diverse metabolic abilities of these microbes and the absence of photosystem II, which immediately solved the problem of inhibition of the hydrogen production by oxygen. Such bacteria can be exemplified by *Rhodobacter sphaeroides*. In the immobilized state (on porous glass), they could steadily evolve H_2 in the amount of 1.1 litre h^{-1} per litre of matrix for 1000 h.[219]

It was shown that water biophotolysis can be carried out by a system containing *Spirulina platensis* cyanobacteria and *Rhodobacter sphaeroides* RV photosynthetic bacteria.[223] Under illumination, a cyanobacterium in a nitrogen-deficient medium accumulated glycogen which later decomposed in darkness to organic acids to afford hydrogen. These organic acids could also be processed in light by photosynthetic bacteria to afford hydrogen. As the result, the total amount of evolved hydrogen was virtually equal to the sum of stoichiometric values calculated based on decomposition of each organic acid.

In another similar system, the cultivation of *Chlamydomonas* sp. cells was first carried out in light.[226] In the process, starch was accumulated in the medium to be decomposed later in darkness under anaerobic conditions to afford such products as acetic acid, ethanol, glycerol and hydrogen. Then, nitroalgae were removed from the medium by means of a microfilter and the photosynthetic bacteria *Rhodovulum sulfidophilum* sp. capable of producing hydrogen in light were added. As the results, this system accumulated 78 litre H_2 in 23 days.

The use of one reactor containing two cultures of cells rather than two separate reactors with two cultures was considered as economically advantageous. It was shown that the growth of two different microorganisms (*Rhodospirillum rubrum* and *Chlamydomonas reinhardtii*) can be controlled by the light intensity. Under the optimal illumination conditions and composition of the medium, each of two cultures could produce H_2 maximally in an amount of 50 ml h^{-1} per litre of medium.[227]

3. Dark fermentation of organic substances

The dark fermentation can proceed with bacteria growing on different substrates, its general scheme is as follows:[218]

$$PYR + CoA + 2\,Fd(ox) \longrightarrow Ac\text{-}CoA + 2\,Fd(red) + CO_2,$$

$$2\,H^+ + Fd(red) \longrightarrow H_2 + Fd(ox),$$

where PYR is pyruvate, CoA is coenzyme A, Fd is ferredoxin, ox and red are the oxidized and reduced forms, respectively. For example, strictly anaerobic *Clostrdium acetobutylicum* bacteria generated the glucose conversion to mainly acetone, butanol, ethanol, hydrogen, carbon dioxide with the hydrogen production rate in the range of 25–55 ml h^{-1} litre^{-1} of the medium.[227]

Experiments with various bacteria and their consortiums usually produced 0.5–4 mol H_2 per mol glucose depending on inoculum composition, pH values, temperature and reactor type,[230] where the maximum amount of H_2 was produced by thermophilic bacteria. For example, *Clostridium thermocellum* cells fermented cellobiose to afford the maximum specific rate of hydrogen accumulation up to 14.6 mmol h^{-1} g^{-1} of dry biomass.[231]

Depending on the carbohydrate taken as the substrate, the hydrogen production rate of *Clostridium saccharoperbutylacetonicum* cells varied in the range of 1.2–2.8 mol H_2 per mole of sugar, where saccharose proved to be preferable.[232] For pH < 6.0, the yield of hydrogen produced by microorganisms was observed to decrease and become zero at pH 5.0. However, the higher hydrogen yields could be obtained with the starting pH $\geqslant$ 7.0.[232]

Hydrogen accumulation by *Clostridium saccharoperbutylacetonicum* cells depended also on their initial concentration in the medium.[233] Yet another promising candidate for the synthesis of hydrogen by fermentation were *Clostridium beijirinckii* cells.[234]

The use of a co-culture consisting of *Clostridium beijirinckii* and *Bacillus thermoamylovorans* cells in the hydrogen production was also reported.[235] A co-culture based on *Clostridium butyricum* and *Rhodobacter sphaeroides* cells was also described.[228]

Anaerobic hydrogen producers were compared with microalgae cells.[236] It was concluded that the dark fermentation should be preferred because required no light and allowed different biological wastes to be efficiently utilized.

Fundamentally new possibilities were opened up by carrying out biophotolysis with systems of microalgae and fermentative cells. The reaction of water photodecomposition to hydrogen and oxygen could be carried out using microalgae and anaerobic hydrogen producers. This scheme of this process is as follows:[10]

$$C_6H_{12}O_6 + 2\,H_2O \xrightarrow{\text{anaerobic hydrogen producers}} 2\,MeCOOH + 2\,CO_2 + 4\,H_2,$$

$$2\,MeCOOH + 2\,CO_2 + 2\,H_2O \xrightarrow{\text{microalgae}} C_6H_{12}O_6 + O_2.$$

The first stage (dark) is a reaction efficiently carried out by anaerobic microorganisms of the *Clostridium* genus and the second stage is the photosynthesis in microalgae.

For the first time, a process of this type was realized back in 1985.[194] A peculiarity of this process is the separate production of hydrogen and oxygen under solar light action. The hydrogen production rate reached 0.5 litre h^{-1} litre^{-1} of the medium.

Attention should be drawn to a study[237] in which *Chlamydomonas reinhardtii* microalgae were first grown for 3 days and then subjected to fermentation with anaerobic bacteria *Clostridium butyricum* with the aim of hydrogen production.

4. Hybrid systems

Side products of hydrogen dark production mainly included volatile fatty acids easily assimilated by purple bacteria; hence, it was proposed to combine the dark and the photo mechanisms of hydrogen production. Thus, in utilizing cellulose-containing waste, the hydrogen yield could be increased up to 11 mol per mol glucose. However, considerable effort was required for optimizing both processes and their integration.[219] Different two-stage processes of hydrogen production involving fermentation and photosynthetic bacteria were compared.[238] It was found that the system of *Clostridium pasteurianum* and *Rhodopseudomonas palustris* cells allowed the target product to be obtained in the amount of 14.2 mol mol^{-1} saccharose.[239] Systems with separate production of hydrogen and oxygen by biophotolysis of water have not yet reached the level of industrial application. However, recently, they were being actively modernized. The qualitative improvement of the operation characteristics of the systems is expected to be associated with the transition to immobilized cells. In case of industrial implementation of systems for solar energy conversion by water photofission, the technologies of this class would supply the society with the energy extremely environmentally clean and renewable. Moreover, it is beyond doubt that the important role will be played by processes based of utilization of different wastes from industry, municipal facilities and agriculture as the main substrates for hydrogen production.[187,240–242]

VIII. New approaches towards biofuel production

Modern development of the technology of biofuel production was characterized by the appearance of new solutions and approaches. Novel solutions were associated with both the use of new combinations of chemical and biotechnological stages and also with new chemical reactions to afford highly energetic products. Some of these approaches are considered below.

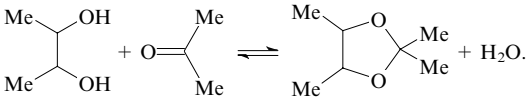
1. Bioketals

In the classical version of a technology for biomass conversion to alcohols (ethanol, butanol), only glucose produced by chemical or enzymatic hydrolysis of cellulose underwent biotechnological transformation. Five-carbon sugars were the wastes in this process. In the biodiesel production, glycerol was a by-product that has not yet found any large-scale application. These substances could be transformed into vehicle fuel by their direct chemical modification and hydrophobization.

Chemical transformation of pentoses involving direct conversion of carbohydrates into the hydrophobic phase of conventional fuels is possible. This approach was used for all organic molecules containing vicinal hydroxyl groups, for example, ethylene glycol, glycerol.[243,244]

As the result of a chemical reaction, ketals were obtained, a water molecule was eliminated and carbohydrates or polyols were thus hydrophobized. Organic products proved to be soluble in the alcohol and hydrocarbon phases, the removal of water increased their relative energy content.

The most practically important ketone is acetone, the accessible and relatively cheap product of a large-scale organic synthesis and a by product of the biotechnological production of biobutanol.



The new method of biofuel production from lignocellulose substrates involved the stages of the chemical or enzymatic hydrolysis of cellulose and hemicellulose followed by the direct chemical conversion of sugars to the hydrocarbon-soluble fuel form.[244] This made the flow diagram of this process substantially simpler, allowed the low-efficient, highly labour- and resource-consuming microbiological stage to be abandoned and increased the biofuel yield by 50% – 80%.

Modern vehicles require high-octane fuel with antiknock properties characterized by the engine octane ratings 92, 95 or 98. The high antiknock characteristics were achieved through either the deep modification of petrol by using cracking, isomerization and alkylation processes or the introduction of special high-octane additive to fuels. The latter method is today the most economic and popular.

At present, aromatic amines, ferrocenes and methyl *tert*-butyl ether (MTBE) were used to increase the octane rating of gasoline, but all these substances are harmful for people and the environment. MTBE was used most widely; however, because of its toxicity and harmfulness for the environmental it was banned in 2004 in California and is to be prohibited in the USA in 2010.

Being added to petrols together with ethanol, the ketals of five-membered sugars and glycerol allowed the octane rating to be substantially increased.[243, 244] The known antiknock substances (aromatic amines, ferrocene and its derivatives, manganese-containing complexes) when used in admissible concentrations allowed the octane rating of gasoline to be increased by 3 – 6 points. A similar effect was observed for MTBE and ethanol.

Cyclic ketals of glycerol, arabinose, biomass hydrolyzates in concentrations of 1% to 10% could stabilize water in alcohol – petrol blends and prevent their separation to layers and the deposition of solid ice phase above -30 °C.

2. Conversion of biomass thermal gasification products to biofuel

One of conventional methods for biomass production was associated with thermal pyrolysis and gasification. Dozens of processes employing thermal decomposition of wood and related substrates to produce liquid and gaseous fuels were developed.[245 – 248] The products of biomass thermal gasification represented the mixture of hydrogen, CO, CO_2 and lower hydrocarbons. Analogous products were synthesized by coal gasification (syngas). Biotechnological processes are known that allow converting syngas into organic synthesis products including biofuels.

The reaction of CO_2 (CO) reduction with molecular hydrogen to afford several organic compounds such as sodium acetate, ethanol and butanol was studied in detail.[247, 249, 250] This involved using free and immobilized cells of *Thermoanaerobacter*, *Clostridium*, *Moorella*, *Carboxydocella* genera as the catalysts.[247, 251] Depending on the nature of microorganism, the process of syngas fermentation was characterized by predominance of one or another product. Thus, *Clostridium ljungdahlii* cells demonstrated the highest ethanol yield. Coskata company (USA) under supervision of General Motors is going to build a plant with the yearly output of 160 thousand litre ethanol by using a technology that combines the thermal production of syngas from virtually all organic substrates (solid domestic waste, worn tyres, wasters of wood and agricultural industries) with its biotechnological conversion to ethanol.

3. Pyrolytic biooils

Fast pyrolysis of various bioorganic substrates (wood, agriculture waste, microalgae biomass[248, 252, 253]) carried out at temperatures of 400 – 500 °C for 5 min led to the formation of the so-called pyrolytic biooils that resemble hydrocarbon fuels obtained from high-gravity oils in their characteristics and composition but in contrast includes no sulfur- or nitrogen-containing components. To date, the production of this pyrolytic biooil was discussed as one of the possible directions of the further development of the biofuel industry aimed at decentralizaton of fuel streams.

4. Diesel fuel from bacterial cells

The development of methods of genetic engineering allowed the appearance of radically new processes to be expected, which includes the developments in the field of biofuel production, particularly, the microbial biodiesel fuel (Microdiesel).[254, 255] Nowadays, a producer was designed based on *E. coli* cells that proved capable of realizing the ethyl oleate production with glucose and oleic acid as the main feedstocks. This was achieved by expressing *pdc* and *adhB* genes from *Zymomonas mobilis* bacteria that code the synthesis of pyruvate carboxylase and alcohol dehydrogenase into the transformed cells and also of the *atfA* gene from *Acinetobacter bayalyi* that codes the synthesis of acyl transferrase.

5. Biogasoline from bioethanol

The studies of a new reaction, reductive dehydration of alcohols seems very promising. In this reaction, an alcohol was transformed to alkane with doubling (at the least) of its carbon chain length as compared with the original alcohol molecule. The reaction occurred at 250 – 350 °C and a pressure of 7 – 50 atm in the presence of polymetallic catalysts.[256] It was shown that ethanol obtained as the result of CMC biochemical conversion can be transformed into a high-octane component of engine fuel, particularly, to $C_4 - C_{10}$ octanes, with the degree of conversion of 60% – 70%.[257] The further development of these processes in combination with biotechnological approaches to processing of renewable energy sources can form a platform for synthesizing biopetrol based on environmentally clean industrial processes.

6. Biohydrogen from carbon monoxide and water

The results of studies of the microbiological synthesis of hydrogen from CO and water (shift reaction) are very promising. Carbon monoxide is one of the main components of syngas derived from renewable biomass.

$$CO + H_2O \longrightarrow H_2 + CO_2\,(-20\ \text{kJ mol}^{-1}).$$

This reaction was effected by bacteria of several genera (*Rhodobacter*, *Rhodopseudomononas*, *Rhodospirillum*, *Eubacterium*, *Butyribacterium*, *Citrobacter*[258, 259]) and the *Methanosarcina barkeri* archaea.[260] It was shown that this process can be conducted under conditions of either con-

tinous of periodic operation of a bioreactor containing microorganisms cells. The maximum yield with respect to hydrogen attained to date was 80%.[259] The maximum CO conversion level could be maintained stable for 27 days in a flow-through reactor with one and the same biocatalyst sample representing suspended cells. The maximum hydrogen production rate in this process was $12-14$ mmol h^{-1}. The optimization of synthetic conditions and designs of bioreactors will apparently become the main direction of the development of this process in close future.

* * *

The active development of studies in the field of synthesis of bioenergy carriers and the development of new technological processes was determined by the growing demands for supplied energy, the environmental advantages of using solar energy and the advancing deficiency of accessible hydrocarbon feedstocks. The motto 'carbohydrates in place of hydrocarbons' lies in the basis of the development of many chemical and fuel companies. This review illustrates the wide diversity of biofuel products, approaches and technologies aimed at the development of a new industry branch, biofuel industry.

The development of biofuel industry as one of the most useful and large-scale methods of converting substances invoked the emergence of new branches of chemical industry. Apparently, the next stage of this development will be the creation of scaled processes for producing environmentally clean materials, biodegradable polymers, bioplastics, new types of bioelastomers and rubber, new products of fine organic synthesis. At present, the prerequisites are being laid for the transition to new environmentally clean technologies.

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