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Promising Directions for Utilization of Glycerol-Containing Waste from Biodiesel Fuel Production

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Abstract—The provided examples of utilization of glycerol and glycerol-containing media are far from completely reflecting the diversity of research works dedicated to this problem. Nevertheless, the presented works provide sufficiently convincing evidence on broad prospects for utilization of excess glycerol for obtainment of practically important products in the course of chemical and biotechnological processes.

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INTRODUCTION

One of the major directions in search of solutions to global problems (including energy, resource, environmental, and other aspects) is the use of renewable sources of energy and raw materials, first of all, plant biomass [1]. Bioethanol and biodiesel fuel (biodiesel) are already applied as biofuels.

In 2030 it is planned to replace ~30% of traditional motor fuels with biofuels on the territory of the United States and 25%, in countries of the European Union. At the same time, while in the United States the attention is focused on bioethanol, European countries prefer biodiesel [1], which raises the problem of excess glycerol, which is a residual product of biodiesel production. The annual quantity of glycerol produced in European countries and in the United States amounts to approximately 1.5 mln t [2]; moreover, the growth in glycerol production volumes is characterized by positive dynamics, as 2/3 of this amount is related to biodiesel production.

In connection with the necessity to create a sales market for glycerol, generated in the process of biodiesel production, a wide range of chemical and biotechnological methods for processing of glycerol and glycerol-containing waste was developed.

This article describes promising directions for processing of glycerol-containing waste and areas of application for the resulting products.

Production of Energy Carriers

One of the achievements of the chemistry of glycerol is the creation of the aqueous-phase reforming process, taking place under relatively mild conditions (225–330°C) with the use of platinum-group catalysts. In the course of this process glycerol is transformed into hydrogen and carbon monoxide (syngas) [3, 4]. The production of hydrocarbons within the framework of the Fischer–Tropsch process using source syngas obtained from glycerol-containing waste is an economically viable way to manufacture alternative fuels [5].

The production of liquid hydrocarbon fuels on the basis of glycerol can rely on a thermally balanced two-stage process, involving endothermic aqueous-phase reforming of glycerol resulting in the production of syngas ($CO + H_2$) and the subsequent exothermic conversion of syngas in compliance with the Fischer–Tropsch reaction as follows:

$$nCO + (2n+1)H_2 \rightarrow C_nH_{2n+2} + nH_2O.$$

Occurrence of the reaction at relatively low temperatures makes it possible to create an economical, integrated process for manufacturing of liquid motor fuels on the basis of the aqueous solution of glycerol obtained as a result of biodiesel production.

Another method to process cheap glycerol-containing waste from biodiesel production in order to manufacture products considered to be promising biofuels is anaerobic fermentation of glycerol by microorganisms resulting in the formation of ethanol and butanol [1].

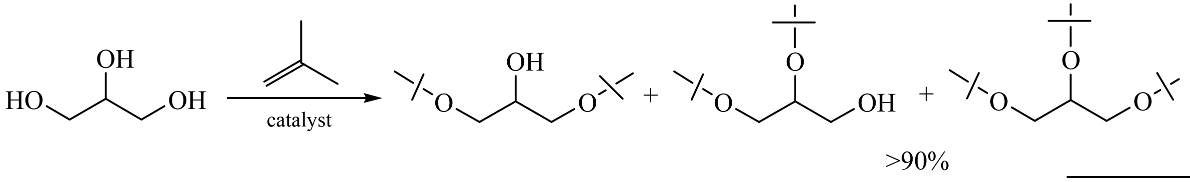
Recombinant strains are cultivated for ethanol production, as natural bacterial strains (*Klebsiella planticola*, *Enterobacter aerogenes*, *Paenibacillus macerans* etc.) growing in a glycerol-containing medium produce low-yield ethanol [6, 7]. A promising strain was created on the basis of Saccharomyces cerevisiae yeast used by the industry for the production of ethanol from carbohydrate raw materials [8]. A high glycerol utilization rate was achieved through overexpression of glycerol dehydrogenase and dihydroxyacetone kinase, as well as of channel protein in the cells of this microorganism. Research works are performed in order to create genetically modified strains on the basis of *Escherichia coli*, ensuring overexpression of genes for utilization of glycerol and elimination of competitive ways of ethanol formation [9, 10]. The most efficient recombinant strain causes the formation of ~22 g l^{-1} of ethanol [10].

The process of biobutanol production using bacteria of *Clostridium acetobutylicum* and *Clostridium pasteurianum* makes it possible to replace hydrocarbon raw materials with glycerol either partially or completely [11, 12]. Within the framework of studies aimed at increasing the efficiency of the process of butanol production from glycerol researchers work on the problems of improving butanol producers' properties through increasing their resistance to high concentrations of the product [13] and blocking the formation of side compounds [14] and develop methods of removing the products of fermentation in situ [15].

One of the most rational biotechnological directions for utilization of glycerol-containing waste from biodiesel production is to use such waste for obtainment of microbial lipids, which can be later processed into biodiesel fuel (micro-biodiesel). Such approach makes it possible to reduce the cost of biodiesel due to glycerol returning into the production cycle [16] and to decrease the consumption of natural raw materials for lipid extraction. Among oleogenic bacteria, algae, yeast, and fungi, capable of converting organic waste into lipids, the most interesting microorganisms are those accumulating large quantities of neutral lipids close to vegetable oils in composition [17–19]. Lipids can be synthesized from glycerol by fungi: *Cunninghamella echinulata* [20] and *Mortierella isabellina* [20, 21]; by yeast: *Yarrowia lipolytica* [21], *Cryptococcus curvatus* [22], and *Rhodotorula glutinis* [23]; and by heterotrophic algae: *Spirulina platensis* [24].

Defatted biomass of microorganisms can be used for further processing. Thus, biomass of mucoralean fungi can be applied for the production of chitin with a higher wound healing activity as compared to crab chitin [17].

Some glycerol derivatives can be applied as fuel additives. Due to glycerol molecule polarity and hydrophilic nature it cannot be used in fuel additives directly; however, a mixture of di- and tri-*tert*-butyl esters of glycerol, which is well soluble in non-polar fuel components, can serve as a perfect fuel additive for standard diesel fuels significantly improving their environmental characteristics. The synthesis of these esters is performed according to the reaction of glycerol with excess isobutylene in the presence of an acid catalyst at temperatures of 50–150°C [25, 26]:



Other glycerol derivatives, such as acetals and glyceryl carbonate, are also perfect additives for biodiesel, which not only improve the environmental characteristics of the fuel but also reduce its viscosity [27]. Esterification of glycerol with acetic acid or transesterification of glycerol with alkyl acetates result in the formation of a mixture of diacetin and triacetin, which is a promising fuel additive [28–30].

Methods of producing hydrogen on the basis of glycerol are intensively developed. The demand for hydrogen as an energy carrier is constantly increasing, in particular, due to the active development of fuel cell technology. The global hydrogen production is estimated at approximately 45 mln t per year; ~95% of the world hydrogen is obtained from fossil fuels.

There are several major ways to convert glycerol into hydrogen. The following processes take place under stringent processing conditions (catalyst, tem-perature, and pressure): aqueous-phase reforming (Pt, 200–270°C, and 16–55 atm) [31, 32], steam reforming (Pt, Co, Ni and 400–650°C) [33–35], and supercritical aqueous reforming (Ru/Al_2O_3, 700–800°C, and 240 atm) [36]:

$$HOCH_2CH(OH)CH_2OH + 3H_2O \rightarrow 7H_2 + 3CO_2.$$

Hydrogen formed as a result of aqueous-phase reforming is sufficiently pure and can be directly used as high-energy fuel for internal combustion engines, gas turbines, and full cells. In works [32, 37–39] optimization of conditions for implementation of the aqueous-phase and steam reforming processes was performed in order to increase the yield of hydrogen and the selectivity of the process.

Other promising ways to produce hydrogen from glycerol include biotechnological methods based on the use of microorganisms [40]. These techniques are less expensive and allow using crude glycerol.

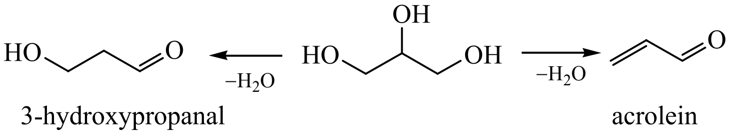
One of the processes for conversion of crude glycerol into hydrogen is anaerobic fermentation with heterotrophic bacteria [6, 40, 41]. However, the yield of hydrogen from these processes is quite low (0.05–1.05 mole/mole [42]), while the amounts of by-products (CO_2, 1,3-propandiol, ethanol, acetic acid etc.) are quite significant [41–43].

In order to obtain additional quantities of the target product it is proposed to carry out the second stage of the process, during which hydrogen is generated from the by-products using photoheterotrophic micro-organisms [41]. Another approach to increasing the yield of hydrogen is electrochemical activation of anaerobic fermentation of glycerol [44]. During the process carried out in a single-chamber reactor with the use of crude glycerol a higher yield of hydrogen, reaching 3.9 mole/mole, was achieved [42]. Apart from that, it is proposed to economically compensate a low yield of hydrogen from the processes of anaerobic fermentation with simultaneous production of 1,3- propandiol, which is a valuable by-product of the process [43].

Another alternative that can prove more rational is the process of hydrogen production based on the use of photoheterotrophic bacteria of *Rhodopseudomonas palustris*, which can directly convert glycerol itself into hydrogen with a high yield reaching 6 mole/mole (75% of the theoretically possible level). The use of crude glycerol in this process reduces this value only negligibly [45].

Obtainment of Practically Important Chemical Products

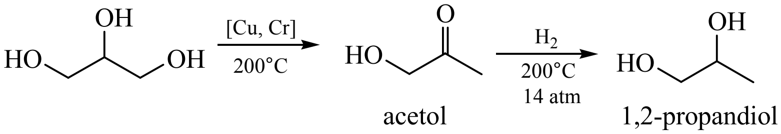
Two extremely important chemical products, namely acrolein and 3-hydroxypropanal, can be obtained through dehydration of glycerol as follows:



Complete conversion of glycerol primarily into acrolein is achieved in the course of the catalytic reaction of gaseous water-glycerol mixture containing 10–40 wt % of glycerol at 250–340°C [46].

Taking into account the continuously rising prices of propylene (traditional raw material for the industrial production of acrolein) and decreased costs of glycerol, the production of acrolein from glycerol is seen as an economically attractive option. In this connection, works on search of active acid catalysts and optimization of conditions for the production of acrolein from glycerol are carried out [47–49].

Glycerol can be hydrogenated into 1,2-propandiol or 1,3-propandiol in the presence of metallic catalysts. The most practically important process is the conversion of glycerol into 1,2-propandiol (propylene glycol), which is an important large-tonnage product of organic synthesis [50]. The resulting 1,2-propandiol with a yield of 90% is characterized by lower production costs as compared to the product obtained from propylene oxide on the basis of the traditional method [51].

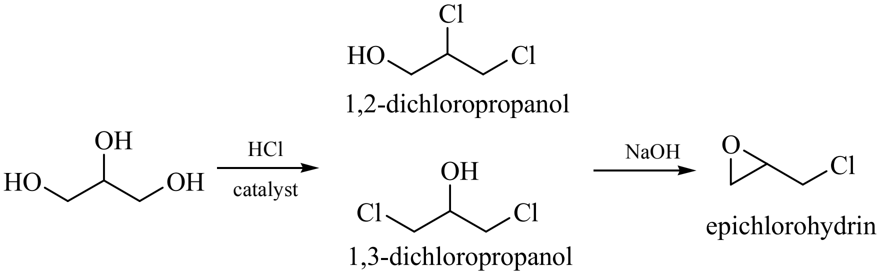


A high-selectivity (over 96%) process for the production of 1,2-propandiol through hydrogenation of glycerol using heterogenic copper catalysts at 200°C and 20 atm was patented [52]. Studies of the reaction of glycerol direct hydrogenation with the use of a wide range of heterogenic catalysts (Cu, Pd, Rh, and Ni–Re) are performed [53–55].

Another valuable product of glycerol reduction is 1,3-propandiol, which is widely applied in the synthesis of polymethylenterephtalates, polyurethanes, and cyclic compounds [56]. The method of producing 1,3-propandiol from glycerol is competitive with the traditional methods of obtaining this product from ethylene oxide and acrolein.

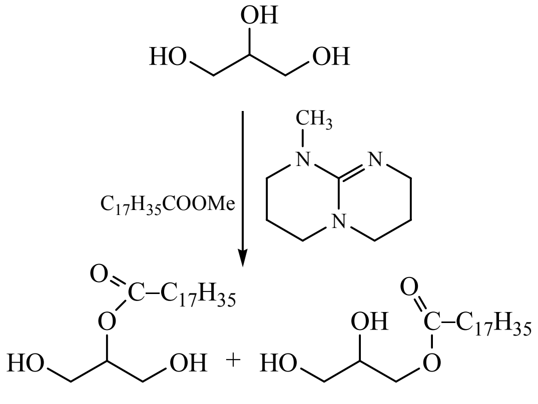
There are methods to produce 1,3-propandiol from crude glycerol using *Clostridium* genus bacteria and *Klebsiella pneumoniae* [21, 57]. Intermediate 3-hydroxypropionic aldehyde can be withdrawn from the culture medium through binding with semicarbazide and used in organic synthesis [2]. It was demonstrated [58] that in the process of growth of a recombinant strain of *Escherichia coli*, expressing glycerol dehydratase and aldehyde dehydrogenase, in glycerol-containing medium 3-hydroxypropionic aldehyde was transformed into 3-hydroxypropionic acid used for the production of heat-resistant polymers and antistatic agents with the yield of 31 g l^{-1} of culture fluid for 72 h of fermentation.

Another technically important product, the production of which from glycerol is economically effective, is epichlorohydrin. Direct hydrochlorination of glycerol produces a mixture of dichloropropanols, alkaline treatment of which results in the formation of the target product as follows:



Traditional processes for synthesis of epichlorohydrin based on glycerol, using aqueous solutions of HCl and acetic acid as a catalyst, have major drawbacks manifesting themselves both at the synthesis stage and at the product extraction stage [59, 60]. A modified process based on the use of gaseous anhydrous hydrogen chloride and non-volatile acid catalysts was developed in order to produce epichlorohydrin from crude glycerol [61]. Through kinetic separation of racemic epichlorohydrin with the use of epoxide hydrolase of *Pseudomonas sp.* and *Rhodococcus sp.* bacteria it is possible to obtain high-purity enantiomers of epichlorohydrin and 3-chlor-1,2-propandiol, which are extremely important synthons of pharmaceuticals [β-adrenergic blockers, (R)-carnitine, (+)-tetrazoline, (S)-ipsenol, (R)-recifiolide, (S,S)-vermiculine, epothilone etc.] [62].

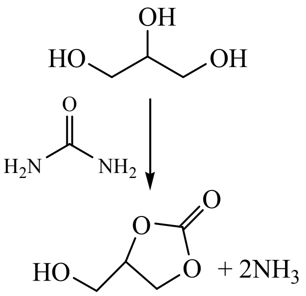
Active research of glycerol esterification, resulting in the formation of a large number of valuable products, is performed [63–69]. Esterification of glycerol with higher fatty acids results in the formation of monoacylglycerides and diacylglycerides applied as nonionic surfactants, emulsifiers, nutritional and cosmetic additives, as well as lubricants [63–65]. In industry these compounds are obtained through glycerolysis of natural vegetable fats and oils or through esterification of glycerol and free fatty acids [66]. Catalytic processes are performed in the presence of hard bases and, as a rule, result in the formation of mixtures of different composition. Monoacylglycerides were obtained with high selectivity at glycerolysis of methyl esters of fatty acids catalyzed by guanidine derivatives as follows [67]:



Alternative catalysts for the reactions of production of glycerol esters are lipases (triacylglyceride hydrolase) [68, 69]. These enzymes capable of working under mild conditions can be used for the production of esters of essential polyunsaturated fatty acids of natural configuration [70]. Biocatalytic methods for synthesis of pharmacologically important monoglycerides and diglycerides were developed on the basis of interaction processes with the participation of glycerol lipase, oleic acid, and ferulic acid, possessing antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties [71]. Esterification of glycerol with benzoic acid using lipase of *Candida antarctica* resulted in the formation of α-monobenzoyl glycerol, a synthon of carvidilol and propanolol used in medical treatment of hypertension and glaucoma [72].

Glyceryl carbonate (4-hydroxymethyl-1,3-dioxolan-2-one) is applied as a specific solvent for cellulose acetate, nylon, nitrocellulose, and polyacrylonitrile, as a synthon for polymer materials, and as a flexible precursor in organic synthesis [73–75]. Due to its low toxicity, low volatility, and moisturizing ability glyceryl carbonate possesses the properties that are necessary for cosmetic components and medical solvents [76]. The general methods of glyceryl carbonate synthesis are based on the interaction of glycerol with phosgene [77] or transesterification of glycerol with other carbonates [78, 79].

A promising approach to the synthesis of glyceryl carbonate involves glycerolysis of carbamide in the presence of catalysts [80–82]. The yield of the product of the reaction performed using zirconium phosphate as a catalyst reaches 80% [83].



Glyceryl carbonate was also obtained through carboxylation of glycerol directly with carbon dioxide with the use of Bu_2SnO dibutyltin oxide as a catalyst; the yield of the product amounted to 35% [84].

Modern works on glycerol nitration are mostly related to obtainment of dinitroglycerin and its further conversion to glycidyl nitrate, which is a raw material for the production of poly(glycidyl nitrate) (PGN) used as an explosive material and rocket fuel [85].

There is a wide range of developed chemical, electrochemical, and biocatalytic methods for synthesis of glycerol oxygenated derivatives [86–90]. Modern developments in the field of chemical methods for glycerol oxidation exclude the use of strong oxidants resulting in the formation of large quantities of toxic waste. Oxidation is performed using atmospheric oxygen in the presence of catalysts based on Pt, Pd, Au etc (Sceme 1).

Chemical oxidation of glycerol often results in the formation of a mixture of oxygenated derivatives, including racemic chiral compounds [91]. It is possible to make the process regio- and enantio-directed using enzymes and cells of microorganisms.

The process of regioselective oxidation of glycerol into 1,3-dihydroxyacetone under the effect of acetic acid bacteria is well-known; 1,3-dihydroxyacetone is the main component of skin toning creams and an important building block in synthesis of a number of chemical compounds, including methotrexate anticancer agent [91–93].

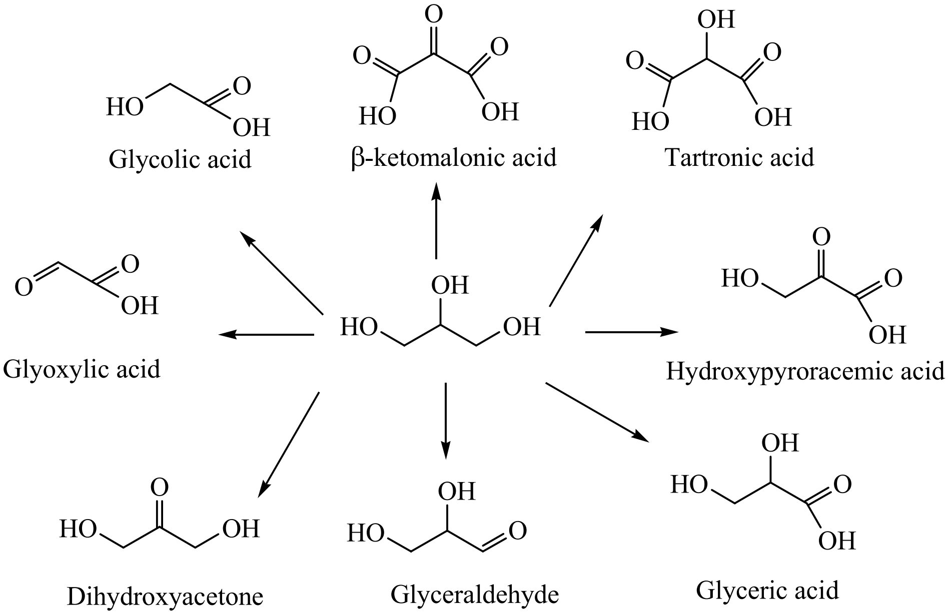
Thermophilic bacteria of *Thermus thermophilus* were used for the extraction of thermostable alcohol dehydrogenase, capable of performing enantioselective oxidation of glycerol into D-glyceraldehyde, thus opening a number of new directions for synthesis. In particular, it is possible to synthesize pharmacologically important tetrahydrofurans from D-glyceraldehyde derivatives [94].

Bio-oxidation of glycerol using bacteria of *Gluconobacter frateurii* NBRC103465 or *Acetobacter tropicalis* NBRC16470 makes it possible to obtain bioactive D-glyceric acid, which can be used as a basis for synthesis of new surfactants and polymers [91, 95].

Multi-stage conversion of glycerol with aerobic and anaerobic microorganisms forms the basis for the production of a number of practically important organic acids, diols, and polyols.

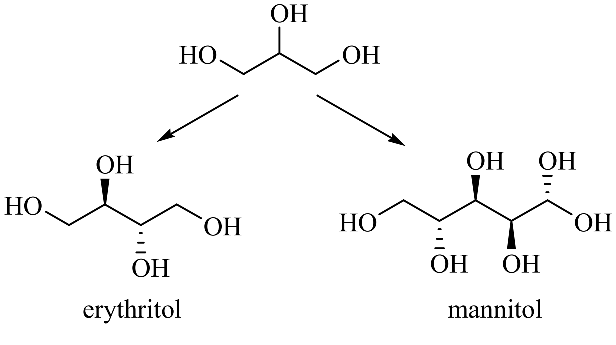
The ability of *Yarrowia lipolytica* yeast to convert glycerol into citric acid with the yield of 0.4–0.6 g/g is demonstrated in works [21, 96, 97]. An efficient process of crude glycerol bioconversion into citric acid using *Yarrowia lipolytica* A-101-1.22 is created; the process envisages multiple recycling of biomass [97].

Scheme 1.



Inhibition of pyruvate dehydrogenase activity in the cells of thiamine auxotrophic yeast of *Yarrowia lipolytica* 374/4 growing in glycerol-containing medium results in the formation of pyruvic acid with the yield of 61.3 g l^{-1} of the medium [98]. *Aspergillus niger* fungi, growing in a medium containing crude glycerol under conditions of nitrogen-source limitations, accumulate oxalic acid in the medium in the amounts reaching 20.5–21.5 g l^{-1} of the culture fluid [99].

Acetate-negative mutant of *Yarrowia lipolytica Wratislavia* K1, growing in a medium containing crude glycerol, produces erythritol (the yield of 170 g l^{-1}, 56% of the initial substrate) alongside with citric acid [100]. The resting cells of osmophilic yeast of *Candida magnoliae* in phosphate buffer solution convert glycerol into a single product, namely mannitol [101].



Glycerol can be applied for the production of succinic acid using bacteria of *Basfia succiniciproducens* DD1 [102] and *Anaerobiospirillum succiniciproducens* [103].

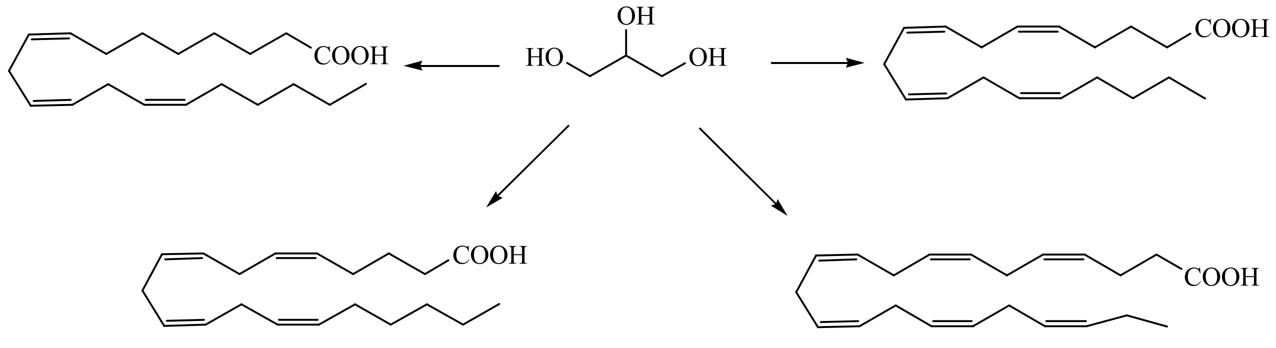
Propionic acid with the yield of 106 g l^{-1} is formed as a result of glycerol fermentation with a recombinant strain of *Propionibacterium acidipropionici* (ACK-Tet), tolerant to high concentrations of this compound [104].

Bacteria of *Klebsiella pneumoniae* G31 convert glycerol into 2,3-butandiol, which under the optimal conditions (pH = 8) is accumulated in the medium as the main product (the yield of 49.2 g l^{-1}) [105]. A principal possibility to convert glycerol into 1,2-propandiol using bacteria of *Escherichia coli* is demonstrated [106].

Glycerol can serve as an initial substrate for production of a number of complex functionally substituted molecules of natural configuration through microbiological synthesis.

The use of glycerol for commercial production of essential amino acids is also promising. Thus, the yield of L-phenylalanine obtained from glycerol as a result of cultivation of the recombinant strain of *Escherichia coli* BL21(DE3), a producer of this amino acid, reaches 0.58 g/g, which is about two times higher than the yield of this product from glucose (0.25 g/g). It is forecasted that in case of dosing of the substrate (glycerol) the final content of this amino acid can reach 50 g l^{-1} [107]. Introduction of *Escherichia coli* genes, coding the enzymes of glycerol kinase (glpK) and glycerol-3-phosphate dehydrogenase (glpD), as well as aquaglyceroporine transport protein (glpF), into the cells of *Corynebacterium glutamicum*, producing L-lysine, resulted in the creation of a recombinant strain capable

Scheme 2.



of synthesizing this amino acid from glycerol [108]. Similar modification of a wild-type strain of *C. glutamicum* makes it possible to obtain L-glutamic acid from glycerol [108].

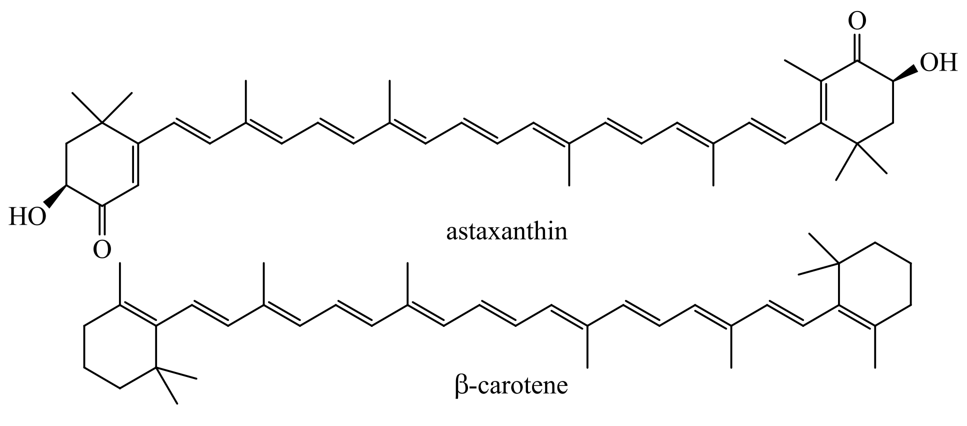
Microbiological methods were used to synthesize a number of essential polyunsaturated fatty acids (Scheme. 2) on the basis of glycerol. These acids are applied as nutritional supplements and pharmaceuticals treating cardiovascular diseases, cancer, schizophrenia, blood clotting disorders, arthritis, Alzheimer's disease, psoriasis, and other diseases [109–115].

In our studies of solid-state fermentation of *Mortierella alpina* 18-1, which is a producer of arachidonic acid, we demonstrated that the fungi grew and synthesized lipids more efficiently in a glycerol-containing medium than in a medium containing glucose [109, 110]. The content of arachidonic acid in fungal lipids synthesized in a glycerol-containing medium reaches 63.6–68.7% (of the fatty acids total) [110]. In a glycerol-containing medium with 5% of sodium chloride the haloresistant mutant of the fungi (*Mortierella alpina* XH-1) produces eicosapentaenoic acid alongside with arachidonic acid (14.1% and 19.8% of the fatty acids total, respectively) [111].

An ability of a number of *Mortierella genus* fungi to grow in a glycerol-containing medium under submerged cultivation conditions and to produce arachidonic and dihomo-γ-linoleic acids at a level comparable to the level achieved in a medium containing glucose was described [112]. The mixotrophic growth of diatoms of *Phaeodactylum tricornutum* UTEX 640 in a glycerol-containing medium resulted in a ten times higher yield of eicosapentaenoic acid as compared to the photoautotrophic growth of this culture [113].

A possibility for efficient use of crude glycerol for production of docosahexaenoic acid using *Schizochytrium limacinum algae* [114] and a possibility for synthesis of eicosapentaenoic acid using *Pythium irregulare* fungi were demonstrated [115].

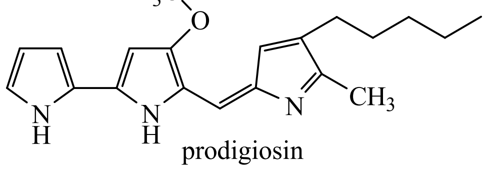
It is proposed to use glycerol-containing media for obtainment of natural food colorants. *Phaffia rhodozyma* yeast produces astaxanthin (33.7 mg l^{-1}), a red-orange pigment, in a glycerol-containing medium [116]. The growth of *Blakeslea trispora* fungi on biodiesel production waste results in the synthesis of β-carotene with the yield of 8 mg g^{-1} of dry biomass [117].



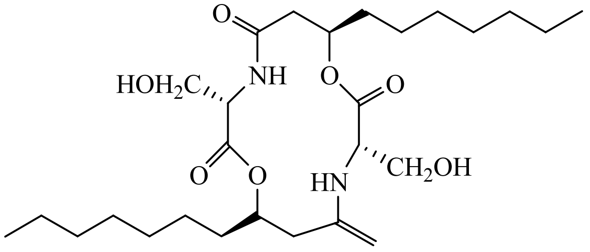
astaxanthin

β-carotene

A possibility to synthesize prodigiosin using *Serratia genus* bacteria in glycerol-containing media is of great interest [118–121] (prodigiosin can induce apoptosis of several cancer cell lines). In the course of a two-stage process using glucose at the first stage and glycerol at the second stage the mutant of *Serratia marcescens* performs the synthesis of prodigiosin with the yield of 583 mg l^{-1} [130]. Cultivation of the mutant obtained by us on the basis of an oil-oxidizing strain of *Serratia sp.* HC-P1 under optimal conditions in a glycerol-containing medium resulted in the formation of prodigiosin with the yield of 1000 mg l^{-1} [122]. The strain of *Serratia sp.* HC-P1 and its mutant synthesize serravettin W1, a biosurfactant of lipopeptide nature, alongside with prodigiosin [122–124]. Serravettin W1 exhibits antimicrobial activity [123], delays cell cycle, and induces apoptosis of several cancer cell lines [125] in the absence of toxicity to normal cells, which makes it possible to consider serravettin W1 as a potential therapeutic agent. The greatest yield of serravettin obtained in a glycerol-containing medium using the mutant of *Serratia sp.* HC-P1 reaches 1800 mg l^{-1} [122].

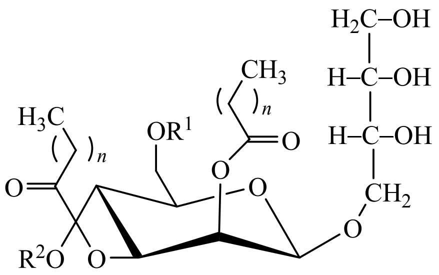


prodigiosin



serravettin W1

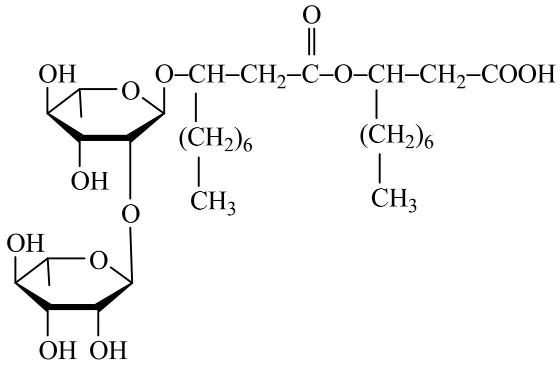
Some yeast and bacterial strains in a glycerol-containing medium produce large quantities of extracellular biosurfactants of glycolipid nature (sophorolipids [126], mannosylerythritol lipids [127], and rhamnolipids [128]), possessing antimicrobial, antiviral, and anticancer properties.



mannosylerythritol lipids

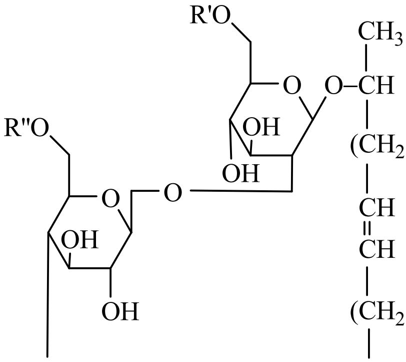
$n = 6–10$

$R^1 = R^2 = Ac$; $R^1 = Ac$, $R^2 = H$; $R^1 = H$, $R^2 = Ac$.

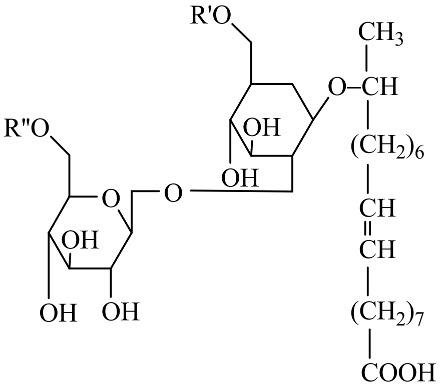


rhamnolipid

Candida bombicola yeast produces sophorolipids mainly in the lactone form in a pure glycerol medium; in case biodiesel production waste is used, the yeast forms open-chain lipids [128a].



lactone sophorolipids



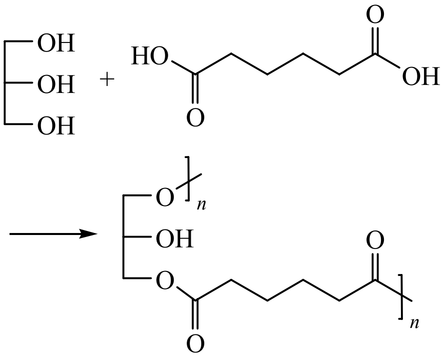
non-lactone sophorolipids

R" = R' = Ac; R' = Ac, R" = H; R' = H; R' = Ac; R" = R' = H.

Production of Polymers

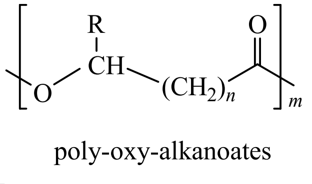
One of the directions for utilization of glycerol-containing waste is the synthesis of polyethers and polyesters through glycerol intermolecular dehydration and polycondensation with dicarboxylic acids in the presence of catalysts, respectively. The resulting polyglycerols and polyesters are used as antistatic, lubricant, and plasticizing agents [129].

Methods to synthesize glycerol polyesters in organic medium using lipases of microorganisms were developed. Thus, regioselective polycondensation of glycerol with adipic acid in dioxane in the presence of B lipase produced from *Candida antarctica* yeast resulted in the formation of polyhydroxylated ester with relatively low molecular weight (1314–1716), which can be used for modification of polyesters and polyurethanes [130].

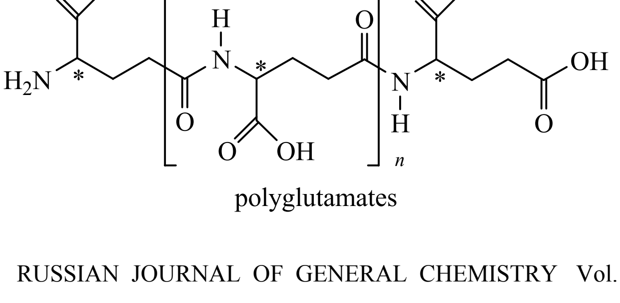


Application of enzymes for the synthesis of polymers makes it possible to obtain new materials of relatively low molecular weight, which can be used as adsorbents, hydrogels, biodegradable materials, liquid crystals, and chiral phases for kinetic separation of racemic mixtures [130].

Glycerol can serve as an economical substrate for the synthesis of a number of biopolymers (poly-oxy-alkanoates, polyglutamates, and polysaccharides) using microorganisms as, as a rule, the major production costs for these biopolymers are related to the costs of the feedstock [131, 132].



poly-oxy-alkanoates



polyglutamates

Many aerobic bacteria growing in glycerol-containing media can catalyze the synthesis of polyesters on the basis of hydroxy acids (hydroxy butanoic acid, hydroxy valeric acid etc.) [133, 134]. These biodegradable polyesters are a promising alternative to plastics produced from petrochemical raw materials [135]. Biocompatibility of these polymers with the human body tissues makes it possible to use them for the creation of bioartificial organs and tissues [136].

A recombinant strain of *Escherichia coli* arcA, synthesizing polyhydroxybutirate from glycerol under microaerophilic conditions, was obtained [137]. Polyhydroxybutirate (molecular weight of 7.9×10^5–9.6×10^5) was synthesized from biodiesel production waste containing glycerol without any preliminary cleaning (delipidation or demethanization) using bacteria of *Cupriavidus necator* DSM 545 [133].

However, usually shorter polymers are formed in glycerol-containing media as compared to glucose media, as glycerol can form covalent bonds with the terminal carboxyl group of the polyester and interrupt the synthesis of the poly-oxy-alkanoate chain [133]. In order to obtain more high-molecular polymers in a glycerol-containing medium it is necessary to perform an additional process, in the course of which condensation of polymers with low molecular weight takes place [133]. It is noted that the processing of poly (3-hydroxybutyrate/valerate) synthesized using a mixture of pseudomonades of *Pseudomonas oleovorans* and *P. Corrugate* resulted in the formation of a polymer with a higher molecular weight, close to polylactylate by properties [2].

Polyglutamic acid applied in various industrial sectors is produced extracellularly by individual strains of *Bacillus genus* bacteria, growing in a medium containing its precursors (amino acids and tricarboxylic acids). When glycerol is used as a co-substrate together with L-glutamic acid and citrate, the yield of polyglutamate is significantly increased due to more efficient secretion of the polymer from the cells of the bacteria. It is believed that the stimulating effect of glycerol it related to its influence on the synthesis of cell membrane phospholipids and fatty acids associated with them [138].

Another important direction for utilization of glycerol-containing waste is related to the synthesis of cheap polysaccharides, which could replace expensive xanthan in processes intensifying oil production under conditions of oil fields depletion. An ability to synthesize extracellular polysaccharides when growing on glycerol-containing waste from biodiesel production was demonstrated for *Enterobacter sp*. bacteria [139] and a bacterial strain of *Pseudomonas oleovorans* NRRL B-14682, forming high-molecular heteropolysaccharide (molecular weight of $1.0–5.0\times10^6$) characterized by good viscosity and pseudoplasticity [140, 141].

In connection with the development of nanotechnologies there is an increase in the demand for bacterial cellulose. Cellulose producers (*Acetobacter strains*) in glucose-containing media form gluconic acid as a by-product. This acid reduces the acidity of the medium and inhibits the synthesis of the polymer. Substitution of glycerol for glucose in the synthesis of bacterial cellulose using bacteria of *Acetobacter sp*. V6 results in a 3.8-fold increase in the yield of the polymer [132].

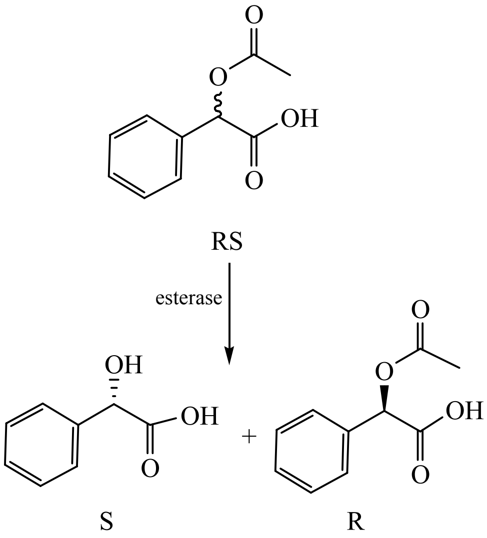
Production of Enzymes and Enzymatically Active Biomass on the Basis of Glycerol

One of the existing problems is the production of pharmaceutical analogues to natural compounds and the synthesis of environmentally friendly new-generation pesticides (controlling the behavior of insects) and plant growth stimulants. There are a lot of well-known examples of successful solutions to such problems involving the application of enzymes and cells of microorganisms in the processes of transformation of natural and synthetic compounds.

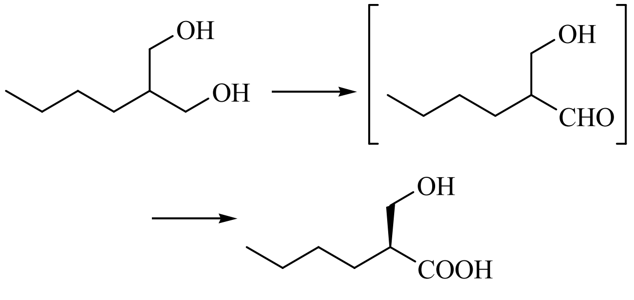
A series of published works speaks for advisability to use glycerol-containing media for obtainment of a number of enzymes using natural strains of microorganisms. Thus, in glycerol-containing media bacteria of *Gluconobacter oxydans* form membrane-bound glycerol dehydrogenase, which is capable of oxidizing sorbitol, gluconic acid, and arabitol [142]. Bacteria of *Staphylococcus caseolyticus* EX17 produce organic solvent tolerant lipase [143]. Glycerol-inducible lipases were found in thermophilic strains of Geobacillus stearothermophilus [144] and Bacillus sp. [145]. In the presence of the inducer (benzonitrile) bacteria of *Streptomyces sp.* MTCC 7546 synthesize nitrilase in a glycerol-containing medium [146]. The expression of 1,3-regiospecific lipase of *Rhizopus oryzae* in methylotrophic yeast of *Pichia pastoris* also significantly increases in case of glycerol additions into the culture medium [147]. Phytase was obtained in crude glycerol using recombinant yeast of *Pichia pastoris* [148]. Recombinant yeast of *Y. Lipolytica* can express alpha-amylase from rice if cultivated in a glycerol-containing medium [149].

The data regarding the application of purified enzymes obtained in glycerol-containing media in the course of organic synthesis are quite limited. However, broad prospects for the application of cell catalysts, cultivated in glycerol-containing media, in the synthesis of practically important compounds were already demonstrated.

Bacteria of *Pseudomonas sp.* ECU1011, the cells of which can perform enantioselective deacylation of (S)-α-acetoxy-phenylacetic acid, were found [150]. Through kinetic separation of racemic α-acetoxy-phenylacetic acid in the presence of cells grown in a glycerol-containing medium it is possible to obtain (S)-mandelic acid, which is a synthon of a number of substituted cyclopentanones and commercial pharmaceuticals, including celecoxib and deracoxib, non-steroidal anti-inflammatory drugs. The residual ester can be used for the production of (R)-mandelic acid, which is a precursor of semi-synthetic penicillin, cephalosporin, and anti-obesity drugs.

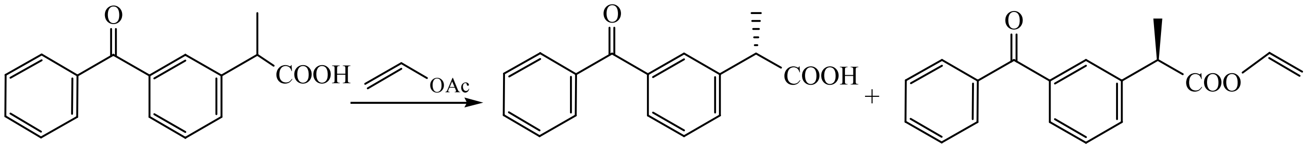


Bacterial biomass of *Acetobacter pasteurianus IAM 12073*, obtained in a medium containing glycerol and glucose, performs enantioselective oxidation of 2-butyl-1,3-propandiol into S-2-hydroxymethyl-hexanoic acid as follows [151]:



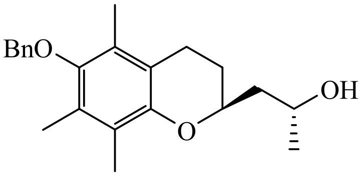
We discovered microorganisms forming intracellular enantioselective hydrolases (lipases/esterases) and oxidoreductases when cultivated in glycerol-containing media. These biocatalysts form the basis for the methods to obtain a number of optically active synthons of extremely important low-molecular bioregulators.

It was demonstrated that introduction of glycerol into a medium for cultivation of bacteria of *Bacillus sp.* K-3-2 significantly increased the activity of the acetone-dehydrated biomass in the process of enantioselective transesterification of ketoprofen with vinyl acetate in the isooctane-acetone system [124, 152]. This fact made it possible to create a chemo-enzymatic method for the production of (S)-ketoprofen, a non-steroidal anti-inflammatory drug, on the basis of affordable raw materials.



R, S-ketoprofen S-(+)-ketoprofen R-(+)-ketoprofen vinyl ester

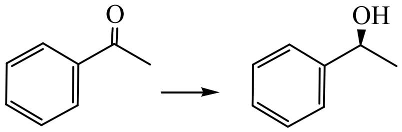
An increase in lipolytic activity in glycerol-containing media was also detected for bacteria of *Rhodococcus sp.* 77-32, which formed the basis for the development of a method to obtain chromanyl ethanol, a synthon of α-tocopherol and α-tocotrienol, which are natural cytoprotectors, as well as of MDL-73404, which is their synthetic analogue possessing anti-oxidant activity [124, 153].



S-(–)-chromanyl ethanol

The biocatalyst makes it possible to obtain the target product from racemic acetate of chromanyl ethanol with the yield of almost 100%.

The catalytic activity of the native biomass of *Geotrichum sp.* 85-1 fungi, cultivated in a glycerol-containing medium, forms the basis for the enantioselective method of acetophenone reduction to (S)-1-phenylethanol, which can be used for the production of liquid crystals and polymeric chromatographic phases, as well as for the separation of racemic mixtures [124, 154].



acetophenone (S)-1-phenylethanol

A promising direction for application of this biocatalyst adapted to transformation of acetophenone functional derivatives is the production of pharmaceuticals with antidiabetic, antidepressant, or antirabic effects.

The growth of *Geotrichum sp.* 85-1 fungi in glycerol-containing media is accompanied with the synthesis of products, capable of stabilizing emulsions of organic solvents (*n*-alkanes, isooctane, chloroform, and ethyl acetate) in water, which was used for intensification of the reduction process for a number of carbonyl-containing compounds [155].

Polytransformation of α-linolenic acid, forming part of linseed oil, into eicosapentaenoic acid under the effect of metabolic products of arachidonic acid was demonstrated using *Mortierella alpina* 1S-4 fungi growing in a glucose medium. We showed that a similar transformation took place in the presence of the biomass of *Mortierella alpina* 18-1 and the haloresistant mutant of the fungi (XH1), which entered the stationary growth phase under conditions of solid-phase cultivation in an oatmeal medium containing glycerol [157]. Moreover, the specified fungi can also convert other vegetable lipids (olive, sunflower, and soybean oils) into eicosapentaenoic acid despite the absence or low content of its precursor (α-linolenic acid). The total content of arachidonic and eicosapentaenoic acids in fungal lipids reaches 48–88% of the fatty acids total [124, 158, 159].

REFERENCES

1. Debabov, V.G., *Biotekhnologiya*, 2008, no. 1, p. 3.
2. Pletnev, M.Yu., *Biotekhnologiya*, 2009, no. 1, p. 3.
3. Soares, R.R., Simonetti, D.A., and Dumesic, J.A., *Angew. Chem. Int. Ed.*, 2006, vol. 45, p. 3982.
4. Cortright, R.D., Davda, R.R., and Dumesic, J.A., *Nature,* 2002, vol. 418, p. 964.
5. Pagliaro, M. and Rossi, M., *The Future of Glycerol: New Uses of a Versatile Raw Material*, Cambridge: RCS-Publishing, 2008.
6. Ito, T., Nakashimada, Y., Senba, K., Matsui, T., and Nishio, N., *J. Biosci. Bioeng.*, 2005, vol. 100, p. 260.
7. Gupta, A., Murarka, A., Campbell, P., and Gonzalez, R.,

Appl. Environ. Microbiol., 2009, vol. 75, p. 5871.

8. Yu, K.O., Kim, S.W., and Han, S.O., *Bioresource Technol*., 2010, vol. 101, p. 4157.
9. Yazdani, S.S. and Gonzalez, R., *Metab. Eng.,* 2008, vol. 10, p. 340.
10. Durnin, G., Clomburg, J., et al., *Biotechnol. and Bioeng*., 2009, vol. 103, p. 148.
11. Andrade, J.C. and Vasconcelos, I., *Biotechnol. Lett*., 2003, vol. 25, p. 121.
12. Biebl, H., *J. Ind. Microbiol. Biotechnol*., 2001, vol. 27, p. 18.
13. Ezeji, T., Milne, C., Price, N.D., and Blaschek, H.P., *Appl. Microbiol. Biotechnol*., 2010, vol. 85, p. 1697.
14. Yu Jiang et al., *Metabolic Engineering*, 2009, vol. 11, p. 284.
15. Ezeji, T.C., Qureshi, N., and Blaschek, H.P., *Curr. Opin. Biotechnol*., 2007, vol. 18, p. 220.
16. Papanikolaou, S. and Aggelis, G., *Lipid Technol*., 2009, vol. 21, p. 83.
17. Sergeeva, Ya.E., Galanina, L.A., Andrianova, D.A., and Feofilova, E.P., *Prikl. Biokhimiya i Mikrobiologiya*, 2008, vol. 44, no. 5, p. 576.
18. Feofilova, E.P., *Biol. Nauki*, 1991, no. 1, p. 5.
19. Li, Q., Du, W., and Liu, D.H., *Appl. Microbiol. Biotechnol*., 2008, vol. 80, p. 749.
20. Fakas, S., Papanikolaou, S., et al., *Biomass and Bioenergy*, 2009, vol. 33, p. 573.
21. Papanikolou, S. et al., *Ibid*., 2008, vol. 32, p. 60.
22. Meesters, P.A.E.P., Huijberts, G.N.M., and Eggink, G., *Appl. Microbiol. Biotechnol*., 1996, vol. 45, p. 575.
23. Easterling, E.R., Todd French, W., Hernandez, R., and Licha, M., *Bioresource Technol*., 2009, vol. 100, p. 356.
24. Narayan, M.S., et al., *Int. J. Food Sci. Nutr*., 2005, vol. 56, p. 521.
25. Karinen, R.S. and Krause, A.O.I., *Appl. Catal. A: General*, 2006, vol. 306, p. 128.
26. Melero, J.A. et al., *Ibid*., 2008, vol. 346, p. 44.
27. Rahmat, N. et al., *Renewable and Sustainable Energy Reviews*, 2010, vol. 14, p. 987.
28. Melero, J.A., van Grieken, R., Morales, G., and Paniagua, M., *Energy Fuels*, 2007, vol. 21, p. 1782.
29. Liao, X., Zhu, Y., Wang, S.G., and Li, Y., *Fuel Processing Technology*, 2009, vol. 90, p. 988.
30. Goncalves, V.L.C, Pinto, B.P., Silva, J.C., and Mota, C.J.A., *Catal. Today*, 2008, vols. 133–135, p. 673.
31. Davda, R.R. et al., *Appl. Catal. B: Environ*., 2005, vol. 56, p. 171.
32. Wen, G., Xu, Y., Ma, H., Xu, Z., and Tian, Z., *Int. J. Hydrogen Energy*, 2008, vol. 33, p. 6657.
33. Buffoni, I.N., Pompeo, F., Santori, G.F., and Nichio, N.N., *Catal. Commun*., 2009, vol. 10, p. 1656.
34. Dou, B., Rickett, G.L., Dupont, V., Williams, P.T., Chen, H., Ding, Y., and Ghadiri, M., *Bioresource Technol*., 2010, vol. 101, p. 2436.
35. Dauenhauer, P.J., Salge, J.R., and Schmidt, L.D., *J. Catal*., 2006, vol. 244, p. 238.
36. Byrd, A.J., Pant, K.K., and Gupta, R.B., *Fuel,* 2008, vol. 87, p. 2956.
37. Iriondo, A., Barrio, V.L., Cambra, J.F., Arias, P.L., Guemez, M.B., et al., *Top. Catal*., 2008, vol. 49, p. 46.
38. Zhang, B., Tang, X., Li, Y., Xu, Y., and Shen, W., *Int. J. Hydrogen Energy*, 2007, vol. 32, p. 2367.
39. Sanchez, E.A., D`Angelo, M.A., and Comelli, R.A., *Ibid*., 2010, doi:10.1016/j.ijhydene.2009.12.115.
40. Kapdan, I. K. and Kargi, F., *Enzyme Microbiol. Technol*., 2006, vol. 38, p. 569.
41. Seifert, K., Waligorska, M., Wojtowski, M., and Laniecki, M., *Int. J. Hydrogen Energy*, 2009, vol. 34, p. 361.
42. Selembo, P.A., Perez, J.M., Lloyd, W.A., and Logan, B.E., *Ibid*., 2009, vol. 34, p. 5373.
43. Akutsua, Y., Lee, D.Y., Li, Y.Y., and Noike, T., *Ibid*., 2009, vol. 34, p. 5365.
44. Hatayama, K. and Yagishita, T., *Appl. Microbiol. Biotechnol*., 2009, vol. 83, p. 749.
45. Sabourin-Provost, G. and Hallenbeck, P.C., *Bioresource Technol*., 2009, vol. 100, p. 3513.
46. US Patent no. 5387720, 1995.
47. Tsukuda, E., Sato, S., Takahashi, R., and Sodesawa, T., *Catal. Commun*., 2007, vol. 8, p. 1349.
48. Wang, F., Dubois, J.L., and Ueda, W., *Appl. Catal. A: General*, 2010, vol. 376, p. 25.
49. Watanabe, M., Iida, T., Aizawa, Y., Aida, T.M., and Inomata, H., *Bioresource Technol*., 2007, vol. 98, p. 1285.
50. Shelley, S., *Chem. Eng. Prog*., 2007, vol. 103, p. 6.
51. Dasari, M., Kiatsimkul, P., Sutterlin, W., and Suppes, G., *Appl. Catal. A: General*, 2005, vol. 281, p. 225.
52. WO no. 2007010299, 2007.
53. Chaminand, J., Djakovitch, L., Gallezot, P., Marion, P., Pinel, C., and Rosier, C., *Green Chem*., 2004, vol. 6, p. 359.
54. Miyazawa, T., Kusunoki, Y., Kunimori, K., and Tomishige, K., *J. Catal*., 2006, vol. 240, p. 213.
55. Furikado, I., Miyazawa, T., Koso, S., Shimao, A., Kunimori, K., and Tomishige, K., *Green Chem*., 2007, vol. 9, p. 582.
56. Stinson, S.C., *Chem. Eng. News*, 1995, vol. 73, p. 10.
57. Moon, C., Ahn, J.H., Kim, S.W., Sang, B.I., and Um, Y., *Appl. Biochem. Biotechnol*., 2010, vol. 161, p. 502.
58. Raj, S.M., Rathnasingh, C., Jung, W.C., and Park, S., *Appl. Microbiol. Biotechnol*., 2009, vol. 84, p. 649.
59. US Patent no. 4620912, 1986.
60. Spadlo, M., Dziwinski, E., and Wasilewski, J., *Przem. Chem*., 1994, vol. 73, p. 306.
61. WO no. 2006111810, 2006.
62. Petukhova, N.I., Khalimova, L.Kh., Mitrofanov, D.V., Gareev, V.M., and Zorin, V.V., *Bashk. Khim. Zh*., 2000, vol. 7, no. 5, p. 43.
63. Stevenson, D.E., Stanley, R.A., and Furneaux, R.H., *Biotechnol. Lett*., 1993, vol. 15, p. 1043.
64. Bornscheuer, U.T., *Enzyme Microbiol. Technol*., 1995, vol. 17, p. 578.
65. Jackson, M.A. and King, J.W., *J. Am. Oil Chem. Soc*., 1997, vol. 74, p. 103.
66. Sonntag, N.O.V., *Ibid*., 1992, vol. 59, p. 795.
67. Jerome, F., Kharchafi, G., Adam, I., and Barrault, J., *Green Chem*., 2004, vol. 6, p. 72.

68. Ghamgui, H., Miled, et al., *Enzyme Microbiol. Technol.*, 2006, vol. 39, p. 717.
69. Martinez, M., Coteron, A., and Aracil, J., *J. Am. Oil Chem. Soc.*, 1998, vol. 75, p. 657.
70. Medina, A.R., Cerdarn, L.E., Gimernez, A.G, Parez, B.C., Gonzarlez, M.J.I., and Grim, E.M., *J. Biotechnol.*, 1999, vol. 70, p. 379.
71. Sun, S., Shan, L., Liu, Y., Jin, Q., Wang, X., and Wang, Z., *Biotechnol. Lett.*, 2007, vol. 29, p. 1947.
72. Ceni, G., Lerin, L.A., et al., *Enzyme Microbiol. Technol.*, 2010, vol. 46, p. 107.
73. Bher, A., Eilting, J., Irawadi, K., Leschinski, J., and Lindner, F., *Green Chem.*, 2008, vol. 10, p. 13.
74. Simao, A.C., Pukleviciene, B.L., et al., *Lett. Org. Chem.*, 2006, vol. 3, p. 744.
75. Clements, J.H., *Ind. Eng. Chem. Res.*, 2003, vol. 42, p. 663.
76. Climent, M.J., Corma, A., De Frutos, P., Iborra, S., Noy, M., Velty, A., and Concepcion, P., *J. Catal.*, 2010, vol. 269, p. 140.
77. McKetta, J.J. and Cunningham, W.A., *Encyclopedia of Chemical Processing and Design*, vol. 20, New York: Marcel Decker, 1984, p. 177.
78. Vieville, C., Yoo, J.W., Pelet, S., and Mouloungui, Z., *Catal. Lett.*, 1988, vol. 56, p. 245.
79. Patel, Y., George, J., Pillai, S.M., and Munshi, P., *Green Chem.*, 2009, vol. 11, p. 1056.
80. Li, Q., Zhang, W., Zhao, N., Wei, W., and Sun, Y., *Catal. Today.*, 2006, vol. 115, p. 111.
81. Japan Patent no. 2007039347, 2007.
82. Japan Patent no. 2008285457 A, 2008.
83. Aresta, M., Dibenedetto, A., Nocito, F., and Ferragina, C., *J. Catal.*, 2009, vol. 268, p. 106.
84. George, J., Patel, Y., Pillai, S.M., and Munshi, P., *J. Mol. Catal. A.: Chemical*, 2009, vol. 304, p. 1.
85. US Patent no. 2004138481, 2004.
86. Corma, A., Iborra, S., and Velty, A., *Chem. Rev.*, 2007, vol. 107, p. 2411.
87. Kimura, H., *Appl. Catal. A*, 1993, vol. 105, p. 147.
88. Demirel, S., Lehnert, K., Lucas, M., and Claus, P., *Appl. Catal. B.*, 2007, vol. 70, p. 637.
89. Fordham, P., Besson, M., and Gallezot, P., *Catal. Lett.*, 1997, vol. 46, p. 195.
90. Kimura, H., *Polym. Adv. Technol.*, 2001, vol. 12, p. 697.
91. Habe, H., Fukuoka, T., Kitamoto, D., and Sakaki, K., *Appl. Microbiol. Biotechnol.*, 2009, vol. 84, p. 445.
92. Gatgens, C., Degner, U., Bringer-Meyer, S., and Herrmann, U., *Ibid.*, 2007, vol. 76, p. 553.
93. Ma, L., Lu, W., Xia, Z., and Wen, J., *Biochem. Eng. J.*, 2010, vol. 49, p. 61.
94. Raghava, S. and Gupta, M.N., *Bioresource Technol.*, 2010, vol. 101, p. 2554.
95. Habe, H., Fukuoka, T., Kitamoto, D., and Sakaki, K., *Appl. Microbiol. Biotechnol.*, 2009, vol. 81, p. 1033.
96. Makri, A., Fakas, S., and Aggelis, G., *Bioresource Technol.*, 2010, vol. 101, p. 2351.
97. Rymowicz, W. et al., *Appl. Microbiol. Biotechnol.*, DOI 10.1007/s00253-010-2561-z.
98. Morgunov, I.G., Kamzolova, S.V., Perevoznikova, O.A., Shishkanova, N.V., and Finogenova, T.V., *Process Biochem.*, 2004, vol. 391, p. 469.
99. Andre, A., Diamantopoulou, P., Philippoussis, A., Sarris, D., Komaitis, M., and Papanikolaou, S., *Industrial Crops and Products*, 2010, vol. 31, p. 407.
100. Rymowicz, W., Rywinska, A., and Marcinkiewicz, M., *Biotechnol. Lett.*, 2009, vol. 31, p. 377.
101. Khan, A., Bhide, A., and Gadre, R., *Bioresource Technol.*, 2009, vol. 100, p. 4911.
102. Scholten, E., Renz, T., and Thomas, J., *Biotechnol. Lett.*, 2009, vol. 31, p. 1947.
103. Lee, P.C., Lee, W.G., Lee, S.Y., and Chang, H.N., *Biotechnol. and Bioeng.*, 2001, vol. 72, p. 41.
104. Zhang, A. and Yang, S.T., *Process Biochem.*, 2009, vol. 44, p. 1346.
105. Petrov, K. and Petrova, P., *Appl. Microbiol. Biotechnol.*, 2009, vol. 84, p. 659.
106. Gonzalez, R., Murarka, A., Dhermadi, Y., and Yazdani, S., *Metab. Eng.,* 2008, vol. 10, p. 234.
107. Khamduang, M., Packdibamrung, K., Chutmanop, J., Chisti, Y., and Srinophakun, P., *J. Ind. Microbiol. Biotechnol.*, 2009, vol. 36, p. 1267.
108. Rittmann, D., Lindner, S.N., and Wendisch, V.F., *Appl. Environ. Microbiol.*, 2008, vol. 74, p. 6216.
109. Rakhmatullina, Yu.R., Petukhova, N.I., Avdeeva, E.N., and Zorin, V.V., *Bashk. Khim. Zh.*, 2005, vol. 12, no. 1, p. 49.
110. Petukhova, N.I., Rakhmatullina, Yu.R., Yakhutova, Ya.R., Spirikhin, L.V., and Zorin, V.V., *Bashk. Khim. Zh.*, 2006, vol. 13, no. 1, p. 95.
111. Petukhova, N.I., Rakhmatullina, Yu.R., Panteleeva, S.N., and Zorin, V.V., *Bashk. Khim. Zh.*, 2007, vol. 14, no. 1, p. 141.
112. Hou, C.T., *J. Ind. Microbiol. Biotechnol.*, 2008, vol. 35, p. 501.
113. Ceron Garcıa, M.C., Fernandez Sevilla, J.M., Acien Fernandez, F.G., Molina Grima, E., and Garcıa Camacho, F., *J. Appl. Phycol.*, 2000, vol. 12, p. 239.
114. Chi, Z., Pyle, D., Wen, Z., Frear, C., and Chen, S., *Process Biochem.*, 2007, vol. 42, p. 1537.
115. Athalye, S.K., Garcia, R.A., and Wen, Z., *J. Agric. Food Chem.*, 2009, vol. 57, p. 2739.
116. Mantzouridou, F., Naziri, E., and Tsimidou, M.Z., *Ibid.*, 2008, vol. 56, p. 2668.
117. Kusdiyantini, E., Gaudin, P., Goma, G., and Blanc, P.J., *Biotechnol. Lett.*, 1998, vol. 20, p. 929.
118. Tao, J., Wang, X., Shen, Y., and Wei, D., *World J. Microbiol. Biotechnol.*, 2005, vol. 21, p. 969.
119. Komleva, E.V., Petukhova, N.I., Badretdinov, A.D., Sultanova, L.M., and Zorin, V.V., *Bashk. Khim. Zh.*, 2007, vol. 14, no. 1, p. 159.
120. Komleva, E.V., Petukhova, N.I., Khuzina, A.V., Faizrakhmanova, L.S., Muslukhov, R.R., and Zorin, V.V., *Bashk. Khim. Zh.*, 2009, no. 1, p. 106.
121. Willams, R.P., *Appl. Microbiol.*, 1973, vol. 25, p. 396.
122. Petukhova, N.I., Komleva, E.V., Faizrakhmanova, L.S.,

Shakirov, A.N., and Zorin, V.V., Abstracts of Papers, *VII Russ. Conf. on Chemistry and Medicine: ORKHIMED-2009*, Ufa, 1–5 July 2009, p. 244.

123. Komleva, E.V., Sharaeva, A.A., Petukhova, N.I., and Zorin, V.V., *Bashk. Khim. Zh.*, 2009, vol. 16, no. 4, p. 65.
124. Zorin, V.V., Petukhova, N.I., Prishchepov, F.A., Vershinin, S.S., Khalimova, L.Kh., and Sultanova, L.M., *Neftegazovoe Delo*, 2008, vol. 6, no. 2, p. 245.
125. US Patent no. 20050239694, 2005.
126. Van Bogaert, I.N.A., Saerens, K., De Muynck, C., Develter, D., Soetaert, W., and Vandamme, E.J., *Appl. Microbiol. Biotechnol.*, 2007, vol. 76, p. 23.
127. Arutchelvi, J.I., Bhaduri, S., Uppara, P.V., and Doble, M., *J. Ind. Microbiol. Biotechnol.*, 2008, vol. 35, p. 1559.
128. Maier, R.M. and Soberon-Chavez, *Appl. Microbiol. Biotechnol.*, 2000, vol. 54, p. 625.

128a. Ashby, R.D., Nunez, A., Solaiman, D.K.Y., and Foglia, T.A., *JAOCS*, 2005, vol. 82, no. 9, p. 625.

129. Pachauri, N. and He, B., *American Society of Agricultural and Biological Engineers*, St. Joseph, MI, 2006, Pap. no. 066223.
130. Iglesias, L.E., Fukuyama, Y., Nonami, H., Erra-Balsells, R., and Baldessari, A., *Biotechnol. Techniques*, 1999, vol. 13, p. 923.
131. Cavalheiro, J.M.B.T., de Almeida, M.C.M.D., Grandfils, C., and da Fonseca, M.M.R., *Process Biochem.*, 2009, vol. 44, p. 509.
132. Jung, H.I., Jeong, J.H., Lee, O.M., Park, G.T., Kim, K.K., Park, H.C., Lee, S.M., Kim, Y.G., and Son, H.J., *Bioresourse Technol.*, 2010, vol. 101, p. 3602.
133. Koller, M., Atlić, A., Dias, M., Reiterer, A., and Braunegg, G., *Microbiology Monographs*, Chen, G.-Q., Ed., Berlin-Heidelberg: Springer-Verlag, 2010, vol. 14, p. 85.
134. Ibrahim, M.H.A. and Steinbuchel, A., *Appl. Environ. Microbiol.*, 2009, vol. 75, p. 6222.
135. Olivera, E.R., Arcos, M., Naharro, G., and Luengo, J.M., *Microbiology Monographs*, Chen, G.-Q., Ed., Berlin-Heidelberg: Springer-Verlag, 2010, vol. 14, p. 133.
136. Shishatskaya, E.I., *Doctoral Sci. (Biol.) Dissertation*, Krasnoyarsk, 2009.
137. Nikel, P.I., Pettinari, M.J., Galvagno, M.A., and Mendez, B.S., *Appl. Microbiol. Biotechnol.*, 2008, vol. 77, p. 1337.
138. Dua, G., Yanga, G., Qub, Y., Chena, J., and Lun, S., *Process Biochem.*, 2005, vol. 40, p. 2143.
139. Alves, V.D., Freitas, F., Torres, C.A.V., Cruz, M., Marques, R., Grandfils, C., Goncalves, M.P., Oliveira, R., and Reis, M.A.M., *Carbohydrate Polymers*, 2010, vol. 80, p. 325.
140. Alves, V.D., Freitas, F., Costa, N., Carvalheira, M., Oliveira, R., Goncalves, M.P., and Reis, M.A.M., *Ibid.*, 2010, vol. 79, p. 981.
141. Freitas, F., Alves, V.D., Carvalheira, M., Costa, N., Oliveira, R., and Reis, M.A.M., *Ibid.*, 2009, vol. 78, p. 549.
142. Gatgens, C., Degner, U., Bringer-Meyer, S., and Herrmann, U., *Appl. Microbiol. Biotechnol.*, 2007, vol. 76, p. 553.
143. Volpato, G., Rodrigues, R.C., Heck, J.X., and Ayub, M.A.Z., *J. Chem. Technol. Biotechnol.*, 2008, vol. 83, p. 821.
144. Bereka, M.M., Zaghloul, T.I., Abdel-Fattah, Y.R., Saeed, H.M., and Sifour, M., *World J. Microbiol. Biotechnol.*, 2009, vol. 25, p. 287.
145. Gupta, N., Mehra, G., and Gupta, R., *Can. J. Microbiol.*, 2004, vol. 50, p. 361.
146. Nigam, V.K., Khandelwal, A.K., Gothwal, R.K., Mohan, M.K., Choudhury, B., Vidyarthi, A.S., and Ghosh, P., *J. Biosci.*, 2009, vol. 34, p. 21.
147. Minning, S., Serrano, A., Ferrer, P., Sola, C., Schmid, R.D., and Valero, F., *J. Biotechnol.*, 2001, vol. 86, p. 59.
148. Tang, S., Boehme, L., Lam, H., and Zhang, Z., *Biochem. Eng. J.*, 2009, vol. 43, p. 157.
149. Kim, J.W., Park, T.J., Ryu, D.D.Y., and Kim, J.Y., *Biotechnol. Prog.*, 2000, vol. 16, p. 657.
150. Ju, X., Yu, H.L., Pan, J., Wei, D.Z., and Xu, J.H., *Appl. Microbiol. Biotechnol.*, 2010, vol. 86, p. 83.
151. Mitsukura, K., Uno, T., Yoshida, T., and Nagasawa, T., *Ibid.*, 2007, vol. 76, p. 61.
152. Kon'shina, I.I., Vershinin, S.S., Petukhova, N.I., Kotlov, V.M., and Zorin, V.V., Abstracts of Papers, *VIII Vseros. konf. s mezhd. uchastiem po khimii i meditsine* (VIII Russian Conf. with Intern. Participation on Chemistry and Medicine), Ufa, 6–8 April, 2010, p. 140.
153. Khabibullina, I.I., Petukhova, N.I., Spivak, A.Yu., Odinokov, V.N., and Zorin, V.V., Abstracts of Papers, *VI Vseros. nauch. sem. po khimii i meditsine* (VI Russian Scientific Seminar on Chemistry and Medicine), Ufa, 26–29 November, 2007.
154. Kalimullina, L.Ya., Petukhova, N.I., and Zorin, V.V., *Bashk. Khim. Zh.*, 2008, no. 1, p. 8.
155. Kalimullina, L.Ya., Mukhitdinov, E.R., Petukhova, N.I., and Zorin, V.V., *Bashk. Khim. Zh.*, 2007, vol. 14, no. 1, p. 151.
156. Shimizu, S., Kawashima, H., Akimoto, K., Shinmen, Y., and Yamada, H., *Appl. Microbiol. Biotechnol.*, 1989, vol. 32, p. 1.
157. Petukhova, N.I., Kalimullina, L.Ya., Syundyukova, Yu.R., and Zorin, V.V., *Bashk. Khim. Zh.*, 2008, no. 1, p. 19.
158. Kalimullina, L.Ya., *Cand. Sci. (Eng.) Dissertation*, Kazan', 2008.
159. Syundyukova, Yu.R. et al., Abstracts of Papers, *VI Russ. Sci. Seminar on Chemistry and Medicine,* Ufa, 26–29 November, 2007.