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TECHNOLOGIES OF SYNTHESIS OF ORGANIC SUBSTANCES BY MICROORGANISMS USING WASTE BIODIESEL PRODUCTION

T. P. PIROG, N. A. GRYTSENKO, A. P. SOFILKANYCH, I. V. SAVENKO

National University of Food Technologies, Kyiv, Ukraine

E-mail: tapirog@nuft.edu.ua

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We describe here literature and our experimental data concerning microbial synthesis using waste biodiesel production, mono- and dihydric alcohols (1,3-propanediol, 2,3-butanediol, butanol, ethanol), polyols (mannitol, erythritol, arabitol), organic acids (citric, succinic, lactic, glyceric), polymers and compounds with a complex structure (polysaccharides, polyhydroxyalkanoates, surfactants, cephalosporin, cyanocobalamin). In some mentioned cases recombinant producer strains were used.

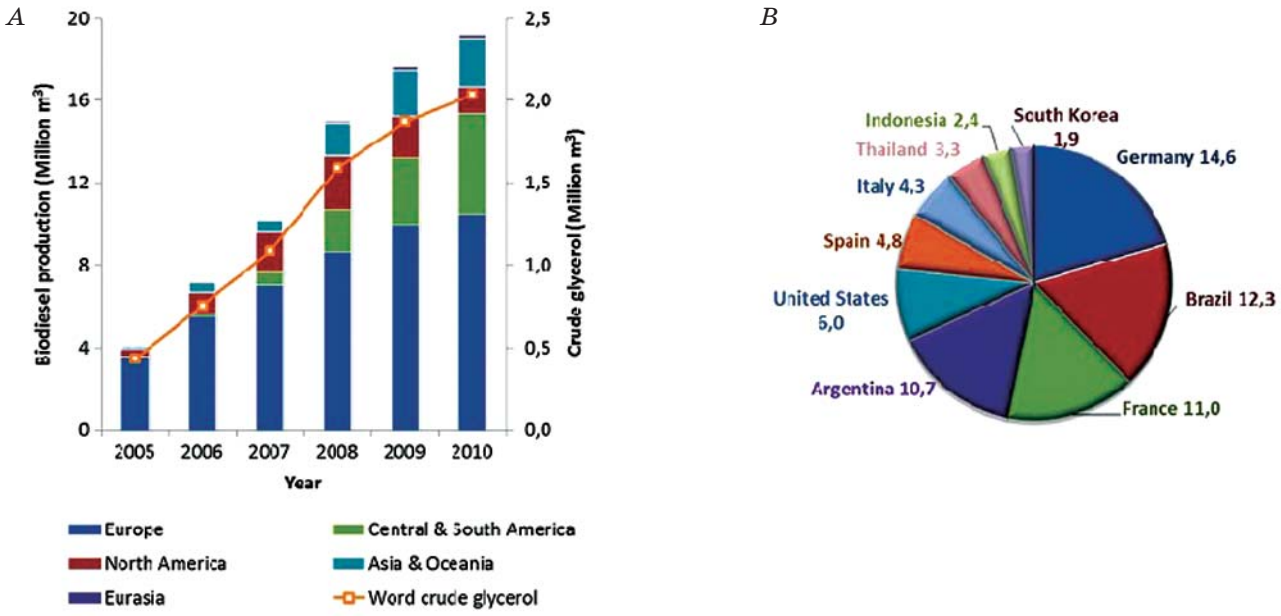
It was shown that due to the presence of potential inhibitors in the composition of technical (crude) glycerol (methanol, sodium and potassium salts), the efficiency of synthesis of most microbial products on such a substrate is lower than on the purified glycerol. However, the need of utilization of this toxic waste (storage and processing of crude glycerol is a serious environmental problem due to the high alkalinity and the content of methanol in it), compensates the lower rates of synthesis of the final product. Furthermore, currently considering the volumes of crude glycerol formed during the production of biodiesel, microbial technologies are preferred for its utilization, allowing realizing biosynthesis of practically valuable metabolites in the environment with the highest possible concentration of this waste.

Using of crude glycerol as a substrate will reduce the cost of products of microbial synthesis and increase the profitability of biodiesel production.

Key words: biodiesel, technical (crude) glycerol, products of microbial synthesis.

The obtaining of biodiesel from vegetable oil or animal fats by transesterification with alcohols (ethanol, methanol) using NaOH (KOH) as a catalyst is accompanied with accumulation of a byproduct such as crude glycerol. Ten liters of waste is formed per 100 liters of biodiesel. The promising way of utilization of the excess glycerol is its using in biotechnological industry as a substrate for cultivation of microorganisms — producers of practically valuable substances. Wide using of fossil fuels led to greenhouse gas emission that brings irreparable harm to the environment. Instability of oil supplies and continual price fluctuation are the reason for the great interest in alternative sources of energy. The mentioned factors, concerning economical, ecological and geopolitical problems, are important to solve the essential problem of recovered energy sources [1]. The biotechnological direction, so-called "white" biotechnology includes bioproduction of fuel and chemical compounds from the reduced sources. Technology based on living cells and enzymes needs less energy. The products of such biotechnology are biodegradable and it was noted the less waste during their production or application as compared with the use of fossil resources [1, 2].

Biofuels, in particular, ethanol and biodiesel are prospective substitutes of fossil fuels. Biodiesel (biodiesel fuel, bio-oil, etc.) is ecologically pure biofuel. It can be obtained from vegetable oil or animal fats and is used to substitute petroleum diesel. The most popular way to obtain biodiesel is transesterification of vegetable oil that can be considered as a mixture of methyl (ethyl) monoalkyl esters of fat acids with a long chain (saturated or unsaturated). Vegetable oil is transesterified with methanol (sometimes with ethanol or isopropanol, one ton of oil per 200 kg of methanol) at the 60 °C and normal pressure for the period from 1 to 8 hours. Sodium or potassium hydroxide can be used as a catalyst [3]. The volumes of biodiesel production have significantly increased in the last decade. It was obtained 4 mln cubic meters of this fuel in 2005 and approximately twenty mln cubic meters — in 2010 (Figure) [4].



Production of biodiesel and crude glycerol which is formed as a byproduct:
A — world production in 2005–2010;
B — production in ten countries which are the most important biodiesel producers (mln m^3) in 2010

As it was noted [5] the annual increase of biodiesel production will be from 8 to 10%, its production will have expected to 37 billion gallons (around 140 million tons) by 2016 [6]. Europe has been the most important producer of this biological fuel. It was obtained 111 mln gallons of biodiesel in European Union countries in May 2013 [7].

However, due to the increased demand for biodiesel, there is a problem concerning by-product (glycerol) utilization. Approximately 10 liters of crude glycerol (so called glycerol fraction) are formed per 100 liters of biodiesel [3, 4]. Glycerol fraction contains the great amount of different impurities that makes impossible its using in traditional fields of glycerol application (production of food, pharmaceutical industry and cosmetology) without expensive purification technologies [3]. It has to be noted that storage and disposal of crude glycerol is a serious ecological problem due to the high alkalinity and the presence of methanol.

Accumulation of waste products of biodiesel production led to serious changes in many industrial fields. For instance, the company Procter and Gamble, as many other cosmetic firms, stopped to produce its own glycerol [3, 4, 8]. The price for pure glycerol decreased in the USA from 0.7 to 0.3 USD per lb. The price for crude glycerol respectively decreased from 0.25 to 0.05 USD per lb [6].

So, to increase economic feasibility and profitability of biodiesel production, it is necessary to develop new ways for utilization of this waste product. Incineration, composting, thermochemical conversion and bioconversion are the possible variants to solve the problem [9].

Glycerol is a simple trivalent alcohol (1,2,3-propanetriol), which is assimilated by many microorganisms. However, for many investigations researchers use high-quality purified glycerol as a substrate, and for obtaining of some products the using of crude glycerol is unprofitable [9].

Now it is considered the possibility of bioconversion of crude glycerol by yeast, filamentous fungi and some bacteria such as *Enterobacteriaceae* and *Clostridiaceae* (the genera of *Klebsiella*, *Enterobacter* and *Clostridium*) into different alcohols (1,3-propanediol; 2,3-butanediol; butanol, ethanol), organic acids and other valuable compounds.

Mono-, dihydric alcohols and polyols

1,3-Propanediol. The main way to obtain 1,3-propanediol is its chemical synthesis. However, there are some disadvantages of this way: formation of toxic intermediates, high expences for production and its dependence on the raw materials, which are obtained from fossil resources. The alternative of chemical

way is the microbiological one when the crude glycerol is converted into 1,3-propanediol [4].

Producers of 1,3-propanediol are *Lactobacillus* sp., *Citrobacter freundii* and genetically modified strains of *Escherichia coli*, *Saccharomyces cerevisiae* and *Pichia pastoris*. However, the most studied process is the obtaining of this alcohol during cultivation of *Klebsiella* spp. and *Clostridium* spp. on the crude glycerol under anaerobic conditions [10–15]. It was investigated the ability of *Clostridium butyricum* AKR102a to synthesize 1,3-propanediol during the cultivation on the pure and crude glycerol [10]. Accumulation of this alcohol by producers depended on the quality of crude glycerol because unsaturated fatty acid, chlorides, sodium ions and ions of heavy metals are the inhibitors of microorganism growth. That is why it was performed the preliminary treatment of crude glycerol (removing of fatty acids by decantation with the following treatment by hydrotalcite) and modification of nutrient medium (replacing ammonium chloride on ammonium sulphate, and sodium hydroxide on ammonium hydroxide).

In the first stage to optimize the cultivation process the strain AKR102a was grown for 32 hours in the fermenter (volume 1 liter) on the medium with yeast autolysate and pretreated crude glycerol (the substrate was added in portions for each 5 hours till the achievement of the final concentration 25 g/l). The maximal concentration of 1,3-propanediol reached 76.2 g/l and the process efficiency was 2.3 (g/l) h^{-1}, that was 1.2 times less than in case of the purified substrate [10]. During the cultivation of *C. butyricum* AKR102a in the fermenter (volume 200 liters) the concentration of added substrate was decreased till 20 g/l and the amount of synthesized 1,3-propanediol under these conditions was 61.5 g/l on the 30^{th} hour of cultivation with the total process efficiency 2.1 (g/l) h^{-1} [10].

González-Pajuelo et al. [11] investigated the influence of different concentrations of crude and pure glycerol on the accumulation of 1,3-propanediol by the strain *C. butyricum* VPI 3266.

It was established that maximal concentration of the final product was 29.7 g/l (efficiency 2.98 (g/l) h^{-1}) when the strain VPI 3266 was cultivated on the purified substrate (58 g/l). In case of using of crude glycerol (62 g/l) the amount of synthesized 1,3-propanediol achieved 31.5 g/l (efficiency 3.15 (g/l) h^{-1}) [11].

Other researchers [12] investigated the ability of *C. butyricum* DSP 1 to synthesize 1,3-propanediol using crude glycerol in the fermenters of different volumes (6.6; 42 and 150 l). The highest alcohol concentration (71 g/l) was observed in case of continuous cultivation in the fermenter with the volume 6.6 l. In case of batch cultivation the product concentration was 37 g/l regardless of the volume of the fermenter [12]. When different concentrations of crude glycerol (20–140 g/l) were added into the medium of cultivation of the strain DSP 1, the maximal concentration of 1,3-propanediol was 32.54 g/l at the substrate concentration 60–80 g/l and efficiency 1.28 (g/l) h^{-1} [13].

It is known that bacteria *Klebsiella* are also active producers of 1,3-propanediol [14, 15]. As it was shown in [14] *Klebsiella pneumoniae* DSM 4799 were able to use crude glycerol and produce 1,3-propanediol. It was found that cultivation of the strain DSM 4799 on this substrate provided concentration of the final product 80 g/l that was 1.8 times higher as compared with the purified glycerol. It was investigated the influence of different concentrations of crude glycerol on the synthesis of 1,3-propanediol by immobilized cells of *Klebsiella* sp. HE-2 [15]. It was established that strain HE-2 is resistant towards high concentrations of the substrate (till 100 g/l). The active synthesis of 1,3 propanediol was observed in case of addition of crude glycerol (5–50 g/l). The maximal concentration (8.8 g/l) of the product was achieved when the strain was cultivated on the medium with the substrate concentration 30 g/l. Under these conditions of HE-2 growing the efficiency of the process was 0.42 (g/l) h^{-1} [15].

According to the ability of some producers to synthesize 1,3-propanediol during their growing on the crude and purified glycerol [16] it was found that in most cases the synthesis was more efficient on the medium with the purified substrate (Table 1).

We should notice that some producers of 1,3-propanediol have some disadvantages which significantly decrease the competitiveness of microbial technologies compared with the chemical synthesis: need for vitamin B_{12} (cofactor of glycerol dehydrogenase); some strains are pathogenic and byproducts could be toxic and suppress the synthesis of the final product [17, 18]. That is why the using of genetically modified microorganisms is a good alternative, which can minimize abovementioned disadvantages. As it was described in [17] the recombinant strain of *E. coli* was obtained by transferring genes of *C. butyricum*

2CR371.5 which encode the synthesis of dihydroxyacetone kinase. It was shown that in case of using of crude glycerol (10 g/l) as the source of carbon and energy by the genetically engineered strain of *E. coli* BL21 the amount of synthesized 1,3-propanediol was increased till 3.7 g/l [17]. Using mathematical methods of planning of the experiments Rujananon et al. [18] showed that maximal amount of the synthesized 1,3-propanediol (2.43 g/l) was observed under cultivation of recombinant strain of *E. coli* BP41Y3 on the medium with the purified glycerol containing 63.65 mM fumarate, 3.80 g/l $(NH_4)_2HPO_4$ and 1.12 g/l peptone. At the same time the using crude glycerol as a source of carbon and energy led to the decrease of 1,3-propanediol of 32%. It can be due to the presence of impurities that are toxic for bacteria. Besides, it was noticed that the significant amount (11.92 g/l) of succinic acid (byproduct) was formed during cultivation on the crude glycerol [18].

Portuguese scientists modified the strain *Clostridium acetobutyricum* DG1 by transferring the genes from *C. butyricum* which are responsible for 1, 3-propanediol synthesis [19]. The further investigations showed that *C. acetobutyricum* DG1 (pSPD5) synthesized 1,104 mM of 1,3-propanediol in case of using of crude glycerol at concentration 1,792 mM.

There are reports of *Citrobacter freundii* ATCC 8090, which synthesizes 1,3-propanediol under conditions of growing on the crude glycerol [20]. Thus, from 20 g/l of substrate strain ATCC 8090 synthesized the 4.85 g/l of the target product.

Along with 1,3-propanediol it was formed 9.72 g/l of acetic acid and small amount of succinic acid. Other authors [21] present data on synthesis of 1,3-propanediol by *Lactobacillus diolivorans* using the crude glycerol that was a byproduct of biodiesel production from palm oil. Studies showed that *L. diolivorans* synthesized 85 g/l of 1,3-propanediol, the efficiency of the process was 0.45 $(g/l)\cdot h^{-1}$.

2,3-Butanediol is used to produce plastics, antifreeze and solvents, as well as an additive to fuel [4]. The strains of *Klebsiella* spp., *Aerobacter aerogenes*, *Bacillus polymyxa* and some genera of *Lactococcus* and *Clostridium* can be its producers. It was established that *K. pneumoniae* SU6 synthesizes 2,3-butanediol using crude glycerol as a substrate [22].

To increase the synthesis of the alcohol it was used methods of mathematical modeling of nutrient medium. The maximum concentration of 2,3-butanediol (9.16 g/l) was achieved in the medium that contained 200 g/l of crude glycerol, 1.96 g/l of yeast extract, 2.87 g/l of ammonium phosphate and 2.16 g/l of sodium fumarate during aerobic cultivation, that was approximately the same as theoretically calculated one (9.54 g/l) [22]. Similar studies were carried out for the strain *K. pneumoniae* G31 [23]. Initial concentration of crude glycerol was 30 g/l, and during the cultivation 25 and 15 g/l of the substrate was additionally added into the medium. It was established that the concentration of the synthesized alcohol under aerobic conditions was 70 g/l (0.39 g/g of glycerol), and under the micro aerobic conditions — 49.2 g/l.

Table 1. **Indicators of synthesis of 1,3-propanediol by bacteria of *Klebsiella* and *Clostridium* on the purified and crude glycerol [16]**

Producers	Concentration of 1,3-propanediol while growing on glycerol (g/l)		Yield from the substrate while growing on glycerol (g/g)	
	purified	crude	purified	crude
K. pneumoniae DSM 2026	61.90	51.30	0.49	0.46
K. pneumoniae DSM 4799	51.86	80.00	0.50	0.67
C. butyricum DSM 15410	9.70	4.10	–	–
C. butyricum DSM 2477	7.90	1.90[1] 2.90[2] 5.80[3] 6.20[4]	–	–
C. butyricum VPI 3266	29.70	31.50	0.62	0.61

Notes: [1] — crude glycerol without purification that was obtained from the waste of biodiesel production from vegetable oil; [2] — crude glycerol without treatment which was obtained from the waste of biodiesel production from soybean; [3] — crude glycerol which was obtained from the waste of biodiesel production from vegetable oil after acidic treatment; [4] — crude glycerol which was obtained from the waste of biodiesel production from soybean after acidic treatment; «–» — the data were not presented.

However, some strains of *K. pneumoniae* are pathogenic, so it is reasonable to use gene modified strains of *E. coli*, which contain genes responsible for the synthesis of 2,3-butanediol [24]. Lee et al. showed that the strain of *E. coli* SGSB03, which was modified by genes budA and meso-budC from *K. pneumoniae*, synthesized 6.9 g/l of target product in the medium with 6% (volume fraction) of crude glycerol at 37 °C and pH 7 at the fourth hour of cultivation [24]. Other authors reported about the ability of the mutant strain of *E. coli* BW25113 modified by genes of *Bacillus subtilis* and *Clostridium beijerinckii* to synthesize 2,3-butanediol (9.54 g/l) using the cultivation on the medium with crude glycerol (30 g/l).

Ethanol. Technologies of microbial synthesis of ethanol are well understood nowadays and they are widely used, but there is one possible niche in this market — obtaining ethanol from glycerol. Taking into consideration the global excess of crude glycerol the alcohol fermentation with glycerol as a substrate can be considered as the promising alternative of ethanol obtaining for biofuel needs [26–32]. The process of obtaining ethanol with methylotrophic yeast *Hansenula polymorpha* is actively investigated. Transferring genes from *Zymomonas mobilis*, encoding pyruvate decarboxylase and alcohol dehydrogenase into *H. polymorpha* allowed to increase the yield of the product by 3.3 times under conditions of microaeration (up to 2.74 g/l) [26]. As it was described in [27] there was a possibility to obtain ethanol by growing yeast *Pachysolen tannophilus* CBS4044 on the crude glycerol. The influence of substrate concentration on the synthesis of ethanol by CBS4044 strain showed that on medium containing 2, 5 and 10% (v/v) of crude glycerol the concentration of synthesized product reached 6.28, 17.5, and 18.6 g/l, respectively [27]. It was described the ability of *E. coli* SY4 to the transformation of crude glycerol into ethanol under both anaerobic and aerobic conditions [28].

Genetically engineered manipulation (inactivation of fumarate reductase, phospho acetyltransferase and formiate lyase and the increase of gene expression of glycerol dehydrogenase and dihydroxyaceton kinase) made it possible to increase the concentration of the synthesized ethanol to 7.8 g/l (efficiency — 0.15 (g/l) h^{-1}) [28].

The metabolism of glycerol in the mutant strain of *K. pneumoniae* GEM 167 is different from that of the original [29]. Thus, 1,3-propanediol and 3-hydroxypropionic acid are not synthesized during the growing of the mutant strain on glycerol, and the level of 2,3-propanediol, ethanol, lactate and succinate, conversely, was increased. It was established that cultivation of GEM 167 strain on the purified and crude glycerol provided ethanol concentration 21.5 g/l and 20.5 g/l respectively. To increase the yield of ethanol adhII and pdc genes from *Z. mobilis*, encoding pyruvate dehydrogenase and aldehyde dehydrogenase were incorporated into the genome of *K. pneumoniae* GEM 167. As a result, the ethanol concentration reached 25.0 g/l in case of using of purified glycerol and 24.6 g/l — in case of crude glycerol [29].

To increase the synthesis of ethanol researchers obtained both: the mutant strain of *K. rneumoniae* GEM 167ΔldhA that was not able to synthesize lactate (there was a deletion of the genes responsible for the synthesis of lactate dehydrogenase) and genetically modified strain GEM 167ΔldhA / pBR-pdc-adh. For the last one the genes of pyruvate decarboxylase and alcohol dehydrogenase from *Zymomonas mobilis* were incorporated into genome [30]. According to the presented data (Table 2), obtained strains produced 29–31 g/l ethanol and the lactate was not practically formed.

It is known that some representatives of the genus *Kluyvera* can use the crude glycerol as a source of carbon and energy for an active synthesis of ethanol. So, it was found that *Kluyvera cryocrescens* S26 synthesized 27 g/l ethanol with efficiency 0.61 (g/l)·h^{-1} [31]. In 2012, Corean scientists reported that *Enterobacter aerogenes* ATCC 29007 was able to synthesize 6.62 g/l of ethanol from 20 g/l of crude glycerol (efficiency — 1.07 mol ethanol/mol glycerol) [32].

Butanol is widely used as a solvent in paint and varnish industry, production of resins and plasticizers, as well as biofuel [33, 34]. Until recently, *Clostridium pasteurianum* has not been considered as an effective producer of butanol.

However, there are some reports about the ability of *C. pasteurianum* to synthesize this alcohol using crude and purified glycerol as a source of carbon and energy [33]. Jensen et al. [33] established the ability of the mutant strain of *S. rasteurianum* MNO6 to consume high concentrations of crude glycerol (till 105 g/l).

It was shown that the concentration of butanol synthesized by the strain MNO6, was 1.5 times higher compared to the concentration that was obtained in case of using of original

Table 2. **Metabolits synthesized in case of growing of *Klebsiella pneumoniae* strains on the crude glycerol [30]**

Metabolites	Concentration of metabolites which were synthesized by strains (g/l)		
	GEM167	GEM167 ΔldhA	GEM167 ΔldhA /pBR-pdc-adh
Acetate	0.4	0.5	1.0
Ethanol	21.5	28.9	31.0
Lactate	11.5	1.4	0.8
1,3-propanediol	0.5	0.5	0.8
Succinate	1.3	2.4	3.1
2,3-butanediol	2.1	5.7	2.3

strain *S. rasteurianum* DMSZ 525. By culturing *S. rasteurianum* MNO6 in the fermenter the maximum concentration of butanol (12.6 g/l) was achieved on the 44th hour of cultivation, but in this case the crude glycerol was modified by activated carbon [33]. Other authors [34] found that after the optimization process of biosynthesis the mutant strain of *S. pasteurianum* MBEL_GLY2 synthesized 17.8 g/l butanol using crude glycerol (82 g/l) that was 2.3 times higher than could be achieved with the original strain of *C. pasteurianum* ATCC 6103. Further investigation showed that the maximal efficiency (7.8 (g/l)·h^{-1}) for the continuous cultivation of strain MBEL_GLY2 could be reached by changing the dilution rate of the environment 0.9 h^{-1} [34]. It was shown the ability of *C. pasteurianum* MTCC 116 to synthesize butanol using the crude glycerol [35]. So, the maximal output of this alcohol (0.28 mol/mol of the substrate) was observed in case of adding of 25 g/l substrate in the medium of cultivation.

Polyols or sugar alcohols are organic compounds that are widely used in food, pharmaceutical and medical industries and as intermediates in the chemical industry [36, 37]. Nowadays polyols are predominantly obtained using chemical approach. There is an alternative microbiological way with applying of such expensive substrates as glucose, fructose and maltose. At the same time the prospective way of investigations is the using of crude glycerol as the source of carbon and energy in the technology of polyol obtaining [36–41].

Mannitol. In 2009 Khan et al. [36] demonstrated the possibility of mannitol production with help of *Candida magnoliae* cultivated on a crude glycerol. It is shown that amount of mannitol synthesized was up to 51 g/l when this substrate was added to the cultivating medium in concentration 100 g/l. Ability of *Yarrowia lipolytica* LFMB 19 or *Y. lipolytica* LFMB 20 to synthesize mannitol when cultured in the medium containing crude glycerol (30 g/l) was also described [37]. Several studies have shown that the concentration of synthesized polyol for both strains did not exceed 6 g/l. However, increasing substrate concentration up to 90 g/l allowed elevating the mannitol yield (3.5 folds) in comparison with that synthesized in the medium with a lower concentration of crude glycerol [37].

Erythritol. In 2008 Rymowicz et al. [38] showed that the maximal amounts of erythritol (81 g/l) and citric acid (110 g/l) are synthesized during cultivation of *Y. lipolytica* Wratislavia K1 on the medium supplemented with 250 g/l crude glycerol at 168th hour of cultivation. However, in 2009, the same authors [39] found that the maximal concentration of polyol (170 g/l) can be achieved when growing *Y. lipolytica* Wratislavia K1 on the medium containing 300 g/l of crude glycerol. In addition, under such cultivation conditions, Wratislavia K1 strain was not able to synthesize citric acid [39]. Later, the synthesis of erythritol during the cultivation of *Y. lipolytica* Wratislavia K1 with constant medium supply and periodic selection of cultural liquid in the fermenter with volume of 5 L was studied [40]. Implementation of such process when 40, 30 and 20% of the medium with pure glycerol (final concentration 250, 333.3 and 500 g/l) were replaced, the amount of the synthesized glycerol was 135.5, 174.8 and 208 g/l respectively. In the similar cultivation conditions with use of crude glycerol, strain synthesized polyol in concentrations 133.6, 110.5 and 155.5 g/l.

Arabitol is an enantiomer of xylitol and can be used as a natural sweetener or sugar

substitute for diabetics [41]. Screening of 214 yeast strains capable of synthesizing of arabitol growing on biodiesel wastes, allowed selecting strain *Debaryomyces hansenii* SBP-1, which synthesized 14 g/l of arabitol during cultivation in medium containing 150 g/l crude glycerol [41].

Organic acids

Citric acid is an important product of microbial synthesis and it is widely used in the food and pharmaceutical industries due to the lack of toxicity. Annually, the worldwide production of this acid is 800,000 tons. Conventional biotechnologies of citric acid production are based on the use of such producer as *Aspergillus niger* using sucrose or molasses as sources of carbon and energy [42]. But today, enquiry for this product of microbial synthesis is constantly growing, so the elaboration of alternative technologies based on waste products of various industries in order to reduce the cost of citric acid production and minimize the amount of by-products is an important issue. There are some papers, reporting the ability of yeast *Y. lipolytica* and some *Candida* species to synthesize citric acid when wastes of biodiesel production are used [42, 43]. *Y. lipolytica* A-101-1.22 converts crude glycerol (total concentration 250 g/l) into citric acid (112 g/l) with rate of 0.71 (g/l) (g/l)·h^{-1} [42]. However, several studies have shown that the duration of the process more than 100 hours is impractical due to the gradual decrease in acid-producing capacity of the strain. Meanwhile, in the case of semicontinuous cultivation, active biosynthesis of citric acid (96–107 g/l) lasted approximately 300 hours, and in some cases — up to 1000 hours (124.2 g/l) [42]. Another strain, *Y. lipolytica* NRRL YB-423, synthesized maximal amounts of citric acid (21.6 g/l) growing on the medium containing 40 g/l of purified glycerol [43]. The authors report that strain NRRL YB-423 cultivated on a crude glycerol produced citric acid with a rate of 94 (mg/l)·h^{-1}. Da Silva et al. [44] found that during cultivation of *Y. lipolytica* IMUFRJ 50682 on the medium containing crude glycerol (45 g/l), the peak levels of both citric and iso-citric acids achieved by 160^{th} hour of cultivation, were 12.94 g/l and 6.66 g/l respectively. Studies of citric acid synthesis by *Y. lipolytica* NCIM 3589 strain showed that the maximal concentration of the end product reached 77.4 g/l, when the initial concentrations of crude glycerol and yeast extract added in the medium were 54.4 g/l and respectively 0.27 g/l, followed by addition of substrate partially in the cultivation medium [45].

Lactic acid is used in the chemical industry for the production of acrylic acid, 1,2-propandiol, polyester resins, polyurethane and antifreeze, as well as in the food industry [4]. It is synthesized chemically and microbiologically, however, the last method has a drawback associated with the use of high-cost media for cultivating lactic acid bacteria. This greatly affects the final price of the product. Therefore, the seeking for alternative strains and substrates for their cultivation is an essential task. It is known that the bacterium *E. coli*, some representatives of *Klebsiella*, *Clostridium*, *Bacillus* and micromyceta *Rhizopus oryzae* are able to synthesize lactic acid by use of crude glycerol as the sole source of carbon and energy [46–49]. According to the report [46], the ability to form lactic acid homoenzymatycally is not an inherent natural feature for *E. coli*. Due to this, *Streptococcus bovis* genes responsible for the synthesis of L-lactate were introduced into the genome of the strain and succinate, acetate and ethanol synthesis ways were also blocked. Several studies have established that the resulting genetically modified strain when cultivated on crude glycerol (56 g/l) was able to synthesize 50 g/l lactic acid of high optical (99.9%) and chemical (97%) purity [46]. Other authors [47] found that the strain of *E. coli* AC-521 is also capable consuming high concentrations of crude glycerol and accumulate up to 85.8 g/l lactic acid with productivity rate 0.49 g/l·h^{-1} under aerobic conditions. It is shown that *E. coli* LA02Δdld homoenzymatically ferments crude glycerol (initial concentration 40 g/l, re-supplementation at the 48^{th} hour of cultivation — 20 g/l) into lactate [48]. Lactic acid obtained in this way using minimal cultivating medium at a concentration of 34 g/l, has 99.9% purity, while the productivity of the process reached 1.5 g/l·h^{-1} [48]. Vodnar et al. [49] investigated the ability of *R. oryzae* NRRL 395 to synthesize lactic acid when growing on biodiesel waste. Thus, the strain NRRL 395 was grown on mineral cultivating medium with glycerol and crude juice of green lucerne, which is a natural source of nutrients. Some studies have shown that the peak level of synthesized acid (48 g/l) was observed in the presence of 75 g/l glycerol and 25 g/l lucerne juice in culture medium [49].

Glycerine acid is used in the chemical and pharmaceutical industries for the

production of polymers and surfactants. It is generally synthesized chemically. The main problem of existing biotechnologies of this acid is a large amounts of simultaneously formed dihydroxyacetone as a byproduct, which is significantly inhibits their large-scale implementation due to high cost of isolation and purification of the end product. The application of crude glycerol, which is a waste of biodiesel production, in microbial technologies of glycerine acid production can significantly reduce its cost [50]. The main producers of glycerine acid are representatives of *Acetobacteraceae*, in particular *Gluconobacter* sp., *Acetobacter* sp. and *Gluconacetobacter* sp. It has been found that the cultivation of *Gluconobacter frateurii* NBRC 103465 on a crude glycerol (250 g/l) yielded 136.5 g/l glycerine acid at 144th hour of growth [50]. *Acetobacter tropicalis* NBRC 16470 synthesized 101.8 g/l end product at substrate concentrations 220 g/l [50]. There are reports indicating *Gluconobacter* sp. NBRC3259 formed 49.5 g/l glycerine acid and 28.2 g/l dihydroxyacetone from 174 g/l crude glycerol pre-purified with use of activated carbon [6].

Succinic acid is an important substance used in the technology of production of plastics, resins, drugs, and as a food additive E363 [51, 52]. In industrial conditions, succinic acid is obtained mainly by chemical synthesis, such as hydrogenation of maleic anhydride. However, at present, microbiological method based on various sugars, agricultural wastes and by-products of biodiesel production is considered to be promising [51]. To develop a recombinant *E. coli* strain-producer of succinic acid, the ways of synthesis of by-products such as lactate, ethanol and acetate had to be blocked. Additionally, this strain has been introduced by a gene isolated from *Lactococcus lactis*, which is responsible for the synthesis of pyruvate carboxylase, the enzyme that catalyzes the reaction of pyruvate carboxylation to form a succinate precursor. Further investigation showed that the strain transforms crude glycerol to succinic acid with a rate of about 400 mg succinate/g cells· h^{-1} and 0.69 g yield of succinate per g glycerol [51]. Carvalho et al. [52] demonstrated the possibility of succinic acid to be produced by *Actinobacillus succinogenes* growing on the medium containing crude glycerol. It has been established that *A. succinogenes* synthesizes 49.62 g/l of succinic acid with efficacy of the process 2.31 g/l·h^{-1}. Other authors found that the peak level of succinic acid achieved 29.3 g/l during cultivation of *A. succinogenes* ATCC 55618 in a mineral medium containing the initial glycerol concentration 36.9 g/l [53].

Oxalic acid is used in the production of paper and the manufacture of detergents [54, 55]. Currently microbial technologies for the production of oxalic acid by *A. niger* with use of crude glycerol are developed [54]. It has been shown that *A. niger* synthesizes 21 g/l of the final product with the efficacy of the process 0.62 g/l·h^{-1} by 240th hour [54]. Musial et al. [55] found that *A. niger* XP synthesizes 49.8 g/l oxalic acid by 168th hour of cultivation with use of crude glycerol in concentration of 50 g/l, and the productivity of the process is 0.88 g/l·h^{-1}.

Other products of microbial synthesis

Polysaccharides. Freitas et al. [56] found that the bacteria *Pseudomonas oleovorans* NRRLB-14682 synthesize high-molecular weight exopolysaccharides during the growth, containing crude glycerol. The greatest amounts of synthesized polysaccharides (12.18 g/l) and efficacy (3.85 g/l per day) and release from substrate (0.36 g/g biomass) was achieved during cultivation of bacteria on a crude glycerol. The same parameters during growth on purified substrate were 11.82 g/l, 2.00 g/l/day, and 0.28 g/g, respectively [56].

Biohydrogen. Since the world's reserves of oil and minerals at this time are limited, the use of alternative energy sources can minimize the burden on natural resources and the environment. One of these sources is biohidrohen formed during thermal or electrochemical treatment of wood and other natural materials or natural resources (oil, gas, coal). Biological hydrogen production method is more environmentally friendly and less energy-intensive, but low yield of the product significantly impedes implementation of its large-scale production. Therefore, improvement of existing biotechnologies of hydrogen and search for alternative substrates are urgent issues [57–60]. In the reports [57], the ability of *Thermotoga neapolitana* DSM 4359 and *Enterobacter aerogenes* HU-101 strains to assimilate crude glycerol with subsequent formation of biohydrogen has been shown. It has been established that, in the presence of substrate at the concentrations 5 and 10 g/l in the culture medium of strains DSM 4359 and HU-101 respectively, 1.98 mol H_2/mol of crude glycerol could be synthesized. Other studies have shown that *Rhodopseudomonas palustris* synthesizes

6 mol H_2/mol crude glycerol by 240-th hour of cultivation in the medium with substrate concentration 9 g/l [58].

New approach for increasing the yield of the final product is the producer cell immobilization, which can reduce the negative impact of some components of crude glycerol [59]. *E. aerogenes* ATCC 29007 cells were immobilized on such materials as agar, alginate, glass beads, k-carrageenan, and gelatin. In the conditions of growth on the crude glycerol, agar-immobilized cells of ATCC 29007 strain produced hydrogen in concentration 4.216 ml/l, whereas alginate-, k-carrageenan-, gelatin- or glass beads-immobilized cells produced 3.290, 3.237, 2.005 and 2.162 ml/l, respectively [59]. It has been established that halophilic bacteria *Halanaerobium saccharolyticum* subsp. *senegalensis* and *Halanaerobium saccharolyticum* subsp. *saccharolyticum* could consume crude glycerol and grow at very high concentrations of salts. It has been also found that the optimum conditions for maximal accumulation of hydrogen is cultivation on the medium with 2.5 g/l of glycerol and 150 g/l of sodium chloride and maintaining the pH value within 7.4 for *H. saccharolyticum* subsp. *saccharolyticum* and 7.0 for *H. saccharolyticum* subsp. *senegalensis* (6.2 and 6.3 mM hydrogen are produced respectively) [60].

Surface-active substances (surfactants). In the paper [61], strains of *Acinetobacter calcoaceticus* NRRL B-59190, NRRL B-59191, *Enterobacter asburiae* NRRL B-59189, *Enterobacter hormaechei* NRRL B-59185, *Pantoea stewartii* NRRL B-59187 and *Pseudomonas aeruginosa* (strain NRRL B-59182, NRRL B-59183, NRRL B-59184, NRRL B-59186, NRRL B-59188, NRRL B-59192, NRRL B-59193) are shown to be able to consume crude glycerol (10 ml/l) and synthesize ramnolipids in concentration 1.9–2.5 g/l.

Candida bombicola ATCC 22214 strain synthesized up to 9 g/l of sophorolipids cultivated on purified glycerol (10%), but 60 g/l of these surface-active compounds were produced in medium containing 10% biodiesel production wastes (40% glycerol, 34% fatty acids, no methanol) [62]. Taking into account that methanol inhibits the growth of microorganisms, investigators in [63] studied the influence of this alcohol (up to 1.5%) on synthesis of sophorolipids of *C. bombicola* ATCC 22214 grown in a mixture of purified glycerol (100 g/l) and oleic acid (2%). This substrate is a modification of biodiesel production wastes. It was established that in the absence of methanol in a medium, amount of synthesized sophorolipids was 12.7 g/l, while increasing methanol concentration to 1.5% reducing level of lipids (to 5.6 g/l) was observed [63].

Production of sophorolipids by *Starmerella (Candida) bombicola* ATCC 22214 strain in a mixture of refined glycerol (15%) and sunflower oil (10%), in which purified glycerol was substituted on glycerol-containing wastes of commercial fat hydrolysis, has been studied [64]. It has been revealed that regardless of the source of glycerol in the mixture, concentration of sophorolipids appeared to be almost the same (6.36–6.61 g/l). It is known that *B. subtilis* LAMI005 and *B. subtilis* LAMI009 synthesize surfactin during growth on crude glycerol [65]. Thus, the presence of 2% substrate (v/v) in the culture medium, amounts of surfactin synthesized by LAMI005 and LAMI009 strains reached 441.06 and 267.56 mg/l, respectively, at 72-nd hours of growth.

It was found that the surface-active compound peak level (1.37 g/l) was synthesized at 60-th hour of growth of *B. subtilis* LSFM-05 on the medium with crude glycerol (5% v/v), process efficacy was 11.42 $(mg/l)\cdot h^{-1}$ [66]. In the report [67], fengicine synthesized at these conditions was shown to be present as two homologues (A and B), which characterized by difference in 6-th amino acidic residue (alanine or valine, respectively). According to mass spectrometry data, lipopeptide represents 4 isoforms of fengicine A and 3 isoforms of fengicine B, which were identified to bear fatty acid moieties, containing from 14 to 17 carbon atoms.

It was found that *Ustilago maydis* consumes crude glycerol followed by glycolipid synthesis [68]. To determine the optimal substrate concentration, crude glycerol in concentrations from 10 to 50 g/l was added in the culture medium. The maximal concentration of glycolipids (32.1 g/l) was monitored on the medium with 50 g/l of the substrate, 20 mg/l ammonium citrate, 10 mg/l asparagine and vitamin B [68]. It is known that *Pseudomonas* bacteria are efficient producers of surfactants [69]. For example, *P. aeruginosa* J16 synthesizes complex of the surface-active compounds, chemically recognized as combination of mono- and di-rhamnolipids. It was found out that the synthesized surfactant concentration achieved 448.3 mg/l, and efficacy of the production was

4.67 (mg/l)·h^{-1} when crude glycerol and NH_4Cl as a source of nitrogen were used.

When replacing NH_4Cl with $(NH_4)_2SO_4$, increasing amount of rhamnolipids to 2121 mg/l (process efficacy 22.1 (mg/l)·h^{-1}) was taken place. However, the maximal concentration of surfactant (3190 mg/l) and process efficacy (44.3 mg/l·h^{-1}) was observed after optimization of J16 strain cultivation using the methods of mathematical planning experiment [69]. Wu et al. [70] investigated the effects of different sources of carbon and nitrogen on the synthesis of rhamnolipids by *P. aeruginosa* EM1. The results showed that the maximal amounts of surface-active compounds (12.6 g/l) was observed in the presence of 30.5 g/l of crude glycerol, 18.1 g/l glucose, and 4.9 g/l of sodium nitrate in culture medium for EM1strain. In the paper [71], the possibility of obtaining of rhamnolipids by *P. aeruginosa* MSIC02 grown on biodiesel production wastes is decribed. Maximal concentration of rhamnolipids (1.27 g/l) was achieved by culturing the strain in medium, containing 18 g/l of crude glycerol, 4 g/l $NaNO_3$, 62 mM KH_2PO_4, pH 7.0 and 37 °C. Under these conditions, the process efficacy was 19.9 (mg/l)·h^{-1}. Synthesized rhamnolipids displayed relatively high emulsifying activity (E_{24} 65%), when mineral and vegetable oils were used as substrates [71]. Our studies showed that, in the presence of components of glycerol fraction (potassium and sodium — 2,5%, methanol and ethanol — 0.3%) in the medium containing refined glycerol (1% v/v), elevation of the relative concentration of surface-active compounds of *Acinetobacter calcoaceticus* IMV V-7241, *Rhodococcus erythropolis* IMV Ac-5017 and *Nocardia vaccinii* IMV V-7405 to 11–68% in comparison with these parameters for salt- and alcohol-free media was found. However, when strains were cultivated on crude glycerol (2.2%), obtained directly from the manufacturer of biodiesel (Biofuels Zaporozhye Plant), the concentration of synthesized extracellular surface-active substances appeared to be twice higher than that produced on the purified substrate [72]. In the followed-up experiments, the possibility of further increase of surfactant synthesis parameters when biodiesel production wastes are used with supplementation by low (0.05–0.1%) concentrations of precursors (glucose, sunflower oil, organic acids) and a mixture of crude glycerol and hexadecane, has been shown [72]. Increasing of inoculum concentrations to 10–15% and elevation by half (compared to the basal medium) content of the nitrogen source, made it possible to implement the process of synthesis of surfactant by IMV Ac-5017, IMV B-7241 and B-7405 IMV strains in the medium containing 7–8% (v/v) of crude glycerol. Under these cultivation conditions, concentrations of extracellular surface-active compounds synthesized by studied strains were 3,4–5,3 g/l. These are 1.4–3-fold higher values than that obtained in the basal medium with the same concentration of substrate [73].

Therefore, parameters of surfactant synthesis provided by *A. calcoaceticus* IMV V-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IIR and B-7405 cultivated on biodiesel production wastes appear to be at least not worse, and often even better, than those described in literature in relation to many well-known producers.

Cephalosporin C. Cephalosporin C is conventionally obtained by culturing *Acremonium chrysogenum* on medium, containing glucose and soybean oil [74]. However, there are several reports, describing using of crude glycerol as an alternative to traditional substrates used in the technology of synthesis of this antibiotic. Korean scientists have found that the amount of synthesized cephalosporin C reached the level of 7,24 g/l, when *A. chrysogenum* M35 was cultivated on the medium containing 4% (v/v) of crude glycerol. This concentration of antibiotic is 10-fold higher than that for cephalosporin produced in the glucose-containing medium [74].

Trehalose is a carbohydrate used in the food industry for the production of confectionery, juices, milk, bread, condiments, etc. [75]. One of the producers of trehalose is *Propionibacterium freudenreichii* subsp. *shermanii*. It is established that *P. shermanii* NCIM 5137 utilizes both refined and crude glycerols. By culturing the studied strain in rocking flasks (200 rotates/min) on the medium with refined or crude substrates (20 g/l), trehalose concentration reached 361 mg/l or 1.3 g/l, respectively. During cultivation in fermenter, *P. shermanii* NCIM 5137 synthesized 1.56 g/l of the target product, when crude glycerol in concentration of 20 g/l was used [75].

Lipids. Study [76] indicates that there is an ability to use crude glycerol to produce lipids and carotenoids by *Rhodotorula glutinis* TISTR 5159 yeast. It was found that the use of ammonium sulphate and Tween-20 increases the concentration of lipids and carotenoids, and optimal conditions for their synthesis is the concentration of glycerol 9.5% and the

ratio C/N = 85. The highest rates of synthesis of lipids (6.10 g/l with intracellular level 60.7%) and carotenoids (135.25 mg/l) were observed during cultivation of *R. glutinis* TISTR 5159 in the bioreactor at pH 6.0 and the aeration 2 rpm. These values were 12.4- and 2.1-folds higher than that obtained at conditions without optimization [76]. Paper [77] demonstrates that *Cryptococcus curvatus* synthesizes 17.1 g/l of lipids when cultivated in the mineral medium, in which crude glycerol was added in portions (initial concentration 30 g/l followed by the addition of every 12 hours) for 288 hours. During cultivation of *Schizochytrium limacinum* SR21, substrate concentration varied within 25 — 35 g/l, while amount of target product reached 9.7 g/l [78].

Chen et al. [79] showed that *Chlorella protothecoides* UTEX 256 was characterized by the ability to assimilate crude glycerol and accumulate lipids and carotenoids. Cultivation was carried out over 216 hours in mineral medium, containing yeast extract (4 g/l) and glycerol in initial concentration 30 g/l (every 24 hours 150 g/l of substrate and 15 g/l of yeast extract were added). Under such conditions, strain UTEX 256 synthesized 24.6 g/l of lipids and had process efficacy 2.99 g/l/day [79]. In the study [80], an ability of wild yeast species to consume pure or crude glycerol were investigated. Of the 40 isolated strains, 4 strains were selected and identified (*Lidnera saturnus* UFLA CES-Y677, *Y. lipolytica* UFLA CM-Y9.4, *R. glutinis* NCYC 2439 and *C. curvatus* NCYC 476), which were able to consume biodiesel production wastes in the different concentrations (10, 20, 30%, v/v). *Y. lipolytica* UFLA CM-Y9.4 appeared to be the most productive strain, which were able to accumulate 63.4% of lipids in its biomass, when cultivated in crude glycerol-containing medium (30%). It is worth mentioned that the most prevailed fatty acids in the produced lipids were palmitic (74.67%) and stearic (87.64%) acids.

Genetically modified *S. cerevisiae* strain, containing nonspecific acyltransferase genes from *Acinetobacter baylyi*, was obtained for the synthesis of fatty acid ethyl esters from glycerol [81]. Genetically modified YPH499 strain synthesized 0.24 g/l esters from endogenous ethanol, which was synthesized from glycerol as the growth substrate. In further studies, another genetically modified YPH499 fps1Δgpd2Δ strain, characterized by increased productivity of ethanol and subsequently fatty acid esters (0.52 g/l), was obtained. The concentration of crude glycerol in the medium was 17 g/l [81].

Polyhydroxyalkanoates. Polyoxybutyrate is a sort of polyhydroxyalkanoates. Its producers are some Gram-negative bacteria, which are able to use glycerol as the sole source of carbon and energy, including *Cupriavidus necator* DSM 545, *Methylobacterium rhodesianum* MB126, *Ralstonia eutropha* DMS 11348, *C. necator* JPM134, *E. coli* CT1061, and *E. coli* ATCC: PTA-1579 [82, 83]. For example, *E. coli* Arc2 synthesized 10.81 g/l of polyhydroxyalkanoates with process efficacy 0.18 $(g/l)\cdot h^{-1}$, cultivated in the medium with crude glycerol (20 g/l) [82]. According to the data described in [83], the main problem associated with the use of crude glycerol is a high concentration of sodium salts, which adversely affect polyhydroxyalkanoate accumulation. However, *Zobellella denitrificans* MW1 strain, which appeared to be resistant to high concentrations of Na^+, has been recently isolated. Due to this, it is considered as promising producer of polyhydroxyalkanoates [83]. This strain was grown on mineral medium with high content of sodium chloride (20 g/l) and crude glycerol (initial concentration 15 g/l with subsequent addition of 10 and 20 g/l of substrate). It was established that maximal amount of synthesized product for *Z. denitrificans* MW1 was 54.3 g/l with process efficacy 1.09 $(g/l)\cdot h^{-1}$ [83].

To study the process of polyoxybutyrate accumulation, *C. necator* JMP 134 [84] assimilating both refined and crude glycerols, which contain methanol and other inorganic impurities, was applied. During JMP 134 strain cultivation, two types of substrate were used (the mass fraction of glycerol was 88 and 98%, corresponding to its concentration in the medium 170.8 and 249 g/l, respectively). It was established that the total quantity of polyoxybutyrate that formed during 44 hours of cultivation was 27.8 g/l for the first variant of substrate and 57.1 g/l for the second one [84].

Teeka et al. [85] found that *Novosphingobium* sp. THA_AIK7 synthesized 1.58 g/l of polyhydroxyalkanoates during 72 hour growing on the medium containing 2% (v/v) crude glycerol, 1.44 g/l nitrogen source, 1.28 g/l phosphorus source, and 1.384 g/l sodium. In [86], an ability of *Haloferax mediterranei* DSM 1411 archaea to form a copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate from refined or crude glycerols is reported. The initial concentration

of substrate in the medium was 10 g/l. During cultivation, the culture medium was additionally supplied with glycerol, and maintenance of its concentration at the level of 10–20 g/l was performed. The concentration of synthesized polyhydroxyalkanoates during cultivation of DSM 1411 strain on crude glycerol appeared to be slightly higher than that obtained on pure substrate (approximately 20 and 15 g/l respectively), however, ratio product/substrate was turned out to be lower (0.19 g/g and 0.37 g/g of crude or purified glycerol, respectively). Poly-3-hydroxypropionate is not synthesized by any of the natural strains of microorganisms [87, 88]. In 2010, obtaining of recombinant *E. coli* strain, which were able to synthesize this polymer from glycerol (concentration of poly-3-hydroxypropionate was about 12% per cell weight), was documented [87]. Genetically modified *Shimwellia blattae* ATCC 33430 strain, accumulating up to 9.8% of poly-3-hydroxypropionate to cell mass when cultured on crude glycerol, was obtained in 2013 [88].

Vitamin B_{12}. The study [89] is the first work, indicating possibility of vitamin B_{12} (cyanocobalamin) synthesis by *Propionibacterium freudenreichii* ssp. *shermanii* 1 bacteria in the medium containing crude glycerol. Before optimizing, the concentration of vitamin was 2.11 mg/l. After two stages of optimization of the culture medium, concentration of vitamin was achieved to 3.542 mg/l. In the same time, concentration of crude glycerol in the producer culture medium was 35.67 g/l (calculated on the purified substrate).

Summary data concerning application of crude glycerol as substrate for the production of valuable microbial metabolites listed in the Table 3.

In summary, it could be assumed that the rapid development of biodiesel production worldwide has led to the need to solve the serious environmental problems associated with utilization of by-product (crude glycerol). One of the ways of solving this problem is to use such waste as a substrate for cultivation of microorganisms in biotechnology. Compared with other industrial wastes, which are used as substrates, crude glycerol is more economically (cheap and available in very large quantities) and technologically (hydrophilic, sterilization is not required) advantageous. Though, due to the presence of inhibitors in its composition, it is less suitable substrate for microorganism growing, compared with purified one. Therefore, in many processes of microbial synthesis, refined glycerol is used, and some biotechnologies based on crude glycerol application appeared to be unprofitable or require its prior purification procedure. As late as five years ago, much attention was focused on anaerobic microbial transformation of glycerol, which was applied for production of alcohols and ketones. However, at present, possibility of using of this substrate for production of organic acids, polyhydroxyalkanoates, surface-active compounds and other products of microbial synthesis has been established.

Bioconversion of glycerol in valuable products of microbial synthesis will allow solving simultaneously two urgent problems: first, to reduce the cost of microbial technology by means of using of cheap raw materials as substrates, and secondly, to increase the profitability of biodiesel production through utilization of one of the by-products, crude glycerol.

Table 3. **Crude glycerol as substrate for microbial synthesis**

End product	Producer	Technical glycerol concentration in the medium	Concentration of the product/yield from the substrate	References
1	2	3	4	5
1,3-Propanediol	*C. butyricum* AKR102a	The initial concentration of 30 g/l followed by adding of fractional parts of 25 g/l	76.2 g/l	[10]
	C. butyricum VPI 3266	62 g/l	31.5 g/l	[11]
	C. butyricum DSP1	60–80 g/l	32.54 g/l	[13]
	Klebsiella sp. HE-2	30 g/l	8.8 g/l	[15]
	E. coli BL21	10 g/l	3.7 g/l	[17]
	C. acetobutyricum DG1	1792 mM	1104 mM	[19]
	C. freundii ATCC 8090	20 g/l	4.85 g/l	[20]

Table 3 (continued)

1	2	3	4	5
	C. butyricum DSM 5431	87.8 g/l	45.0 g/l	[90]
	C. *butyricum* VPI 1718	80 g/l	67.9 g/l	[91]
	C. diolis GSHM 2	1440 mM	706 mM	[92]
2,3- Butanediol	*K. pneumoniae* SU6	200 g/l	9.16 g/l	[22]
	K. pneumoniae G31	70 g/l	49.2 g/l	[23]
	E. coli SGSB03	6% (volume fraction)	6.9 g/l	[24]
	E. coli BW25113	30 g/l	9.4 g/l	[25]
Ethanol	*P. tannophilus* CBS4044	10% (volume fraction)	18.6 g/l	[27]
	E. coli SY4	–	7.8 g/l	[28]
	K. pneumoniae GEM 167	6%	6.9 g/l	[29]
	K. cryocrescens S26	–	27 g/l	[31]
	E. aerogenes ATCC 29007	20 g/l	6.62 g/l	[32]
Butanol	*C. pasteurianum* DMSZ 525	–	0.280 mol/mol	[33]
	C. pasteurianum MNO6	–	0.252 mol/mol	[33]
	C. pasteurianum MBEL-GLY2	82 g/l	17.8 g/l	[34]
	C. pasteurianum MTCC 116	25 g/l	0.28 mol/mol	[35]
Mannitol	*C. magnoliae* NCIM 3470	100 g/l	51 g/l	[36]
	Y. lipolytica LFMB 19 and LFMB 20	30 g/l	6 g/l	[37]
Erythritol	*Y. lipolytica* Wratislavia K1	300 g/l	170 g/l	[38, 39]
Arabitol	*D. hansenii* SBP-1	150 g/l	14 g/l	[41]
Citric acid	*Y. lipolytica* IMUFRJ 50682	45 g/l	12.96 g/l	[42]
	Y. lipolytica NRRL YB-423	40 g/l	35 g/l	[43]
Lactic acid	*E. coli* AC-521	95 g/l	85.8 g/l	[47]
	E. coli LA02Δdld	40 g/l	32 g/l	[48]
Glycerol acid	*A. tropicalis* NBRC 16470	220 g/l	101. 8 g/l	[50]
	G. frateurii NBRC 103465	250 g/l	136.5 g/l	[50]
Succinic acid	*E. coli*	–	14 g/l	[51]
	A. succinogenes DMSO	60 g/l	49.62 g/l	[52]
Oxalic acid	*A. niger* XP	50 g/l	49.8 g/l	[54]
Biohidrohen	*T. neapolitana* DSM 4359	5 g/l	1.98 mol/mol	[57]
	E. aerogenes HU-101	10 g/l	1.98 mol/mol	[57]
	R. palustris CGA009	9 g/l	6 mol/mol	[58]
Surfactants	*A. calcoaceticus* NRRL B-9191	10 ml/l	2.2 g/l	[61]
	E. asburiae NRRL B-59189	10 ml/l	2 g/l	[61]
	E. hormaechei NRRL B-59185	10 ml/l	2.4 g/l	[61]
	P. stewartii NRRL B-59187	10 ml/l	2.2 g/l	[61]

Table 3 (finished)

1	2	3	4	5
	P. aeruginosa NRRL B-9184	10 ml/l	2.5 g/l	[61]
	U. maydis L8	50 g/l	32.1 g/l	[68]
	B. subtilis LSFM-05	5% (volume fraction)	1.37 g/l	[66, 67]
	P. aeruginosa MSIC02	18 g/l	1.27 g/l	[71]
	A. calcoaceticus IMB B-7241	7% (volume fraction)	5.0 g/l	[73]
	R. erythropolis IMB Ac-5017	8% (volume fraction)	3.4 g/l	[73]
	N vaccinii IMB B-7405	8% (volume fraction)	5.3 g/l	[73]
Cephalosporin C	*A. chrysogenum* M 35	4% (volume fraction)	7.92 g/l	[74]
Trehalose	*P. shermanii* NCIM 5137	20 g/l	1.3 g/l	[75]
Lipids	*R. glutinis* TISTR 5159	9,5% (volume fraction)	6.10 g/l	[76]
	S. limacinum SR21	35 g/l	9.7 g/l	[78]
	C. protothecoides UTEX 256	–	24.6 g/l	[79]
Polihidroksy-alkanoates	*E. coli* Arc2	20 g/l	10.81	[82]
	C. necator JMP 134	249 g/l	57.1	[84]
	Novosphingobium sp. THA_AIK7	2% (volume fraction)	1.58 g/l	[85]
	H. mediterranei DSM 1411	–	20 g/l	[86]
Cyano-kobalamin	*P. freudenreichii* ssp. *shermanii* 1	35,67 g/l (in terms of refined glycerol)	3.542 mg/l	[89]

Note: «–» — data are not shown.

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ТЕХНОЛОГІЇ СИНТЕЗУ ОРГАНІЧНИХ СПОЛУК МІКРООРГАНІЗМАМИ НА ВІДХОДАХ ВИРОБНИЦТВА БІОДИЗЕЛЯ

Т. П. Пирог
Н. А. Гриценко
А. П. Софілканич
І. В. Савенко

Національний університет харчових технологій, Київ, Україна

E-mail: tapirog@nuft.edu.ua

Наведено дані літератури і власних експериментальних досліджень щодо мікробного синтезу на основі відходів виробництва біодизеля одно- і двоатомних спиртів (1,3-пропандіол, 2,3-бутандіол, бутанол, етанол), поліолів (манітол, еритритол, арабітол), органічних кислот (лимонна, бурштинова, молочна, гліцеролова), полімерів і сполук зі складною структурою (полісахариди, полігідроксіалканоати, поверхнево-активні речовини, цефалоспорин, ціанокобаламін), зокрема з використанням рекомбінантних штамів-продуцентів. Показано, що через наявність потенційних інгібіторів у складі технічного гліцеролу (метанол, натрієві та калієві солі) ефективність технологій одержання більшості продуктів мікробного синтезу на такому субстраті є нижчою, ніж на очищеному. Проте необхідність утилізації цього токсичного відходу (через підвищену лужність і вміст метанолу зберігання і перероблення технічного гліцеролу є серйозною екологічною проблемою) компенсує нижчі показники синтезу цільового продукту. Окрім того, на сьогодні, враховуючи обсяги утворюваного під час одержання біодизеля технічного гліцеролу, перевагу віддають мікробним технологіям, що дають змогу реалізувати біосинтез практично цінних метаболітів у середовищі з максимально можливою концентрацією відходів.

Використання технічного гліцеролу як субстрату сприятиме зниженню собівартості продуктів мікробного синтезу і підвищенню рентабельності виробництва біодизеля.

Ключові слова: біодизель, технічний гліцерол, продукти синтезу органічних сполук мікроорганізмами.

ТЕХНОЛОГИИ СИНТЕЗА ОРГАНИЧЕСКИХ СОЕДИНЕНИЙ МИКРООРГАНИЗМАМИ НА ОТХОДАХ ПРОИЗВОДСТВА БИОДИЗЕЛЯ

Т. П. Пирог
Н. А. Гриценко
А. П. Софилканич
И. В. Савенко

Национальный университет пищевых технологий, Киев, Украина

E-mail: tapirog@nuft.edu.ua

Приведены данные литературы и собственных экспериментальных исследований, касающихся микробного синтеза на основе отходов производства биодизеля одно- и двухатомных спиртов (1,3-пропандиол, 2,3-бутандиол, бутанол, этанол), полиолов (маннитол, эритритол, арабитол), органических кислот (лимонная, янтарная, молочная, глицероловая), полимеров и соединений со сложной структурой (полисахариды, полигидроксиалканоаты, поверхностно-активные вещества, цефалоспорин, цианокобаламин), в том числе с использованием рекомбинантных штаммов-продуцентов. Показано, что из-за наличия потенциальных ингибиторов в составе технического глицерола (метанол, натриевые и калиевые соли) эффективность технологий получения большинства продуктов микробного синтеза на таком субстрате ниже, чем на очищенном. Однако необходимость утилизации этого токсичного отхода (из-за повышенной щелочности и содержания метанола хранение и переработка технического глицерола представляет серьезную экологическую проблему) компенсирует более низкие показатели синтеза целевого продукта. Кроме того, в настоящее время, учитывая объемы образующегося при получении биодизеля технического глицерола, предпочтение отдается микробным технологиям, позволяющим реализовать биосинтез практически ценных метаболитов в среде с максимально возможной концентрацией отходов.

Использование технического глицерола в качестве субстрата позволит снизить себестоимость продуктов микробного синтеза и повысить рентабельность производства биодизеля.

Ключевые слова: биодизель, технический глицерол, продукты синтеза органических соединений микроорганизмами.