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MICROBIOLOGICAL CONVERSION OF PURE AND CRUDE GLYCEROL TO 1,3-PROPANEDIOL

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Abstract. 1,3-propanediol (1,3-PD) is the important product used in chemical industry. Microbiological synthesis of 1,3-PD from crude glycerol is a good solution, both from an economic and environmental point of view. The aim of this work was to investigate the effect of raw material (pure and crude glycerol) on the efficiency of the synthesis of 1,3-PD by the bacteria *Clostridium butyricum* DSP1 and *Clostridium butyricum* DO14 isolated from the samples taken from natural environment. Two strains of *C. butyricum* were simultaneously investigated. The obtained results showed that the concentration of 1,3-PD was slightly lower in the case of crude glycerol than in pure glycerol, for both strains. Moreover, waste glycerol was not completely utilized.

Key words: 1,3-PD, *Clostridium* spp., pure glycerol, crude glycerol

INTRODUCTION

The observed increase of biodiesel production involves a serious problem of disposal of by-product, which is a crude glycerol. One of the possibilities of further use of the crude glycerol is its use as a carbon source in the culture medium for microorganisms of industrial potential. A good example is the microbiological conversion of glycerol to 1,3-PD [Hao et al. 2008, Amaral et al. 2009, Leja et al. 2011a]. 1,3-PD, also known as trimethylene glycol, 1,3-dihydroxypropane, propylene glycol and propane-1,3-diol, is an alcohol which consists of two hydroxyl groups with the molecular formula $C_3H_8O_2$ [Amaral et al. 2009]. It is a valuable chemical intermediate used in the production of polymers, lubricants, cosmetics, medicines, and as an intermediate compound in the synthesis of heterocyclic compounds [Liu et al. 2007, Zhang et al. 2007, Kubiak et al. 2012]. The rapid development of biodiesel production leads to the formation large amounts of waste glycerol. Depending on the type of raw material and production technology, the by-product varies in its composi-

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tion. The crude glycerol besides glycerol can include various impurities [Chatzifragkou, Papanikolaou 2012]. The crude glycerol is a mixture of glycerol, alcohol, inorganic salts, free fatty acids, non-reacted mono-, di- and triacylglycerols, methyl esters, variety of other non-glycerol organic materials, and water [Pagliaro, Rossi 2010]. The pharmaceutical and cosmetic industries use only purified glycerol. The above mentioned industries require the crude glycerol to be refined, which means higher costs. An interesting alternative to the standard and commonly used methods of treatment and the use of glycerol is the bioconversion of glycerol to 1,3-PD. The significant advantage of this process is not only the use of untreated waste product, but also a low temperature of microbial synthesis, high selectivity and no toxic by-products [Kośmider et al. 2011].

The glycerol can be metabolized to 1,3-PD by means of microorganisms such as *Klebsiella pneumoniae*, *Bacillus welchia*, *Lactobacillus* spp., *Enterobacter* spp., *Citrobacter* spp., and *Clostridium* spp. [Liu et al. 2007, Leja et al. 2011b]. Unfortunately, most of 1,3-PD producers are pathogenic, which limited it in terms of industrial use. Therefore, non-pathogenic bacteria of the species *C. butyricum* seem attractive microorganisms which may be used in the microbial conversion of glycerol to 1,3-PD. Literature increasingly shows information about the synthesis of this diol from crude glycerol, because it is an important element of prospective industrial applications [Chatzifragkou, Papanikolaou 2012, Metsoviti et al. 2012a, Venkataramanan et al. 2012, Wilkens et al. 2012].

The aim of this work was to investigate the effect of raw material (pure and waste glycerol) on the efficiency of the synthesis of 1,3-PD by the bacteria *C. butyricum* DSP1 and *C. butyricum* DO14 isolated from the samples taken from natural environment.

EXPERIMENTAL PROCEDURES

Microorganisms

Bacteria of the species *C. butyricum* DSP1 and *C. butyricum* DO14 came from the collection of the Department of Biotechnology and Food Microbiology, University of Life Sciences in Poznań, Poland.

Raw material

The raw material was pure, anhydrous glycerol (POCH S.A.). In addition, a crude glycerol A (pharmaceutical II quality) and crude glycerol B (technical) were used. The characteristic of crude glycerol A and B is shown in Table 1.

Table 1. The characteristic of crude glycerol A and B
Tabela 1. Skład odpadowego glicerolu A i B

Component Składniki	Concentration [%] – Stężenie	
	Glycerol A – Glicerol A	Glycerol B – Glicerol B
Glycerol – Glicerol	96,5	83,0
Methanol – Metanol	0,01	0,02
M.O.N.G.*	3,0	4,0
Ash as NaCl – Popiół jako NaCl	0,7	8,0
Water – Woda	1,0	8,0
pH	6,6	6,0

* M.O.N.G. – matter organic non-glycerin – związki organiczne z wyłączeniem glicerolu

Culture medium

Pre-cultures for glycerol utilization experiments were grown in Reincorfed Clostridial Medium – RCM (BIOCORP Poland Sp.). Production medium was prepared as described by Himmi et al. [1999]. Compositions of the production medium is showed in Table 2.

Table 2. The compositions of fermentation medium [Himmi et al. 1999]
Tabela 2. Skład pożywki produkcyjnej [Himmi i in. 1999]

Component – Składniki	Concentration [g/dm^3] – Stężenie
$K_2HPO_4*3H_2O$	3,4
KH_2PO_4	1,3
$(NH_4)_2SO_4$	2,0
$MgSO_4*7H_2O$	0,2
$CaCl_2$	0,02
$FeSO_4*7H_2O$	0,05
Yeast extract – Ekstrakt drożdżowy	2,0

Culture medium was supplemented with a pure or waste glycerol at the concentrations of 70 g/dm^3. In addition, a mixture of the minerals was used in the form of the solution SL_7 [Papanikolaou et al. 2000], at amount 2 cm^3/dm^3 and indicator of pH – 0.1% bromocresol purple (2 cm^3/dm^3). The initial pH of culture medium was 7.05.
Culture media were sterilized at 121°C for 20 minutes.

Fermentation parameters

The inoculum was grown in the RCM medium. Precultivation was carried out in Hungate tube (10 ml). Precultured bacteria were inoculated to the fermentation medium containing either pure or crude glycerol. The pH (7.0) was controlled manually in certain time intervals (every 10 hours) using 20% NaOH and the pH indicator – 0.1% bromocresol purple. The fermentations were carried in Hungate tube (10 ml) in the culture chamber for anaerobes (Whitley MG500 by Scientific) at the temperature of 32°C. Samples were taken every 10 hours.

Analytical Methods

In order to obtain supernatants cultures of analyzed strains were centrifuged for 10 min at 10000 x g. The cell free supernatants was collected and used for estimation of 1,3-PD production and other by-products (butyric acid, acetic acid, formic acid, lactic acid, succinic acid, ethanol) production via high liquid performance chromatography (HPLC) technique. In the experiments Hewlett Packard system consisted of autosampler, pump, oven and refractive index detector was carried out. Analysis were performed isocratically at flow rate 0.6 ml/min. at 65°C, on column Aminex HPX-87H 300 mm x 7.8 mm (BIO-RAD), with 0.25 mM H_2SO_4 as a mobile phase. Standards were applied to identify peaks in chromatograms, and peak areas were used to determine the concentration (ChemStation, Agilent).

RESULTS AND DISCUSSION

Synthesis of 1,3-PD is one of the promising ways of biotechnological conversion of crude glycerol [Zeng, Sabra 2011, Posada et al. 2012]. The results presented in Table 3 showed that the efficient synthesis of 1,3-PD both from pure and crude glycerol was observed.

Table 3. The final products and the parameters of microbial synthesis of 1,3-PD from pure and crude glycerol

Tabela 3. Produkty końcowe i parametry mikrobiologicznej syntezy 1,3-propanodiolu z czystego i odpadowego glicerolu

Raw material Surowiec	Pure glycerol Czysty glicerol		Crude glycerol A Glicerol odpadowy A		Crude glycerol B Glicerol odpadowy B	
Strain Nazwa szczepu	C. butyricum					
	DSP1	DO14	DSP1	DO14	DSP1	DO14
1,3-PD [g/dm^3]	36.2±1.22	34.63±1.32	31.07±1.07	26.23±1.02	21.89±0.92	23.72±1.01
Butyric acid [g/dm^3] Kwas masłowy	7.51±0.51	6.88±0.47	4.81±0.21	6.35±0.42	3.14±0.16	6.61±0.36
Acetic acid [g/dm^3] Kwas octowy	4.03±0.22	3.36±0.16	2.71±0.11	3.05±0.19	1.81±0.08	2.11±0.09
Formic acid [g/dm^3] Kwas mrówkowy	0.93±0.06	0.85±0.09	0.51±0.05	0.73±0.11	0.31±0.03	0.88±0.10
Lactic acid [g/dm^3] Kwas mlekowy	3.34±0.12	0.52±0.11	2.66±0.31	0.08±0.02	1.56±0.11	0.14±0.03
Succinic acid [g/dm^3] Kwas bursztynowy	0.31±0.01	0.20±0.03	0.19±0.02	0.96±0.11	0.13±0.01	0.43±0.06
Ethanol [g/dm^3] Etanol	1.87±0.09	1.15±0.04	0.76±0.02	1.97±0.08	0.55±0.03	3.02±0.09
Total time of fermentation [h] Czas całkowity fermentacji	92	72	79	72	71	72
Utylization of glycerol [%] Wykorzystanie glicerolu	100.0	100.0	100.0	99.66	89.0	99.71
Yield [$mol_{1,3\text{-}PD}/mol_{glycerol}$] Wydajność	0.66	0.63	0.56	0.47	0.42	0.42

Both *C. butyricum* DSP1 and *C. butyricum* DO14 allowed to obtain high concentrations of 1,3-PD as well as the utilization of glycerol at nearly of 90–100%. The highest concentration of 1,3-PD 36.2 g/dm^3 and 34.6 g/dm^3 was achieved with pure glycerol for *C. butyricum* DSP1 and *C. butyricum* DO14, respectively. Lower values obtained for crude glycerol B, can be explained by higher content of impurities as compared to the crude glycerol A. In *Clostridium* strains two products are formed in addition to 1,3-PD. They are acetic and butyric acids. Butyric acid is formed after condensation of two molecules of acetyl-CoA in a reaction chain that involves two NADH-oxidizing steps and the generation of ATP. Kusharyoto et al. [2011], who investigated the utilization of both, pure and crude glycerol for the microbial conversion of glycerol to 1,3-PD used *C. butyricum* p50b1, and obtained 81% utilization of both materials. 1,3-PD concentration for pure and crude glycerol was equal to 33.9 and to 31.7 g/dm^3, respectively. Wilkens et al. [2012] during fed-batch fermentation, in 1 dm^3 scale, with *C. butyricum* AKR102a strain obtained for pure and crude glycerol 93.7 and 76.2 g/dm^3 1,3-PD, respectively. Metsoviti et al. [2012 b]

during fed-batch fermentation, using the strain of *Citrobacter freundii* FMCC-B 294 with pure and crude glycerol they achieved 68.1 and 66.3 g/dm^3 of 1,3-PD, respectively. Venkataramanan et al. [2012] used *C. pasteurianum* ATCC 6013 strain, and received a slightly lower concentration of 1,3-PD for crude glycerol as a substrate in comparison with the results obtained for pure glycerol.

Chatzifragkou et al. [2011] with the strain of *C. butyricum* VPI 1718, showed no differences in the 1,3-PD concentrations obtained with crude or pure glycerol 11.1 and 11.5 g/dm^3, respectively. Jun et al. [2010] with bacterial strain *Klebsiella pneumoniae* DSM 4799 achieved a higher efficiency of 1,3-PD synthesis from crude glycerol as compared with pure glycerol. Inhibition effect of impurities from crude glycerol on microorganisms also seems to be strain-dependent phenomenon. It should also be noted that the composition of the crude glycerol varied and depended on both the technology in the production of biodiesel and the raw material used [Moon et al. 2010].

The type and concentration of impurities present in crude glycerol have a significant influence on the metabolism of microorganisms and on a final concentration of 1,3-PD (Table 3). Impurities, such as methanol, salts, fatty acids, and heavy metal ions, had a negative effect on the course of biochemical reactions in microorganisms [Chatzifragkou, Papanikolaou 2012]. Alcohol affected the cell microorganism membranes by increasing membrane's liquidity. The intensity of interaction depended on the length of the carbon chain and the concentration of aliphatic alcohol. Despite the fact that the bacteria possessed defense mechanism (involving the adjustment of the ratio of saturated and unsaturated fatty acids in the tail of the lipid bilayer of the membrane) when the limit of alcohol concentration exceed, that will have a negative influence on the growth and metabolism of the bacterial cells [Venkataramanan et al. 2012]. Impurities in the form of high concentration of monovalent salts caused swelling of the membrane by the weakening the Van der Waals forces in the tail of the lipid membrane. The salts effect on the energy barrier in the lipid layer lead to a change in biochemical processes [Petrache et al. 2006].

Biosynthesis of 1,3-propanediol from crude glycerol was current and an important issue in the valuation of raw material so as to use it in industry. Biotechnological production of 1,3-PD in by-product of biodiesel production, namely crude glycerol is a promising and encouraging alternative to the traditional chemical synthesis.

CONCLUSIONS

The strains *C. butyricum* DSP1 and *C. butyricum* DO14 were enabled to convert efficiently crude glycerol to 1,3-propanediol. The next step should be study on the effects of impurities present in the crude glycerol on synthesis of 1,3-PD to a greater extent.

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MIKROBIOLOGICZNA KONWERSJA CZYSTEGO I ODPADOWEGO GLICEROLU DO 1,3-PROPANODIOLU

Streszczenie. 1,3-propanodiol (1,3-PD) jest ważnym związkiem chemicznym stosowanym w przemyśle chemicznym. Mikrobiologiczna synteza 1,3-PD z wykorzystaniem odpadowego glicerolu jest atrakcyjnym rozwiązaniem zarówno z ekonomicznego, jak i ekologicznego punktu widzenia. Celem niniejszej pracy było zbadanie wpływu surowca (czystego i odpadowego glicerolu) na efektywność syntezy 1,3-PD przez bakterie *Clostridium butyricum* DSP1 i DO14. Bakterie wyizolowano ze środowiska naturalnego. Końcowe stężenia 1,3-PD były zbliżone dla obydwu badanych szczepów. W przypadku odpadowych gliceroli stężenie 1,3-PD było nieco niższe niż dla czystego glicerolu. Zastosowanie odpadowych gliceroli nie pozwoliło na całkowitą utylizację glicerolu.

Słowa kluczowe: 1,3-PD, Clostridium spp., czysty glicerol, odpadowy glicerol

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