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Research Article

Biodegradation of deproteinized potato wastewater and glycerol during cultivation of *Rhodotorula glutinis* yeast

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ABSTRACT

Background: Deproteinized potato wastewater and glycerol are two by-products which are difficult to dispose. The objective of this study was to determine the ability of *Rhodotorula glutinis* to use glycerol and nitrogen compounds present in deproteinized potato wastewater and to evaluate the ability of simultaneous biodegradation of potato wastewater and glycerol via microbiological methods.
Results: It has been found that *R. glutinis* used glycerol and potato wastewater as a source of carbon and nitrogen, respectively. The highest degree of glycerol content (70.6%) reduction was found after cultivation of the investigated strain using a medium with 5% glycerol. In this medium, a significant reduction in the total protein content, estimated at 61%, was observed. The process of 72 h cultivation of yeast in a medium containing potato wastewater and 5% glycerol reduced the chemical oxygen demand (COD) more than 77%. Supplementation of media with high doses of glycerol (i.e. 20 and 25%) led to decreased metabolic activity in the yeast strain tested.
Conclusion: It has been found that there is a possibility of simultaneous biodegradation of potato wastewater and glycerol during the cultivation of *R. glutinis*.

1. Introduction

In many industrial units, production processes always generate by-products. For these by-products, no rational management concepts exist and their disposal under natural conditions may lead to a progressive degradation of the natural environment. Examples of such wastes are deproteinized potato wastewater generated during the production of potato starch and glycerol fraction occurring during the production of biodiesel. Problems arising during the disposal of these two by-products have led to the search for new biotechnological methods for their treatment.

Potato wastewater is the primary waste generated during the production of potato starch. It has been estimated that during the processing of 1000 tons of potatoes, about 600 m^3 of potato wastewater is produced [1]. Due to the high content of organic substances, potato wastewater from the production of potato starch creates waste disposal problems. To reduce the content of nitrogen compounds, potato-protein precipitation is performed during the thermal-acid coagulation process. Deproteinized potato wastewater contains 2.9–4.3% of dry matter on average, of which protein, sugar and fat (emulsified with water) contents are estimated at 0.93–1.57%, 0.5–0.8% and approximately 0.2%, respectively. It also contains significant amounts of minerals (about 1%), of which potassium (600 mg/L) and phosphorus (about 300 mg/L) are dominant. Moreover, this waste is characterized by a high value of chemical oxygen demand (COD), which is equal to at least 20,000 mg O_2/L [2]. Potato wastewater is mostly utilized during field sprinkling [3], which may lead to adverse eutrophication of water [2] and soil sealing [3] in the natural environment.

The main by-product generated during the production of biodiesel is a glycerol fraction. This consists mainly of pure glycerol (50–65%), and among the remaining components one can distinguish methanol, free fatty acids, mono- and diacylglycerols, phospholipids, tocopherols, colorants, soaps, water, and catalyst residues [4]. In 2012, biodiesel production was estimated at 23.40 million tons, of which about 40% was produced in the European Union, a leading manufacturer of this fuel for years. In comparison to 2009, the global production of biodiesel has increased by more than 30% [5]. It is expected that in the future, biodiesel production will increase, which in turn will generate higher amounts of waste glycerin. The process of refining crude glycerol to high purity glycerol is energy intensive and expensive; therefore, new methods for the disposal and management of this waste are emerging [6].

Biodegradation is an alternative method of decomposition of pollutants using living organisms [7]. The fundamental issue concerning biotechnological disposal is the selection of micro-organisms, which

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must decompose contaminants in a short time without producing toxic metabolites [8]. So far, molds have particularly been used for this purpose [9,10,11]. In this paper, an attempt was made to use *Rhodotorula glutinis* for simultaneous biodegradation of potato wastewater and glycerol.

R. glutinis (*syn. Rhodotorula gracilis*) yeasts are anamorphic stages of *Rhodosporidium toruloides* [12,13,14,15]. They are aerobes and their optimum growth temperature lies in the range of 20–40°C. They have the ability to use many substrates as a carbon source, e.g. glucose, galactose, sucrose, maltose, trehalose, ethanol, glycerol, or hexadecane [16]. *R. glutinis* yeasts are capable of the biosynthesis of various important compounds, including carotenoids [17], phenylalanine ammonia lyase (PAL) [18] and microbial lipids [19].

The objective of this study was to evaluate the ability of *R. glutinis* yeast to use nitrogen compounds present in deproteinized potato wastewater and glycerol, and to determine the possibility of simultaneous biodegradation of these two wastes via microbiological methods.

2. Materials and methods

2.1. Microorganism

R. glutinis LOCK 0051 yeast strain derived from the Pure Culture Collection of the Department of Biotechnology and Food Microbiology, Warsaw University of Life Sciences, constituted the biological material used for the study. Yeasts were stored on slants of YPD medium at 4°C and were subcultured every month.

2.2. Potato wastewater

Deproteinized potato wastewater was collected from the technological line of potato starch production (PEPEES S.A., Łomża, Poland). The waste was sterilized (117°C for 10 min, HICLAVE HG-80, HMC Europe) and stored at room temperature. To remove residual solids (starch and cellulose fibers) before use, potato wastewater was centrifuged at 12,900 × g for 10 min (Centrifuge 5804R, Eppendorf). The dry matter content (g/L) was determined by the gravimetric method through drying a certain volume of the sample to constant weight at 105°C for 24 h (SML 32/250, Zatmet). Total protein content was determined by the Kjeldahl method. Samples were subjected to burning in concentrated sulfuric (VI) acid with the addition of a catalyst (Büchi Digestion Unit K-435), followed by alkalinization and distillation with steam water (Büchi Distillation Unit K-355). The resulting product (g/L) was calculated per total protein content using a factor equal to 6.25. The content of reducing sugars (calculated per glucose) in potato wastewater was determined spectrophotometrically ($\lambda = 550$ nm) using 3,5-dinitrosalicylic acid [20]. The result was given in g/L. The rate of chemical-oxygen demand (g O_2/L) in potato wastewater was determined by the dichromate method using the Hach Lange cuvette tests in the Water Center of the Warsaw University of Life Sciences. All the obtained results are listed in Table 1.

2.3. Preparation of inoculum

To prepare inoculum, YPD medium containing glucose (20 g/L), peptone (20 g/L), and yeast extract (10 g/L) was used [21]. The initial active acidity was determined at 5.0 ± 0.1. Cultivation was carried out in flat-bottomed flasks containing 100 mL of the medium at 28°C for 24 h at 200 rpm/min (SM-30 Control, Edmund Bühler).

Table 1
Characteristics of potato wastewater after the process of thermal-acid coagulation (PEPEES S. A., Łomża, Poland).

Dry matter (g/L)	Total protein (g/L)	Reducing sugars (g/L)	COD (g O_2/L)
35.8 ± 0.5	9.0 ± 0.66	2.96 ± 0.13	37.4 ± 2.8

2.4. Control and experimental cultures

For submerged yeast cultures, two culture media were used, YPD of an optimal composition for yeasts [21] and non-enriched potato wastewater (PW). For experimental cultures, five experimental media were used containing glycerol as a carbon source (POCH, Poland) and potato wastewater as a source of nitrogen. Glycerol was added to the medium at an amount of 50, 100, 150, 200, and 250 g/L. In later parts of the experiment, culture media with glycerol were denoted by the following abbreviations: PW + G5%, PW + G10%, PW + G15%, PW + G20% and PW + G25%. Inoculation was estimated at 10% (v/v). Cultivation of yeast was carried out on a reciprocating shaker (SM-30 Control, Edmund Bühler) for 72 h, at a speed of 200 cycles/min and temperature equal to 28°C. The experiment was carried out in triplicate.

2.5. Growth characteristics of yeast

Yield of dry cellular biomass was determined by the gravimetric method. A specified volume of culture was centrifuged for 10 min at 6000 × g (Centrifuge 5804R, Eppendorf), and rinsed with distilled water. The wet-cell biomass was dried at 85°C (SML 32/250, Zatmet) to a constant weight. The result was given in $g_{d.w.}$/L.

The optical density of the culture (OD) was determined using the spectrophotometry technique. Two milliliters of culture medium were collected and centrifuged at 5000 g for 5 min (MiniSpin Plus, Eppendorf). Post-culture medium was removed and 2 mL of deionized water was added to the biomass, followed by careful stirring and centrifugation of the solution using the same parameters. The supernatant was decanted and the biomass was suspended in 2 mL of deionized water, followed by the measurement of absorbance of the cell suspension at a wavelength of $\lambda = 600$ nm (UV1800 spectrophotometer, Rayleigh) against deionized water.

2.6. Determination of the potential of yeast for biodegradation of wastewater

To determine the ability to use the added glycerol and the protein present in the potato wastewater, their concentrations were determined in the latter hours of cultivation, followed by the determination of COD in selected culture media.

Glycerol content was determined via the chemical method by using oxidative properties of *meta*-periodate acid. As a result of its activity toward two adjacent hydroxyl groups in a glycerol molecule, decomposition of the main carbon chain is observed. As a result, two molecules of formaldehyde and a formic acid molecule are formed. The latter is titrated with 0.1 M sodium hydroxide in the presence of bromothymol blue. The result was given in g/100 mL [22].

Total protein content in post-culture media was determined by the Kjeldahl method, using a conversion factor equal to 6.25. The results were expressed in g/100 mL.

The rate of chemical-oxygen demand in post-culture media, based on the conducted experiments, was determined using Hach Lange cuvette tests in the Water Center of the WULS and the results were expressed in g O_2/L.

The experiment was carried out in triplicate and the values represent in the figures and tables are mean ± SD. Based on the obtained results, the degree of utilization of glycerol and nitrogen compounds per total protein, as well as the degree of reduction of COD, were determined.

2.7. Statistical analysis of results

The results were analyzed in R statistical software (version i386 2.15.3). One-way analysis of the variance (one-way ANOVA) and Tukey's test at a significance level ($\alpha = 0.05$) were performed.

3. Results and discussion

3.1. Growth of R. glutinis in a medium with potato wastewater and glycerol

Based on the results of the optical density change of the culture and the biomass yield, it was found that *R. glutinis* yeasts could be grown in the control medium (YPD) and in all variants of experimental media tested. It can therefore be concluded that glycerol was used as a carbon source and potato wastewater as a nitrogen and minerals source.

Concentrations of compounds which constituted a carbon source acted as factors determining the growth of the yeast strain studied. After analyzing the results of measurements of the optical density of culture biomass yield, it may be concluded that the most favorable condition for the growth of *R. glutinis* was provided by media containing potato wastewater and glycerol at the concentrations of 5 and 10%, respectively. In both these cases, OD values of the biomass yield were significantly higher compared to the YPD control medium after 72 h (Fig. 1, Table 2). After 72 h of cultivation in PW + G5%, PW + G10% and YPD media, biomass yield was estimated at 20.34, 19.63 and 8.71 g $_{d.w.}$/L, respectively (Table 2). During cultivation, in medium containing 15% glycerol, intensive growth of the analyzed yeast strain was observed. After 72 h the value of OD reached the level of 2.4 (Fig. 1), and cell biomass yield was 15.08 g $_{d.w.}$/L. These values were slightly higher than those determined after the same time in the YPD control medium. Glycerol at the concentrations of 20 and 25% showed inhibitory effects toward the growth of the yeast strain studied. After 72 h, with such addition of glycerol, cellular biomass yield was estimated at only 6.06 and 4.22 g $_{d.w.}$/L. Yeast cultured in medium containing potato wastewater demonstrated the highest growth at the 24th h of cultivation. It can be assumed that by this time the cells had exhausted the residual simple sugars present in the potato wastewater (about 0.3%). After depletion of the carbon substrate, proliferation was inhibited and autolysis processes were intensified over time, as indicated by the decreasing OD value and biomass yield.

Schneider et al. [23] grew cultures of *R. glutinis* ATCC 15125 using media containing wastewater from the brewing industry. As a result of the cultivation which lasted for 168 h, biomass yields of 5.22 g $_{d.w.}$/L and 7.38 g $_{d.w.}$/L, respectively, were obtained in media containing wastewater from brewing industry and sewage enriched with 1% glucose. Similar low levels of yeast biomass yield of *R. glutinis* TISTR 5159 were reported by Saenge [24]. The authors used ammonium sulfate as a nitrogen source, and glycerol at the concentrations of 5%, 9.5% and 14% as a carbon source. Similarly, no significant differences were observed between the results obtained after 72 h of cultivation for media supplemented with 5% and 9.5% of glycerol addition (5.15–5.65 g $_{d.w.}$/L). However, the concentration of 14% was found to have an inhibitory effect on growth of the analyzed yeast strain (4.30 g $_{d.w.}$/L). The results of biomass yields cited in the above-mentioned papers were as much as four times lower than the results obtained in the present study (Table 2), which demonstrates the stimulating activity of the potato wastewater on *R. glutinis* growth.

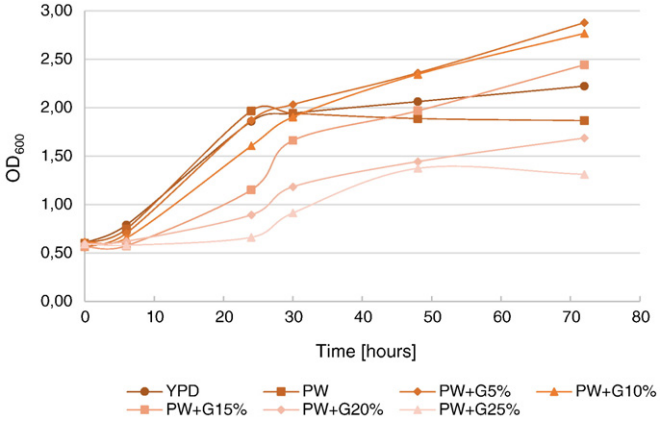

Fig. 1. Changes in optical density of *R. glutinis* yeast in the control and experimental media.

Table 2
Changes in the yield of *R. glutinis* yeast cell biomass during the cultivation in control and experimental culture media. The values of the standard deviation were considered and the results of Tukey's test ($\alpha = 0.05$) within the same hours of cultivation (a, b, c, d, e-indexes denote homogeneous groups) were reported.

Medium	Biomass yield [g $_{d.w.}$/L]			
	0 h	24 h	48 h	72 h
YPD	0.55 ± 0.07^{a}	4.91 ± 0.27^{b}	8.46 ± 0.75^{b}	8.71 ± 0.49^{c}
PW	0.58 ± 0.03^{a}	6.49 ± 0.52^{a}	5.27 ± 0.41^{c}	4.71 ± 0.35^{e}
PW + G5%	0.44 ± 0.12^{a}	6.34 ± 0.38^{a}	12.69 ± 0.74^{a}	20.34 ± 1.08^{a}
PW + G10%	0.50 ± 0.05^{a}	4.71 ± 0.69^{b}	12.33 ± 0.62^{a}	19.63 ± 0.97^{a}
PW + G15%	0.48 ± 0.06^{a}	2.11 ± 0.15^{c}	7.71 ± 0.83^{b}	15.08 ± 0.83^{b}
PW + G20%	0.44 ± 0.07^{a}	0.93 ± 0.21^{d}	3.60 ± 0.41^{d}	6.06 ± 0.51^{d}
PW + G25%	0.58 ± 0.03^{a}	0.84 ± 0.17^{d}	2.38 ± 0.36^{e}	4.22 ± 0.25^{e}

3.2. Determination of biodegradation potential of R. glutinis yeast

R. glutinis yeast demonstrated the ability to utilize glycerol as a carbon source. After 72 h of cultivation in PW + G5% medium, the glycerol content decreased to 1.5%. The total degree of utilization of glycerol was above 70%, while in terms of other experimental cultures, it did not exceed 35% (Table 3). Based on the results obtained, it was found that the microbial biodegradation process of glycerol by *R. glutinis* runs most efficiently, if its concentration in the environment is not higher than 5%.

Different results for the degree of reduction for the same concentrations of glycerol in media with potato wastewater were obtained by Błażejak et al. [25]. They used a *R. gracilis* yeast strain derived from the Pure Culture Collection of the University of Life Sciences in Lublin. In medium supplemented with 20% of carbon substrate yeast utilized almost 59% of its content. This indicates that tested in this work *R. glutinis* yeast strain exhibit higher sensitivity to increased glycerol content in the environment.

The maximum reduction of nitrogen compounds from the medium was detected during the cultivation in media supplemented with glycerol at concentrations of 5 and 10%, respectively. After 72nd h of cultivation in PW + G5% medium, over 0.3 g/100 mL of total protein still remained, and the degree of reduction was estimated at 61%. If cultivation was conducted using PW + 10% medium after 72 h, total protein content was reduced to 0.3 g/100 mL, and the total degree was 63%. In terms of cultivations conducted in media supplemented with the highest doses of glycerol, i.e. 20 and 25%, degree of reduction

Table 3
Changes in the content of glycerol in the experimental media after inoculation and after 72 h of submerged cultivation, and total degree of utilization of glycerol by *R. glutinis*.

Medium	Concentration of glycerol in medium (g/100 mL)		Glycerol use (%)
	0 h	72 h	
PW + G5%	5.14 ± 0.12	1.51 ± 0.06	70.62
PW + G10%	10.26 ± 0.08	6.85 ± 0.15	33.23
PW + G15%	14.92 ± 0.04	13.11 ± 0.20	12.13
PW + G20%	19.73 ± 0.21	19.33 ± 0.34	2.03
PW + G25%	24.82 ± 0.25	24.64 ± 0.18	0.72

of protein content was negligible, and was estimated at 15 and 9%, respectively (Fig. 2).

It was found that the concentration of carbon substrates determined the metabolic activity of the yeast strain tested. At its high concentrations (20% and 25%), yeasts practically did not utilize nutrients present in the medium, which could be a result of the increased osmotic pressure of the environment. The osmotic pressure of the culture medium is in fact one of the most important parameters determining microbial growth and propagation [26]. Glycerol, as a substance characterized by a high viscosity [27], may also, to some extent, adhere to yeast cells, hampering the use of nutrients present in the environment. In medium containing only potato wastewater, yeasts did not efficiently use nitrogen compounds due to the low content of simple sugars constituting a source of carbon.

In the last part of the study, chemical rate of oxygen demand was determined in the selected post-culture media. Cultivation carried out in a medium containing only the potato wastewater led to a reduction of the COD of 37.4 ± 2.8 to 17.38 ± 1.75 g O_2/L after 72 h of process (53% reduction rate). Nowak et al. [11] found that *Aspergillus niger* 334 and *Rhizopus oligosporus* 2710 mold strains were capable of reducing the COD value of the potato wastewater by 58 and 52%, respectively, during the 72 h of cultivation.

The chemical-oxygen demand indicator was also determined in the medium with potato wastewater supplemented with 5% of glycerol (99.52 ± 3.63 g O_2/L), because in this medium yeasts showed highest growth and metabolic activity (Fig. 3). It was observed that 72 h cultivation of *R. glutinis* yeast in the PW + G5% medium caused reduction of the chemical oxygen demand indicator by 77% and the value of the COD to 22.58 ± 1.20 g O_2/L. This amount was higher than in PW, what was probably a result of the glycerol residues. Because of the higher degree of utilization of nitrogen compounds from the medium PW + G5%, it can be concluded that cultivation yeast in medium containing additional carbon source has enabled more efficient use of ingredient potato wastewater. The cultivation in the medium PW + G5% time should be extended for another day to metabolize glycerol completely.

4. Conclusions

R. glutinis yeasts exhibited the ability to metabolize protein substances in the deproteinized potato wastewater, and to assimilate carbon originating from glycerol for cell biomass proliferation. It was found that there is a possibility of simultaneous biodegradation of these two wastes via microbiological methods. The efficiency of waste disposal was dependent on the concentration of compounds

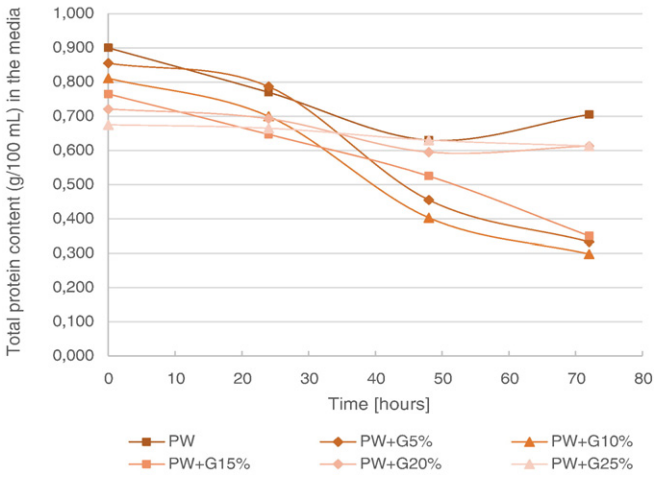

Fig. 2. Changes in the content of total protein in the control and experimental media during cultivation of submerged *R. glutinis* yeast.

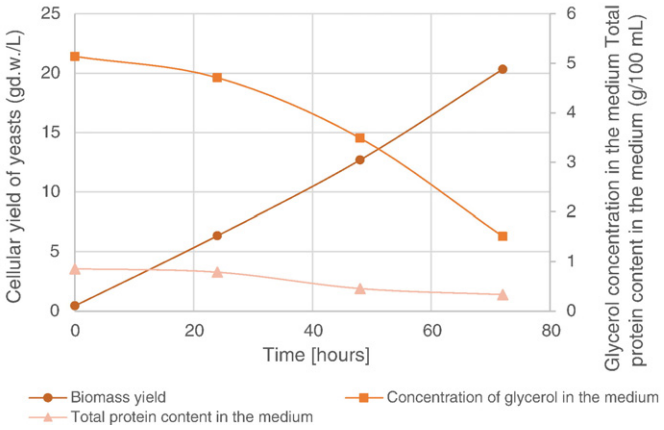

Fig. 3. Changes in cellular yield and the content of glycerol and total protein during 72 h of submerged *R. glutinis* cultivation in the PW + G5% medium.

constituting a source of carbon for yeasts. The enrichment of potato wastewater in an additional carbon source at the level of 5% allowed the efficient use of glycerin (70%) and nitrogen substances per protein amount (61%) and caused a significant decrease in COD (77%). High doses of glycerol inhibited growth and metabolism of the yeast strain studied, which resulted in the reduction of nutrient utilization from the environment.

From the literature data, it can be concluded that *R. glutinis* yeasts are capable of synthesis of intracellular metabolites, such as fat and carotenoids, and this may find an application in the food industry. Future studies should involve the determination of these two components in yeast cell biomass obtained after cultivation in media prepared from these waste materials.

Conflict of interests

The authors declare no conflict of interest.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

References

[1] Bzducha-Wróbel A, Błażejak S, Molenda M, Reczek L. Biosynthesis of β (1,3)/(1,6)-glucans of cell wall of the yeast *Candida utilis* ATCC 9950 strains in the culture media supplemented with deproteinated potato juice water and glycerol. Eur Food Res Technol 2015;240:1023–34. http://dx.doi.org/10.1007/s00217-014-2406-6.

[2] Lubiewski Z, Śmigielska H, Lewandowicz G, Balcerek W. Charakterystyka odcieku po koagulacji białka pozyskiwanego w toku kampanii krochmalniczej. Zesz Probl Postep Nauk Rol 2006;511:617–26 [in Polish].

[3] Kosiek E. Applicability of potato juice in baker's yeast production. Zeszyty Naukowe Politechniki Łódzkiej Technologia Chemia Spożywcza 1993;648:31–41 [in Polish].

[4] Gaca J. Faza glicerynowa po produkcji biodiesla - odpad czy cenny surowiec? Czysta Energia 2006;11:34–5 [in Polish].

[5] Oil World. ISTA Mielke GmbH. http://www.oilworld.biz; 2015. [cited March 15, 2015, Available from Internet].

[6] Yen HW, Yang YC, Yu YH. Using crude glycerol and thin stillage for the production of microbial lipids through the cultivation of *Rhodotorula glutinis*. J Biosci Bioeng 2012; 114:453–6. http://dx.doi.org/10.1016/j.jbiosc.2012.04.022.

[7] Miranda RC, Souza CS, Gomes EB, Lovaglio RB, Lopes CE, Souza MFVQ. Biodegradation of diesel oil by yeasts isolated from the vicinity of Suape port in the state of Pernambuco–Brazil. Braz Arch Biol Technol 2007;50:147–52. http://dx.doi.org/10.1590/S1516-89132007000100018.

[8] Romero M, Reinoso E, Kiernan AM, Urrutia M. Chlorinated biphenyl degradation by wild yeasts pre-cultured in biphasic systems. Electron J Biotechnol 2006;9. http://dx.doi.org/10.2225/vol9-issue3-fulltext-12.

[9] Sinegani AAS, Emtiazi G, Hajrasuliha S, Shariatmadari H. Biodegradation of some agricultural residues by fungi in agitated submerged cultures. Afr J Biotechnol 2005;4: 1058–61.

[10] Sánchez C. Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnol Adv 2009;27:185–94. http://dx.doi.org/10.1016/j.biotechadv.2008.11.001.

[11] Nowak J, Górna B, Nowak W. Applying filamentous fungi to biodegradation of wastewater from potato industry with simultaneous production of mould biomass for forage. Zywnosc 2013;6:191–203. http://dx.doi.org/10.15193/zntj/2013/91/191-203 [in Polish].
[12] Buck JW, Andrews JH. Attachment of the yeast *Rhodosporidium toruloides* is mediated by adhesives localized at sites of bud cell development. Appl Environ Microbiol 1999;65:465–71.
[13] Höfer M, Misra PC. Evidence for a proton/sugar symport in the yeast *Rhodotorula gracilis* (*glutinis*). Biochem J 1978;172:15–22.
[14] Yoon SH, Rhee JS. Lipid from yeast fermentation: Effects of cultural conditions on lipid production and its characteristics of *Rhodotorula glutinis*. J Am Oil Chem Soc 1983;6:1281–6. http://dx.doi.org/10.1007/BF02702101.
[15] Zhu Z, Zhang S, Liu H, Shen H, Lin X, Yang F, et al. A multi-omic map of the lipid-producing yeast *Rhodosporidium toruloides*. Nat Commun 2012;3:1112–23. http://dx.doi.org/10.1038/ncomms2112.
[16] Kurtzman CP, Fell JW. The yeasts, a taxonomic study. 4th ed. Amsterdam: Elsevier; 1998 814–5.
[17] Tinoi J, Rakariyatham N, Deming RL. Simplex optimization of carotenoid production by *Rhodotorula glutinis* using hydrolyzed mung bean waste flour as substrate. Process Biochem 2005;40:2551–7. http://dx.doi.org/10.1016/j.procbio.2004.11.005.
[18] Takac S, Akay B, Ozdamar TH. Bioconversion of *trans*-cinnamic acid to L-phenylalanine by L-phenylalanine ammonia-lyase of *Rhodotorula glutinis*: Parameters and kinetics. Enzyme Microb Technol 1995;17:445–52. http://dx.doi.org/10.1016/0141-0229(94)00072-Y.
[19] Easterling ER, French WT, Hernandez R, Licha M. The effect of glycerol as a sole and secondary substrate on the growth and fatty acid composition of *Rhodotorula glutinis*. Bioresour Technol 2009;100:356–61. http://dx.doi.org/10.1016/j.biortech.2008.05.030.
[20] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426–8. http://dx.doi.org/10.1021/ac60147a030.
[21] Suizu T, Tsutsumi H, Kawado A, Murata K, Suginami K, Imayasu S. Methods for sporulation of industrially used sake yeasts. J Ferment Bioeng 1996;81:93–7. http://dx.doi.org/10.1016/0922-338X(96)87583-0.
[22] Polish standard BN-76/6026-02. Gliceryna surowa. (in Polish).
[23] Schneider T, Graeff-Hönninger S, French WT, Hernandez R, Merkt N, Claupein W, et al. Lipid and carotenoid production by oleaginous red yeast *Rhodotorula glutinis* cultivated on brewery effluents. Energy 2013;61:34–43. http://dx.doi.org/10.1016/j.energy.2012.12.026.
[24] Saenge C, Cherisilp B, Suksaroge T, Bourtoom T. Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from biodiesel plant to lipids and carotenoids. Process Biochem 2011;46:210–8. http://dx.doi.org/10.1016/j.procbio.2010.08.009.
[25] Błażejak S, Gientka I, Bzducha-Wróbel A, Stasiak-Różańska L, Maszewska M. Evaluation of the ability of the intracellular fat biosynthesis by *Rhodotorula gracilis* yeast in media containing potato wastewater enriched with glycerol. Zesz Probl Postep Nauk Rol 2014;576:3–12 [in Polish].
[26] Sochocka M, Boratyński J. Osmoregulacja - ważny parametr rozwoju bakterii. Postepy Hig Med Dosw 2011;65:714–24. http://dx.doi.org/10.5604/17322693.966604 [in Polish].
[27] Quispe CAG, Coronado CJR, Carvalho JA. Glycerol: Production, consumption, prices, characterization and new trends in combustion. Renew Sust Energ Rev 2013;27:475–93. http://dx.doi.org/10.1016/j.rser.2013.06.017.