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ORIGINAL PAPER

Evaluation of waste products in the synthesis of surfactants by yeasts‡

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The highest yields of biosurfactants were obtained by: (i) *Pseudozyma antarctica* (107.2 g L^{-1}) cultivated in a medium containing post-refining waste; (ii) *Pseudozyma aphidis* (77.7 g L^{-1}); and (iii) *Starmerella bombicola* (93.8 g L^{-1}) both cultivated in a medium with soapstock; (iv) *Pichia jadinii* (67.3 g L^{-1}) cultivated in a medium supplemented with waste frying oil. It was found that the biosurfactant synthesis yield increased in all strains when the cell surface hydrophobicity reached 70–80 %, enabling the microbial cells to make good contact with hydrophobic substrates. The lowest surface tension of the post-cultivation medium was from 32.0 mN m^{-1} to 37.8 mN m^{-1}. However, this parameter (which was also determined by a drop collapse assay) was of limited use in monitoring biosurfactant synthesis in this study. The crude glycerol was not a good substrate for biosurfactant synthesis although, in the case of *P. aphidis*, 67.4 g L^{-1} of biosurfactants were obtained after cultivation in the medium supplemented with glycerol fraction (GF2). In a low-cost medium containing soapstock and whey permeate or molasses, about 90 g L^{-1} of mannosylerythritol lipids were synthesised by *P. aphidis* and approximately 40 g L^{-1} by *P. antarctica*.

Keywords: biosurfactants, waste lipids, glycerol, glycolipids, surface tension

Introduction

Transparency Market Research valued the global biosurfactant market at $ 1735.5 million in 2011; in 2018 it is expected to reach $ 2210.5 million, growing at a compound annual growth rate (CAGR) of 3.5 % from 2011 to 2018. In the overall global market, the European region is expected to maintain its leading position in terms of volume and revenue up to 2018. Europe is expected to achieve 53.3 % of global biosurfactant market revenue share in 2018, followed by North America (Transparency Market Research, 2012).

Biosurfactants can play an important role in industry because of their many valuable physico–chemical properties and biological activities (Cameotra & Makkar, 2004). These biological compounds can be used in the agricultural, cosmetic, detergent, pharmacological, medical, food, and paint industries (Rodrigues et al., 2006, Singh et al., 2007). Furthermore, biosurfactants have their uses in agriculture, especially in the formulation of herbicides and pesticides (adjuvants) to disperse active compounds in an aqueous solution. Biosurfactant applications in the environmental industries associated with environmental protection and waste management have received more attention owing to their biodegradability, low toxicity, and effectiveness in enhancing the biodegradation and solubility of hydrophobic compounds (Calvo et al., 2009).

Surfactants synthesised by microorganisms can be classified by their location in cells, chemical structure, and molecular mass (Rosenberg & Ron, 1999). The biochemical pathways of biosurfactant synthesis are diverse and generally can be divided into either de novo or carbon substrate-dependent synthesis. In the latter case, changes in the medium composition in-

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fluence the chemical composition of the biosurfactant (Bednarski et al., 2006; Thanomsub et al., 2007). The diverse structures of biosurfactants allow them to possess different properties and to play different roles. The synthesis of surfactants by microorganisms is usually performed in order to increase both the bioavailability of almost inaccessible substrates and their survival in low-water conditions or in environments containing heavy metals and antibiotics. Biosurfactants play an essential natural role in the swarming motility of microorganisms and participate in the cellular physiological processes of signalling and differentiation as well as in biofilm formation (Van Hamme et al., 2006). However, the physiological role of biosurfactants remains unclear because it was demonstrated that biosurfactants could also be synthesised by microorganisms under optimum growth conditions.

The synthesis of biosurfactants usually requires a hydrophobic and hydrophilic carbon source in the cultivation medium. The process is economically and environmentally attractive if it uses waste products. Following Makkar et al. (2011), the perfect scenario would be to have biosurfactants priced at $ 4–10 per kg. Production of waste fat, a renewable material has increased; this can be used as a substrate for processing after hydrolysis (Willke & Vorlop, 2004). Increased biodiesel production requires methods for valorising glycerol, a by-product generated during transesterification (Coombs, 2007; Pagliaro et al., 2009), but few publications present the possibility to utilise waste glycerol for biosurfactant synthesis (Giannopoulos et al., 2011; Morita et al., 2007a).

The aim of the current study was to evaluate biosurfactant synthesis by selected yeast strains in a media containing waste lipids, post-refining products or a crude glycerol, as well as in low-cost media containing selected waste products and whey permeate or molasses.

Experimental

The strains and waste products

The cultivation of *Pseudozyma antarctica* ATCC 28323 (*Candida antarctica*), *Pseudozyma aphidis* DSM 70725, *Starmerella bombicola* (*Candida bombicola*) ATCC 70163, and *Pichia jadinii* DSM 70163 was performed by the submerge-shaking method in a YPD medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} glucose) (Merck, Poland) containing 8 different waste products (Table 1). The waste products were obtained from local companies.

The amount of waste products in the medium was established by preliminary experiments (data not presented). The waste products were characterised by the determination of acid, iodine, and peroxide values based on standard procedures. The total amount of lipids in the waste products was determined by the Folch extraction method (Folch et al., 1957). The free glycerol content in the glycerol fractions was determined using an enzymatic test (Sigma–Aldrich, USA).

All chemicals used in the experiments were the highest purity and purchased from Polish Chemical Reagents (Poland).

Selection of conditions for biosurfactant synthesis

The yeast culture was stored at 4 ℃ in a YPD medium with agar (20 g L^{-1}), pH 4.5. The inoculum was prepared in a 500 mL Erlenmeyer flask containing 100 mL of the YPD medium, sterilised at 121 ℃ for 20 min. The cultivation was performed at 30 ℃ and 300 min^{-1}, for 24–48 h in an incubator shaker G–25 (New Brunswick Scientific, USA). The inoculum was standardised based on the optical density determination at 650 nm and added to the cultivation medium in the amount of 100 mL L^{-1} of the medium. The synthesis of biosurfactants was performed for 7 days under the conditions as for inoculum preparation and the samples were collected at the end of cultivation. The reference culture was prepared by cultivation of the yeasts in a YPD medium without waste products.

Kinetics of biosurfactant synthesis

The kinetics of biosurfactant synthesis was analysed under optimal conditions chosen during the selection procedure over 10 days of cultivation of *P. antarctica*, *P. aphidis*, *S. bombicola*, *P. jadinii* in the YPD medium supplemented with: PostRW, soapstock, soapstock, WFO, respectively. To analyse the influence of hydrophobic and hydrophilic compounds on biosurfactant synthesis, the cultivation was also performed in the YPD medium and YP (without dextrose) medium with selected waste products.

Biosurfactant synthesis was also analysed by determining the parameters listed below during the cultivation of *P. antarctica* or *P. aphidis* (the best producers of biosurfactants) in a cultivation medium containing PostRW or soapstock, respectively, and whey permeate or molasses (10 g L^{-1} of lactose or saccharose).

The cultures were prepared as indicated previously and samples were collected aseptically every 24 h.

Analytical methods

The yeast culture was analysed by determining the following parameters: biomass concentration (Y; g of dry matter per L), concentration of glycolipids by the extraction method with ethyl acetate (B; g L^{-1}), cell hydrophobicity (HPH; %), and diameter of the medium drop (DMD; mm), pH, and surface tension (σ; mN m^{-1}) of the medium.

Biomass concentration

The biomass was separated from the medium by centrifugation (4 ℃, 5000*g*, 10 min), washed with ethanol/butanol/chloroform mixture ($\varphi_r = 10 : 10 : 2$) and deionised water. The biomass concentration was determined gravimetrically after overnight drying at 105 ℃.

Glycolipid concentration

The glycolipid assay was carried out by triple extraction with ethyl acetate from the cultivation medium, without biomass separation (Smyth et al., 2010). The excess lipid carbon sources were removed by washing the extract three times with hexane. The solvents were removed in a rotor vacuum evaporator at 50 ℃, 24 kPa. The crude biosurfactants containing mainly extra-cellular (but also intra-cellular) compounds were determined gravimetrically.

Cell hydrophobicity assay

Cell hydrophobicity was determined by the microbial adhesion to hydrocarbon method (MATH) using the procedure described by Rosenberg et al. (1980). After centrifugation, the cells were washed with PUM buffer (19.70 g L^{-1} K_2HPO_4, 7.26 g L^{-1} KH_2PO_4, 1.80 g L^{-1} urea (H_2NCONH_2), and 0.20 g L^{-1} of $MgSO_4 \cdot 7H_2O$) and the optical density (A_0) of the suspension was adjusted to 1.0–1.5 at 600 nm. 0.4 mL of tetrahydrofuran (THF) was added to 2 mL of the cell suspension; after 10 min of incubation at 30 ℃ the sample was vortex-mixed (3000 min^{-1}) for 2 min. After 15 min, the optical density of the aqueous phase was measured (A_1). The degree of hydrophobicity (HPH) was calculated by using Eq. (1):

$$\mathrm{HPH} = (1 - (A_0 - A_1)/A_0) \times 100\ \% \qquad (1)$$

Surface activity determination

The surface activity of the medium was determined by the ability to collapse medium droplets on the hydrophobic surface of Parafilm® M laboratory film (Kuiper et al., 2004). The diameter of the medium drop (50 μL) on Parafilm® M was determined using AnalySIS software (Soft Imaging System; Olympus, Poland).

The surface tension was quantified at 25 ℃ with the Du Noüy ring method using a K-9 tensiometer (Krüss, Germany).

Microscopic observation of intracellular glycolipids

The content of intracellular glycolipids was confirmed by microscopic observation with Nile red (Sigma–Aldrich) as a fluorescence probe (Pinzon et al., 2011; Siloto et al., 2009). Nile red solution in acetone (500 μg mL^{-1}) was added to a cell suspension to a final concentration of 0.1 μg mL^{-1}. After 5 min of incubation at 37 ℃, the cells were separated by centrifugation and washed with saline solution. Fluorescence microscopy was carried out using an Olympus BX51 microscope equipped with a fluorescence filter cube (460–495 nm excitation filter and a 505 nm dichroic mirror, emission filter 510 nm) and a cooled charge-coupled device camera (CCD) using objective lens (magnification × 100).

The results of all experiments are the mean of (at least) triplicate experiments and the standard deviation did not exceed 5 % of the recorded value.

Results and discussion

The synthesis of biosurfactants in the medium supplemented or prepared based on the waste products could render the process economically and environmentally attractive (Makkar et al., 2011). The synthesis of sophorolipids by *S. bombicola*, mannosylerythritol lipids by *P. antarctica*, or *P. aphidis* was reported previously, e.g. in a medium with waste frying oil (Fleurackers, 2006), poultry fat (Adamczak & Bednarski, 2000a, 2000b), post-refinery fatty acids (Bednarski et al., 2004), and fatty acid residues (Felse et al., 2007).

Selection of conditions for biosurfactant synthesis

The waste products for the experiments were chosen based on availability in Poland and were not subjected to any purification or separation procedures prior to use. The utilisation of waste products can cause problems with the reproducibility of the process conditions, as their composition could differ significantly. The acid value and amount of lipids extracted by the Folch method in the waste products were 7.3–88.6 mg of KOH per g and 8.9–263.1 g L^{-1}, respectively (Table 1). The lipid-rich waste products used for the synthesis of biosurfactants are frequently not characterised due to the complex chemical composition. The crude glycerol used by Papanikolaou et al. (2004) for the synthesis of 1,3-propanediol consisted of 650 g kg^{-1} of glycerol and the impurities of the industrial feedstock were largely composed of potassium and sodium salts (40–50 g kg^{-1}), methanol (10 g kg^{-1}), heavy metals–lignin (10 g kg^{-1}), non-glycerol organic materials (5 g kg^{-1}), and water (280 g kg^{-1}). Crude glycerol used for the production of citric acid and erythritol was obtained from a pilot plant installation producing ethyl ester of fatty acids and contained 550 g L^{-1} of glycerol and 50 g L^{-1} of KCl (Rymowicz et al., 2008).

The diverse composition of the waste products

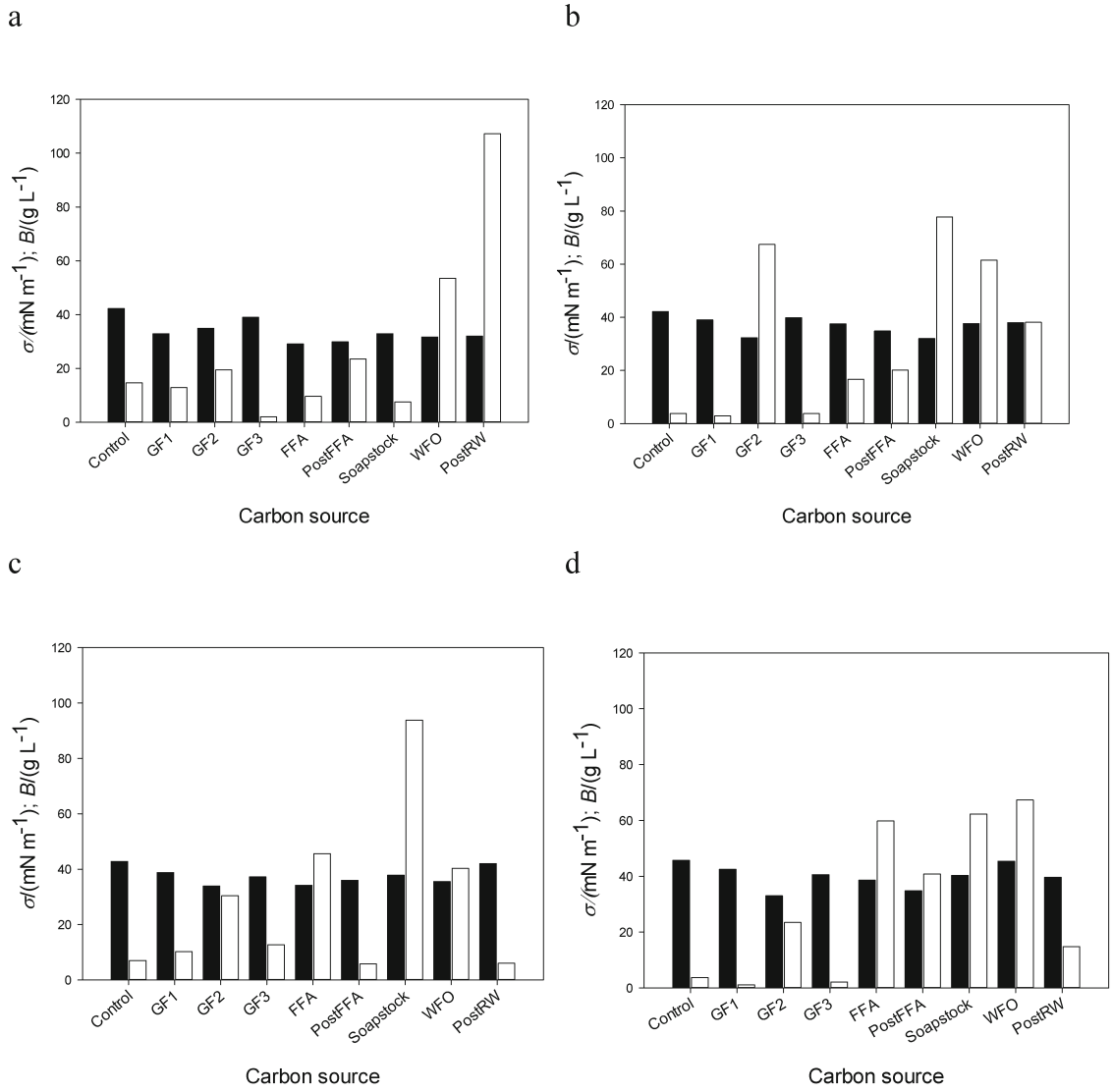

Fig. 1. Influence of carbon source in the medium (■) on surface tension value (σ) and concentration (□) of biosurfactants (B) by *P. antarctica* (a), *P. aphidis* (b), *S. bombicola* (c), and *P. jadinii* (d) (30 °C, 168 h); GF1–GF3 – glycerol fractions, FFA – free fatty acids, PostFFA – post-refining fatty acids, WFO – waste frying oil, PostRW – post-refining waste.

influenced the results of the preliminary selection (Fig. 1). The highest yields of biosurfactants were obtained by: (i) *P. antarctica* (107.2 g L^{-1}) cultivated in a medium containing post-refining waste; (ii) *P. aphidis* (77.7 g L^{-1}); and (iii) *S. bombicola* (93.8 g L^{-1}), both cultivated in a medium with soapstock; and (iv) *P. jadinii* (67.3 g L^{-1}) cultivated in a medium supplemented with waste frying oil. The separated raw extracts of biosurfactants were a mixture of glycolipids and contained other lipid compounds (data not presented).

These hydrophobic compounds from the waste induced the synthesis of biosurfactants more effectively than the waste glycerol. There were also preliminary indications that *P. jadinii* synthesised glycolipids like the other yeasts used, which are well-known producers of mannosylerythritol lipids or sophorolipids (Desai & Banat, 1997).

Although the physiological role of biosurfactants is not fully understood, they emulsify water-insoluble substrates; this increases the interfacial surface area and improves the mass transfer (Kitamoto et al., 2002). For all the strains tested, the synthesis of biosurfactants was detected when only glucose was used as a carbon source (3.8–14.6 g L^{-1}) (Fig. 1). These observations are in accordance with data in the literature on the synthesis of biosurfactants from hydrophilic substrates (mainly carbohydrates), e.g. glucose or lactose (Adamczak & Bednarski, 2000a, 2000b) and sugarcane molasses (Takahashi et al., 2011).

Utilisation of the glycerol fractions in the culture medium resulted in synthesis of the biosurfactants in concentrations of 1.1 g L^{-1} to 67.4 g L^{-1} (Fig. 1). A waste glycerol product (GF2), was selected from wastes containing glycerol as the best suited for the synthesis of biosurfactants, probably because of the

Table 1. Selected parameters of waste products used for biosurfactant synthesis

Waste products	Waste concentration in medium	Acid value	Lipid concentration
	g L^{-1}	mg of KOH per g	g L^{-1}
Glycerol fraction (GF1)	100	69.5	14.5
Glycerol fraction (GF2)	100	11.8	41.4
Glycerol fraction (GF3)	100	88.6	8.9
Free fatty acids (FFA)	200	29.2	671.2
Post-refining fatty acids (PostFFA)	200	34.8	527.1
Soapstock	200	7.9	795.5
Waste frying oil (WFO)	100	21.9	717.3
Post-refining waste (PostRW)	200	8.1	684.3

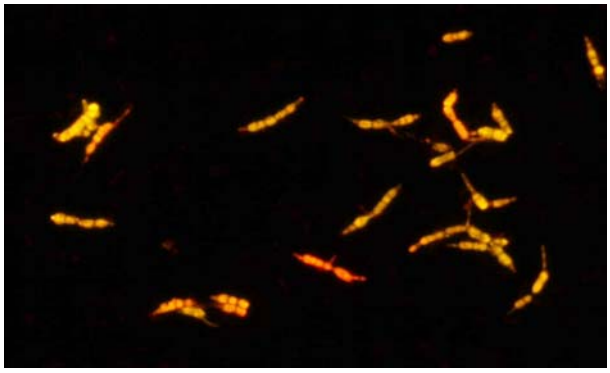

Fig. 2. Examples of fluorescent microscopy observation of *P. antarctica* cells after cultivation in YPD medium with post-refining fatty acids (cells stained with Nile red, excitation wavelengths (λ_{ex} = 460–495 nm) and emission wavelengths (λ_{em} = 510 nm), respectively). Orange–red colour indicates the presence of glycolipids.

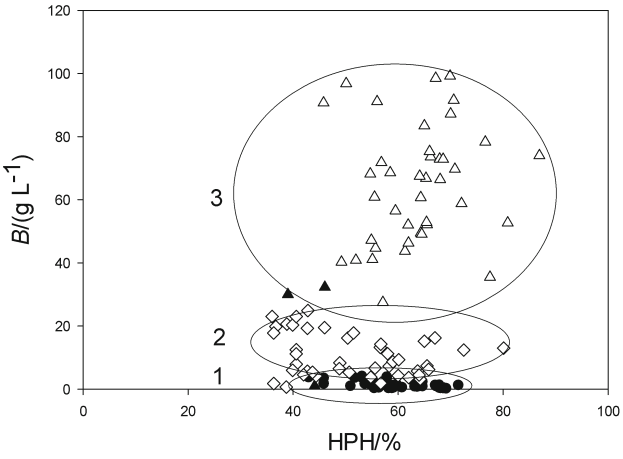

Fig. 3. Changes in biosurfactant concentration (B) in relation to cell hydrophobicity (HPH) during cultivation of *P. antarctica*, *P. aphidis*, *S. bombicola*, *P. jadinii,* in medium; 1 – YPD (●), 2 – YP (yeast extract, peptone medium) (◊), or 3 – YPD (△) with post-refining waste (PostRW), soapstock, soapstock, waste frying oil (WFO), respectively.

highest content of lipids, 41.4 g L^{-1} (Table 1). Also the highest concentration of free glycerol was found in GF2 (791 g L^{-1}); in products GF1 and GF3 it was 624 g L^{-1} and 711 g L^{-1}, respectively.

The concentration of mannosylerythritol lipids attained only 16.3 g L^{-1} using step-wise supplementation of pure glycerol during the cultivation of *P. antarctica* JCM 10317 (Morita et al., 2007a). Ashby and Solaiman (2010) also indicated that the methanol present in crude glycerol could significantly reduce sophorolipid biosynthesis.

The surface tension of the post-culture medium was in the range from 30.0 mN m^{-1} to 45.0 mN m^{-1}. The lowest value of surface tension of 29.1 mN m^{-1} was obtained after cultivation of *P. antarctica* in the medium with post-refining fatty acids (Fig. 1a).

Kinetics of biosurfactant synthesis

The biosurfactants synthesised by microorganisms were extra- and intracellular; an example of *P. Antarctica* cells stained with Nile red is presented in Fig. 2. Nile red is known to exhibit yellow–gold fluorescence (582 nm) and orange–red fluorescence (617 nm) in the presence of neutral lipids or polar lipids, e.g. glycolipids. The function of intracellular surfactants is not known but it could be that they alter the cell hydrophobicity and, together with extracellular surface-active compounds, support the microorganisms' utilisation of medium compounds.

A cell surface hydrophobicity assay is used as a screening method for selecting biosurfactants-producing microorganisms (Walter et al., 2010). It was demonstrated that adherence of the microbial cells to hydrocarbons is a characteristic feature of biosurfactant producers. Increasing the cell surface hydrophobicity increases the adhesion and contact of the cells with hydrophobic substrates. According to the data obtained in this study, cell hydrophobicity depends on the strain and the cultivation conditions. A relationship was found between this parameter and the synthesis of biosurfactants (Figs. 3 and 4). Increased cell hydrophobicity resulted in increased biosurfactant synthesis, which could be ex-

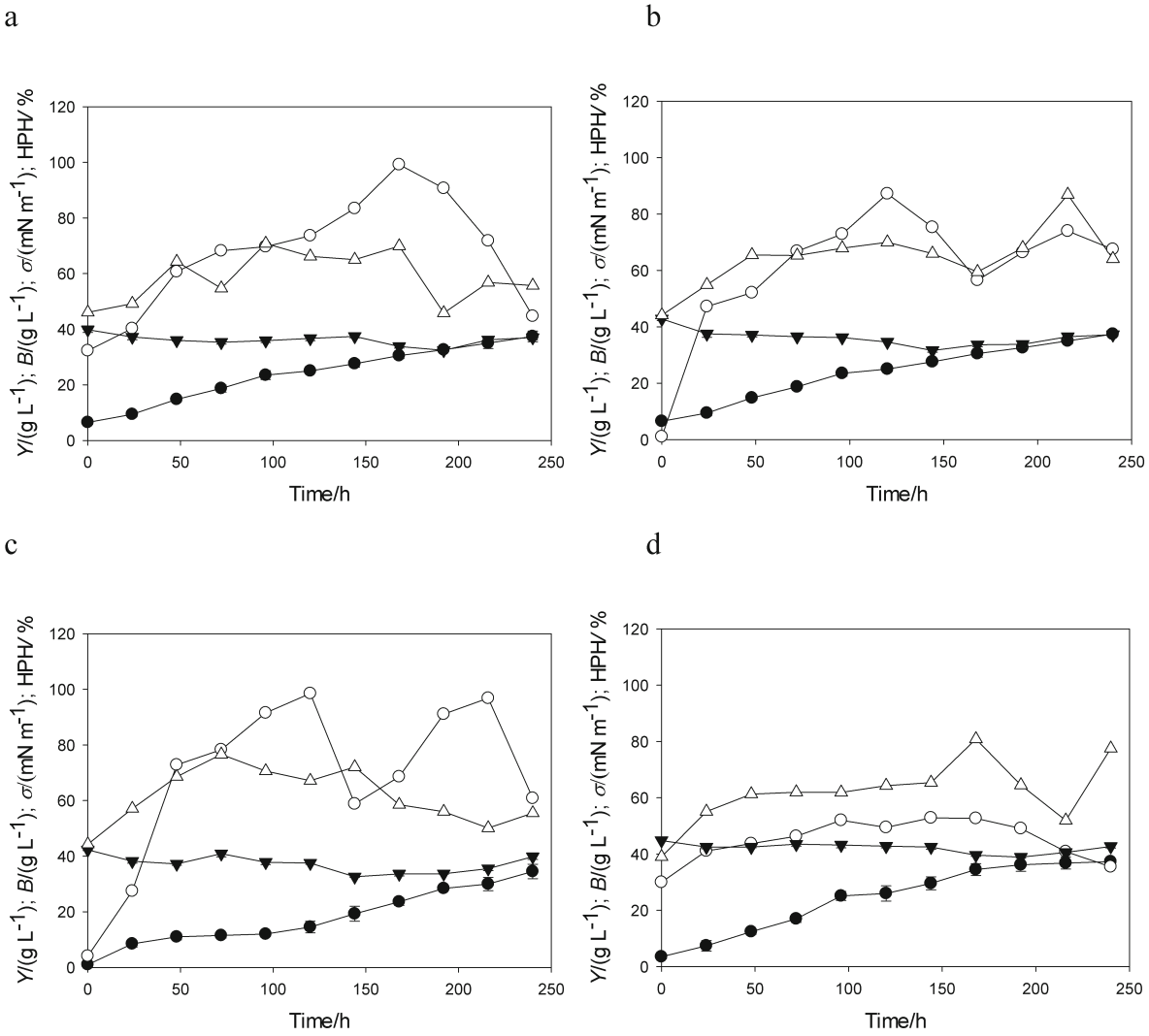

Fig. 4. Synthesis of biosurfactants in a shake-flask culture by: *P. antarctica* (a), *P. aphidis* (b), *S. bombicola* (c), and *P. jadinii* (d), in medium with with post-refining waste (PostRW), soapstock, soapstock, waste frying oil (WFO), respectively (30 °C, 240 h); ● – biomass (Y), ○ – concentration of biosurfactants (B), ▼ – surface tension (σ), and △ – cells hydrophobicity (HPH).

plained by the better contact of the microbial cells with hydrophobic substrates (Vasileva-Tonkova & Gesheva, 2004). Only in the case of *P. jadinii* were the changes in cell hydrophobicity significant, but the synthesis of biosurfactant was low. This could indicate either that the biosurfactants synthesised by this strain could be very effective or that cell contact with the hydrophobic substrate does not induce biosurfactant synthesis. Interesting conclusions could be drawn based on the analysis of a scatter graph presenting the changes in biosurfactant yield in relation to cell hydrophobicity during the cultivation in different media (Fig. 3). The data obtained could be divided into three groups representing the data obtained after the cultivation of yeast strains in the following media: YPD (Group 1), YP (without dextrose) (Group 2), or YPD (Group 3) with hydrophobic waste products (Fig. 3). It appears that the critical parameter for the effective synthesis of biosurfactant by the microorganisms studied is the medium composition and even changes in cell hydrophobicity could not contribute to obtaining a high concentration of surface-active compounds. For the effective synthesis of biosurfactant by the yeasts studied, the hydrophilic and hydrophobic substrate should be present in the cultivation medium (Group 3, Fig. 3). The lowest yield of biosurfactant was obtained in the medium with only the hydrophilic substrate (Group 1, Fig. 3) and was moderate in the medium with just the hydrophobic substrates (Group 2, Fig. 3). From directly after the start of cultivation, the HPH of the cells and the concentration of biosurfactant was higher for all yeasts in the YPD medium with hydrophobic substrate (Group 3, Fig. 3).

The highest concentrations of biosurfactants obtained at this stage of the research were similar to

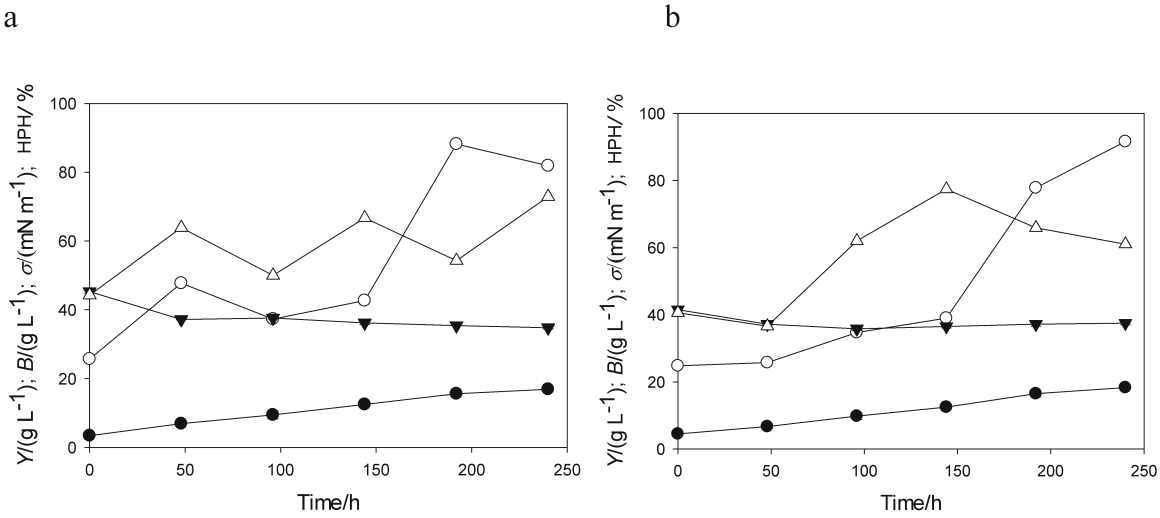

Fig. 5. Synthesis of biosurfactants by *P. aphidis* growing in medium with soapstock and whey permeate (a) or molasses (b); ● – biomass (Y), ○ – concentration of biosurfactants (B), ▼ – surface tension (σ), △ – cells hydrophobicity (HPH).

those obtained during the selection procedure. The strains of *P. antarctica*, *P. aphidis*, *S. bombicola*, and *P. jadinii* synthesised biosurfactants at maximum concentrations of 99.2 g L^{-1} (168 h), 87.2 g L^{-1} (120 h), 98.5 g L^{-1} (120 h), and 52.8 g L^{-1} (144 h), respectively (Fig. 4).

Analysis of the culture indicated that the kinetics of biosurfactant synthesis is related to the growth of microorganisms (Fig. 4). The time of cultivation selected in the first stage of the experiments appears to be optimal for the synthesis of biosurfactants in the culture conditions analysed. It was also found that the synthesis of biosurfactants occurred mainly during the stationary phase of growth. Biosurfactant production by resting cells is important for reducing product separation costs, because the growth, and product formation phases can be separated. Depending upon the nature of the biosurfactant and the producing microorganisms, several patterns of biosurfactant production by fermentation are possible (Desai & Banat, 1997). In some cases, the biosurfactants produced during a part of the growth cycle are subsequently either inactivated or incorporated into other metabolites, e.g. with no surface activity. It was also shown that the decrease in biosurfactant concentration could be a result of its uptake by the cells. This could explain changes or decrease in biosurfactant concentration during yeast growth (Figs. 3–5).

The surface changes in the cultivation medium were controlled by using two methods, i.e. the Du Noüy ring method (which is accurate, but requires about 50 mL of the sample) and a drop-collapse assay (which is simple and quick and is very often used for screening purposes). The synthesis of surface-active compounds does not significantly influence the changes in the surface tension of the medium, probably because of the mixture of the compounds present in the medium and their quantitative changes influencing the value of this parameter. However, all the microorganisms studied meet the common requirements for good biosurfactant producers: that the surface tension of the liquid medium should be $\leq$ 40 mN m^{-1} or the surface tension of the growth medium is reduced by $\geq$ 20 mN m^{-1} in comparison with distilled water (Willumsen & Karlson, 1996).

The analysis of the medium surface tension by drop-size analysis was not a sufficiently sensitive method to determine the synthesis of biosurfactants, but was useful for screening and selection purposes. The diameter of the medium drop increased from 6.05–9.69 mm to 7.31–9.73 mm. Morita et al. (2007b) also did not find a good relationship between the synthesis of biosurfactants and medium drop diameter.

The yield of biosurfactant synthesis and chemical composition (properties) of biosurfactants depend on the microorganisms and cultivation conditions, e.g. medium composition, temperature, aeration, and acidity (Glenns & Cooper, 2006). A common problem during the biosynthesis of surface-active compounds is intensive foam formation due to the low medium surface tension. On the other hand, the synthesis of biosurfactants usually requires intensive aeration and mixing, which makes the engineering problem difficult to solve. The synthesis of biosurfactants in a shake flask avoids the foaming problems but does not provide the optimal conditions for medium aeration and limits the possibility of controlling the process.

Application of low-cost media for biosurfactant synthesis

Waste fat, glycerol fraction, whey, and molasses usually contain all the compounds required by microorganisms for growth and could result in the

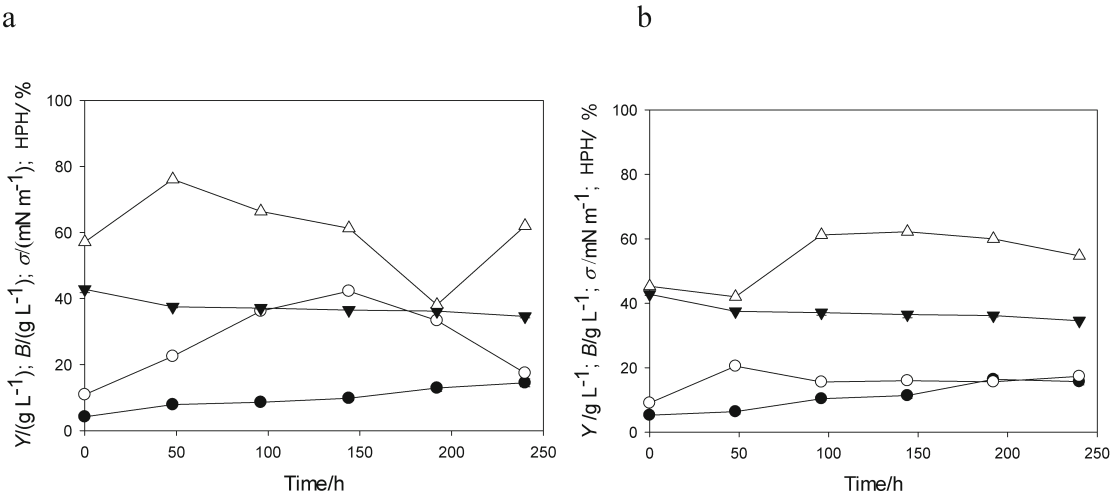

Fig. 6. Synthesis of biosurfactants by *P. antarctica* growing in medium with post-refining waste (PostRW) and whey permeate (a) or molasses (b); ● – biomass (Y), ○ – concentration of biosurfactants (B), ▼ – surface tension (σ), △ – cells hydrophobicity (HPH).

biosynthesis of valuable products, e.g. biosurfactants, biofuel, enzymes, and polyhydroxyalkanes (Tang et al., 2009, 2007; Yin et al., 2009). In a medium containing only soybean oil and molasses, the yield of sophorolipids synthesised by *S. bombicola* was (23.25 ± 1.07) g L^{-1} (Daverey & Pakshirajan, 2009). Another low-cost medium used for the synthesis of biosurfactants was composed of only distilled water supplemented with 5.0 % ground-nut oil refinery residue and 2.5 % corn-steep liquor. However, the yield of biosurfactant synthesis in this medium by *Candida sphaerica* UCP0995 was just 4.5 g L^{-1} (Sobrinho et al., 2008).

In a low-cost medium containing soapstock and whey permeate or molasses, approximately 90 g L^{-1} (Fig. 5) of biosurfactant was obtained from a culture of *P. aphidis*, *P. antarctica* synthesised approximately 40 g L^{-1} of mannosylerythritol lipids in a medium with post-refining waste and whey permeate (Fig. 6a) and a 2-times smaller amount of biosurfactants when the whey permeate was substituted by molasses (Fig. 6b).

During the cultivation, no substantial differences in the surface tension of the medium were observed and biosurfactant synthesis depended on yeast growth (Figs. 5 and 6). Under the conditions analysed, the cell hydrophobicity increased from 40.6–57.1 % to 62.2–77.4 %, similar to the YPD medium with waste products.

The mannosylerythritol lipids obtained have many interesting properties and applications, including an anti-agglomeration effect on ice particles in ice slurry and the capacity to increase the efficiency of gene transfection into mammalian cells; they can also self-assemble to form different lyotropic liquid crystalline phases, such as sponge (L_3), bicontinuous cubic (V_2), and lamellar (L_α) (Kitamoto et al., 2002, 2009).

Conclusions

The application of waste products in the synthesis of biosurfactants may be an attractive method for the environmentally friendly conversion of waste to valuable compounds. Lipids present in the waste products are better carbon sources for microorganism growth and biosurfactant synthesis than glycerol from the glycerol fraction.

A good correlation was found between cell hydrophobicity and the yield of biosurfactant synthesis. This suggests that hydrophobic cells are in better contact with hydrophobic substrates, which induced the synthesis of biosurfactants. However, this mechanism may not be effective for all microorganisms and requires analysis.

The biosurfactants isolated can find many applications and can be used as a raw material or after purification. This technology requires an analysis of the waste products obtained after biosurfactant separation and needs to eliminate the use of organic solvents for their separation. The results of this research will be used to optimise biosurfactant synthesis in bioreactors and to analyse the influence of aeration on effective synthesis. A mixture of substrates will be used to improve biosurfactant synthesis in a medium containing waste products.

References

Adamczak, M., & Bednarski, W. (2000a). Influence of medium composition and aeration on the synthesis of biosurfactants produced by *Candida antarctica*. *Biotechnology Letters*, *22*, 313–316. DOI: 10.1023/a:1005634802997.

Adamczak, M., & Bednarski, W. (2000b). Properties and yield of synthesis of mannosylerythritol lipids by *Candida antarctica*. In S. Bielecki, J. Tramper, & J. Polak (Eds.), *Food Biotechnology* (pp. 229–234). Amsterdam, The Nertherlands: Elsevier.

Ashby, R. D., & Solaiman, D. K. Y. (2010). The influence of increasing media methanol concentration on sophorolipid biosynthesis from glycerol-based feedstocks. *Biotechnology Letters*, *32*, 1429–1437. DOI: 10.1007/s10529-010-0310-0.

Bednarski, W., Adamczak, M., Tomasik, J., & Płaszczyk, M. (2004). Application of oil refinery waste in the biosynthesis of glycolipids by yeast. *Bioresource Technology*, *95*, 15–18. DOI: 10.1016/j.biortech.2004.01.009.

Bednarski, W., Narwojsz, M., Adamczak, M., & Nawotka, R. (2006). Carbon-source-dependent synthesis and composition of biosurfactant synthesized by *Pseudozyma antarctica*. *Environmental Biotechnology*, *2*, 31–36.

Calvo, C., Manzanera, M., Silva-Castro, G. A., Uad, I., & González-López, J. (2009). Application of bioemulsifiers in soil oil bioremediation processes. Future prospects. *Science of the Total Environment*, *407*, 3634–3640. DOI: 10.1016/j.scitotenv.2008.07.008.

Cameotra, S. S., & Makkar, R. S. (2004). Recent applications of biosurfactants as biological and immunological molecules. *Current Opinion in Microbiology*, *7*, 262–266. DOI: 10.1016/j.mib.2004.04.006.

Coombs, A. (2007). Glycerin bioprocessing goes green. *Nature Biotechnology*, *25*, 953–954. DOI: 10.1038/nbt0907-953.

Daverey, A., & Pakshirajan, K. (2009). Production of sophorolipids by the yeast *Candida bombicola* using simple and low cost fermentative media. *Food Research International*, *42*, 499–504. DOI: 10.1016/j.foodres.2009.01.014.

Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*, *61*, 47–64.

Felse, P. A., Shah, V., Chan, J., Rao, K. J., & Gross, R. A. (2007). Sophorolipid biosynthesis by *Candida bombicola* from industrial fatty acid residues. *Enzyme and Microbial Technology*, *40*, 316–323. DOI: 10.1016/j.enzmictec.2006.04.013.

Fleurackers, S. J. J. (2006). On the use of waste frying oil in the synthesis of sophorolipids. *European Journal of Lipid Science and Technology*, *108*, 5–12. DOI: 10.1002/ejlt.200500237.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Giannopoulos, A., Makri, A., & Aggelis, G. (2011). Production of biosurfactants from yeasts cultivated on glycerol. In *FEBS Workshop: Microbial Lipids from Genomics to Lipidomics*, May 13–15, 2010 (pp. 92). Vienna, Austria: Graz University of Technology.

Glenns, R. N., & Cooper, D. G. (2006). Effect of substrate on sophorolipid properties. *Journal of the American Oil Chemists' Society*, *83*, 137–145. DOI: 10.1007/s11746-006-1186-y.

Kitamoto, D., Isoda, H., & Nakahara, T. (2002). Functions and potential applications of glycolipid biosurfactants — from energy-saving materials to gene delivery carriers —. *Journal of Bioscience and Bioengineering*, *94*, 187–201. DOI: 10.1016/s1389-1723(02)80149-9.

Kitamoto, D., Morita, T., Fukuoka, T., Konishi, M., & Imura, T. (2009). Self-assembling properties of glycolipid biosurfactants and their potential applications. *Current Opinion in Colloid & Interface Science*, *14*, 315–328. DOI: 10.1016/j.cocis.2009.05.009.

Kuiper, I., Lagendijk, E. L., Pickford, R., Derrick, J. P., Lamers, G. E. M., Thomas-Oates, J. E., Lugtenberg, B. J. J., & Bloemberg, G. V. (2004). Characterization of two *Pseudomonas putida* lipopeptide biosurfactants, putisolvin I and II, which inhibit biofilm formation and break down existing biofilms. *Molecular Microbiology*, *51*, 97–113. DOI: 10.1046/j.1365-2958.2003.03751.x.

Makkar, R. S., Cameotra, S. S., & Banat, I. M. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB Express*, *1*, 5. DOI: 10.1186/2191-0855-1-5.

Morita, T., Konishi, M., Fukuoka, T., Imura, T., & Kitamoto, D. (2007a). Microbial conversion of glycerol into glycolipid biosurfactants, mannosylerythritol lipids, by a basidiomycete yeast, *Pseudozyma antarctica* JCM 10317^{T}. *Journal of Bioscience and Bioengineering*, *104*, 78–81. DOI: 10.1263/jbb.104.78.

Morita, T., Konishi, M., Fukuoka, T., Imura, T., & Kitamoto, D. (2007b). Physiological differences in the formation of the glycolipid biosurfactants, mannosylerythritol lipids, between *Pseudozyma antarctica* and *Pseudozyma aphidis*. *Applied Microbiology and Biotechnology*, *74*, 307–315. DOI: 10.1007/s00253-006-0672-3.

Pagliaro, M., Ciriminna, R., Kimura, H., Rossi, M., & Della Pina, C. (2009). Recent advances in the conversion of bioglycerol into value-added products. *European Journal of Lipid Science and Technology*, *111*, 788–799. DOI: 10.1002/ejlt.200800210.

Papanikolaou, S., Fick, M., & Aggelis, G. (2004). The effect of raw glycerol concentration on the production of 1,3-propanediol by *Clostridium butyricum*. *Journal of Chemical Technology and Biotechnology*, *79*, 1189–1196. DOI: 10.1002/jctb.1103.

Pinzon, N. M., Aukema, K. G., Gralnick, J. A., & Wackett, L. P. (2011). Nile red detection of bacterial hydrocarbons and ketones in a high-throughput format. *mBio*, *2*, e00109-11. DOI: 10.1128/mbio.00109-11.

Rodrigues, L., Banat, I. M., Teixeira, J., & Oliveira, R. (2006). Biosurfactants: potential applications in medicine. *Journal of Antimicrobial Chemotherapy*, *57*, 609–618. DOI: 10.1093/jac/dkl024.

Rosenberg, E., & Ron, E. Z. (1999). High- and low-molecular-mass microbial surfactants. *Applied Microbiology and Biotechnology*, *52*, 154–162. DOI: 10.1007/s002530051502.

Rosenberg, M., Gutnick, D., & Rosenberg, E. (1980). Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters*, *9*, 29–33. DOI: 10.1111/j.1574-6968.1980.tb05599.x.

Rymowicz, W., Rywińska, A., & Gładkowski, W. (2008). Simultaneous production of citric acid and erythritol from crude glycerol by *Yarrowia lipolytica* Wratislavia K1. *Chemical Papers*, *62*, 239–246. DOI: 10.2478/s11696-008-0018-y.

Siloto, R. M. P., Truksa, M., He, X. H., McKeon, T., & Weselake, R. J. (2009). Simple methods to detect triacylglycerol biosynthesis in a yeast-based recombinant system. *Lipids*, *44*, 963–973. DOI: 10.1007/s11745-009-3336-0.

Singh, A., Van Hamme, J. D., & Ward, O. P. (2007). Surfactants in microbiology and biotechnology: Part 2. Application aspects. *Biotechnology Advances*, *25*, 99–121. DOI: 10.1016/j.biotechadv.2006.10.004.

Smyth, T. J. P., Perfumo, A., Marchant, R., & Banat, I. (2010). Isolation and analysis of low molecular weight microbial glycolipids. In K. N. Timmis (Ed.), *Handbook of hydrocarbon and lipid microbiology* (pp. 3705–3723). Heidelberg, Germany: Springer-Verlag.

Sobrinho, H. B. S., Rufino, R. D., Luna, J. M., Salgueiro, A. A., Campos-Takaki, G. M., Leite, L. F. C., & Sarubbo, L. A. (2008). Utilization of two agroindustrial by-products for the production of a surfactant by *Candida sphaerica* UCP0995. *Process Biochemistry*, *43*, 912–917. DOI: 10.1016/j.procbio.2008.04.013.

Takahashi, M., Morita, T., Wada, K., Hirose, N., Fukuoka, T., Imura, T., & Kitamoto, D. (2011). Production of sophorolipid glycolipid biosurfactants from sugarcane molasses using *Starmerella bombicola* NBRC 10243. *Journal of Oleo Science*, *60*, 267–273.

Tang, S., Boehme, L., Lam, H., & Zhang, Z. S. (2009). *Pichia pastoris* fermentation for phytase production using crude glycerol from biodiesel production as the sole carbon source. *Biochemical Engineering Journal*, *43*, 157–162. DOI: 10.1016/j.bej.2008.09.020.

Thanomsub, B., Pumeechockchai, W., Limtrakul, A., Arunrattiyakorn, P., Petchleelaha, W., Nitoda, T., & Kanzaki, H. (2007). Chemical structures and biological activities of rhamnolipids produced by *Pseudomonas aeruginosa* B189 isolated from milk factory waste. *Bioresource Technology*, *98*, 1149–1153. DOI: 10.1016/j.biortech.2005.10.045.

Thavasi, R., Jayalakshmi, S., Balasubramanian, T., & Banat, I. M. (2007). Biosurfactant production by *Corynebacterium kutscheri* from waste motor lubricant oil and peanut oil cake. *Letters in Applied Microbiology*, *45*, 686–691. DOI: 10.1111/j.1472-765x.2007.02256.x.

Transparency Market Research (2012). Biosurfactants market – global scenario, raw material and consumption trends, industry analysis, size, share and forecasts, 2011 – 2018. Retreived December 18, 2012, from http://www.transparency marketresearch.com/biosurfactants-market.html

Van Hamme, J. D., Singh, A., & Ward, O. P. (2006). Physiological aspects. Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. *Biotechnology Advances*, *24*, 604–620. DOI: 10.1016/j.biotechadv.2006.08.001.

Vasileva-Tonkova, E., & Gesheva, V. (2004). Potential for biodegradation of hydrocarbons by microorganisms isolated from Antarctic soils. *Zeitschrift für Naturforschung C, Journal of Biosciences*, *59*, 140–145.

Walter, V., Syldatk, C., & Hausmann, R. (2010). Screening concepts for the isolation of biosurfactant producing microorganisms. In R. Sen (Ed.), *Biosurfactants* (pp. 1–13). New York, NY, USA: Springer.

Willke, T., & Vorlop, K. D. (2004). Industrial bioconversion of renewable resources as an alternative to conventional chemistry. *Applied Microbiology and Biotechnology*, *66*, 131–142. DOI: 10.1007/s00253-004-1733-0.

Willumsen, P. A., & Karlson, U. (1996). Screening of bacteria, isolated from PAH-contaminated soils, for production of biosurfactants and bioemulsifiers. *Biodegradation*, *7*, 415–423. DOI: 10.1007/bf00056425.

Yin, H., Qiang, J., Jia, Y., Ye, J. S., Peng, H., Qin, H. M., Zhang, N., & He, B. Y. (2009). Characteristics of biosurfactant produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater. *Process Biochemistry*, *44*, 302–308. DOI: 10.1016/j.procbio.2008.11.003.