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Liquid membrane extraction of bio-active amphiphilic substances: Recovery of surfactin

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A B S T R A C T

The interest of application of liquid membrane (pertraction) processes for recovery of biosurfactants from aqueous media was demonstrated. Transport of pure surfactin in three-liquid-phase system was studied. Surfactin was successfully extracted from slightly acid media (pH 5.65–6.05) applying batch pertraction in a rotating discs contactor and using *n*-heptane as liquid membrane. The process efficiency was found to be strongly affected by the feed solution acidity (83% at pH_F 6.05 and 97% at pH_F 5.65 after 4 h pertraction).

An atypical pH effect was observed when the behaviour of surfactin extraction from aqueous media by non-polar solvents (*n*-heptane and *n*-octane) was studied. The obtained high extraction degrees from both acid and basic media and the clearly reduced degree of extraction from neutral media could be attributed to the different conformations of surfactin in these media.

Keywords:

Surfactin
Extraction
Liquid membranes
Biosurfactants
Pertraction
Process integration

1. Introduction

In literature, there has been considerable interest on the micro-biological productivities [1–7], properties [1,8–12] and applications [12–17] of biosurfactants, including heptapeptide surfactin, which is one of the most powerful ones [18]. However, few reports have been mentioned concerning surfactin separation, purification and concentration [19–22]. The amphiphilic character of such compounds presents some limitations to their efficient recovery. Practically, even at low concentration, the produced surfactin generates extensive foaming. This phenomenon causes substrate and product outflow and the surfactin production becomes hard to control [19]. Thus, development of efficient production and separation operations as well as processes integration are of growing interest. Recently, Montastruc et al. have shown that an adsorption process may be effective for continuous surfactin isolation from culture media [20]. From large scale point of view the same authors have proposed the use of a fixed bed adsorption column in the process known as in situ product removal (ISPR) [19]. The purity of surfactin isolated after desorption with methanol and further solvent evaporation is high, but the process is relatively long: 85% recovery in 24 h at 38 mg L⁻¹ initial concentration of surfactin.

An interesting potential advance of the ISPR technology for surfactin recovery is the application of solvent extraction and more especially the low-cost liquid membrane technique. The classical solvent extraction provides relatively high surfactin purity [21], but its main inconvenient is the necessity of further regeneration of the loaded solvent, and therefore the use of important quantities of solvent. Moreover, the most efficient and generally used for biosurfactants recovery solvents, such as chloroform, methanol, and acetone, are known to be toxic and harmful to the environment and human health [23].

The liquid membrane separation process, based on solvent extraction, is called pertraction and operates in three-liquid-phase systems. Two aqueous solutions, i.e., a feed solution F and a receiving solution R, are separated by an organic liquid M, representing the “liquid membrane”, insoluble in both aqueous solutions. The target species are transported from the feed to the receiving solution across the organic liquid membrane thanks to appropriately selected and different equilibrium conditions at the interfaces F/M and M/R. In fact, pertraction process is a combination of extraction and stripping operations performed simultaneously in one stage [24]. The main advantages of pertraction towards conventional liquid–liquid extraction are the use of smaller quantities of organic solvent due to continuous regeneration of the organic solvent, as well as the possibility to recover the target species even in cases of low distribution coefficients [25]. The use of liquid membranes presents an attractive approach to produce valuable products of high quality at reduced costs, giving the opportunity to use as

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liquid membranes less powerful but more selective, less toxic and less expensive solvents than in the case of classical solvent extraction. The interest of liquid membrane process for recovery of fermentation products have grown rapidly because a substantial part of the technological and financial success of bioprocesses depends on the post-fermentation steps [26]. Liquid membrane technique was applied for recovery of fructose [27], citric acid [28], lactic acid [29], butyric acid [30], and cephalosporin C [31] from fermentation broths. However, there are no data on biosurfactants recovery by using liquid membrane processes.

The aim of the present work was to study liquid membrane permeation of surfactin in three-liquid-phase system. To confirm the applicability of pertraction process on biosurfactant recovery, we focused on the pH effect upon equilibrium repartition of surfactin between aqueous solutions and various organic solvents.

2. Experimental

2.1. Materials (reagents, solvents and aqueous solutions)

Surfactin was purchased from Fluka and used as received. The product was synthesized by *Bacillus subtilis* and was 98% pure. As organic solvents *n*-heptane, *n*-octane and 1-octanol (all p.a. grade reagents from Merck) were used. To adjust the acidity of the aqueous solutions KH_2PO_4 and K_2HPO_4 (both p.a. grade reagents from Merck) were used. The acidity of the aqueous solutions was measured with a pH-meter InoLab pH/ION Level 2 P (WTW GmbH & Co., KG, Germany).

2.2. Procedure of equilibrium studies

Prior to study surfactin transport across a liquid membrane, its equilibrium distribution between organic and aqueous phases was established using separating funnels. Model aqueous solutions of surfactin were preliminary prepared. Surfactin concentration was between 30 and 50 mg L^{-1} and pH between 5.5 and 8.8. As organic liquids *n*-heptane, *n*-octane and 1-octanol, as well as their mixtures were used. In each experiment equal volumes of surfactin aqueous solution and organic solvent (each of 10 mL) were shaken moderately for 15 min. In order to achieve the equilibrium and complete phase decantation the flasks were reposed for 12 h minimum. Initial concentrations of surfactin in the aqueous solutions were determined prior the contact between the two phases and its equilibrium concentrations after complete phase separation.

2.3. Procedure of pertraction

Among the large variety of liquid membrane techniques [24,31–33], the pertraction in rotating discs contactor (RDC) was selected due to its stable and efficient continuous operation [34–37]. Kinetics of surfactin transport in three-liquid-phase system was studied in laboratory RDC, presented schematically in Fig. 1. The lower part of the contactor is divided into four compartments: two for the feed and two for the receiving solution. The liquid organic membrane covers both aqueous solutions and occupies the common upper part of the contactor. A polymer disc (1 mm thick, 18 cm in diameter) coated by hydrophilic material rotates in each compartment. The distance between two discs is 15 mm. The lower part of each disc is immersed in the corresponding aqueous solution. The discs rotation provides a formation and continuous renewal of aqueous films of solutions F and R on discs surfaces as well as the agitation of all three phases. The contact surfaces F/M and M/R are of 0.07 m^2 . The overall mass transfer coefficients in the RDC contactor used are in the range of 10^{-5} to 10^{-6} m s^{-1} [36].

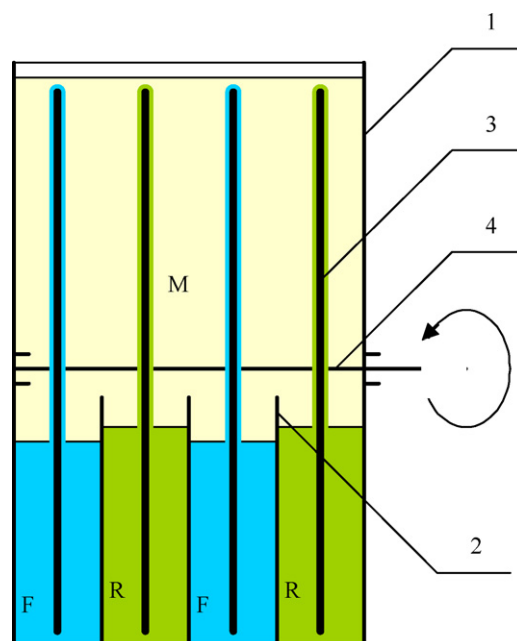


Fig. 1. Schematic diagram of the experimental set-up: (1) contactor body; (2) cell separation plates; (3) hydrophilic discs; (4) rotation shaft.

To homogenise the aqueous solutions and to provide samples, both liquids were circulated by means of peristaltic pumps with flow rates of 1.5 L h^{-1} .

The volumes of both feed and receiving solutions were of 270 mL and the liquid membrane phase was 1250 mL. The initial concentration of surfactin in the feed solution was 40.0 mg L^{-1} . The velocity of discs rotation was fixed at 10 min^{-1} .

All experiments were carried out at room temperature of 293 K.

2.4. Analysis of surfactin

Surfactin samples from aqueous solutions were analysed after preliminary preparation following a procedure, described elsewhere [19]. Finally samples are dissolved in methanol and surfactin concentrations were determined by reverse phase C_{18} HPLC (600s, Waters, USA) equipped with a Merck C_{18} column (5 μm , Merck, Germany). The injection samples were of 20 μL . Surfactin was eluted for 25 min at a rate of 1 mL min^{-1} over a mixture of ACN/ H_2O /TFA, e.g., 80% of acetonitrile, 20% of water, 0.1% of trifluoroacetic acid, by volume. The spectrum was analysed using second derivative values.

3. Results and discussion

3.1. Choice of solvents: extraction of surfactin by organic solvents

To apply liquid membrane process for surfactin recovery from aqueous media, including fermentation broth, it is necessary to find conditions suitable for its extraction by an organic liquid, but also conditions favourable for its back extraction into an aqueous solution (regeneration of the organic solvent). Usually, the *B. subtilis* strains used for surfactin production have been cultivated in medium with an initial pH adjusted between 6.0 and 8.5 [19]. Mostly the media have been maintained at pH 7.0 [2,38], but in some cases final pH values decreased in the range of 6.3–6.7 [5,19]. Hence, in order to isolate surfactin, its recovery from such media was sought out. To improve surfactin extraction, a possible small correction of pH was also envisaged. However, acidification of the aqueous solutions containing surfactin, including fermenta-

Table 1

Degree of surfactin extraction from model aqueous solutions to various organic solvents (phase ratio 1:1, surfactin initial concentration in the aqueous solutions was of $40 \pm 10 \text{ mg L}^{-1}$)

Organic phase	% Extraction		
	At pH 5.50	At pH 7.15	At pH 8.80
<i>n</i> -Heptane	83.44	7.84	61.11
<i>n</i> -Octane	87.73	9.42	66.24
1-Octanol	100.00	100.00	100.00
<i>n</i> -Heptane/1-octanol 80/20	87.70	86.50	80.49
<i>n</i> -Heptane/1-octanol 50/50	100.00	100.00	100.00
<i>n</i> -Octane/1-octanol 50/50	93.30	100.00	100.00

tion broth, was quite limited, because of its tendency to precipitate at low pH. Wei and Chu have reported a precipitation at $\text{pH} < 5.5$ [39].

Equilibrium partition of surfactin between various organic solvents and aqueous solutions was studied at three different equilibrium pH values of the aqueous phase: 5.50, 7.15, and 8.80, respectively. Table 1 lists the studied organic solvents (mixtures) and the equilibrium data on surfactin extraction in these solvents from aqueous solutions. Obviously, 1-octanol, the most polar from the studied solvents, is an excellent solvent for surfactin extraction. Pure 1-octanol and its mixtures with *n*-heptane (e.g., 20/80, 50/50) or *n*-octane (50/50) provided a very efficient surfactin recovery from aqueous media. However, in the studied pH interval, there are not conditions favourable for back extraction of surfactin into an aqueous solution and regeneration of the loaded organic solvent (1-octanol or its mixtures). Therefore 1-octanol is not very appropriate for surfactin recovery by pertraction. An inhibition of biological activity of fungi in presence of 1-octanol has been also reported [40]. Usually the *n*-alkanes are considered as not suitable solvents for surfactin extraction [21,41]. Table 1 confirms that the non-polar *n*-heptane and *n*-octane are less efficient than 1-octanol but surfactin extraction in these solvents is strongly affected by the change of aqueous phase acidity. For both studied alkanes, surfactin extraction was relatively high from slightly acid (over 80% at pH 5.50) or slightly basic (over 60% at pH 8.80) aqueous solutions, while from neutral aqueous solutions (pH 7.15) the extraction was less than 10%. Consequently, the studied alkanes are suitable for liquid membrane permeation of surfactin, providing conditions favourable for surfactin extraction into the organic solvent (from slightly acid or basic media), but also conditions for its back extraction into an aqueous solution (at the neutral zone of pH).

3.2. Solvent extraction of surfactin by *n*-heptane and *n*-octane

The effect of pH on surfactin extraction from its model aqueous solutions into selected organic solvents, namely *n*-heptane and *n*-octane, was studied in details over the whole range of pH between 5.5 and 8.8. The extraction degrees obtained at various equilibrium pH are presented in Fig. 2. The results are almost identical for both solvents: the increase of pH provokes continuous decrease of degree of extraction from acid medium; then extraction degree passes through a minimum at neutral zone of pH, and grows up again progressively in basic medium. Such behaviour is quite strange comparing to the usual pH effects on solvent extraction: increase of extraction degree for cations and decrease for anions with pH increase, for example [42,43]. It could be explained by amphiphilic character of surfactin molecules, but mostly by the micropolarity of the different conformations of these molecules in acid, neutral and basic media. Concerning pure surfactin, it is well known that it forms large rod micelles even at low concentrations close to the CMC ($\text{CMC} = 9.4 \times 10^{-6} \text{ M}$ in pure water at 25°C)

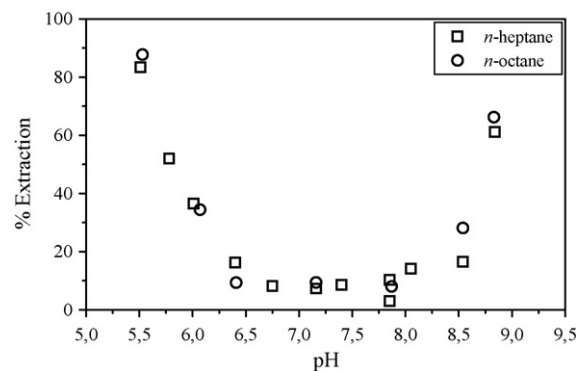


Fig. 2. Influence of equilibrium pH on surfactin extraction by *n*-heptane and *n*-octane (surfactin initial concentration in the aqueous solutions was of $40 \pm 10 \text{ mg L}^{-1}$).

[8]. Its properties are closely related to its molecular organization in micelles. For best choice and understanding the optimal conditions for pertraction process we will analyse the effects of pH on surfactin conformation in both micellar and non-micellar solutions.

In aqueous media various surfactin conformations, with gradual shifting from one to another, have been reported [9]. At neutral zone of pH (6.5–7.5), surfactin molecules are organized mainly in β -sheet micelles. This configuration is characterized by an exposure of a large number of carboxylic groups on the micelle surface which could explain more polar character of surfactin. At these conditions, surfactin is in anionic form, with two negative charges, one at the aspartate residue and another at the glutamate one, and it is able to bind metal cations [44]. Thus, the anionic character of surfactin in such media predetermines its limited solubility in non-polar solvents and, therefore, its insignificant extraction by *n*-heptane and *n*-octane. Outside this neutral zone, other conformations have been observed [9]. With increasing the pH values, at pH 8.5 or more, below CMC surfactin monomers have mainly unordered conformation, but above CMC α -helices are predominantly formed. Osman et al. have reported a strong reduction of surfactin micropolarity with pH increase in basic media, related to the changes of its conformation [10]. At slightly acid solutions, transition of β -sheet to α -helices micelles have been also observed [9]. Thus, the strongly reduced micropolarity of α -helices comparing to β -sheet micelles could explain the higher surfactin extraction in *n*-heptane and *n*-octane obtained from both basic and acid solutions. In such media, the lipophylic ends of surfactin molecules in unordered conformation or as α -helices are more accessible to non-polar solvents used.

3.3. Pertraction of surfactin

Kinetics of surfactin transport through a liquid membrane was studied in the above described laboratory RDC (Fig. 1). As far as the two tested alkanes showed very similar extraction behaviour, as liquid membrane *n*-heptane was selected because of its easier evaporation. In order to provide appropriate and constant pH values of the aqueous solutions during whole pertraction process, they were buffered by potassium phosphate buffers. For kinetic studies on surfactin pertraction the acidity of the feed solution was estimated to be optimal at $\text{pH} \sim 6.0$, because of the highest extraction degree at the conditions reported to be suitable for surfactin production by fermentation (pH 6.0–8.5) [19]. Moreover, Wei and Chu have reported a very efficient fermentation process at $\text{pH} \sim 6.0$ (2.0 g L^{-1} surfactin) [39]. As receiving solu-

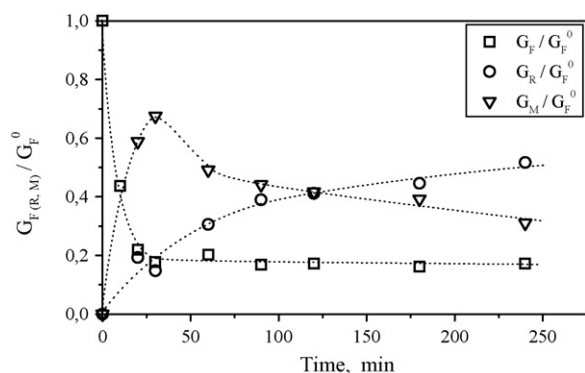


Fig. 3. Evolution of dimensionless surfactin amount in feed, membrane and receiving solutions versus time ($C_0^F = 10.8 \text{ mg}$, $\text{pH}_F = 6.05 \pm 0.05$, $\text{pH}_R = 7.30 \pm 0.05$, discs rotation velocity of 10 min^{-1} , liquid membrane : *n*-heptane).

tion distilled water, buffered to $\text{pH}_R = 7.30 \pm 0.05$, was used. Taking into consideration the presence of surfactant substance with risk of deterioration of the pertraction process by droplet formation and mechanical transportation of surfactin, the studies were carried out at moderate and constant velocity of discs rotation of 10 min^{-1} .

Fig. 3 shows the evolution of surfactin repartition between the three liquid phases during a batch pertraction process at constant acidity of the feed solution $\text{pH}_F = 6.05 \pm 0.05$. The interaction between surfactin molecules of the feed solution and *n*-heptane occurred at the first interface F/M where surfactin was partially extracted to organic phase. The process beginning is characterized by a sharp decrease of surfactin amount in the feed solution and its instantaneous accumulation in the organic liquid membrane. Due to the concentration gradient created surfactin molecules extracted in the organic membrane phase were transferred to the second interface M/R, where the pH of the receiving solution favoured the formation of β -sheet micelles and, therefore, surfactin transfer and accumulation into this solution. The permanent stripping of the organic solution provoked a continuous surfactin removal from the feed solution. At the end of experimental run (after 4 h), the majority of surfactin was accumulated in the receiving solution. However, the extraction from the feed solution was not complete: about 17% of surfactin remained in F phase. The relatively high amount of surfactin in the liquid membrane (about 30%) results to the higher volume of this solution and the thermodynamic conditions at the interfaces F/M and M/R which could not provide a complete membrane stripping.

Higher surfactin recovery was obtained when feed solution acidity was slightly increased. Fig. 4 shows the effect of feed solution acidity on the kinetic profiles of surfactin concentrations in both aqueous solutions at different initial acidities of the feed solution. As expected, the extraction efficiency increases at lower pH_F because of the more favourable equilibrium conditions at the interface F/M. At $\text{pH}_F = 5.65 \pm 0.05$ surfactin recovery from feed solution was almost complete (about 97%). The faster and more efficient

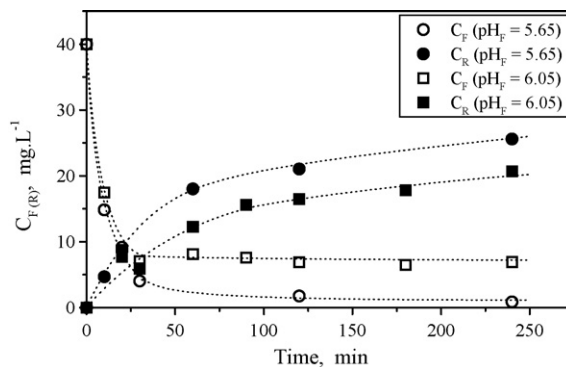


Fig. 4. Influence of feed solution acidity on surfactin pertraction ($C_0^F = 40.0 \text{ mg L}^{-1}$, $\text{pH}_R = 7.30 \pm 0.05$, discs rotation velocity of 10 min^{-1} , liquid membrane : *n*-heptane).

extraction of surfactin from F solution in this case induced also an enhanced surfactin transfer to R solution. At the end of experimental run (after 4 h), almost 2/3 of surfactin was accumulated in the receiving solution. Table 2 regroups the data on surfactin repartition in all three phases at the end of pertraction process and at equilibrium between the three liquids in contact, calculated on the basis of the two two-phases equilibriums estimated at the interfaces F/M and M/R, respectively. One can conclude that even at the moderate agitation applied (10 min^{-1}) pertraction process was quite rapid, because the final concentrations in the three phases were very close to the equilibrium ones.

It should be mentioned that in all reported studies on fermentation products recovery by pertraction [26–30] the transport of targeted substances was facilitated by extracting agents (carriers), dissolved in an organic diluent. The carriers used (Alamine 336, Aliquat 336, TOMAC, Hostarex A327, tri-laurylamine) are harmful and toxic. In contrast, in our studies no carriers were used and surfactin transport in the model system used was provided by using of pure *n*-heptane as a liquid membrane.

The observed relatively high surfactin permeability through the *n*-heptane liquid membrane offers a new opportunity to isolate the biosurfactant from fermentation broth. A further coupling of pertraction to fermentation process for in situ removal of produced surfactin could contribute to resolve the problem with foam formation during fermentation process. Obviously, an optimization of the conditions suitable for both fermentation and pertraction processes is required. In fact, the conditions at $\text{pH} < 6.0$ are more favourable for surfactin pertraction, but less suitable for its production by fermentation. Nevertheless, Wei et al. have reported a satisfactory surfactin production at $\text{pH} 5.75$ (0.85 g L^{-1}) [6]. Processes integration fermentation–pertraction seems to be applicable for surfactin production, since liquid membrane purification has been successfully coupled to fermentation in phenol production [45], as well as to solid–liquid extraction for continuous isolation of alkaloids from medicinal plants [46,47].

Table 2

Surfactin distribution in all three phases at the end of pertraction process and at equilibrium between the three liquid phases in contact, estimated on the basis of the two two-phases equilibriums at the interfaces F/M and M/R ($\text{pH}_F = 5.65$, $\text{pH}_R = 7.30$)

Solution	After 4 h pertraction in RDC	At equilibrium between the three liquid phases in contact (calculated)		
	Surfactin content (mg)	Surfactin concentration (mg L^{-1})	Surfactin content (mg)	Surfactin concentration (mg L^{-1})
Phase F	0.35	1.28	0.30	1.10
Phase M	3.54	2.95	2.93	2.44
Phase R	6.91	25.60	7.57	28.05

4. Conclusion

When the behaviour of surfactin extraction from aqueous media by various organic solvents (mixtures) at equilibrium was studied, the non-polar *n*-heptane and *n*-octane were found to be the most suitable for surfactin recovery by pertraction process. The degree of surfactin removal into these two alkanes was found to be strongly affected by the aqueous solution acidity. The observed minimum of degree of extraction from neutral media could be attributed to the higher hydrophility of the β -sheet micelles formed by surfactin molecules at these conditions. In both acid and basic media, surfactin conformation alters from β -sheet to α -helices. At these conditions, the non-polar ends of surfactin molecules are more exposed to contact the organic solvents used and, as result, higher extraction degrees were obtained.

The obtained results on surfactin transport in three-liquid-phase system show that it can be successfully recovered from slightly acid media by means of pertraction in a rotating discs contactor, using *n*-heptane as liquid membrane. The process efficiency was found to be strongly affected by the feed solution acidity (about 83% at pH_F 6.05 and about 97% at pH_F 5.65 after 4 h pertraction). The pertraction process was relatively fast: about 90% of surfactin was removed from F phase in 30 min and, in 2 h, more of 50% was already transferred in R phase.

The obtained efficient surfactin recovery by pertraction suggests a new prospect to isolate the biosurfactant from fermentation broth. A further coupling of liquid membrane purification to fermentation process for in situ removal of produced surfactin is envisaged. However, the integrated process should be accomplished at the conditions optimal for both fermentation and pertraction processes.

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References

- [1] K. Arima, A. Kakinuma, A. Tamura, Surfactin a crystalline peptidolipid surfactant produced by *Bacillus subtilis*: isolation, characterization and its inhibition of fibrin clot formation, *Biochem. Biophys. Res. Commun.* 31 (1968) 488–494.
- [2] D.A. Davis, H.C. Lynch, J. Varley, The production of surfactin in batch culture by *Bacillus subtilis* ATCC 21332 is strongly influenced by the conditions of nitrogen metabolism, *Enzyme Microb. Technol.* 25 (1999) 322–329.
- [3] E. Akpa, P. Jacques, B. Wathelet, M. Paquot, R. Fuchs, H. Budzikiewicz, P. Thonart, Influence of culture conditions on lipopeptide production by *Bacillus subtilis*, *Appl. Biochem. Biotechnol. A: Enzyme Eng. Biotechnol.* 91 (2001) 551–561.
- [4] J.M. Bonmatin, O. Laprévoté, F. Peypoux, Diversity among microbial cyclic lipopeptides: iturins and surfactins. Activity–structure relationships to design new bioactive agents, *Comb. Chem. High Throughput Screen* 6 (2003) 541–556.
- [5] M.S. Yeh, Y.H. Wei, J.S. Chang, Enhanced production of surfactin from *Bacillus subtilis* by addition of solid carriers, *Biotechnol. Prog.* 21 (2005) 1329–1334.
- [6] Y.H. Wei, L.F. Wang, J.S. Chang, S.S. Kung, Identification of induced acidification in iron-enriched cultures of *Bacillus subtilis* during biosurfactant fermentation, *J. Biosci. Bioeng.* 96 (2003) 174–178.
- [7] C.L. Queiroga, L.R. Nascimento, G.E. Serra, Evaluation of paraffins biodegradation and biosurfactant production by *Bacillus subtilis* in the presence of crude oil, *Braz. J. Microbiol.* 34 (2003) 321–324.
- [8] Y. Ishigami, M. Osman, H. Nakahara, Y. Sano, R. Ishiguro, M. Matsumoto, Significance of β -sheet formation for micellization and surface adsorption of surfactin, *Colloids Surf. B: Biointerf.* 4 (1995) 341–348.
- [9] M. Osman, H. Hoiland, H. Holmsen, Y. Ishigami, Tuning micelles of a bioactive heptapeptide biosurfactant via extrinsically induced conformational transition of surfactin assembly, *J. Pept. Sci.* 4 (1998) 449–458.
- [10] M. Osman, H. Hoiland, H. Holmsen, Micropolarity and microviscosity in the micelles of the heptapeptide biosurfactant “surfactin”, *Colloids Surf. B: Biointerf.* 11 (1998) 167–175.
- [11] M. Morikawa, Y. Hirata, T. Imanaki, A study on the structure–function relationship of lipopeptide biosurfactants, *Biochim. Biophys. Acta* 1488 (2000) 211–218.
- [12] F.F.C. Barros, C.P. de Quadros, M.R. Marostica, G.M. Patore, Surfactin: chemical, technological and functional properties for food applications, *Quim. Nova* 30 (2007) 409–414.
- [13] P. Singh, S.S. Cameotra, Potential application of microbial surfactants in biomedical science, *Trends Biotechnol.* 22 (2004) 142–146.
- [14] J.D. Desai, I.M. Banat, Microbial production of surfactants and their commercial potential, *Microbiol. Mol. Biol. Rev.* 61 (1997) 47–64.
- [15] S.C. Lin, Biosurfactants: recent advances, *J. Chem. Technol. Biotechnol.* 66 (1996) 109–120.
- [16] K.D. Schaller, S.L. Fox, D.F. Bruhn, K.S. Noah, G.A. Bala, Characterization of surfactin from *Bacillus subtilis* for application as an agent for enhanced oil recovery, *Appl. Biochem. Biotechnol. A: Enzyme Eng. Biotechnol.* 115 (2004) 827–836.
- [17] C.N. Mulligan, Environmental applications for biosurfactants, *Environ. Pollut.* 133 (2005) 183–198.
- [18] F. Peypoux, J.M. Bonmatin, J. Wallach, Recent trends in the biochemistry of surfactin, *Appl. Microbiol. Biotechnol.* 51 (1999) 553–563.
- [19] T. Liu, L. Montastruc, F. Gancel, L. Zhao, I. Nikov, Integrated process for production of surfactin. Part 1. Adsorption rate of pure surfactin onto activated carbon, *Biochem. Eng. J.* 35 (2007) 333–340.
- [20] L. Montastruc, T. Liu, F. Gancel, L. Zhao, I. Nikov, Integrated process for production of surfactin. Part 2. Equilibrium and kinetic study of surfactin adsorption onto activated carbon, *Biochem. Eng. J.* 38 (2008) 349–354.
- [21] H.L. Chen, R.S. Juang, Recovery and separation of surfactin from pretreated fermentation broths by physical and chemical extraction, *Biochem. Eng. J.* 38 (2008) 39–46.
- [22] R. Sen, T. Swaminathan, Characterization of concentration and purification parameters and operating conditions for the small-scale recovery of surfactin, *Proc. Biochem.* 40 (2005) 2953–2958.
- [23] S. Mukherjee, P. Das, R. Sen, Towards commercial production of microbial surfactants, *Trends Biotechnol.* 24 (2006) 509–515.
- [24] L. Boyadzhiev, Liquid pertraction or liquid membranes—state of the art, *Sep. Sci. Technol.* 25 (1990) 187–205.
- [25] L. Boyadzhiev, Z. Lazarova, Liquid membranes (liquid pertraction), in: R.D. Noble, S.A. Stern (Eds.), *Membrane Separation Technology. Principles and Applications*, Elsevier, Amsterdam, Netherlands, 1995, pp. 283–352.
- [26] P.R. Patniak, Liquid emulsion membranes: principles, problems and applications in fermentation processes, *Biotechnol. Adv.* 13 (1995) 175–208.
- [27] M. Di Luccio, B.D. Smith, T. Kida, T.L.M. Alves, C.P. Borges, Evaluation of flat sheet and hollow fiber supported liquid membranes for fructose pertraction from a mixture of sugars, *Desalination* 148 (2002) 213–220.
- [28] D.T. Friesen, W.C. Babcock, D.J. Brose, A.R. Chambers, Recovery of citric acid from fermentation beer using supported-liquid membranes, *J. Membr. Sci.* 56 (1991) 127–141.
- [29] C. Scholler, J.B. Chaudhuri, D.L. Pyle, Emulsion liquid membrane extraction of lactic acid from aqueous solutions and fermentation broth, *Biotechnol. Bioeng.* 42 (1993) 50–58.
- [30] J. Zigova, E. Sturdik, D. Vandak, S. Schlosser, Butyric acid production by *Clostridium butyricum* with integrated extraction and pertraction, *Process. Biochem.* 34 (1999) 835–843.
- [31] G.C. Sahoo, N.N. Dutta, N.N. Dass, Liquid membrane extraction of cephalosporin-C from fermentation broth, *J. Membr. Sci.* 157 (1999) 251–261.
- [32] S. Schlosser, R. Kertesz, J. Martak, Recovery and separation of organic acids by membrane-based solvent extraction and pertraction: an overview with a case study on recovery of MPCA, *Sep. Purif. Technol.* 41 (2005) 237–266.
- [33] M.L.F. Gameiro, P. Bento, M.R.C. Ismael, M.T.A. Reis, J.M.R. Carvalho, Extraction of copper from ammoniacal medium by emulsion liquid membranes using LIX 54, *J. Membr. Sci.* 293 (2007) 151–160.
- [34] N.M. Kocherginsky, Q. Yang, L. Seelam, Recent advances in supported liquid membrane technology, *Sep. Purif. Technol.* 53 (2007) 171–177.
- [35] S. Schlosser, E. Kossaczky, Pertraction through liquid membranes, *J. Radioanal. Nucl. Chem.* 101 (1986) 115–125.
- [36] K. Dimitrov, V. Rollet, A. Saboni, Cobalt recovery from sulfate media applying a liquid membrane containing cyanex 302, *Chem. Eng. Technol.* 29 (2006) 625–630.
- [37] S. Alexandrova, A. Saboni, L. Boyadzhiev, N. Mouhab, L. Estel, Récupération de substances par pertraction à films tournants, *Chem. Eng. J.* 79 (2000) 155–163.
- [38] V. Leclère, R. Marti, M. Béchet, P. Fickers, P. Jacques, The lipopeptides mycosubtilin and surfactin enhance spreading of *Bacillus subtilis* strains by their surface-active properties, *Arch. Microbiol.* 186 (2006) 475–483.
- [39] Y.H. Wei, I.M. Chu, Enhancement of surfactin production in iron-enriched media by *Bacillus subtilis* ATCCV 21332, *Enzyme Microb. Technol.* 22 (1998) 724–728.
- [40] J. Martak, E. Sabolova, S. Schlosser, M. Rosenberg, L. Kristofikova, Toxicity of organic solvents used in situ in fermentation of lactic acid by *Rhizopus arrhizus*, *Biotechnol. Tech.* 11 (1997) 71–75.
- [41] K. Arima, G. Tamura, A. Kakinuma, Surfactin, US Patent 3,687,926 (1972).
- [42] E. Rodríguez de San Miguel, J.C. Aguilar, J.P. Bernal, M.L. Ballinas, M.T.J. Rodríguez, J. de Gyves, K. Chimmel, Extraction of Cu(II), Fe(III), Ga(III), Ni(II), In(III), Co(II), Zn(II) and Pb(II) with LIX® 984 dissolved in *n*-heptane, *Hydrometallurgy* 47 (1997) 19–37.
- [43] F. Xun, L. Junling, M. Ying, Z. Li, W. Debao, H. Zhengshui, Amino acid extraction with AOT reverse micelle, *Colloids Surf. A: Physicochem. Eng. Aspects* 179 (2001) 1–10.

- [44] L. Thimon, F. Peypoux, G. Michel, Interactions of surfactin, a biosurfactant from *Bacillus subtilis*, with inorganic cations, *Biotechnol. Lett.* 14 (1992) 713–718.
- [45] L. Heerema, M. Roelands, J.H. Hanemaaijer, J. de Bont, D. Verdoes, In-situ phenol removal from fermentation broth by pertraction, *Desalination* 200 (2006) 485–487.
- [46] K. Dimitrov, D. Metcheva, L. Boyadzhiev, Integrated processes of extraction and liquid membrane isolation of atropine from *Atropa belladonna* roots, *Sep. Purif. Technol.* 46 (2005) 41–45.
- [47] L. Boyadzhiev, K. Dimitrov, D. Metcheva, Integration of solvent extraction and liquid membrane separation: an efficient tool for recovery of bio-active substances from botanicals, *Chem. Eng. Sci.* 61 (2006) 4126–4128.