LIQUID MEMBRANE EXTRACTION OF LIPOPEPTIDES

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Surfactants of biological origin are of increasing interest for many industries due to their chemical diversity, multifunctional characteristics and low toxicity in comparison to synthetic, petrochemical-derived surfactants. A lipopeptide surfactin is one of the most powerful biosurfactants. Microbiological productivities, properties and applications of lipopeptides, including surfactin, have been extensively studied [1-3]. However, there is a lack of information about their separation, purification and concentration. In fact biosurfactants are not yet widely available because of their high production costs, which results primarily from low strain productivities and high recovery expenses.

The selective recovery and concentration of such lipopeptides from fermentation broth largely determines the production cost. The low concentrations and the amphiphilic character of these compounds pose serious limitations to their efficient recovery. Thus, development of efficient separation technologies is of growing interest. The commonly used methods for biosurfactants recovery are foam separation, acid precipitation, and solvent extraction [4]. The latter technique provides higher biosurfactant purity comparing to the other two methods [5]. The main inconvenient of solvent extraction is the problem with regeneration of the loaded solvent, and therefore the use of important quantities of solvent. In addition, the most efficient and generally used for lipopeptides recovery solvents, such as chloroform, methanol, and acetone, are known to be toxic and harmful to the environment and human health.

A prospective trend in biotechnological production of lipopeptides is the process known as in situ product removal (ISPR) in which the product is removed from the bioreactor during its production by an appropriate separation technique. Several integrated bioprocesses have been proposed to optimise productivity and cost-effectiveness of low and high molecular weight molecules [6]. Recently, the interest of coupling of production of surfactin by fermentation with its simultaneous recovery by adsorption was demonstrated [7, 8]. The purity of surfactin isolated after desorption with methanol and solvent evaporation is high, but the process is relatively long.

A potential advance for lipopeptides recovery from fermentation broth is the application of the low-cost liquid membrane process. This separation technique, based on solvent extraction, is called pertraction and operates in three-liquid-phase systems. Pertraction process is a combination of extraction and stripping operations performed simultaneously in one stage [9]. The main advantages of pertraction towards classical liquid-liquid extraction are the use of smaller quantities of organic solvent due to continuous regeneration of the solvent, as well as the possibility to recover the target species even in cases of low distribution coefficients [9]. Pertraction allows producing of valuable products of high quality at reduced costs, because of possibility to use as liquid membranes less powerful but more selective, less toxic and less expensive solvents than in the case of conventional solvent extraction. The interest of liquid membrane process for recovery of fermentation products have grown rapidly. Liquid membrane technique was successfully applied for recovery of some bioactive substances from fermentation broths [10, 11], but there are no data on lipopeptides recovery by using pertraction processes.

The recovery of the microbial lipopeptide surfactin from model aqueous solutions was studied. To confirm the applicability of the liquid membrane process to surfactin isolation from aqueous media, including fermentation broth, some properties, in particular, solubility and pH stability of the surfactin were studied. Usually, the *B.subtilis* straines used for surfactin production have been cultivated in medium with pH = 6.0-8.5 [7]. Consequently, lipopeptide extraction from such media was studied. To improve surfactin recovery, a possible small modification of pH was envisaged, too. However, acidification of the aqueous media containing surfactin was quite limited, because of its precipitation at pH < 5.5.

The effect of pH on the equilibrium distribution of surfactin between various organic solvents and aqueous solutions was studied. The most polar from studied solvents 1-octanol provided practically complete lipopeptide extraction from aqueous media in all studied pH-interval (pH = 5.5-9.0). However, the back extraction of lipopeptide into an aqueous solution and therefore, the regeneration of the loaded organic phase after the extraction was very difficult. In contrast, the non-polar *n*-heptane and *n*-octane were clearly less efficient solvents, but the degree of surfactin removal into these solvents was found to be strongly affected by the aqueous solution acidity. For both studied alkanes, the degree of surfactin extraction was relatively high from slightly acid (over than 80 % at pH = 5.5) or slightly basic (over than 60 % at pH = 9.0) aqueous solutions, while from neutral aqueous solutions the extraction was limited (less than 10 % at pH = 7.0-7.5). Consequently, the studied alkanes are suitable for liquid membrane permeation of surfactin, providing conditions suitable for lipopeptide

extraction into organic solvent (at pH = 5.5-6.0), but also conditions favourable for its back extraction into an aqueous solution (at pH = 7.0-7.5). The observed unusual pH effect of relatively high extraction degrees from both acid and basic media and noticeably reduced degree of extraction from neutral media could be explained to the different conformations of lipopeptide in these media. The observed minimum of degree of extraction from neutral media could be attributed to the higher micropolarity of the β-sheet micelles formed by surfactin molecules at these conditions. This configuration is characterised by an exposure of a large number of carboxylic groups on the micelle surface which could explain the relatively polar character of surfactin. In both acid and basic media, surfactin conformation alters from β-sheet to α-helices [3]. At this configuration, the non-polar ends of lipopeptide molecules are more exposed to contact the organic solvents and, as result, higher extraction degrees were obtained.

Surfactin permeation through a liquid membrane of *n*-heptane was studied in a laboratory rotating discs contactor. Batch pertraction process was carried out at different acidities of the feed solution. The obtained results of lipopeptide transport in the three-liquid-phases system show that surfactin can be successfully recovered from slightly acid media (pH = 5.5-6.0), including fermentation broth, by means of pertraction. The process efficiency grows with decrease of pH of the feed solution (83 % recovery at pH_{feed} = 6.05 and 97 % at pH_{feed} = 5.65 after 4 h pertraction). The pertraction process was very rapid: about 90 % of surfactin was removed from feed solution in 30 min only.

The efficient permeation of surfactin through a liquid membrane offers a new opportunity to isolate lipopeptide from fermentation broth. A further coupling of fermentation with pertraction in a new ISPR process could provide a relatively low-cost production of lipopeptides with high purity. This integrated bioprocess could also contribute to resolve the problem with foam formation during fermentation of biosurfactants.

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