SUPERCRITICAL CO_2 EXTRACTION AS A METHOD OF SURFACTIN SEPARATION AFTER BIOSYNTHESIS

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Biosurfactants are surface active compounds, which are produced by microorganisms or are obtained by biotransformation. Surfactin (Fig.) is cyclic biosurfactant secreted by various strains of *Bacillus subtilis*. It is one of the most studied and well characterized biosurfactant [1].

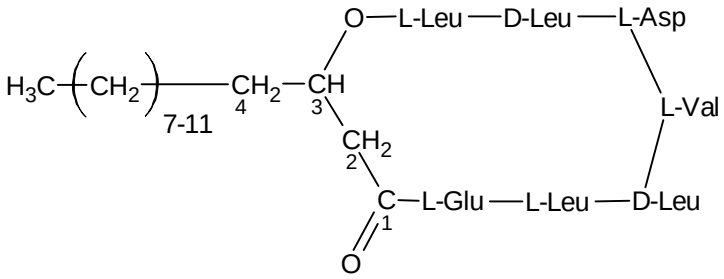

Fig. 1 Surfactin molecule.

When compared to synthetic surfactants, biosurfactants exhibit numerous advantages such as biodegradability, low toxicity, and tolerance of extreme conditions of pH, temperature, and salinity [2]. Due to these features they can potentially replace synthetic surfactants in many industries, such as cosmetics, agricultures, detergents, and food. The most numerous patents concern their utilization in a petroleum industry (33%), where they are used to facilitate transportation of viscous hydrocarbons and for crude oil extraction from reservoirs [3]. Despite these benefits, their industrial scale production is hindered by low manufacturing output and expensive downstream processing [2].

We propose downstream method for separation and purification of surfactin after solid state fermentation which utilizes supercritical fluid extraction (SFE). Another method we proposed is supercritical fluid desorption of surfactin from activated carbon. Adsorption on activated carbon has been examined as a potential method for recovery of surfactin from liquid fermentation broth [4–7]. Only organic solvent desorption of surfactin from activated carbon has been proposed so far [5]. Supercritical fluids are excellent solvents due to density and dissolving ability such as liquid solvents and diffusivity, viscosity and penetration ability such as gases [8]. Extraction yields depends not only on type of supercritical fluid, but also on temperature, pressure and time of extraction. As supercritical fluids can serve aliphatic hydrocarbons, such as propane, pentane or hexane, aromatic hydrocarbons, such as toluene, and alcohols, such as methanol, ethanol or 2-propanol. The most interesting supercritical fluid is carbon dioxide, because it has numerous advantages: it is cheap, nontoxic and easy accessible [9]. Moreover, it has low critical temperature and pressure of 31.1°C and 73.8 bar, respectively. Its main disadvantage is that it is nonpolar and

thus weekly dissolves polar compounds. To overcome this problem, polar cosolvents, such as methanol or ethanol are added.

Supercritical CO_2 extractions of surfactin adsorbed on activated carbon and produced by *Bacillus subtilis* natto KB1 strain grown on rapeseed cake have been conducted on a semi-automatic Waters MV-10 ASFE System for SFE. Effect of various parameters on desorption yield have been evaluated: temperature and pressure of extraction, as well as type and mole fraction of cosolvent. Surfactin extraction yield has been assessed based on HPLC-UV analysis of solutions.

We found that no surfactin extraction occurs when pure CO_2 is used, both from activated carbon and from rapeseed cake. The probable cause is nonpolar nature of CO_2. Therefore we added polar cosolvents to supercritical fluid: methanol and ethanol. It turned out that both of them significantly improve extraction yield of surfactin from activated carbon (Fig. 2a). Extraction of fermented rapeseed cakes lead to release of numerous compounds with similar retention time as surfactin, thus making quantitative analysis of surfactin impossible. Effect of extraction conditions on surfactin yield have been checked only for activated carbon. We observed decrease of surfactin quantity desorbed from 1g of activated carbon with increase of the extraction process temperature in the range of 40-60°C (Fig. 2b). Methanol and ethanol have similar critical pressure as CO_2 (81 and 63 bar) but significantly higher critical temperature (239.85 and 240.85°C). Critical temperature of CO_2 and methanol/ethanol mixture is higher than for pure CO_2, and is 41.5°C (x_{alc} = 0.05), 52°C (x_{alc} = 0.1) and 62°C (x_{alc} = 0.15). Thus, we can notice that extraction yield is better in subcritical region, *i.e.* just below critical point (T = 40 and 50°C), than in supercritical (T = 60°C) (Fig. 2b). We performed extractions in 3 cycles, each for 20 minutes. Regardless of extraction temperature, 83-85% of total extracted surfactin has been recovered in first cycle (Fig. 2b).

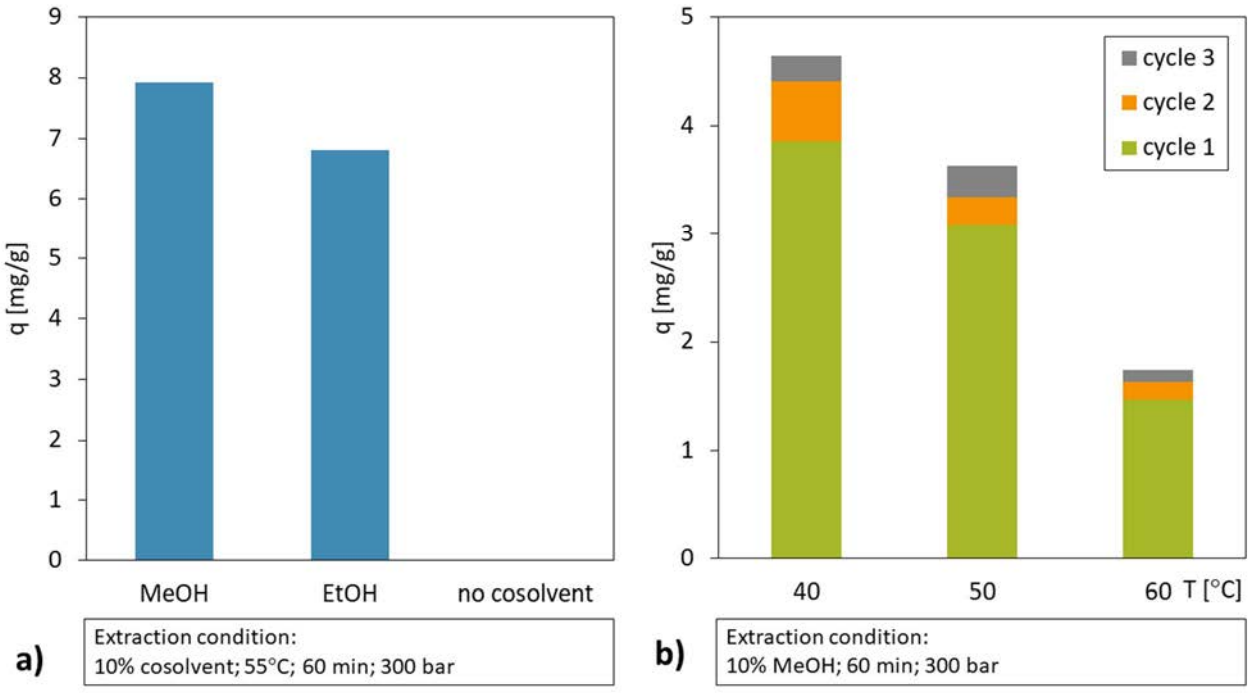

Fig. 2 Supercritical CO_2 extraction yield of surfactin from activated carbon: a) effect of cosolvent type; b) effect of extraction temperature.

More pronounced effect than temperature on surfactin desorption yield, had mole fraction of cosolvent as indicated by predominant horizontal arrangement of zones in Fig. 2a. Surfactin desorption increased with mole fraction of cosolvent. Extraction at 100 bar was almost as high as at 300 bar, but at 200 bar we observed unexpected decrease of extraction yield (Fig. 2b). The highest amount of surfactin (16.1 mg/g) has been desorbed at 300 bar, when ethanol has been employed as cosolvent (x_{alc} = 0.15), temperature was 55°C, and time 60 min.

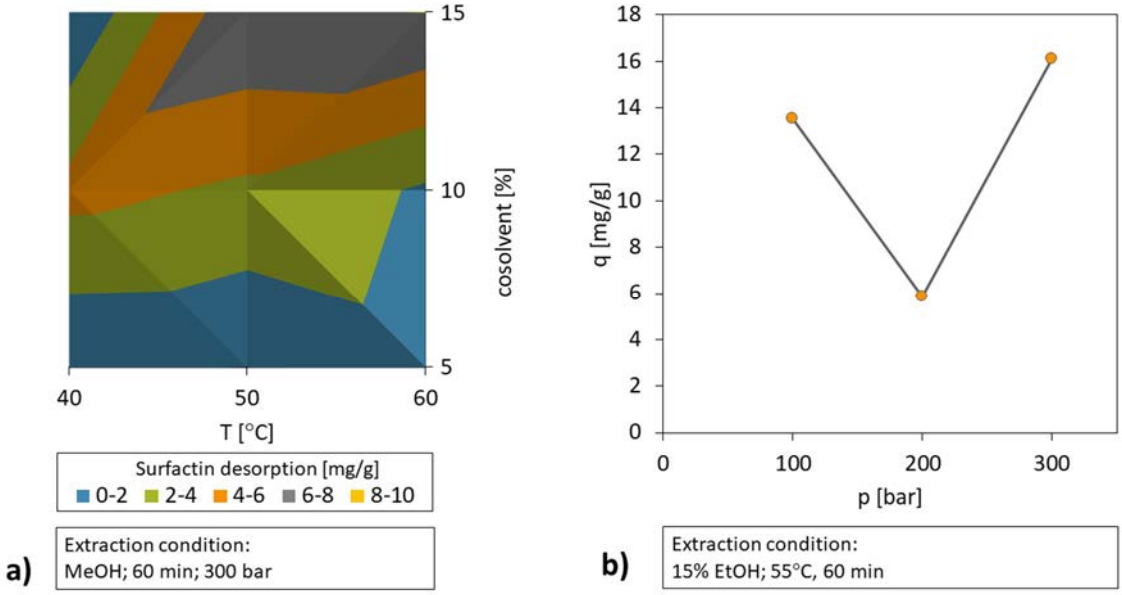

Fig. 3 Supercritical CO_2 extraction yield of surfactin from activated carbon: a) combined effect of extraction temperature and mole fraction of cosolvent; b) effect of extraction pressure.

Supercritical CO_2 extraction is suitable method for surfactin extraction after biosynthesis from rapeseed cake or desorption from activated carbon only if polar cosolvent, such as methanol or ethanol, has been employed.

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