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# PHD THESIS: „BIOSURFACTANTS SEPARATION ON ACTIVATED CARBON”

## SUMMARY

Biosurfactants is a promising group of surface active compounds which possess numerous advantages over synthetic surfactants. Their biodegradability make them environment-friendly and their low toxicity make them also consumer-friendly. However, only limited number of them are mass produced. Surfactin is one of biosurfactants with the highest surface activity and interesting biological properties. Despite these benefits, its industrial scale production is hindered by low biosynthesis yield and expensive downstream processing. To overcome these constraints, optimization of production process is carried out, as well as looking for cheaper and more efficient methods of surfactin recovery from fermentation broth.

In presented thesis, a new method of surfactin separation is proposed, which combines activated carbon (AC) adsorption and supercritical fluid extraction (SFE) with carbon dioxide and ethanol mixture as supercritical fluid. Activated carbon is cost-efficient adsorbent commonly used for purification of air and water contaminated with various organic and inorganic compounds. In turn, supercritical fluid extraction allows to significantly reduce utilization of organic solvents when compared with classical solvent extraction. Moreover, extraction of thermolabile compounds is possible thanks to low temperature of extraction by supercritical fluids.

Effect of properties of commercially available activated carbons on surfactin adsorption have been evaluated. Activated carbon were characterized by means of SEM-EDS, proximate analysis and sorption measurements, on which basis porous texture parameters were determined. Also pH of aqueous suspension of active carbons was determined. It turned out that a key factor for efficient surfactin adsorption is high fraction of mesopores in total pores volume and pH of AC aqueous suspension in the range of 6.5-8. It was observed that on activated carbons with higher pH than 8, hydrolysis lactone bond in surfactin molecule occurs, which leads to depsipeptide ring opening and resulting with new linear analogues of surfactin. The bond which undergoes hydrolysis and structures of new analogues were confirmed by UHPLC/MS/MS analysis.

On the basis of found relationships, activated carbons with higher mesoporosity (32-57% compared with 8-29% in previously tested ACs) and pH of aqueous suspension close to neutral were prepared. Coniferous tree wood pellet were chemically activated with orthophosphoric acid. Resulted activated carbons exhibit higher surfactin adsorption capacity (124 mg/g) than commercially available activated carbons (30 mg/g), and also than it had been previously reported in the literature (28 mg/g).

Also, it has been checked how activation conditions influenced activated carbon properties and, in a consequence, surfactin adsorption on resultant sorbents. Effect of activation temperature in the range of 400-700°C and impregnation ratio (2:1 and 1.5:1) have been evaluated. Examples of observed relationships are as follows: ash and phosphorous content in activated carbons rise with activation temperature; volatile matter and oxygen content is the lowest in the sorbents activated in 550°C – both in lower and higher temperature, activated carbons with significantly higher content were prepared. Effect of activated carbon chemical structure on surfactin adsorption were not observed. Adsorption yield depends only on porous texture parameters – it is higher when fraction of mesopores with 5-50 nm width is higher and when fraction of micropores and narrow mesopores with 2-3 nm width is lower.

Optimal parameters of surfactin desorption from activated carbons by supercritical carbon dioxide extraction in continuous system were determined. It was necessary to add polar cosolvent to supercritical carbon dioxide in order to increase its polarity, because pure sc-$CO_2$ desorbed polar surfactin in a minimal way. Optimal conditions of surfactin desorption from activated carbon was temperature of 40°C, pressure of 100 bar, ethanol as cosolvent in concentration of 15%. Desorption yield in these conditions was still low and after 40 min it was 30%. Probable reason was high mass transfer limitations in pores of highly microporous sorbent used in the experiments. Due to high amount of activated carbon needed for process optimization I decided to use commercially available sorbent Novicarbon, instead of prepared mesoporous activated carbon.

Supercritical fluid extraction were used more successfully for surfactin extraction from solid fermentation medium, which means rapeseed meal. Maximum extraction yield was 91%. Optimal parameters of the process were temperature of 60°C, pressure of 100 bar, ethanol as cosolvent in concentration of 15% and supercritical fluid flow of 8 ml/min.

Surfactin concentration at every stage of all processes were analyzed by means of HPLC/UV method. It allowed to determine mutual ratio of surfactin five main analogues, which differ in alkyl chain length ($C_{12}$-$C_{15}$), in solutions after adsorption, hydrolysis and supercritical fluid extraction. As a result of all these processes surfactin analogue composition has changed when compared with original surfactin used for experiments.

As a result of conducted research relationships in systems of activated carbon-surfactin-water, activated carbon-surfactin-supercritical carbon dioxide and rapeseed meal-surfactin-supercritical carbon dioxide were revealed. That makes optimization of surfactin separation from fermentation medium with utilization of activated carbon and supercritical fluid extraction possible.

Feynman said: ‘When a scientist doesn’t know the answer to a problem, he is ignorant. When he has a hunch as to what the result is, he is uncertain. And when he is pretty darn sure of what the result is going to be, he is still in some doubt”[1]. Doubts are still present in many issues discussed in the thesis. It leaves broad field for research continuation and further improvement of proposed method for surfactin separation from fermentation media.

[1] Richard P. Feynman, The Value of Science, http://www.faculty.umassd.edu/j.wang/feynman.pdf, accessed: 29-06-2019, based on: Richard P. Feynman, What Do You Care What Other People Think? Further adventures of a curious character, W W Norton, 1988.