Abstract

Enzymes represent a very important segment among the products of biotechnology because they are used both in food, pharmaceutical, chemical, paper, textile and cosmetics industry, and in medicine for therapeutic purposes and diagnostics. Availability and cost of enzymes and other bioproducts is largely dependent on the isolation method, because the final processes can represent up to 90% of the cost of biotechnological process. Extractions of a two-phase water systems are an interesting alternative to conventional methods mainly due to lower environmental burdens compared with the use of organic solvents, maintaining the stability and activity of biomolecules and low energy consumption. A goal of this doctoral dissertation is to develop a continuous extraction with two-stage water systems in microfluidic devices and further integration of the process of isolation with membrane processes. For the isolation of selected commercial proteins (bovine serum albumim (BSA), α - amylase and laccase) and filtrate obtained by submerged cultivation of the fungus *Trametes versicolor* Tv6 an aqueous two-phase extraction in a variety of microchannel geometries was studied. A new type of aqueous two-phase systems with a selected ionic liquid and D-fructose was used for the first time for the extraction of BSA. For the protein extraction different configurations of the microfluidic devices with diverse flow regimes were tested. The extraction with the parallel flow enabled separation of phases at the exit of Y- and ψ -shaped inlet and outlet microchannels. Within the microfluidic device with the X- and T- junction segmented flow regimes were established and for the separation of the two phases a microsettler was developed.

Furthermore, an extraction of proteins with an aqueous two-phase systems in a microfluidic devices was mathematically described and a model equations were solved by means of appropriate numerical methods. The mathematical model was further validated, with the data obtained from experimental measurements. For a detailed study of transport phenomena in microfluidic devices in parallel and segmented flow, an analytical method for *on-line* concentration measurements within microchannels with thermal lens microscopy was developed.

In the final part of this dissertation, an integrated system for the primary of the isolation of laccase from the filtrate submerged culture of *Trametes versicolor* Tv6 extraction using a two-phase water system with segmented flow, and phase separation in a microsettler was used. Phase with the desired protein was further concentrated and purified in a miniaturized ultrafiltration device.

Additionally an integrated process in a miniaturized device was developed for the continuous production of isoamyl acetate. The reaction of esterification was performed in a two-phase system ([Bmpyr][dca]/*n*-heptane) with a lipase B from yeast *Candida Antarctica* as a catalyst. Reaction takes place at the two-phase interface, which enabled *in-situ* product extraction into the organic phase, which was further separated in an integrated membrane separator. In this way the reuse of ionic liquid with the dissolved enzyme was possible, which lead to the sustainable way of producing flavour with a widespread potential use.

Keywords: aqueous two-phase system, extraction, microfluidic device, proteins, mathematical model