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Multi-stage laccase extraction and separation using aqueous two-phase systems: Experiment and model

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ABSTRACT

This work presents results of experimental and model investigation of continuous multi-stage enzyme extraction using aqueous two-phase systems for the first time. The aqueous two-phase system comprised polyethylene glycol 3000 and phosphate with additional sodium chloride buffered to pH 7. Two different laccases served as model enzymes. One of the laccases was directly taken from fungal culture supernatant, while the other laccase was solubilized lyophilisate. The modeling is based on an equilibrium stage approach. Equilibrium data were taken from single-stage experiments and approximated by different correlation equations. The model describes densities, phase equilibrium, enzyme activity partitioning between the phases. Moreover it allows to consider activity changes due to the aqueous two-phase system. Eight multi-stage mixer-settler experiments under varying operation conditions were performed to validate the proposed model; whereas the total throughput of all multi-stage extraction experiments was about 350 g h^{-1}. The average relative deviation of modeled activities from experimentally measured activities was 23%. Therefore, the model is able to calculate the behavior of the phases as well as the partitioning of the two enzymes between the two phases for a multi-stage process based on single-stage data.

1. Introduction

In the pharmaceutical as well as in the chemical industry biotechnological products are gaining more and more importance. Every year new enzymes and bio-based drugs such as monoclonal antibodies (mAbs) are discovered, developed and produced. Increasing capacities and titers in biotechnological production processes pose a challenge to the existing downstream processes of biotechnological products in general [1]. The challenge can be met by either improving existing processes or establishing new, innovative ones. Aqueous two-phase extraction (ATPE) belongs to the latter category. This process bases on aqueous-two phase systems (ATPS), which can be formed by the solution of two hydrophilic, but incompatible components in water [2]. Examples of such ATPS are the aqueous solutions of two polymers (e.g. polyethyleneglycol (PEG)-dextran) or the aqueous solution of a polymer and a salt (e.g. PEG – phosphate). Fig. 1 shows a rather rough and general scheme of the alternatives and steps in downstream processing of biotechnological products and how the ATPE can replace existing process options. In general, process capacity decreases, while the purity increases. Due to its high capacity and reasonable purity gain, aqueous two-phase extraction is considered as an option in early downstream processing. In case of reduced purity demands, e.g. for some industrial enzymes, the downstream process may stop at an earlier stage with less purity.

It is well known that multi-stage purification can lead to improved product recovery and/or purity. Already at the end of the 1970s, the group of Kula [3–5] has reported enzyme isolation and separation using ATPS in a centrifugal extractor comprising multiple separation stages. More recently, Rosa et al. have investigated multi-stage antibody purification in ATPS experimentally in test tubes [6], a mixer-settler apparatus [7] and a packed extraction column [8].

In spite of extensive knowledge of fundamentals of ATPS and their applicability in purification and separation processes, reports on industrial application are scarce. Available publications report about strong influences of parameters, such as components forming the phase system, ionic strength, molar mass of polymers, concentrations, temperature, additives and the product itself, on extraction performance [9,10,2]. The multiplicity of parameters

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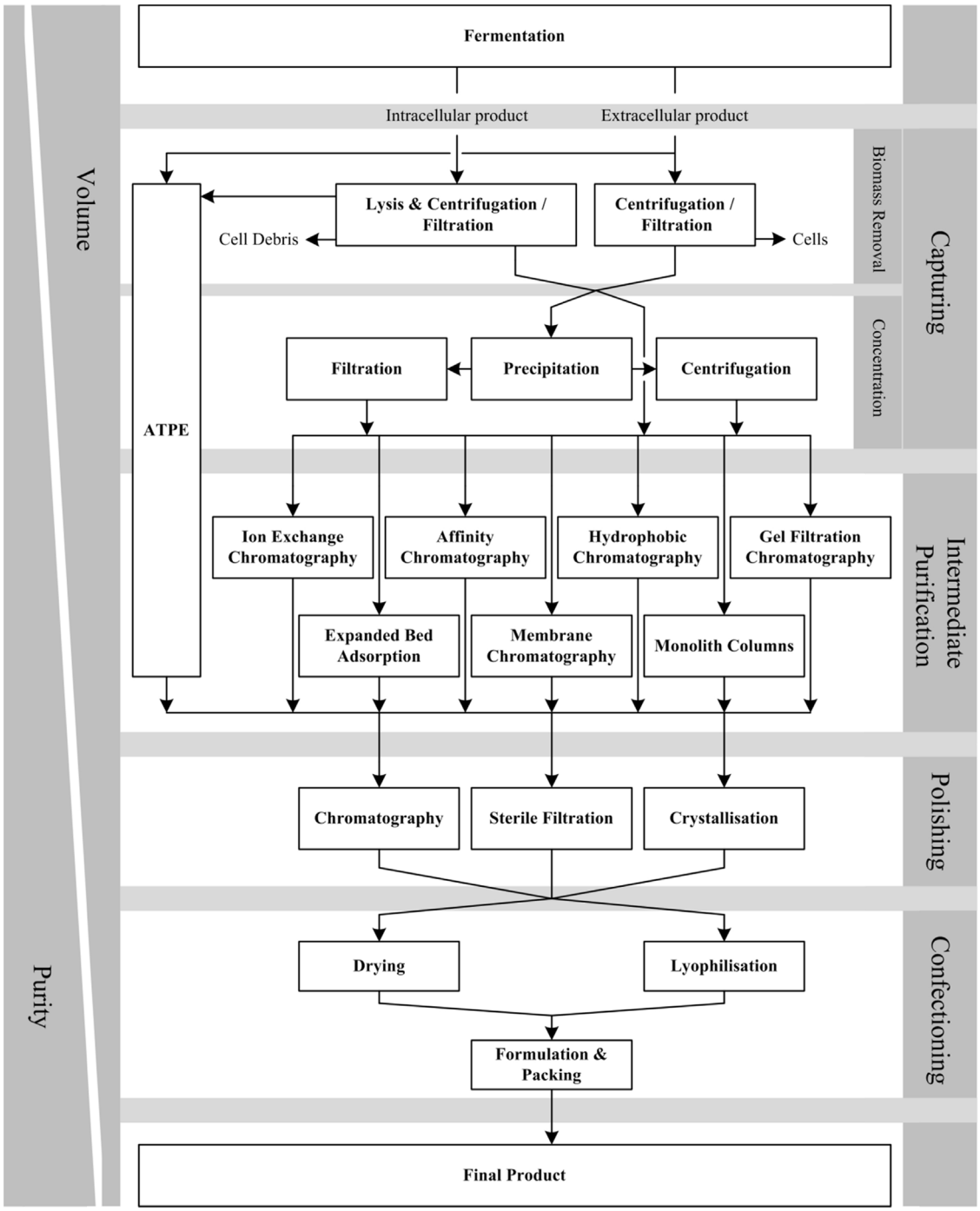

Fig. 1. General scheme of the purification of biotechnological products.

causes a high experimental effort for developing ATPE processes. Process modeling contributes to reducing experimental effort and enables an efficient process design- and variant evaluation. Mistry et al. [11,12], Ahmad et al. [13,14] and Samatou et al. [15] have proposed process models using ATPS. Mistry's and Ahmad's models lack experimental validation and Samatou validates only the partitioning of a mAbs and not for an enzyme which brings several new challenges along. The scope of this work was to model a multi-stage aqueous two-phase enzyme extraction process based on single-stage data and to validate it experimentally. *Pleurotus sapidus* culture supernatant containing laccase was spiked with laccase from *Trametes versicolor*. This spiked supernatant containing two different laccases was proceed in an ATPS consisting of PEG3000 and phosphate in order to show the separation potential of this technology. Moreover this ATPS was chosen for the extraction of the used enzymes, as the concentration of PEG is comparable low for the formation of two phases and so these systems have a lower viscosity. As the phases have to be pumped from one stage to another, it is an important property of an ATPS used for multistage extraction. Sodium chloride, as a partitioning influencing additive, was included in the modeling framework and the experiments. In [18] single-stage separation of the laccases was investigated thoroughly resulting in clearance factors of 5.23 for laccase from *P. sapidus* and 6.45 from *T. versicolor* serving as a bench mark for the evaluation of the multi-stage separation.