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Partitioning and purification of cellulases in aqueous two-phase system

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Introduction and objectives

Aqueous two-phase systems (ATPS) can be formed by mixing the solutions of two mutually incompatible polymers or polymer and salt above critical concentrations and represent media that are very well suited for the separation and purification of biomolecules. The basis of separation is uneven distribution of biomolecules between two phases both having high water content. This so-called biocompatibility of phases allows preservation of biomolecules' native structure while the presence of polymer can even improve their stability. Partitioning is governed by numerous factors that can be manipulated to achieve desired separation and purification results, which makes aqueous two-phase system very flexible for application.

Cellulases, enzymes belonging to family of glycosyl hydrolases, play key role in organic carbon turnover and have important and wide application in industry. Extraction of enzymes in aqueous two-phase systems has been recognized as useful technique in downstream processing for their isolation and purification. In this study, partitioning of cellulases in polyethylene glycol/dextran and polyethylene glycol/salt two-phase systems was investigated with the aim to determine the most appropriate molecular weight of polymers, kind of salt and concentration of aqueous two-phase constituents at which the highest possible yield and purification factor in the top phase can be achieved.

Methodology

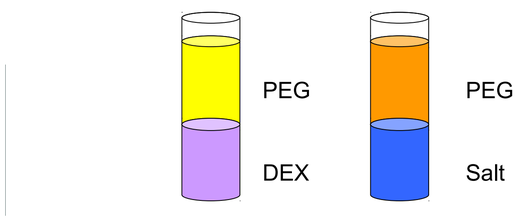
Experiments

The enzyme solution used in partitioning experiments was prepared from commercial cellulases Celluclast 1.5 L™ (Novozyme). Cellulases preparation was diluted in 10 mmol/l acetate buffer, pH 5.0 to make basal enzyme solution (BES) and the cellulases activity was determined by measuring amount of glucose liberated from CM-cellulose at 40 °C.

Concentration of protein was determined by Bradford method using BSA as standard.

In preparation of polymer/polymer ATPS polyethylene glycol (PEG) having different molecular weights and dextran 500,000 were used. PEGs and $(NH_4)_2SO_4$, Na_2SO_4 and KH_2PO_4 were used for polymer/salt ATPS creation.

Polymer/polymer and polymer/salt ATPS



Calculations

Yield in the top phase was calculated as:

$$Y_t(\%) = \frac{100 \cdot V_t \cdot K}{V_t \cdot K + V_b}$$

The partition coefficient was determined as:

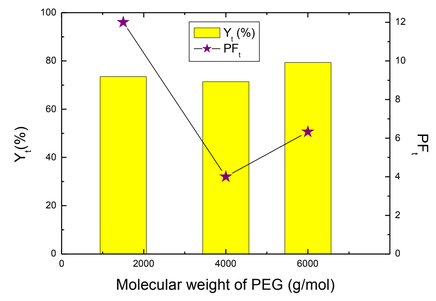
$$K = \frac{activity_{top\ phase}}{activity_{bottom\ phase}}$$

The purification factor was calculated according to:

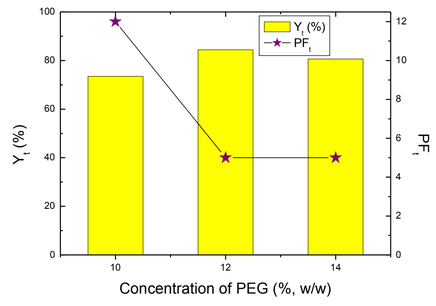
$$PF_t = \frac{specific\ activity_{top\ phase}}{specific\ activity_{BES}}$$

Selected results

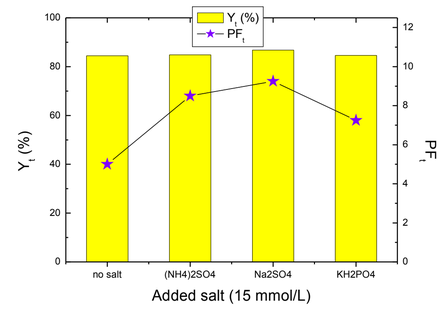
Partitioning and purification in PEG/DEX ATPS



Composition of ATPS: 10% (w/w) PEG/5% DEX (w/w) /35% (w/w) BES



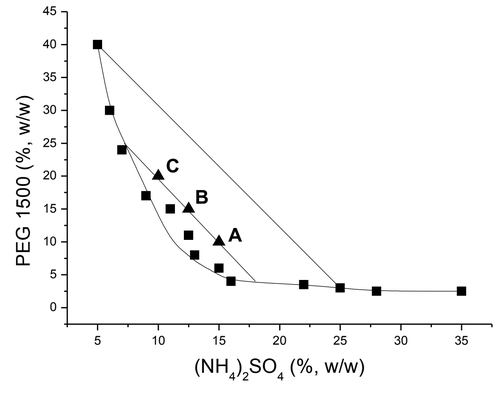
Composition of ATPS: PEG 1500/5% (w/w) DEX//35% (w/w) BES



Composition of ATPS: 12% (w/w) PEG 1500/5% (w/w) DEX//35% (w/w) BES

Aqueous two-phase system is suitable medium for the extraction and purification of cellulases by their partitioning in the top phase.
Generally, higher results of enzymes yield were obtained in PEG/DEX ATPS while partitioning in PEG/salt system can be improved by optimizing phase volume ratio.
In selected PEG/DEX system which gave the highest yield, purification can be enhanced by the addition of salt.

Conclusions



Phase diagram of PEG 1500/$(NH_4)_2SO_4$ ATPS

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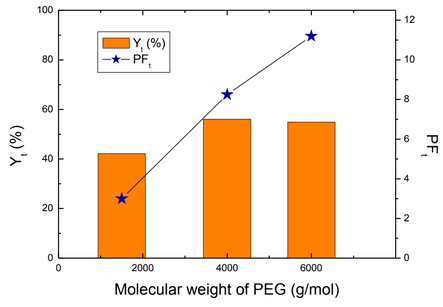
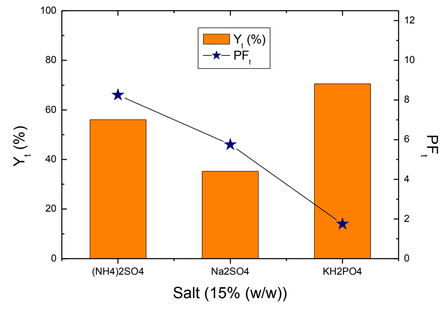
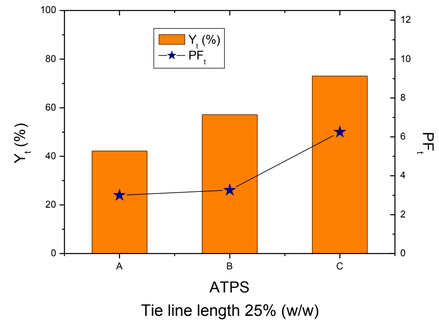
Partitioning and purification in PEG/salt ATPS



Composition of ATPS: 10% (w/w) PEG/15% (w/w) $(NH_4)_2SO_4$//35% (w/w) BES



Composition of ATPS: 10% (w/w) PEG 4000/15% (w/w) salt//35% (w/w) BES



Tie line length 25% (w/w)