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 enzymes in aqueous two-phase systems.

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 preparation.

In preparation of polymer/polymer ATPS polyethylene glycol (PEG) having different molecular weights and dextran 500,000 were used. PEG and (NH₄)₂SO₄, Na₂SO₄ and KH₂PO₄ were used for polymer/salt ATPS creation.

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:

\[ K = \frac{\text{activity}_{\text{top phase}}}{\text{activity}_{\text{bottom phase}}} \]

The purification factor was calculated according to:

\[ PF_t = \frac{\text{specific activity}_{\text{top phase}}}{\text{specific activity}_{\text{BES}}} \]

Selected results

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.

Conclusions

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

\[ K = \frac{\text{activity}_{\text{top phase}}}{\text{activity}_{\text{bottom phase}}} \]

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

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

Composition of ATPS: 10% (w/w) 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