

Experimental Design in Extraction of *Pseudomonas* sp. Protease from Fermented Broth by Polyethylene Glycol/Citrate Aqueous Two-Phase System

Authors : Omar Pillaca-Pullo, Arturo Alejandro-Paredes, Carol Flores-Fernandez, Marijuly Sayuri Kina, Amparo Iris Zavaleta

Abstract : Aqueous two-phase system (ATPS) is an interesting alternative for separating industrial enzymes due to it is easy to scale-up and low cost. Polyethylene glycol (PEG) mixed with potassium phosphate or magnesium sulfate is one of the most frequently polymer/salt ATPS used, but the consequences of its use is a high concentration of phosphates and sulfates in wastewater causing environmental issues. Citrate could replace these inorganic salts due to it is biodegradable and does not produce toxic compounds. On the other hand, statistical design of experiments is widely used for ATPS optimization and it allows to study the effects of the involved variables in the purification, and to estimate their significant effects on selected responses and interactions. The 24 factorial design with four central points (20 experiments) was employed to study the partition and purification of proteases produced by *Pseudomonas* sp. in PEG/citrate ATPS system. ATPS was prepared with different sodium citrate concentrations [14, 16 and 18% (w/w)], pH values (7, 8 and 9), PEG molecular weight (2,000; 4,000 and 6,000 g/mol) and PEG concentrations [18, 20 and 22 % (w/w)]. All system components were mixed with 15% (w/w) of the fermented broth and deionized water was added to a final weight of 12.5 g. Then, the systems were mixed and kept at room temperature until to reach two-phases separation. Volumes of the top and bottom phases were measured, and aliquots from both phases were collected for subsequent proteolytic activity and total protein determination. Influence of variables such as PEG molar mass (MPEG), PEG concentration (CPEG), citrate concentration (CSal) and pH were evaluated on the following responses: purification factor (PF), activity yield (Y), partition coefficient (K) and selectivity (S). STATISTICA program version 10 was used for the analysis. According to the obtained results, higher levels of CPEG and MPEG had a positive effect on extraction, while pH did not influence on the process. On the other hand, the CSal could be related with low values of Y because of the citrate ions have a negative effect on solubility and enzymatic structure. The optimum values of Y (66.4 %), PF (1.8), K (5.5) and S (4.3) were obtained at CSal (18%), MPEG (6,000 g/mol), CPEG (22%) and pH 9. These results indicated that the PEG/citrate system is accurate to purify these *Pseudomonas* sp. proteases from fermented broth as a first purification step.

Keywords : citrate, polyethylene glycol, protease, *Pseudomonas* sp

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