CONTINUOUS BIOLOGICAL PRODUCTS EXTRACTION IN AQUEOUS TWO PHASE SYSTEMS

Edith Espitia-Saloma, Patricia Vázquez-Villegas, Marco Rito-Palomares, Oscar Aguilar Instituto Tecnológico y de Estudios Superiores de Monterrey Centro de Biotecnología-FEMSA, Monterrey C.P.64849, e.espitia.phd.mty@itesm.mx

Key words: aqueous two phase systems, continuous, scale-up

Introduction. Aqueous two-phase systems (ATPS) are a liquid-liquid extraction strategy recognized as an advantageous technique in bioproducs recovery[1]. Continuous operation advantages are more than acknowledged within industrial processes: process time and costs decrease and yields increase[2]. Noteworthy research has been achieved concerning batch ATPS, nevertheless continuous mode, showing industrial potential has been left behind.
Therefore, the objective of this work is to design, implement and characterize a continuous aqueous two-phase system at pilot scale, showing continuous large-scale aqueous two phase systems as a viable and reproducible extraction/purification system.

Methods. A laboratory prototype was scaled up from a capacity of 50 ml/min to 1L/min and semiautomatized. A 2^5 experimental design considering top (TP) and bottom phase (BP) feeding flow, number of static mixers, coalescence stage length and TLL (tie line length) for PEG1000/phosphates systems was made in order to model and optimize ATPS operation in the prototype. Scaled-up equipment performance was tested for Gentian violet, whey protein isolate (WPI) and spent brewer's yeast suspension. Hold-up and partition behavior were monitored via spectrophotometric, protein and enzymatic assays. In the case of the brewer's yeast, alpha amylase activity was measured by starch-iodine method[3], invertase by Miller method[4] and proteases by Kunitz assay[5].

Results. ATPS for batch and continuous mode were performed for crystal violet and WPI. Figure 1 compares the settling time for the batch system with the hold up stabilization time in the continuous mode for WPI. A noteworthy shorter stabilization time (10 min shorter) is appreciated in the continuous mode.

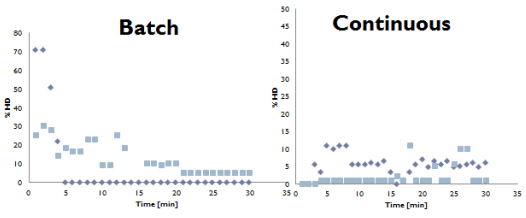

Fig.1 Hold up behavior TP (squares), BP(diamonds)

A better partition performance was also observed due to a better mass transference and more stable product profile in the continuous mode for crystal violet and WPI. (Fig 2)

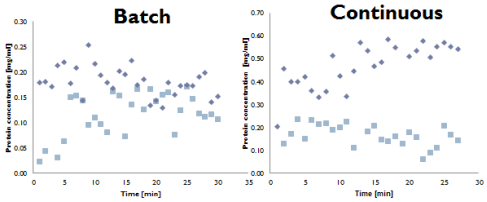

Fig.2 WPI protein concentration (TP (squares), BP (diamonds))

The spent brewer's yeast suspension, with the presence of interphase has a minimal stabilization time for TP and BP and hold up is completely minimized (Fig 3). Different partition viability is evidenced by recovery of alpha amylase in TP, and invertase in the interphase.

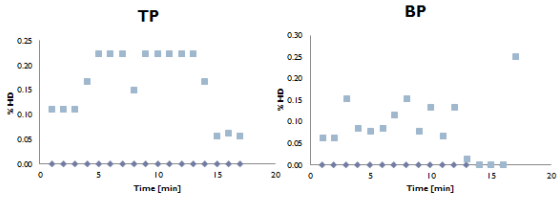

Fig.3 Hold up behavior. Interphase (squares), BP and TP (diamonds).

Conclusions. A continuous scaled-up ATPS plant was developed. Its performance robustness was shown for the recovery of low-molecular weight molecules up to complex cell matrixes such as spent brewer's yeast. Continuous operation showed noteworthy advantages over batch operation: shorter process and stabilization times and improved mass transfer behavior.

Acknowledgements. To Tecnológico de Monterrey Bioprocess Research Chair (Grant CAT161) and CONACyT for the doctoral fellowships of E. Espitia-Saloma No. 246739 and P. Vázquez-Villegas No. 228073.

References.

(1). Raghavarao,K, Ravganath, T, Srinivas, N, Barhate, R. (2003). *Clean Techn Environ Policy.* 5:136-141. (2). Igarashi, L, Kieckbusch, T, Franco, T. (2004). *J. Chromatogr B.* 26. (3): 151-157. 3. Xiao, Z, Storms, R, Tsang, A. (2006). *Anal Biochem.* 351. (1): 146-148. (4). Miller, G. (1960). *Anal Biochem.* 1. (2): 127-132. (5). Kunitz, M. (1947). *Gen Physiol.* 30. (4): 291-310.