

Lipase immobilization in PHBV using carbon dioxide as anti-solvent with SEDS technique

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Introduction

This work investigates the application of supercritical carbon dioxide as anti-solvent for the immobilization of lipase Amano PS in poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) with dichloromethane as organic solvent using the Solution Enhanced Dispersion by Supercritical fluids (SEDS) technique. For the precipitation of pure PHBV the parameters investigated were the concentration of PHBV (30 mg.mL^{-1}) in the organic solution, pressure (from 80 to 200 bar), solution flow rate fixed at 1 mL.min^{-1} , anti-solvent flow rate at 40 mL.min^{-1} and constant temperature of 313 K. Pure precipitation of PHBV indicates that an increase in pressure led in most cases to particles with reduced sizes. The morphology of precipitated PHBV particles was spherical and was not influenced by increasing pressure. For the co-precipitation/immobilization experiments it was evaluated the effect of PHBV concentration (5 to 20 mg.mL^{-1}) and enzyme content (0.5 to 2.0 mg.mL^{-1}) keeping fixed the system pressure (80 bar), temperature (40°C), organic solution flow rate (1.50 mL.min^{-1}) and anti-solvent flow rate (20 mL.min^{-1}).

Results e Discussion

The experimental apparatus used in this work for the precipitation of pure PHBV and co-precipitation lipase Amano PS with PHBV is shown in Figure 1 [1].

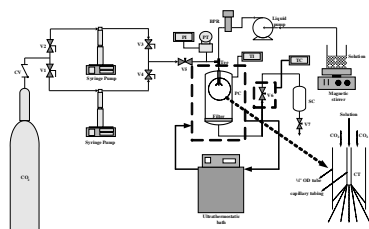


Figure 1. Schematic diagram of the precipitation apparatus. CV - check-valve; V1, V2, V3 and V4 - ball valve; V5, V6 and V7 - needle valve; BPR - back pressure regulator; PT - pressure transducer; PI - Pressure indicator; TI - temperature indicator; TC - temperature control; PC - precipitation chamber; SC - separation chamber; CT - capillary tube.

A more detailed description of the apparatus and experimental procedure can be found elsewhere [1-3].

Table presents the results obtained from the co-precipitation of lipase Amano PS in PHBV in terms of enzyme activity before (original, crude) and after the immobilization. It can be seen from this table that for some experimental conditions the enzyme maintained the original activity, a high desirable characteristic, once the immobilized enzyme may be applied repeatedly in real processes, allowing its re-use and thus providing the establishment of cost-effective processes.

Table 1. Results of lipase Amano PS immobilization in PHBV through SEDS technique.

Run	[PHBV] (mg.mL^{-1})	Pressure (bar)	Enzyme activity (U/g)	
			Initial	Final
1	-1 (10)	-1 (80)	217.5	30.4
2	+1 (20)	-1 (80)	-	-
3	-1 (10)	+1 (160)	168.3	157.8
4	+1 (20)	+1 (160)	118.8	55.9
5	0 (15)	0 (120)	223.8	4.8
6	0 (15)	0 (120)	-	-
7	0 (15)	0 (120)	-	-

Conclusions

In this work a commercial free lipase, Amano PS, was immobilized in a biodegradable, biocompatible biopolymer, PHBV, using the supercritical fluid technology. Preliminary results (not published before) indicate a promising and new route to towards enzyme immobilization.

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