Lactone ring-opening polymerization catalyzed by *Yarrowia lipolytica* lipase: effects of solvent, temperature and immobilization matrices on polymerization kinetics and molecular weight

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1. Introduction

Recently, research on enzymatic polymerizations in non-aqueous or nontraditional media has been receiving increased attention as a new tool for building polymer chains. Polymers with well-defined structures can now be prepared by *in vitro* enzyme catalysis. In contrast, attempts to attain similar levels of polymer structural control by conventional methods might not be possible, or may not be practical, due to requirements of multiple protection-deprotection steps [1]. Application of immobilized enzymes in biocatalytic practice offers unique advantages over soluble enzymes, such as enhanced activity, increased selectivity, improved stability, and reusability [2].

2. Experimental conditions

Lipase production by *Yarrowia lipolytica* was made as previously reported by Barrera *et al* [3]. Lewatit beads were purchased form Sigma-Aldrich. Before immobilization, resin was activated with ethanol and washed with distilled water. Enzyme immobilization was carried out according to the procedure reported by Sandoval *et al* [4]. The immobilized enzyme preparations were dried under vacuum for 24 h at room temperature. ε-Caprolactone (ε-CL) (Aldrich Chemicals Co.) was dried over calcium hydride and distilled under reduced pressure before use. In a typical run, 1.08 mmol of ε-CL, 1 mL of heptane or decane and 12 mg of *Y. lipolytica* lipase were placed in a 10 mL vial previously dried and purged with dry nitrogen. Vials were stoppered with a teflon silicon septum and placed in a thermostated bath at predetermined temperatures and predetermined time. After the reaction was stopped, the enzyme was filtered off and the residue was analyzed for molecular weights and conversions by ^{1}H-NMR.

3. Results and discussion

Polymerizations at 70 ºC were carried out in bulk and in heptane (see figure 1). It was observed that immobilized lipase on Lewatit 1026 and K2629 showed a higher stability as a function of time and solvent. As figure 1 illustrates the polymerization rate strongly depends upon the matrix used for YL lipase immobilization. The polymerization rates are faster for YL on Lewatit K2629, TP214 and 1026, which have styrene-divinylbenzene (macroporous) and crosslinked polystyrene (macroporous) as matrix. The lowest polymerization rate was observed when lipase was immobilized on Lewatit K3433, whose matrix consists of styrene-divinylbenzene (macroporous) with tertiary ammonium as active groups and palladium doped. One of the reasons for the large differences in polymerization rate may be the orientation of YL on the matrix.

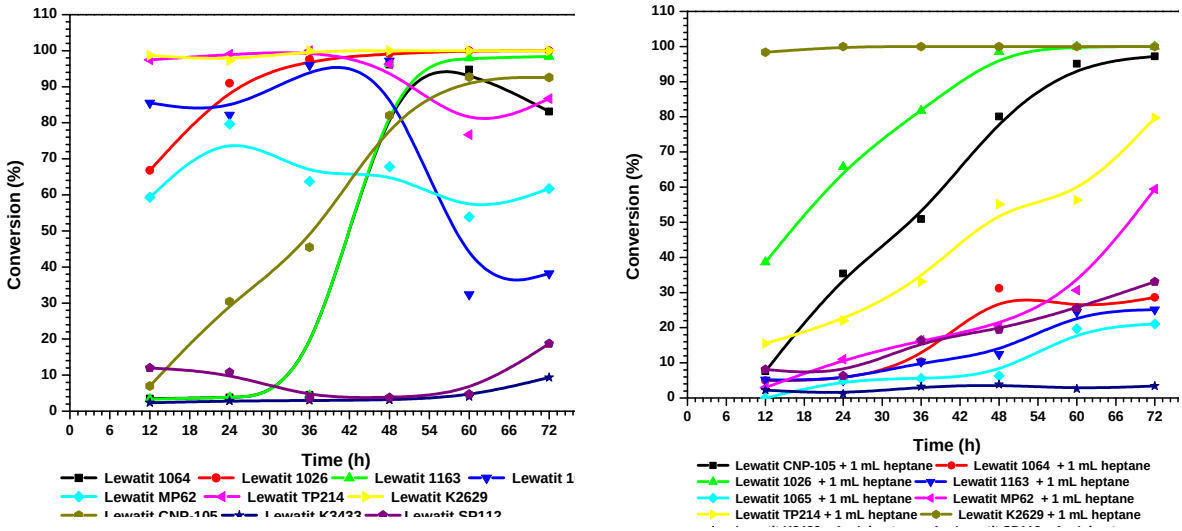

Figure 1*. Monomer conversion as a function of time for the enzyme-catalyzed ε-caprolactone polymerizations at 70 ºC. R= 1.08 mmol ε-CL/12 mg immobilized lipase. Effect of immobilization matrix and solvent.*

The effect of reaction temperature on LY-catalyzed ε-CL ring opening was investigated. Figure 2 shows that the percent conversion increased from ~65 to 95 % as the reaction temperature was increased from 70 to 120 ºC with a reaction time of 12 and 2 h respectively. To determine if polymerization at 120 ºC was due to enzyme-catalyzed processes as opposed to non-enzyme-mediated reactions, a control was performed where no enzyme was used. Under these conditions, ring opening of ε-CL was not detected by ^{1}H-NMR spectroscopy. Based on these results, we concluded that ROP of ε-CL occurs at 120 ºC due to enzyme catalysis. Figure 2 also shows that the polymer molecular weight increases slowly with

conversion, suggesting that polymerization mechanism involves polymerization with rapid initiation and slow propagation steps.

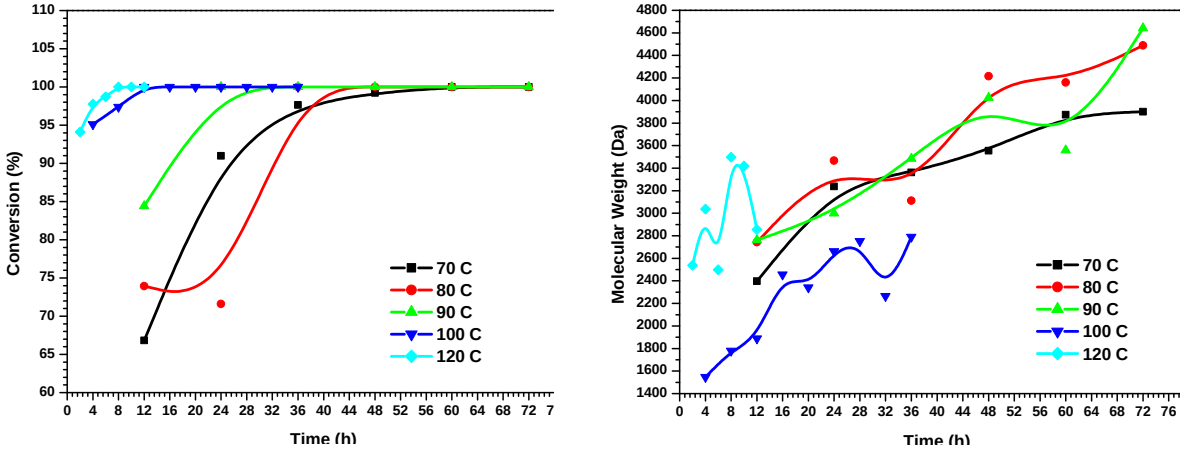

Figure 2*. Monomer conversion and molecular weight as a function of time for the enzyme-catalyzed ε-caprolactone polymerizations at different reaction temperatures. R= 1.08 mmol CL/12 mg immobilized lipase on Lewatit 1026.*

4. Conclusions

In this study, the effect of immobilization matrix on CL polymerization was evaluated. Immobilized lipase from *Yarrowia lipolytica* is an efficient catalyst in the ROP of lactones. The fastest reactions rates resulted by using lewatit K2629, 1026 and TP214. The effects of reaction temperature and solvent on the rate of conversion of ε-CL to PCL were evaluated. It was concluded that polymerization were suitable carried out in decane at 120 ºC. The reaction solvent, monomer concentration had effects on the polymerization rate. As expected from the current accepted mechanism for ring-opening polymerization of CL by lipases, final polymers are asymmetric telechelic α-hydroxy-ω-carboxylic acid poly (ε-caprolactones), as determined by proton and carbon-13 NMR.

References

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