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Lipase-catalyzed synthesis of poly-L-lactide using supercritical carbon dioxide

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ABSTRACT

The present work shows that the enzyme mediated ring-opening polymerization of L-lactide at 65 °C can be achieved using supercritical carbon dioxide. It is reported a biphasic media system where the supercritical phase coexists with a liquid organic phase, which is mainly composed of melted monomer, wherein the growing poly-L-lactide chains are soluble. The immobilized lipase B from *Candida antartica* was used as the biocatalyst. The results indicated that semi-crystalline polymers with a molecular weight (M_w) up to 12,900 g mol^{-1} can be attained and that the monomer conversion is related to the biocatalyst concentration and its initial water activity (a_{wi}). Experiments carried out with denatured enzyme gave no monomer conversion which confirms that the enzymatic mechanism is only involved in our system.

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1. Introduction

The polylactides (PLA) have received increasing attention in the last 20 years [1–3] as biorenewable and bioresourceable biomaterials. PLA are thermoplastic and biodegradable polymers with similar mechanical properties to those of polystyrene (PS) or polyethylene tetraphtalate (PET), therefore they have been pointed out as alternative materials for many applications, principally in packaging and biomedicine [2,3]. PLA can be obtained from different commercial isomers of lactide acid (LA), L-LA, *rac*-LA and meso-LA, giving rise to different materials with significant variations in their mechanical and chemical properties [4]. Poly-L-LA (PLLA), which is obtained from pure L-LA isomer, has a semi-crystalline structure and the mechanical properties of PLLA make this material more attractive with wider applications than amorphous PLA [5].

Besides, the development of new technologies based on the use of solvents following the criteria of the so-called green chemistry has shown a growing interest over the last decades, particularly, clean processes involving the use of compressed fluids (CF). Compressed carbon dioxide, especially in its supercritical state ($scCO_2$) is the most versatile and widely studied CF as it is non-expensive, non-flammable and practically non-toxic, with accessible critical constants (T_c = 31 °C; P_c = 73 bar). The $scCO_2$ presents very low viscosity and high diffusion coefficient along with similar density to common solvents, therefore $scCO_2$ has been proposed as the eco-friendly replacement of the volatile organic compounds (VOCs) in many engineering and synthetic processes.

Furthermore, the enzymatic syntheses of polymers have been reported as a green alternative to those involving inorganic or toxic catalysts [6,7]. Over the last decade, many researchers have investigated the enzymatic activity of lipases in alternative media for polyester syntheses, such as $scCO_2$ as well as other non-toxic CF [8–10] or ionic liquids [11]. The latter might present some toxicity [12], which would restrict further applications of the biomaterials although they are at present in agreement with the concept of green chemistry.

The enzymatic ring-opening polymerization (eROP) of *epsilon*-caprolactone (CL) by the immobilized lipase B from *Candida antartica* (CALB) using $scCO_2$ media was reported to give similar yields and molecular weight polymers to those in toluene [8].

The ROP of L-LA in $scCO_2$ at 70 °C and 300 bar using the inorganic catalyst dibutyltin dimethoxide was reported by Stassin and Jérôme with a polymer molecular weight of 9500 g mol^{-1} [13]. Also, Pack et al. reported up to 160,000 g mol^{-1} using DOH/Sn(Oct)$_2$ in supercritical chlorodifluoromethane (R22), which is a depleting ozone layer substance [14]. In 2003, Bratton et al. reported 14,500 g mol^{-1} of PLLA by BuOH/Sn(Oct)$_2$ with the presence of a fluorinated triblock copolymer in $scCO_2$ [15]. One year later, the same research group reported 13,500 g mol^{-1} of PLLA in $scCO_2$ media using an esterification promoting agent and 4-dimethylaminopyridine catalyst [16].

On the enzymatic side, Matsumura et al. were first to report the eROP of PLLA, using lipase from *Burkholderia cepacia* (former *Pseudomonas cepacia)* (Lipase PS) at 100 °C in bulk for 7 days. They also reported unsuccessful polymerization of either L-LA or D-LA in bulk by immobilized CALB [17]. Recently, Fujioka et al. claimed the

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first eROP of L-LA with M_w of 2440 g mol^{-1} using immobilized CALB in bulk, but polymer yields were not reported [18]. The authors also reported significant polymerization (1440 g mol^{-1}) at identical conditions but without enzyme. Huijser et al. indicated that the polymerization observed in bulk ROP of L-LA without the enzyme might be attributed to cationic polymerization mechanism induced by traces of hydroxyacid from the monomer [19], which might be formed at such operational temperatures.

The enzymatic synthesis of PLLA in VOC media is not encouraging due to the poor solubility of the substrates in commonly used non-polar and hydrophobic solvents adequate for enzymatic polymerization reactions. In addition, the relatively high temperature to reach the melting point of L-LA (92–95 °C) might cause partial enzyme deactivation, since the 55–70 °C temperature range is reported as the most suitable for the synthesis of polymers mediated by lipases [6–10].

An alternative approach to address this issue can be the use of $scCO_2$ to reduce the melting point of the cyclic monomer. It has been reported that at 300 bar the melting temperature of L-LA is above 60 °C [13]. Then, in the presence of $scCO_2$ at 300 bar and 65 °C the non-dissolved liquid monomer fraction produces an emulsion-like system under stirring, as reported by Hile and Pishko in their polymerization studies [20], although, other authors referred to it as suspension polymerization [21]. Nevertheless, this condition would allow for the eROP of L-LA at an adequate temperature for the enzymatic activity.

This article aims at reporting an approach to the eROP L-LA using $scCO_2$, which has never been reported to the best of our knowledge, towards the production of PLLA throughout a green process.

2. Materials and methods

2.1. Materials

(L,L)-Lactide (3*S*-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione, Aldrich, 98%) was purified by re-crystallization in methanol and stored in a dry place at 5 °C until use. Chloroform, hexane and methanol (technical grade, Química Barsa, Mexico) were used as received. Chloroform HPLC grade was purchased from JT Baker (Mexico) and used as supplied. Carbon dioxide (research grade; 99.98% purity) was purchased from Praxair (Mexico). The biocatalyst Novozym 435 (~10 wt% lipase B from *C. antartica* supported on a macroporous acrylic resin, specific activity 7000 PLU/g; a_{wi} = 0.34 at atmospheric conditions) was a kind gift from Novozymes (Mexico). The enzyme biocatalyst was denatured for the blank experiments by steam heating at 120 °C for 1 h in an Autoclave (Hirayama, HA 300 MII, Japan). Polystyrene narrow polydispersity standards were purchased from Varian (US).

2.2. Polymerization procedure

All polymerizations were carried out in house built 100 mL stainless steel high-pressure reactors equipped with an external ceramic body heating jacket, a manometer, two high-pressure valves (Swagelock, US), a safety rupture disk (Swagelock, US) and a cross-bar magnetic stirrer. The reaction temperature was carefully monitored by two independent thermocouples, one measuring the temperature at the heating jacket and the other measuring the temperature inside the reactor. The agitation was provided by external stirring plates. Different amounts of the biocatalyst beads and L-LA were added to each reactor. The reactor was then loaded with liquid CO_2 throughout an ISCO high-pressure syringe pump (ISCO Corp., US). The pressurizing column of the ISCO syringe pump was cooled by a refrigerant bath (American Heto Labs., Inc., US). For experiments at initial dried conditions the reactor contents (monomer and biocatalyst) and the ISCO column were previously dried overnight using an oil vacuum pump (4×10^{-4} mbar). At this condition the amount of water available for the biocatalyst determined as water activity was $a_{wi} < 0.16$, which was the minimum detectable by the hygrometer (Awquick, Rotronic Instrument Corp., USA). Then, heat was applied to the reactor up to the final operation temperature of 65 ± 2 °C. The final operating pressure (300 ± 5 bar) was finally adjusted by the ISCO syringe pump. After the desired period of time, the reaction was halted by cooling the reactor. After equilibration at room temperature the reactor was opened and the CO_2 was vented to atmospheric pressure. The reaction mixture was dissolved in chloroform and the insoluble biocatalyst beads were removed by filtration. The solution was then precipitated in cold hexane (1/10, v/v). The precipitate was collected after filtration as a white powder, which was dried to quantify the monomer conversion. The dried powder was re-dissolved in chloroform and re-precipitated in cold methanol (1/10, v/v) to yield the PLLA as a white-yellowish coarse powder which was dried and weighed to determine the polymer yield. The soluble fractions in methanol were assumed as low molecular weight products or non-reacted monomer.

2.3. Polymer characterization

The molecular weight distributions of the synthesized PLLA were determined by SEC using two different Shodex KF-604 and KF-806 M columns with a Waters Model 1525 binary pump and a dual absorbance detector (Waters 2487). The columns were eluted with chloroform (0.8 mL min^{-1} at 308.15 K) and calibrated with polystyrene standards. ^{1}H NMR spectra were recorded with a Varian Unity Inova (400 MHz) in $CDCl_3$ or d^6-DMSO. The conversion of L-LA was determined by comparison of the ^{1}H NMR spectral integration intensities of the δ = 5.0 ppm signal corresponding to the methine group of the cyclic monomer with the methine proton of the open or hydroxiacid forms (δ = 5.2 ppm), as reported elsewhere [13,17,18]. Differential scanning calorimetry (DSC) analyses were conducted in a DuPont DSC 2100. Thermogravimetric (TGA) data were obtained in a TA Instruments Hi-Res TGA 2950 under nitrogen atmosphere using a heating rate of 10 °C min^{-1}. Powder diffraction X-ray spectra were recorded at λ = 1.5406 Å in a Siemens D500.

3. Results and discussion

The isotherm phase diagrams for L-LA/CO_2 binary mixtures have been reported elsewhere [13]. The solubility of L-LA in $scCO_2$ is rather low (6.15%, w/v at 60 °C and 200 bar), however, at 300 bar and above 60 °C the non-dissolved L-LA monomer fraction becomes liquid. On the basis of this, we could have a system in which the monomer substrate is both partially soluble in the $scCO_2$ (9.5%, w/v) and partially in a separate organic phase [13,20], both available for the lipase-catalyzed ROP reaction, which scheme is shown in Fig. 1. This emulsion-like system under stirring allows performing this reaction at the adequate operational temperature for the immobilized CALB [20], which is among the most important commercially available biocatalyst and widely studied in lipase-catalyzed polyester syntheses [6,7]. Moreover, this relatively low

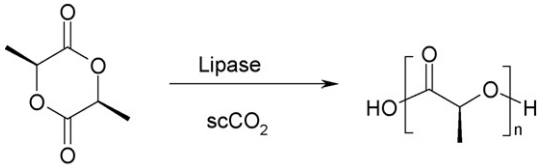

Fig. 1. Reaction scheme of the eROP of L-LA using $scCO_2$.

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