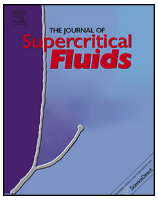
J. of Supercritical Fluids 96 (2015) 334–348

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu



Enzymatic synthesis of poly(ε-caprolactone) in liquified petroleum gas and carbon dioxide

Sibele R. Rosso Comim, Josamaique G. Veneral, Débora de Oliveira,
Sandra R.S. Ferreira, J. Vladimir Oliveira*

Department of Chemical and Food Engineering, UFSC, Florianópolis, SC, 88040-900, Brazil

ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form 3 July 2014
Accepted 4 July 2014
Available online 12 July 2014

Keywords:
Polymerization
ε-CL
Novozym 435
Liquefied petroleum gas
Packed-bed reactor (PBR)

ABSTRACT

Polycaprolactone (PCL) is a biodegradable, bioresorbable and biocompatible polymer widely used in biomedical applications. Unlike the classical polymerization methods that include the use of a metallic catalyst in organic solvent medium, the main goal of this work is to report the synthesis of polycaprolactone using a commercial enzyme, Novozym 435, as catalyst, together with liquefied petroleum gas (LPG) and carbon dioxide (CO_2) as solvent medium in order to establish a clean-technology process. The enzymatic production of PCL was carried out in pressurized LPG medium trough a set of experiments assessing the influence of pressure (120–280 bar), solvent/monomer ratio (2:1–1:2 mass ratio) and enzyme content (by monomer weight, 5–15 wt%) on the reaction yield, number-average molecular weight (M_n), weight-average molecular weight (M_w) and polidispersity index (PI). Kinetic experiments were also conducted in batch using LPG as solvent to evaluate the influence of catalyst content and pressure on reaction yield, M_n, M_w, PI with the reaction time. Furthermore, the enzyme reuse was also evaluated in order to reduce the impact of enzyme cost on the process. Continuous mode polymerizations using CO_2 as solvent were also carried out for reactions at 120 and 200 bar, 65 °C and solvent/monomer mass ratios of 2:1 and 1:2. Results from ANOVA statistical analysis for the first set of experiments show that the pressure has no significant influence over the parameters evaluated, while the solvent/monomer mass ratio and enzyme content presented significant effect on reaction yield. Polymerization results for the kinetic assays indicated reaction yields up to 81 wt% with M_n up to 15,000 Da and M_w up to 23,000 Da for 20 h of reaction, and PI ranging from 1.2 to 1.7 for the batch reactions and reaction yields up to 60.1 wt% with M_n 21,700 Da and M_w up to 36,800 Da for 10.7 min of reaction, and PI ranging from 1.7 to 2.1 for the PBR (Packed Bed Reactor) reactions. The condition of 25 bar, 2:1 solvent/monomer mass ratio, 3 wt% of enzyme, 65 °C and 8 h of reaction was selected for the enzyme batch reuse experiments, which indicate technical feasibility of enzyme reuse. Finally, future directions and some perspectives on the field of enzyme-catalyzed biopolymer production in compressed or supercritical fluid media are addressed.

1. Introduction

Polycaprolactone is a biodegradable, bioresorbable and biocompatible polymer used in a wide variety of high-value biomedical applications [1–7]. Several methods for obtaining polycaprolactone have been published in literature, such as the use of a metallic catalyst and an organic solvent in a polycondensation reaction or in a ring-opening polymerization (e-ROP) reaction [8]. However, biomedical and food applications require highly pure polymers. In order to produce a polymer free of toxic residues, the use of enzymes as catalysts and supercritical carbon dioxide as solvent in the ring-opening polymerization reaction has been proposed by several researchers [9,10].

Literature data for polycaprolactone enzymatic-ring opening polymerization (e-ROP) in carbon dioxide show that the results obtained in terms of reaction yield and molar mass are in the same order of magnitude as those obtained by the use organic solvents and/or metallic catalysts [9]. The best results of molecular weight cited by Albertsson and Srivastava [11] in their review about polyester enzymatic production are the ones obtained by Thurecht et al. [9] with 100% conversion of ε-caprolactone producing polycaprolactone with a molecular weight of 50 kDa using CO_2 as solvent. The best result for an organic solvent was obtained by Gross et al. [12] which produced polycaprolactone in toluene with

---

* Corresponding author. Tel.: +55 48 37212508; fax: +55 48 37219687.
E-mail address: vladimir@enq.ufsc.br (J.V. Oliveira).

http://dx.doi.org/10.1016/j.supflu.2014.07.004

molecular weight of 44.8 kDa with 85% of conversion. The shortcoming of this process is that enzymes such as Novozym 435 which provides good results for e-ROP are expensive. Therefore, the feasibility of the process most likely depends on the catalyst reuse that might be limited to two cycles [13] with the use of carbon dioxide as solvent.

Several studies have shown that the use of carbon dioxide as solvent leads to the reduction of enzymatic activity by three different sorts of interaction as explained by Rosso et al. [13]: (1) pH reduction due to the production of carbonic acid derived from the reaction between CO_2 and water present in the enzyme micro-environment [14]; (2) CO_2 and lysine residues tend to form carbamates [14–16], and (3) enzyme essential water removal by the hydrophilic CO_2 [17,18].

To overcome these drawbacks this work proposes the use of liquefied petroleum gas as polymerization solvent medium for polycaprolactone production through e-ROP of the monomer. When compared to carbon dioxide, the major components of LPG, propane and n-butane, present positive effect on enzymatic activity [18] while presenting a similar dielectric constant (solvent polarity) which is in the range from 1.6 to 1.9 for LPG and 1.6 for carbon dioxide [19]. Since liquid propane and n-butane exhibit low compressibility and very low solubility in water, the literature indicates that they have a hydrostatic behavior working as mechanical press or piston fluid that increases the system pressure changing favorably the enzyme conformation [20].

Besides, LPG is plenty available, cheaper and it can be used in much lower pressures compared to carbon dioxide. For example, at 65 °C and 25 bar, LPG exhibits a density of 9.27 mol/L, while carbon dioxide will reach a similar density only at 124 bar [21]. In addition, as any pressurized gas, LPG has the advantage over liquid organic solvents of being easily separated from the final product by system depressurization. Furthermore, compared to pure propane and n-butane, LPG has a very much lower price and its composition is almost invariant with time, which guarantees safe working with expected reliable polymerization results.

Still, the selection of the reactor type, to drive the polymerization process in pressurized medium, is noteworthy because it exerts a strong influence on the quality of the final polymer obtained and the economic viability of the process [22]. Studies on the production of polycaprolactone in $scCO_2$ with enzyme as catalyst have been performed in batch mode, in reactors/autoclaves of constant volume [9,10,23] and, more recently, using a variable volume reactor [13]. However, processes performed in continuous mode are generally more advantageous because of its good process control, high productivity and low cost (per mass of product) [24]. Moreover, the use of the packed bed reactor (PBR) allows the continuous removal of inhibitory substances thereby facilitating the reuse of the enzymes, this being the most suitable reactor for long-term production on industrial scale as well as for the use of ratios higher of enzyme/substrate, being therefore more cost effective than those conventional operations carried out by batch reactors [25]. In this context, the only works found in the literature for e-ROP of ε-caprolactone in continuous mode were published by Zhang et al. [24] and Kundu et al. [26], both performed at atmospheric pressure using toluene as solvent in the reactions.

In this context, the aim of this work was to investigate the enzymatic production of polycaprolactone by ring opening polymerization (e-ROP) in batch mode using pressurized LPG and in continuous process using CO_2 as solvent. The present research employed a variable-volume reactor that allows the control of solvent/monomer ratio with high precision of temperature and pressure settings [13], equipped with a sapphire window that enables reaction medium visualization. The main objectives were the optimization of the polymerization reaction conditions considering the influence of pressure, solvent/monomer ratio and enzyme content and also provide a comparison with data on e-ROP using carbon dioxide [13]. A kinetic evaluation of the lipase catalyzed synthesis of polycaprolactone in supercritical medium was also investigated as well as the possibility of enzyme reuse.

2. Experimental

2.1. Materials

The ε-caprolactone (ε-CL) (97% purity) supplied by Sigma-Aldrich was used without additional purification. The ε-caprolactone water content was measured by Karl-Fischer (Mettler Toledo, Model DL 50, Columbus, USA) titration method, resulting in 0.56 ± 0.06 wt%. The monomer with this moisture content was kept in sealed containers (no head space) with the amount enough for one reaction to avoid gain of water. The commercial immobilized Novozym 435 (lipase B from *Candida antarctica* or simply CALB) was supplied by Novozymes S/A, Brazil. For all experiments, the enzyme was previously dried in an oven (Odontobrás, EL003, Araraquara, Brazil) at 40 °C for 1 h where the water content was reduced from 2.5 wt% ± 0.3 to 2.0 wt% ± 0.3. This procedure was done right before the reaction. LPG purchased from Liquigás S.A (Brazil) and is constituted by a mixture of propane (50.3 wt%), n-butane (28.4 wt%), isobutane (13.7 wt%), ethane (wt% 4.8 wt%), other minor constituents (methane, pentane, isopentane, etc.) and 140 ppm of sulfur. Carbon dioxide (99.9% purity) used was purchased from White Martins S/A, Brazil.

2.2. High pressure batch polymerization experimental apparatus and procedure

Experimental apparatus and the procedure, applied to evaluate three classes of assays (influence of operating conditions, kinetics and enzyme reuse) here described is also explained elsewhere [13]. Polymerization experiments were conducted in a high-pressure variable-volume cell based on a high pressure phase equilibrium apparatus [27–29]. The variable-volume cell has 27 cm^3 of internal volume and contains a movable piston, which permits the pressure control inside the cell through manipulation of the pressure using the syringe pump (260 D, ISCO, Lincoln, USA) and the solvent (carbon dioxide) as pneumatic fluid. The cell is connected to a thermostatic bath (Nova Ética, Vargem Grande, Brazil) and a pressure transducer (LD 301, Smar, Sertãozinho, SP, Brazil). It also contains two sapphire windows (Swiss Jewel Company, Philadelphia, EUA) for visual observation.

For polymerization experiments, the monomer (ε-caprolactone) and the enzyme (Novozym 435) were weighed on a precision scale balance (Ohaus Analytical Standard, New Jersey, EUA, with 0.001 g accuracy) and placed inside the reactor. A calculated amount of solvent was loaded into the reactor using the syringe pump until a desired global composition was achieved to reach the mass ratio conditions specified by the assays evaluating the effect of process variables, kinetic or reuse conditions. Then, the temperature (controlled within 0.1 K), and the system pressure were set. The cell content was mixed using a magnetic stirrer (Velp Scientifica, Usmate, Italy) and a teflon-coated stirring bar. After polymerization, the polymer–monomer–enzyme mixture was solubilized in tetrahydrofuran (THF) (Fmaia, 99%, Cotia, Brazil), in order to separate the enzyme using a conventional cellulose filter. Then, the polymer was precipitated in cold ethanol (−10 °C) (Merck, 99.9%, Darmstadt, Germany), filtered and dried by solvent evaporation overnight. THF and ethanol were used at the volumetric proportion of 1:6. The polymer was kept in a desiccator with silica and under constant temperature (in a domestic refrigerator at 1–5 °C) up to constant mass. The reaction yield was calculated