Journal of Molecular Catalysis B: Enzymatic 92 (2013) 7–13

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Biocatalytic preparation of dichloropropyl acrylates. Application to the synthesis of poly(dichloropropyl acrylates)

Yara-Varón Edinson[a], Eras Joli Jordi[a], Torres Mercè[b], Balcells Mercè[a], Villorbina Gemma[a], Canela-Garayoa Ramon[a,*]

[a] Department of Chemistry, University of Lleida, Rovira Roure 191, 25198 Lleida, Spain
[b] Department of Food Science and Technology, University of Lleida, Rovira Roure 191, 25198 Lleida, Spain

ARTICLE INFO

Article history:
Received 15 October 2012
Received in revised form 5 February 2013
Accepted 8 March 2013
Available online 15 March 2013

Keywords:
Biocatalytic transesterification
Dichloropropyl acrylates
CALB
Packed bed reactor
Poly(dichloropropyl acrylate)

ABSTRACT

The synthesis of dichloropropyl acrylates from dichloropropyl dodecanoates through a transesterification process using diverse commercial lipases and whole cells (fungal resting cells) is presented. The synthesis was carried out in a solvent-free media using a conventional batch system and a packed bed reactor (PBR). The effect of water activity on the process depended on the lipase used. The commercial enzyme CALB (*Candida antarctica* lipase B immobilized onto a macroporous acrylic resin) showed the best performance as a biocatalyst, achieving a yield of 50% and productivity of 7.2 μmol min^{-1} g^{-1} in the batch reactor and 33% and 35.8 μmol min^{-1} g^{-1} in the PBR. Finally, polymeric material was prepared by suspension polymerization of the dichloropropyl acrylates synthesized using PBR. Particles with diameters between 170 and 380 μm were obtained with a yield of 85% after 18 h reaction.

1. Introduction

Chlorohydrin acrylates are used as a raw material in the preparation of a range of polymeric [1] and optical [2] materials. They also have applications in the production of adhesive polymers with resistance to exposure to X-rays [3], photosensitive resin [4], fire-retardant synthetic fibers [5], and hair care products [6], as well as in imprint lithography [7]. These acrylic esters can be prepared in the following ways, (i) by esterification of 2,3-dichloropropanol with acrylic acid in a non-polar aprotic solvent in the presence of an acid catalyst [2]; (ii) by means of acrylyl chloride; and (iii) by alcoholysis of ethyl acrylate [8]. In addition, we recently described the capacity of diverse commercial lipases and whole cells (fungal resting cells) to synthesize allyl and dichloropropyl acrylates from allyl dodecanoate and dichloropropyl dodecanoates through transesterification in a batch reactor [9]. In that study lipases were used as received without water activity adjustment. Of the biocatalysts tested, CALB was found to be best followed closely by MmL (lipozyme from *Mucor miehei* immobilized onto ion-exchange resin). The maximum yield was obtained using a 1:1 mole ratio of reagents. In turn, allyl dodecanoate and dichloropropyl dodecanoates can be synthesized from crude glycerol [10]. This process is shown in Fig. 1.

Fossil oil and acylglycerides are sources of glycerol. As a result of the escalating prices of petroleum and its derivatives in recent decades, fossil fuels are increasingly being substituted by fuels of plant origin, such as biodiesel (fatty acid alkyl esters). Glycerol (1,2,3-propanetriol) is usually the main by-product in the biodiesel process, accounting for approximately 10% of the total product by mass [11]. Although glycerol is used in a number of industries, such as the cosmetic, paint, automotive, food, tobacco, pharmaceutical, pulp and paper, leather, and textile sectors [12], biodiesel production will continue to rise in the coming years. This growth will result in a large crude glycerol surplus that cannot be absorbed by current markets for this product [13]. Consequently, the price of glycerol will fall. Therefore, the development of processes to convert low-priced glycerol into higher value products is an excellent strategy to add value to the production of biodiesel.

To increase the value of glycerol, our group has adopted new approaches based on the transformation of this polyol into halohydrin esters. 2-Chloro-1-(chloromethyl)ethyl esters are halohydrin esters that can be synthesized from glycerol and carboxylic acid by an esterification-substitution reaction using chlorotrimethylsilane (CTMS) [14]. In turn, microwave irradiation allows the use of higher reaction temperatures that render mixtures of 2-chloro-1-(chloromethyl)ethyl esters and 2,3-dichloro-1-propyl esters in variable ratios [15]. These products can be used as building blocks for the synthesis of a range of biologically active natural and

Abbreviations: PBR, packed bed reactor; CALB, *Candida antarctica* lipase B immobilized onto a macroporous acrylic resin.

* Corresponding author. Tel.: +34 973 702843; fax: +34 973238264.
E-mail address: canela@quimica.udl.cat (C.-G. Ramon).

1381-1177/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molcatb.2013.03.005

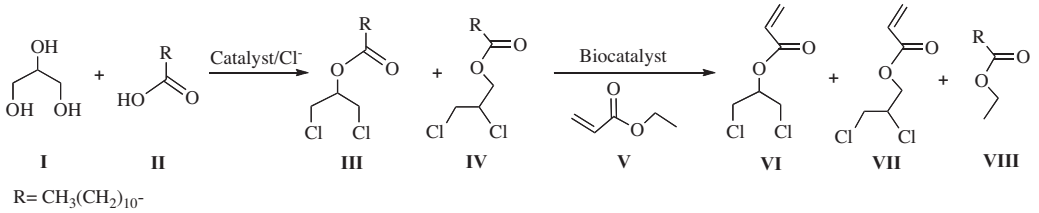

Fig. 1. Biocatalytic production of dichloropropyl acrylates from glycerol.

synthetic products [16]. Nevertheless, this procedure is not feasible with acrylic acid and acrylic esters.

Conversely, the enzymatic preparation of chlorohydrin acrylates using lipases (EC 3.1.1.3) allows reactions to be performed under mild conditions. The lipases can be used in pure form or in an immobilized form on a support. These conditions prevent the formation of by-products because the reactions take place at low temperatures, thus greatly reducing the risk of acrylate polymerization. In addition, enzymes have greater specificity and high selectivity than conventional chemical catalysts [17].

Transesterification processes can be performed using either a batch reactor or a packed-bed reactor (PBR), the latter being one of the most commonly used reactors in biotechnology [18]. A PBR has several advantages over a batch reactor, including a relatively short reaction time [19], easy separation of products [20], and reuse of biocatalyst. Moreover, the ratio between substrate and enzyme is much lower in a PBR than in a conventional batch reactor, thus resulting in higher reaction performance [21].

Here, the enzymatic transesterification of ethyl acrylate (**V**) with 2-chloro-1-(chloromethyl)ethyl dodecanoate (**III**) and 2,3-dichloro-1-propyl dodecanoate (**IV**) using diverse biocatalysts in a batch reactor and in a PBR has been studied. The process was carried out in a solvent-free manner. The PBR allowed the synthesis of monomers at a sufficient gram scale to prepare a poly(dichloropropyl acrylate).

2. Methods

2.1. Materials

Candida antarctica lipase B immobilized onto a macroporous acrylic resin (CALB) from Novozymes A/S, Amano lipase PS-IM immobilized on diatomaceous earth (PS-IM), lipase immobilized on Immobead 150 from *Rhizopus oryzae* (IM-150), lipozyme immobilized from *Mucor miehei* (MmL) from Novo Nordisk A/S Corp. Amano lipase PS (*Burkhloderia cepacia*) (PSL). All lipases were purchased from Sigma–Aldrich. Dodecanoic acid and ethyl dodecanoate were purchased from Sigma–Aldrich (Sigma–Aldrich Quimica, S.A., Madrid, Spain). Ethyl acrylate and butyl acrylate were from Fluka (Sigma–Aldrich, Madrid, Spain). Hexane and tert-butyl methyl ether (MTBE) were supplied by J.T. Baker (Quimega, Lleida, Spain).

2.2. Procedure for obtaining resting cells

The strain of *Rhizopus oryzae* (RoL) was isolated from plants of *Foeniculum vulgare* as endophytic fungi. The plants were collected at The Montsec Natural Park in Catalonia (Spain). Healthy plant tissues were harvested. The tissues were washed and cut in pieces (2–3 cm long). All pieces were surface-sterilized by sequential washes in 0.53% sodium hypochlorite (2 min) and 70% ethanol (2 min) and rinsed with sterile distilled water. The surface-sterilized pieces were placed on 2% malt extract agar in Petri dishes. All plates were incubated at room temperature for a maximum of 2 weeks. Fungi growing out from the plant tissues were transferred to mycological agar [22]. *Aspergillus fumigatus* (Af) was isolated from the soil of olive (*Olea europaea*) orchards in Lleida Catalonia (Spain). The soil was plated on Petri plates containing tributyrin. After five days the carboxylesterase activity of the tested fungal strains was determined and *A. fumigatus* isolated. The fungal strains isolated were cultured in a synthetic liquid medium as previously described [23]. Mycelia were harvested from the whole culture broth by filtration using a Buchner funnel and washed with distilled water followed by acetone. Mycelia were then dried under vacuum for 18 h and ground into a powder. The enzymatic units (*U*) for each resting cell were determined beforehand on the basis of the enzymatic hydrolysis rate of methyl stearate [9].

2.3. Procedure for the preparation of 2,3-dichloro-1-propyl dodecanoate (*VII*)

2-Chloro-1-(chloromethyl)ethyl dodecanoate (**III**) was prepared from glycerol (**I**) and dodecanoic acid (**II**) following a previously described method [24]. The isomerization reaction of **III** to **IV** was achieved by microwave irradiation (300 W max, 17 atm max, 243–247 °C) for 1 h in a solvent-free system. The isomerization process was previously evaluated [15].

2.4. Equilibration of water activity

The water activity (a_w) in the experiments was set by independently equilibrating isomeric mixtures **III**–**IV**, reagent **V**, and biocatalyst with aqueous saturated solutions of LiCl (a_w = 0.12), $MgCl_2$ (a_w = 0.33), K_2CO_3 (a_w = 0.42), $Mg(NO_3)_2$ (a_w = 0.54) and NaCl (a_w = 0.75). Separate closed containers were used for each reactant and biocatalyst [25]. Equilibration was performed at room temperature for at least 48 h. The water activity of the biocatalyst was measured using an Aqua Lab series 3TE from Decagon Devices Inc. (Pullman, WA, USA).

2.5. Enzymatic transesterification in the batch reactor

A 1:1 mixture of **V** (1 mmol, 100.12 mg) and dichloropropyl dodecanoates (100:0 or 32:68 **III** and **IV** isomeric mixtures, 1 mmol, 311.29 mg) was stirred continuously at 1200 rpm in an orbital shaker (Eppendorf® Thermomixer Comfort) and at atmospheric pressure in a reaction vial (1.5 mL) fitted with a PTFE-lined cap. Reaction vials were used as received. Two sets of experiments were carried out in a solvent-free system using either 3.3% (10.3 mg) or 15% (46.7 mg) of biocatalyst (commercial lipases and fungal resting cells) based on the weight of dichloropropyl dodecanoates. Blank experiments were carried out for each a_w studied without biocatalysts. Samples were collected at 1 h and 24 h, depending on the experiment performed. The reaction temperature was chosen on the basis of the previously described optimum temperature for each commercial enzyme [9]. For resting cells, the temperature used was 40 °C. Blank assays were conducted at 50 °C. Once the experiment had ended, an aliquot of 10 mg of the crude product was dissolved in hexane containing an internal standard (typically butyl acrylate). The resulting solution was analyzed using gas chromatography

(GC-FID). Quantification of each compound was achieved using the internal standard. Experiments were performed in triplicate.

2.6. Continuous packed-bed reactions

The PBR consisted of a glass column (inner diameter: 3 mm; total length: 100 mm; packed length: 90 mm; inner volume: 0.70 mL) filled with *ca.* 240 mg of the corresponding biocatalyst. The glass column was packed manually with the biocatalyst, which was then fixed in place using glass wool plugs. All the mixtures were pumped through the column reactor filled with the corresponding biocatalyst at a constant flow rate of 10 μL/min (FRX System, Syrris Ltd, Royston, UK). The column reactor was heated with an FRX Volcano Column Adaptor (Syrris) and a digitally controlled RCT Basic 40 hotplate (IKA-Werke GmbH & Co., KG, Staufen, Germany) with an external Pt 100 sensor for optimum control of temperature. A digital control with an external sensor ensured that the temperature setting was correctly maintained in the column. Reactions were performed in a solvent-free system. Two experiments were carried out. The first experiment consisted on using each described biocatalyst, and a mixture of **V** (2.5 g, 25 mmol) and the **III**–**IV** mixture (7.77 g, 25 mmol). Each experiment was conducted for 24 h, crude products were recovered and aliquots of 10 mg were dissolved in hexane containing an internal standard (typically butyl acrylate). Samples were analyzed as described above. Reaction crude products were vacuum-distilled at 26 °C and 0.3 Torr. This distillate was used for polymerization.

A final experiment was conducted for 72 h, using CALB as biocatalyst, and a mixture of **V** (7.5 g, 75 mmol) and the **III**–**IV** mixture (23.31 g, 75 mmol). Samples were withdrawn at the indicated times, dissolved in hexane containing an internal standard (typically butyl acrylate) and analyzed as described above.

2.7. Preparation of a poly(dichloropropyl acrylate)

Suspension polymerization of a distilled mixture of products **VI** and **VII** was carried out following the described methodology [26]. Distilled water (5.6 g), poly(vinyl pyrrolidone) (19.8 mg), and ascorbic acid (7.89 mg) were added to a reaction vial fitted with a PTFE-lined cap. The reaction medium was heated to 50 °C and the corresponding chemical mixture of dichloropropyl acrylates (620 mg) and benzoyl peroxide (BPO) (3.41 mg), used as initiator, were added under vigorous magnetic stirring (900 rpm). The system was allowed to react for 18 h at 70 °C. The solid formed were recovered and washed until no monomer was detected in the washing solution.

2.8. Analysis

The progress of each reaction was determined by gas chromatography (GC) using an Agilent HP6890 series gas chromatograph (Barcelona, Spain) coupled to a flame ionization detector (FID). The analytical column was a 30 m × 0.25 mm fused silica capillary coated with a 0.20 μm film of poly(80% biscyanopropyl 20% cyanopropylphenyl siloxane) (SP-2330; Supelco, Madrid, Spain). The temperature program used was 40 °C for 5 min, followed by an increase of 20 °C/min until reaching the final temperature of 225 °C, which was then held for 3 min A 1:20 split injection ratio was used. Hydrogen was used as the carrier gas at a constant pressure of 620 kPa The injection volume was 1 μL. The injection system was held at 250 °C and the FID system at 280 °C. Quantification was performed by a conventional internal standard method using the corresponding acrylic ester standards.

The enantioselectivity of enzymes was determined through total hydrolysis of 2,3-dichloro-1-propyl dodecanoate and 2,3-dichloro-1-propyl acrylate using lipozyme immobilized from *Mucor miehei* (MmL). A mixture of 0.172 mmol of each dichloropropyl ester (independently), 30 mg of biocatalyst and 10 μL of water in 1 mL of MTBE was stirred (1200 rpm) for 24 h at 40 °C. 2,3-Dichloro-1-propanol was analyzed by chiral GC in an Agilent 7890 A series gas chromatograph (Barcelona, Spain) using a CP Chirasil–DEX CB column (25 m × 0.25 mm diameter, 0.25 μm film thickness). Hydrogen (3 ml/min) was used as carrier gas; the injection system was held at 250 °C, and the FID system at 280 °C. The oven temperature was isothermally programmed at 60 °C and held for 120 min

Polymer was vacuum-dried and analyzed using a JASCO FT/IR-6300 spectrometer, a JASCO NRS-7100 Confocal Laser Raman spectrometer, and a Varian 400 NMR spectrometer. The C:H:Cl ratio of each solid was determined by combustion elemental analysis in a Carlo Erba Instruments EA 1108.

3. Results and discussion

3.1. Synthesis of 2-chloro-1-(chloromethyl)ethyl acrylate

Initially, the transesterification reaction between 2-chloro-1-(chloromethyl)ethyl dodecanoate (**III**) and ethyl acrylate in a batch reactor using several lipases was carried out using a 1:1 mole ratio of **III** and **V** reagents. Five commercially available lipases and two resting cells at $a_w = 0.33$ at the optimum temperature for each biocatalyst and either 3.3% or 15% of biocatalyst were used. The highest yield (22%) was achieved by MmL at 24 h using 15% of enzyme, followed by CALB (17%) and PSL (15%) under the same conditions (Fig. 2a). The yields obtained using the other biocatalysts were lower than 15%. Although yields were generally improved compared to previous study [9] by adjusting a_w, they were still moderate. This prompted us to study the effect of isomerizing the 1,3-dichloropropyl radical.

3.2. Transesterification process using a regioisomeric mixture of *III* and *IV*

Given the results described above, the transesterification using a dichloropropyl dodecanoate mixture with a high content of 2,3-dichloro-1-propyl dodecanoate (**IV**) regioisomer was studied. The yield should be improved considering that the biocatalysts might react with the primary ester bond much more readily than the secondary ester bond. The isomerization of **III** to **IV** was achieved by microwave irradiation, obtaining a **III**:**IV** regioisomeric ratio of 32:68. This result is in agreement with that described by Escribà et al. [15]. Fig. 2b shows the results of a set of experiments carried out with this regioisomeric mixture. The maximum yield, 33%, was achieved by 15% of CALB at 24 h of reaction. This yield increase might be explained by the fact that although CALB is a highly versatile lipase for esters, it has very limited available space in the active site pocket compared to other lipases [27]. These features may explain why CALB shows higher activity when the regioisomer **IV** is present in the reaction mixture. Similar behavior was observed for MmL. Now yield was 30% higher than when the transesterification was carried out using MmL and the regioisomer **III**. MmL is considered a 1,3-specific lipase [28], which might explain the yield increase favored by the formation of product **VII**. The resting RoL cells showed the best performance, with 15.8% of yield using 15% of biocatalyst. The other biocatalysts showed yields lower than 15% in all cases. Consequently, all subsequent experiments were conducted at 24 h using 15% of biocatalyst.

3.3. Effect of a_w on catalytic activity

In an attempt to improve the yield of dichloropropyl acrylates, we evaluated the influence of several water activity values on the

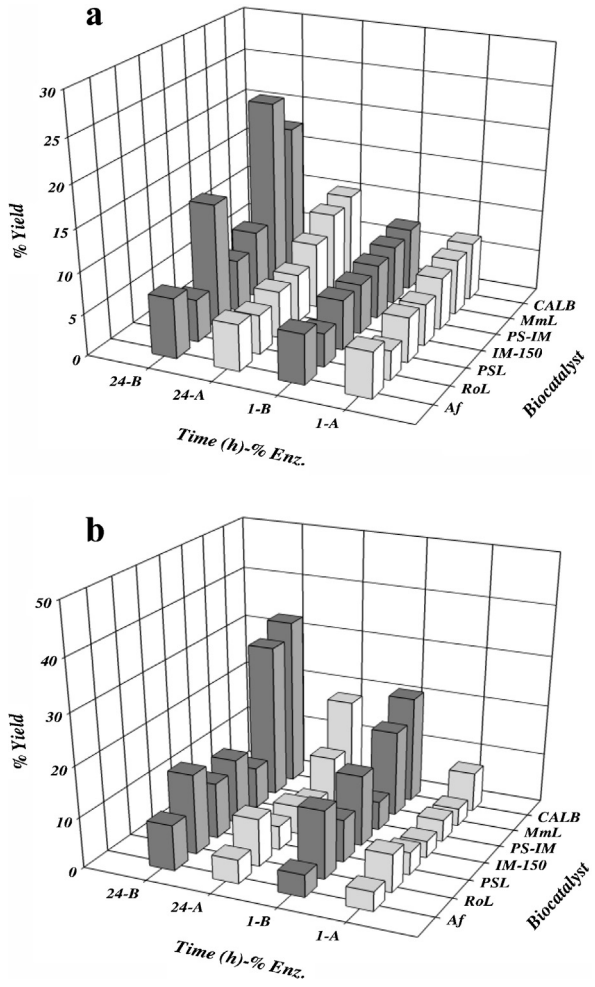

Fig. 2. Yield of dichloropropyl acrylates using lipases at concentrations of $A = 3.3\%$ and $B = 15\%$ based on the weight of dichloropropyl dodecanoates. The reaction was carried out in batch for 1 h and 24 h. $a_w = 0.33$ ($MgCl_2$). (a) 2-Chloro-1(chloromethyl)ethyl acrylate, (b) mixture of the two regioisomers of dichloropropyl acrylate.

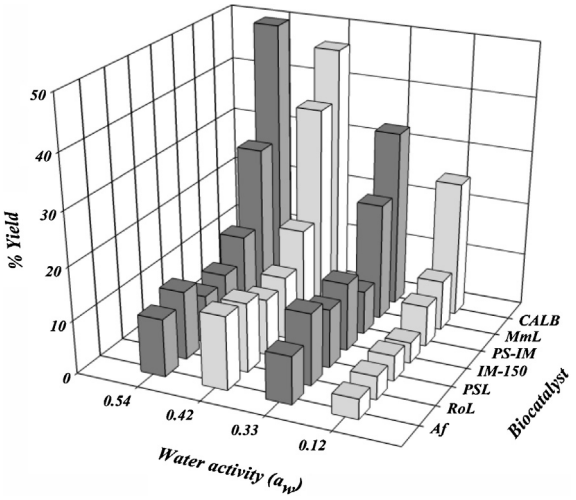

Fig. 3. Yield of dichloropropyl acrylates obtained using diverse commercial enzymes and resting cells in the batch reactor. Reaction conditions: reaction time 24 h, lipases at a concentration of 15% based on the weight of dichloropropyl dodecanoates. a_w: LiCl (0.11), $MgCl_2$ (0.33), K_2CO_3 (0.42), $Mg(NO_3)_2$ (0.54).

reaction. Water activity (a_w) is one of the most important factors affecting enzyme activity. Given that excess water favors hydrolysis over condensation reactions, water content may affect reaction rate, yield and enzyme stability [22]. Fig. 3 shows the influence of a_w on the yield of dichloropropyl acrylates. No transesterification was observed in the blank assays (data not shown). Again, CALB showed the best performance. Maximum yield rose from 33 to 50%. Indeed, this lipase showed the highest yield at all the water activity values tested. Thus, 50, 47, 33 and 25% yields were obtained for $a_w = 0.54$, 0.42, 0.33 and 0.11 respectively. The behavior of CALB at various a_w values showed that the catalytic activity and stability of enzymes are markedly influenced by hydration levels [29]. CALB shows its highest activity at $a_w = 0.54$. Nevertheless, we checked CALB activity at $a_w = 0.75$ and observed a clear decrease in yield (data not shown). In contrast, the dependency of the yield on water activity for MmL followed a classical bell-shaped curve in the range of water activity values assayed initially. These results are in agreement with those described by Wehtje et al. [30]. Karra-Chaabouni et al. [31] proposed that this dependency is due to the simultaneous occurrence of two opposite phenomena: the first ascending part of the curve (22% yield, $a_w = 0.33$) reflects the increasing hydration of the enzyme up to its optimum value (38% yield, $a_w = 0.42$) while the descending part at high a_w values (28% yield, $a_w = 0.54$) comes from apparent variations of the K_M values of the substrate. Most lipases have their active center secluded from the medium by a polypeptide chain called lid or flap [32]. These lipases follow a well-known mechanism so-called interfacial activation. This phenomenon occurs when, the lipase becomes adsorbed on a hydrophobic surface and the lid moves. This process allows the interaction between reagents and the active center (open form). CALB has a very small and simple lid [33]. The active center is not fully isolated and the enzyme does not show interfacial activation [34]. In contrast, MmL has a great lid and its interfacial activation is very well known [35]. Consequently, substrates might easily reach the active center into CALB than into MML. PS-IM showed the highest yield (18%) at $a_w = 0.42$ while IM-150, PSL and RoL lipases transformed ethyl acrylate to dichloropropyl acrylate in a greater proportion at $a_w = 0.33$. These results could be attributed to each enzyme displaying its highest activity at a different water activity value [29]. Moreover, mass transfer limitations may also occur as a consequence of the aggregation of the biocatalysts at high water activity values [36].

3.4. Enzymatic transesterification in a PBR

In an attempt to improve the reaction performance, we performed a new set of experiments using a PBR. For this purpose, we used the enzymes that showed the highest activities in the batch reactor. Transesterification studies were started using a 1:1 mole ratio of ethyl acrylate:dichloropropyl dodecanoates, 240 mg of biocatalyst (3.3%), the optimal water activity for each enzyme, and the minimum flow rate allowed for our system (10 μL/min). We had previously determined in an equivalent PBR that transesterification with several acrylates reached the highest yields at this flow rate [26].

The biocatalysts showed the same behavior as in the batch reactor (Fig. 4). Although CALB was again the best lipase in terms of yield (33%, $a_w = 0.54$), followed by MmL (21%, $a_w = 0.42$), PS-IM (7%, $a_w = 0.42$) and RoL (6.2%, $a_w = 0.33$), yields were moderately lower those obtained in the batch reactor. These results may be attributable to the fact that the amount of biocatalysts in the batch reactor was five-fold that used in the PBR. Moreover, to evaluate the extent of enzyme deactivation in the PBR, a 72 h experiment was carried out using CALB. Fig. 5 shows that yield remained around 32% for 30 h reaction time. However, the yield decreased to 25% after 54 h and 15% after 72 h. A quadratic polynomial adjustment was

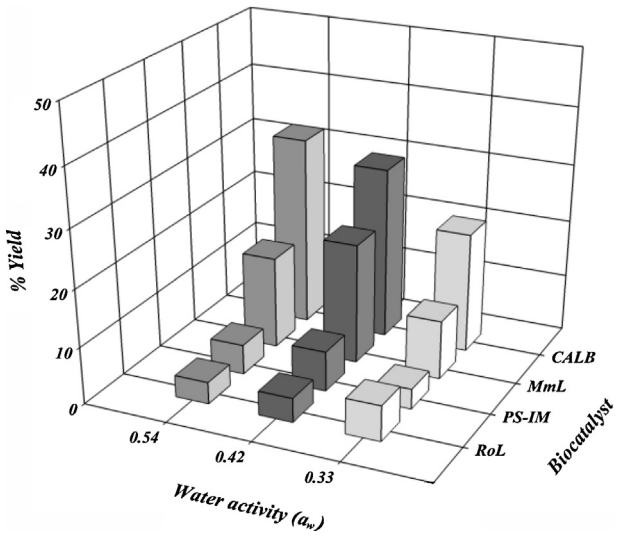

Fig. 4. Yield of dichloropropyl acrylates obtained using a PBR. Reaction conditions: continuous flow rate at 10 μL/min, 1:1 mole ratio ethyl acrylate:regioisomeric mixture of dichloropropyl dodecanoates, lipases at a concentration of 3.3% based on the weight of dichloropropyl dodecanoates. a_w: $MgCl_2$ (0.33), K_2CO_3 (0.42), $Mg(NO_3)_2$ (0.54).

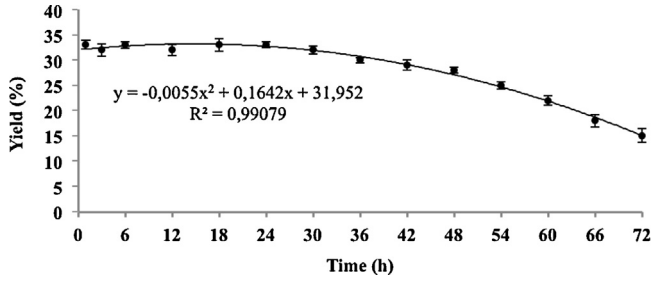

Fig. 5. Effect of the reaction time on the yield of dichloropropyl acrylate prepared using a PBR. Reaction conditions: continuous flow rate at 10 μL/min, 1:1 mole ratio ethyl acrylate:regioisomeric mixture of dichloropropyl dodecanoates, CALB (3.3% based on the weight of dichloropropyl dodecanoates). a_w: $Mg(NO_3)_2$ (0.54).

achieved by the minimum square method. Indeed, this observation suggests that there is an enzyme inactivation, a limited access of the substrate to the enzyme caused by blockage with product, or both [37].

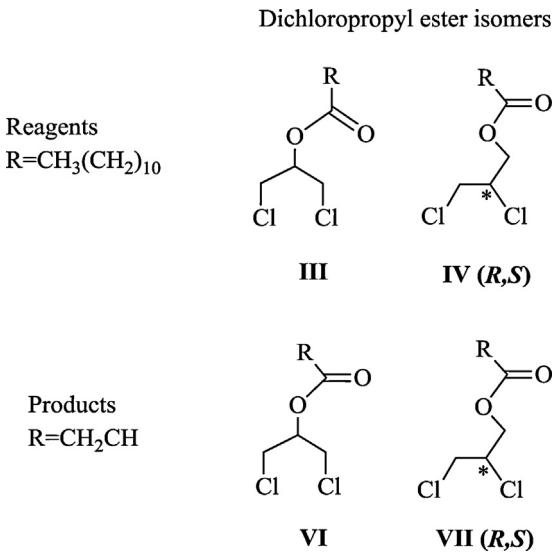

Fig. 6. Mixture of regioisomers: reagents (III and IV) and products (VI and VII).

3.5. Catalytic activity in a batch reactor versus a PBR

Productivity of the reactions using the so-called specific reaction rate (r) was determined in order to compare continuous flow and batch reactions. This parameter indicates the amount of product that can be formed in one minute by 1 g of enzyme [38]. The productivity of a given enzyme was higher in the continuous-flow system than in the corresponding batch reaction (Table 1). This result is in agreement with that described by Boros et al. [39]. CALB showed an r in PBR (r_{PBR}) about five-fold higher than in the batch reactor (r_{batch}). MmL showed a r_{PBR} more than three-fold higher than r_{batch}. For a comparison between the productivity of the two systems, the same degree of conversion should be considered [40]; however, given that yields were higher in the batch reactor than in the PBR, the productivity for the latter might be even higher if conversion were the same.

3.6. Regio and enantio selectivity of the biocatalysts

Enzymes can catalyze a variety of reactions with high specificity. Furthermore, regioselective synthesis is amenable using enzymatic approaches. Therefore, we studied the transformation capacity of regioisomers (Fig. 6). Table 2 shows the ratio of regioisomers **VI** and **VII** achieved by diverse lipases. In all cases **VII** was formed in higher

Table 1
Effects of biocatalyst and reactor system on the yield and specific reaction rate (r = μmol min^{-1} g^{-1}). A 1:1 mole ratio ethyl acrylate:regioisomeric mixture of dichloropropyl dodecanoates was used in all experiments.

Biocatalyst	Biocatalyst support	Specific activity (U g of protein^{-1} × $10^{3)}$	T (°C)	Yield (%)		r (μmol min^{-1} g^{-1})	
				Batch[a]	PBR[b]	r_{batch}	r_{PBR}
CALB	Acrylic resin	111	50	50 ± 1.2[c]	33 ± 0.9[c]	7.2 ± 0.5	35.8 ± 1.3
MmL	Ion-exchange resin	0.943	50	38 ± 1.1[d]	21 ± 1.2[d]	4.3 ± 0.4	13.9 ± 1.1
PS-IM	Diatomaceous earth	0.143	50	18 ± 1.4[d]	7 ± 0.8[d]	2.2 ± 0.6	9.1 ± 0.6
IM-150	Immobead 150	0.367	40	13 ± 0.7[e]	–	1.5 ± 0.3	–
PSL	Powder, free enzyme	34.5	50	11 ± 0.9[e]	–	1.3 ± 0.2	–
RoL	Resting cells	6340	40	14 ± 1.1[e]	6 ± 1.1[e]	1.9 ± 0.4	8.2 ± 0.8
Af	Resting cells	175	40	14 ± 0.6[d]	–	1.6 ± 0.5	–

[a] Batch reactions at 24 h (1000 rpm, lipases at a concentration of 15% based on the weight of dichloropropyl dodecanoates).
[b] Flow reactions in the PBR (flow = 10 μL/min, samples taken 60 min after starting, column reactor filled with lipases at a concentration of 3.3% based on the weight of dichloropropyl dodecanoates).
[c] a_w = 0.54.
[d] a_w = 0.42.
[e] a_w = 0.33.

Table 2
Yield, product ratio of each regioisomer of dichloropropyl acrylate, and standard deviations obtained in the two reactor systems.

Biocatalyst	Batch			PBR		
	Yield (%)	VI (%)	VII (%)	Yield (%)	VI (%)	VII (%)
CALB	50 ± 1.2[a]	19 ± 0.6	81 ± 1.2	33 ± 0.9[a]	20 ± 0.8	80 ± 1.6
MmL	38 ± 1.1[b]	29 ± 1.1	71 ± 1.3	21 ± 1.2[b]	35 ± 1.3	65 ± 1.3
PS-IM	18 ± 1.4 [b]	39 ± 0.7	61 ± 0.8	7 ± 0.8[b]	36 ± 1.4	64 ± 0.9
IM-150	13 ± 0.7[c]	25 ± 1.3	75 ± 0.9	–	–	–
PSL	11 ± 0.9 [c]	34 ± 1.0	66 ± 1.3	–	–	–
RoL	14 ± 1.1[c]	39 ± 0.8	61 ± 1.5	6 ± 1.1[c]	42 ± 1.2	58 ± 1.2
Af	14 ± 0.6[b]	21 ± 1.2	79 ± 0.7	–	–	–

[a] $a_w = 0.54$.
[b] $a_w = 0.42$.
[c] $a_w = 0.33$.

yields in the batch reactor and in the PBR. Although CALB, PS-IM and PSL are non-specific lipases [41], these biocatalysts showed high formation of **VII**. Nonetheless, CALB was the best enzyme in terms of activity and regioselectivity. When MmL an 1,3-specific lipase [28] was used, a ratio of 29:71 of **VI:VII** products was obtained in spite of using a 32:68 ratio of **III**:**IV** reagents. The activity of Af resting cells was lower than that of CALB; however, the formation of regioisomer **VII** was similar to this lipase.

On the basis of the formation of the chiral 2,3-dichloro-1-propyl acrylate, we evaluated the enantioselective capacity of diverse enzymes during the transesterification reaction. Our initially attempts to separate the enantiomer esters by chiral gas chromatography were unsuccessful. Consequently, an indirect approach was attempted. The hydrolysis of racemic mixtures of reagent **IV** and product **VII** was performed using MmL. Hydrolysis of **IV** and **VII** was carried out to corroborate that this lipozyme does not show enantiopreference during hydrolysis reactions. Once this was confirmed, reagent **IV** and product **VII** recovered from the diverse enzymatic studies were hydrolyzed using the same procedure described above. The results showed that racemic mixtures of 2,3-dichloro-1-propanols were obtained in all the transesterifications carried out with the biocatalysts, thereby indicating the lack of stereoselectivity during the transesterification reactions.

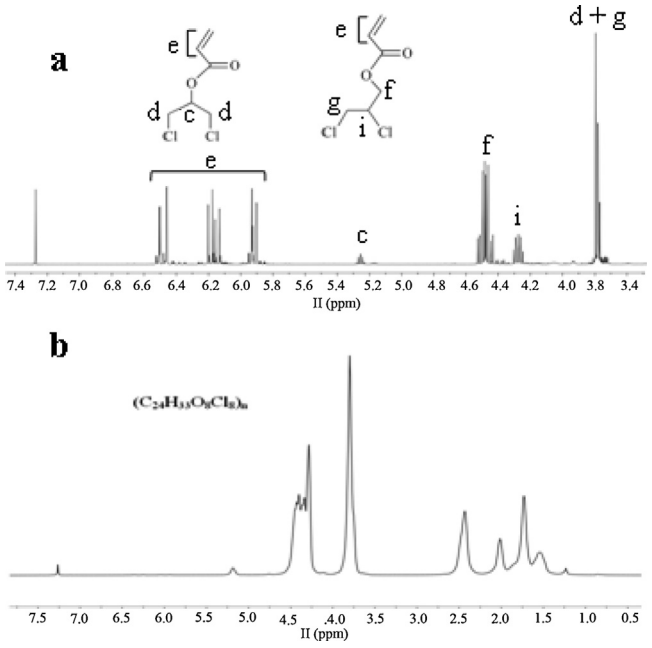

Fig. 7. (a) 1H NMR spectra of initial mixture of dichloropropyl acrylate regioisomers; (b) 1H NMR spectra of poly(dichloropropyl acrylate).

3.7. Preparation of poly(dichloropropyl acrylate)

Crude reaction mixtures obtained using the PBR were distilled and the liquid recovered was analyzed by GC-FID and ^{1}H NMR. Using CALB as a biocatalyst, we prepared 3.6 g of a mixture of regioisomers **VI**:**VII** (2:8) (Fig. 7a). A typical radical catalyzing polymerization method was followed using this mixture. This process allowed the preparation of solid particles with a wide range of diameters (170–380 μm). The yield was 85% after 18 h of polymerization. Fig. 7b shows the ^{1}H NMR spectra of the polymer obtained. NMR signals at δ 3.7, 4.4 and 5.1 confirm the presence of the dichloropropyl ester in the polymer.

Fig. 8 shows the FT-IR spectra (a) and Raman spectra (b) of the polymer obtained after 18 h of reaction. Characteristic IR bands corresponding to the presence of non-conjugated carboxylic esters (1732 cm^{-1}, C=O stretching; 1175–1160 and 1046 cm^{-1}, C—O stretching), alkyl saturated chains (2960 cm^{-1}, C—H stretching), and aliphatic chloro bond (740 cm^{-1} C–Cl stretching) can be observed. The presence of these functional groups was also confirmed by the Raman spectra (1740 cm^{-1}, C=O stretching; 1172 and 982 cm^{-1}, C—O stretching; 2982–2966 cm^{-1} and aliphatic chloro bond 744 cm^{-1} C—Cl stretching; 300 cm^{-1} δ C—C aliphatic chains).

The elemental analysis of the polymer revealed an empirical formula of $C_{24}H_{33}Cl_8O_8$ (Elem. Anal. calculated for $C_{24}H_{33}Cl_8O_8$: C, 39.32; H, 4.54; Cl, 38.69; O, 17.46. Found: C, 39.65; H, 4.50; Cl, 38.47; O, 17.38).

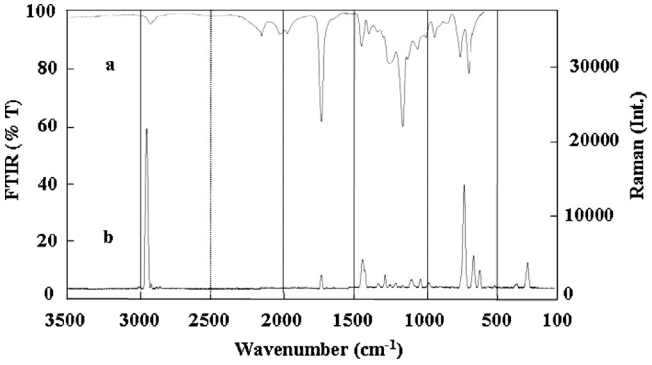

Fig. 8. FT-IR spectra (a) and Raman spectra (b) of poly(dichloropropyl acrylate).

4. Conclusions

This study shows that the biocatalytic transesterification of dichloropropyl acrylates can be carried out with commercial enzymes and fungal resting cells in batch reactor and a PBR. The biocatalysts showed distinct behaviors in response to water activity. CALB was the best biocatalyst, achieving its highest activity at $a_w = 0.54$. CALB can be used in a PBR system up to 30 h without a yield decrease. The resting cells with lipase-like activity were

found to catalyze the reaction, but always with low to moderate yields. Finally, a water-dispersive polymerization procedure was carried out to prepared polymeric material. The particles of poly(dichloropropyl acrylate) achieved reveal that the proposed process starting from a renewable resource as glycerol can be used for polymer preparation purposes.

Acknowledgements

This work was supported in part by a Grant-in-Aid for the Secretaría de Estado de Política Científica y Tecnológica of the Spanish Ministry of Education and Culture (contract grant number: CTQ2009-14699-C02-01). The authors are grateful to the Vicerrectorat de Recerca de l'Universitat de Lleida for the UdL grant of Edinson Yara Varón. We also thank Tania Yates for the proofreading of the manuscript.

References

[1] B. Gruning, G. Hills, W. Josten, D. Schaefer, S. Silber, C. Weitemeyer, US6268521, 2001.
[2] H. Kazuhiko, JP1301648, 1989 1–5.
[3] J.M. Moran, G.N. Taylor, J. Vac. Sci. Technol. 16 (1979) 2014–2019.
[4] S. Arimatsu, K. Konishi, Y. Ichinose, T. Hase, US5397675, 1995.
[5] K. Takeya, H. Suzuki, T. Ichimaru, US4022750, 1977.
[6] D.G. Anderson, A. Nashat, M.J. DeRosa, D.T. Puerta, R.P. McLaughlin, B.S. Akcasu, S.A. Williams, M.R. Ramirez, US20100284953, 2010.
[7] S.F. Wuister, J.F. Dijksman, Y.W. Kruijt-Stegeman, I. Schram, US2011/0266255, 2011.
[8] C.E. Rehberg, M.B. Dixon, W.A. Faucette, J. Am. Chem. Soc. 72 (1950) 5199–5200.
[9] E. Yara-Varón, J. Eras Joli, M. Torres, N. Sala, G. Villorbina, J.J. Méndez, R. Canela-Garayoa, Catal. Today (2012), doi:10.1016/j.cattod.2012.02. 2012 055.
[10] M. Escribà, J. Eras, G. Villorbina, M. Balcells, C. Blanch, N. Barniol, R. Canela, Waste Biomass Valor. 2 (2011) 285–290.
[11] S. Hu, X. Luo, C. Wan, Y. Li, J. Agric. Food Chem. 60 (2012) 5915–5921.
[12] G.P. da Silva, M. Mack, J. Contiero, Biotechnol. Adv. 27 (2009) 30–39.
[13] A. Vlysidis, M. Binns, C. Webb, C. Theodoropoulos, Biochem. Eng. J. 58–59 (2011) 1–11.
[14] J. Eras, J.J. Méndez, M. Balcells, R. Canela, J. Org. Chem. 67 (2002) 8631–8634.
[15] M. Escriba, J. Eras, M. Duran, S. Simon, C. Butchosa, G. Villorbina, M. Balcells, R. Canela, Tetrahedron 65 (2009) 10370–10376.
[16] C. Solarte, M. Escriba, J. Eras, G. Villorbina, R. Canela, M. Balcells, Molecules 16 (2011) 2065–2074.
[17] P. Srimhan, K. Kongnum, S. Taweerodjanakarn, T. Hongpattarakere, Enzyme Microb. Technol. 48 (2011) 293–298.
[18] I. Itabaiana Jr., L.S. de Mariz e Miranda, R.O.M.A. de Souza, J. Mol. Catal. B: Enzym. 85–86 (2013) 1–9.
[19] T. Zhao, B.H. Kim, S.I. Hong, S.W. Yoon, C.-T. Kim, Y. Kim, I.-H. Kim, J. Food Sci. 77 (2012) C267–C271.
[20] L. Babich, A.F. Hartog, M.A. van der Horst, R. Wever, Chem. Eur. J. 18 (2012) 6604–6609.
[21] S.F.A. Halim, A.H. Kamaruddin, W.J.N. Fernando, Bioresour. Technol. 100 (2009) 710–716.
[22] M. Torres, M.M. Dolcet, N. Sala, R. Canela, J. Agric. Food Chem. 51 (2003) 3328–3333.
[23] M. Torres, E. Barbosa, V. Loscos, R. Canela, Biotechnol. Lett. 22 (2000) 1265–1268.
[24] J. Eras, M. Escriba, G. Villorbina, M. Oromi-Farrus, M. Balcells, R. Canela, Tetrahedron 65 (2009) 4866–4870.
[25] M. Nordblad, P. Adlercreutz, Biotechnol. Bioeng. 99 (2008) 1518–1524.
[26] E. Yara-Varón, J. Eras Joli, M. Balcells, M. Torres, R. Canela-Garayoa, RSC Adv. 2 (2012) 9230.
[27] A. Idris, A. Bukhari, Biotechnol. Adv. 30 (2012) 550–563.
[28] P.K. Pal, D.K. Bhattacharyya, S. Ghosh, JAOCS 78 (2001) 31–36.
[29] G.V. Chowdary, S.G. Prapulla, Process Biochem. 38 (2002) 393–397.
[30] E. Wehtje, P. Adlercreutz, Biotechnol. Lett. 19 (1997) 537–540.
[31] M. Karra-Chaabouni, S. Pulvin, D. Thomas, D. Touraud, W. Kunz, Biotechnol. Lett. 24 (2002) 1951–1955.
[32] Y. Bourne, C. Martinez, B. Kerfelec, D. Lombardo, C. Chapus, C. Cambillau, J. Mol. Biol. 238 (1994) 709–732.
[33] M. Skjøt, L. De Maria, R. Chatterjee, A. Svendsen, S.A. Patkar, P.R. Østergaard, J. Brask, ChemBioChem 10 (2009) 520–527.
[34] R.D. Schmid, R. Verger, Angew. Chem. Int. Ed. 37 (1998) 1608–1633.
[35] R.C. Rodrigues, R. Fernandez-Lafuente, J. Mol. Catal. B: Enzym. 62 (2010) 197–212.
[36] J.C. Jeong, S.B. Lee, Biotechnol. Tech. 11 (1997) 853–858.
[37] L.L. Woodcock, C. Wiles, G.M. Greenway, P. Watts, A. Wells, S. Eyley, Biocatal. Biotransform. 26 (2008) 501–507.
[38] E.J. Tomotani, M. Vitolo, J. Food Eng. 80 (2007) 662–667.
[39] Z. Boros, P. Falus, M. Márkus, D. Weiser, M. Oláh, G. Hornyánszky, J. Nagy, L. Poppe, J. Mol. Catal. B: Enzym. 85–86 (2013) 119–125.
[40] C. Csajagi, G. Szatzker, E.R. Toke, L. Uerge, F. Darvas, L. Poppe, Tetrahedron Asymmetry 19 (2008) 237–246.
[41] E. Hernandez-Martin, C. Otero, Bioresour. Technol. 99 (2008) 277–286.