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

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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.

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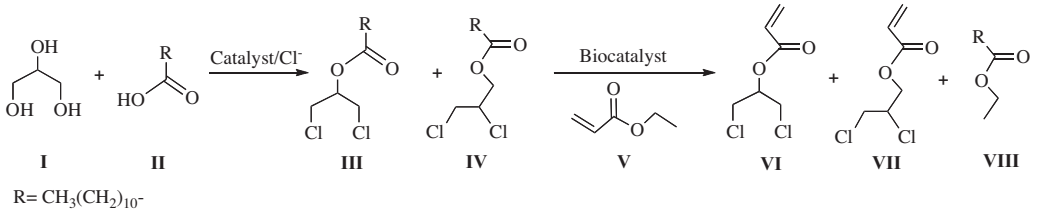

Fig. 1. Biocatalytic production of dichloropropyl acrylates from glycerol.

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