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Ultrasound enhances lipase-catalyzed synthesis of poly (ethylene glutarate)

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ABSTRACT

The present work explores the best conditions for the enzymatic synthesis of poly (ethylene glutarate) for the first time. The start-up materials are the liquids; diethyl glutarate and ethylene glycol diacetate, without the need of addition of extra solvent. The reactions are catalyzed by lipase B from *Candida antarctica* immobilized on glycidyl methacrylate-ter-divinylbenzene-ter-ethylene glycol dimethacrylate at 40 °C during 18 h in water bath with mechanical stirring or 1 h in ultrasonic bath followed by 6 h in vacuum in both the cases for evaporation of ethyl acetate. The application of ultrasound significantly intensified the polyesterification reaction with reduction of the processing time from 24 h to 7 h. The same degree of polymerization was obtained for the same enzyme loading in less time of reaction when using the ultrasound treatment. The degree of polymerization for long-term polyesterification was improved approximately 8-fold due to the presence of sonication during the reaction. The highest degree of polymerization achieved was 31, with a monomer conversion of 96.77%. The ultrasound treatment demonstrated to be an effective green approach to intensify the polyesterification reaction with enhanced initial kinetics and high degree of polymerization.

1. Introduction

Enzyme-based polymerization reactions provide greener alternative to the conventional chemical processes [1]. Enzymatic reactions minimize the formation of undesirable by-products; avoid harsh conditions and the requirement of metallic catalysts [2,3]. Enzymes can be used to produce specialty functional polymers [4]. When immobilized on firm, solid and porous supports enzymes can have better activity and stability [5,6]. Lipases are described as bio-catalysts in solvent [7–10] and in emulsion systems [9,11,12]. Potentially a solvent-free enzymatic system can offer better processing conditions without further complex purification process [10,11,13,14]. The use of solid immobilized enzymes in solvent free system where the reactants are the solvents itself might yield slow reaction rates due to the limiting diffusion between reactants and mass transfer limitations. The use of ultrasound might overcome these disadvantages [12,15]. The observed intensification is mainly due to the cavitational effects, such as increased local temperature and pressure as well as the generation of micro level mixing and turbulence conditions [16,17]. The ultrasonic irradiation produces alternate compression and rarefaction cycles at frequency of 20,000 cycles/s. That fortifies the interaction between phases by cavitation and/or formation of micro flows in liquids, which is initiated by violent collapse of small bubbles or voids in liquids as a result of pressure fluctuation. Additionally, ultrasound also improves mixing, shearing, and mass transfer in the reaction solution or suspensions [14–20]. Moreover cavitation may enhance polymerization by temporarily dispersing aggregates and preventing the formation of layers of compounds surrounding the immobilized enzyme due the micro scale turbulence generated by the cavitational effects [17,18,21–24]. In the case of polymerization, the heterogeneity in the system due to the use of different

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phases results in significant mass transfer resistances which can be eliminated by the use of ultrasonic irradiations.

The use of immobilized lipases as catalysts in ultrasound-assisted esterification reactions of short carboxylic acids to obtain esters has been reported by Rodrigues et al. [19]. Under optimized conditions there was an increase on the conversion of the reagents using ultrasounds. Despite the improvement of the esterification reaction using immobilized enzyme and ultrasounds the processes was not solvent free with a single ester as final product.

Although ultrasound-assisted lipase-catalyzed synthesis of polyester is an innovative approach, very few works have been reported in the literature showing better efficacy. For instance, studies related to the ultrasound assisted lipase-catalyzed synthesis of poly-6-hydroxyhexanoate revealed that sonication improved the monomer conversion by 63% and resulted in a polymer product with a narrower molecular weight distribution and a higher degree of crystallinity as compared to the conventional non-sonicated route [19]. In another study of enzymatic synthesis of poly-ε-caprolactone, it was reported only slightly of ultrasound enhancement of the rate constant for chain propagation (K_p) of the poly-ε-caprolactone. The acoustic effects also allowed the reaction to continue longer based on the control over the viscosity as compared to the non-sonicated process where it became impossible to operate due to the highly elevated reaction mixture viscosity (>2000 times increase from initial viscosity) [25].

In the present study, we investigated for the first time the synthesis of aliphatic polyester from diethyl glutarate and ethylene glycol diacetate using immobilized lipase B from *Candida antarctica* in a solvent free reaction. The effect of ultrasound on the initial reaction kinetics (concentrations analyzed by gas chromatography-flame ionization detector (GC-FID) was established in terms of the comparison with the conventional approach of using a shaker with controlled temperature. The effect of reaction operating parameters on the time of reaction, degree of polymerization and monomer conversion rate were analyzed using the measurements based on the use of nuclear magnetic resonance (NMR).

2. Materials and methods

2.1. Materials

Fermase CALB™ 10,000, a commercial *C. antarctica* lipase B (CALB) immobilized on glycidyl methacrylate-ter-divinylbenzene-ter-ethylene glycol dimethacrylate (particle size of 150–300 μm, pore volume of 1.32 cm^3/g, bulk density of 0.54 g/cm^3 and an activity of 8000 propyl laurate units) was obtained as a gift sample from Fermenta Biotech Ltd., Mumbai, India. Diethyl glutarate (DG) (purity ⩾99%), ethylene glycol diacetate (EGD) (purity 99%), chloroform (AR), ethyl acetate (HPLC, 99.9%), petroleum ether (AR, 40–60 °C), tetrahydrofuran (AR) were obtained from Sigma–Aldrich, Co., Sintra, Portugal. Magnesium sulfate (anhydrous technical grade) was obtained from Appli Chem GmbH, Darmstadt, Germany. Whatman® filter papers were obtained from Whatman International Ltd., England. All the chemicals and enzymes were used directly as received from the supplier without any further modification.

2.2. Experimental setup

The experimental setup involved the use of water bath with mechanical stirring, rotary vacuum bath and ultrasonic bath. The water bath (WB) used in the work was obtained from Grant Instruments (Cambridge) Ltd., England (model OLS 200) equipped with an orbital shaker and temperature controller. An ultrasonic bath (US) (USC600TH, VWR International Ltd., USA) with frequency 45 kHz and power of 120 W was also provided. The rotary vacuum bath (Vac) (Hei-VAP Advantage, Heidolph Instruments GmbH & Co., Germany) equipped with water bath and temperature controller was also used in the reactions. During the experiments, both the water bath and the vacuum bath were operated at 100 rpm.

The reaction mixture consisted of equimolar ratio of diethyl glutarate (10 mmol, 1.90 g) and ethylene glycol diacetate (10 mmol, 1.40 g) for a total reaction volume of 3 mL without any addition of solvents.

In the conventional approach, the reaction mixture with biocatalyst was subjected to a desired temperature in a water bath set at 100 rpm and the reaction time was 18 h at 40 °C [3]. The ultrasound assisted approach was performed by incorporating ultrasonic bath instead of water bath with mechanical stirring to establish the impact of ultrasound on the polyesterification reactions. The required reaction time in the ultrasonic bath operating at frequency of 45 kHz, 120 W power rating and duty cycle of 75% (15 min on/5 min off) was observed to be 1 h. Temperature was controlled at 40 °C. The set of reactions are summarized on Fig. 1. Both reaction mixtures (containing the enzyme) were then subjected to vacuum in bath at 40 °C for 6 h with stirring at 100 rpm for the evaporation of byproduct ethyl acetate in order to favor the forward polyesterification reaction. For both the approaches, the effect of enzyme loading was investigated over the range of 1% (w/v) to 20% (w/v).

Controls of the polyesterification reaction were performed for both approaches without enzyme. To study the effect of vacuum on the polymerization reaction, a set of reactions in water bath with mechanical stirring and in ultrasonic bath were ended before the vacuum step.

For the improvement of the polymerization with different enzyme loadings, a 72 h reaction was performed in vacuum bath in order to check an increase of the reaction turn over. The reaction time was based on previous polymerization reactions performed by Liu et al. [3].

To study the effect of ultrasound during reaction improvement, 2 reactions in water bath were interrupted by 1 h of ultrasound every 24 h of reaction time. However both reactions were performed with ultrasound/vacuum bath cycles, the first cycle varied. One of the reactions was initiated with 1 h of ultrasound while the other with 24 h of vacuum bath. Reactions with 1 h of ultrasound prior to 71 h in vacuum bath and only 72 h in vacuum bath were performed as control of the effect of the ultrasound/vacuum bath cycles on the poly(ethylene glutarate) reaction.

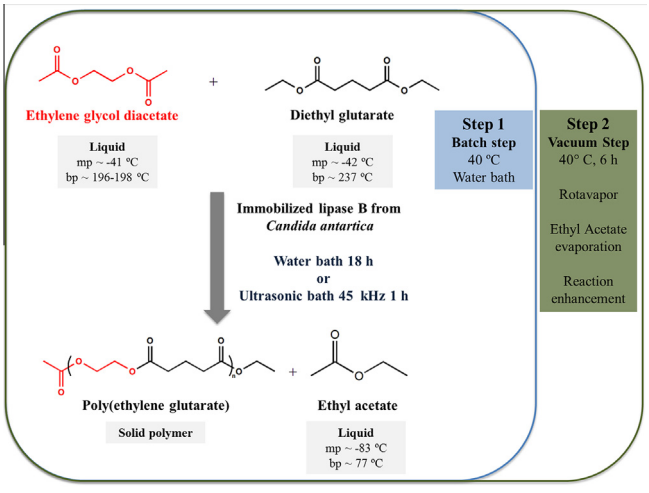

Fig. 1. Scheme of lipase-catalyzed solvent-free synthesis of poly (ethylene-glutarate).