



ELSEVIER

Contents lists available at ScienceDirect

Ultrasonics - Sonochemistry

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

Short communication

Lipase-ultrasound assisted synthesis of polyesters

Prerana D. Tomke^{a,b,1}, Xiaoman Zhao^{a,c,d,1}, Pranali P. Chiplunkar^{a,e}, Bo Xu^a, Hongbo Wang^{c,d}, Carla Silva^f, Virendra K. Rathod^b, Artur Cavaco-Paulo^{a,f,*}^a International Joint Research Laboratory for Textile and Fiber Bioprocesses, Jiangnan University, Wuxi 214122, China^b Department of Chemical Engineering, Institute of Chemical Technology, Matunga (E), Mumbai 400019, India^c Jiangsu Engineering Technology Research Center for Functional Textiles, Jiangnan University, Wuxi 214122, China^d Key Laboratory of Science and Technology of Eco-Textiles, Ministry of Education, Jiangnan University, Wuxi 214122, Jiangsu, China^e Department of Oils, Oleochemicals and Surfactants Technology, Institute of Chemical Technology, Matunga (E), Mumbai 400019, India^f Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

ARTICLE INFO

Keywords:

Ultrasound

Lipase

Polyester

PET polymeric beads

ABSTRACT

Poly (ethylene glutarate), poly (ethylene malonate) and poly (ethylene phthalate), were enzymatically synthesized by immobilized *Candida antarctica* lipase B in solvent free conditions. The synthesis of these polyesters was based on the ester-ester exchange reaction between ethylene glycol diacetate and di-ethyl glutarate, di-benzyl malonate, di-n-octyl phthalate to produce poly (ethylene glutarate), poly (ethylene malonate) and poly (ethylene phthalate), respectively. The effect of ultrasound and PET polymeric beads was evaluated and showed to improve the synthesis of all polyesters. Ultrasound, as a green solvent-free technology, showed high potentiality for the polyester synthesis intensification.

1. Introduction

Polyesters are polymers comprising of repeating ester groups as backbone of the chain structure being the most popular biodegradable polymers. Many studies related to synthesis of aliphatic and aromatic polyesters by using various fermentation and chemical processes have been carried out [1]. Poly (ethylene glutarate) (PEG), Poly (ethylene malonate) (PEM), Poly (ethylene phthalate) (PEP) are attractive polyesters by virtue of their ease in synthesis and widely diverse of applications such as textile manufacturing, microelectronics, bioprocessing, food packaging as well as in bio medical like surgical threads, contact lenses, supporting material in bone repairing, treat air leaks in lung injury [2].

Chemical synthesis of these polyesters requires high temperature and pressure conditions, longer reaction times and costly downstream processing. Though, numerous new heterogeneous catalysts have been developed in recent years to improve the yield and selectivity of the reaction. However, they possess common flaws like requirement of harsh reaction conditions. Many strategies such as the designing of recyclable catalysts, alteration of reaction conditions and the use of biocatalysts have been implemented to overcome these problems and make them green and environmentally friendly processes [3,4]. Re-

cently, a new approach for synthesizing these type of polyesters has been developed by enzyme catalyzed polymerization.

Enzymatic polymerization offers a new synthetic method to produce polyesters whose synthesis is difficult [5]. Enzymes, mainly lipase catalyzed reactions, are among the most promising alternatives to traditional chemical catalyzed processes. They are superior to chemical catalysis, due to their wide-ranging substrate specificity. They do not require co-factors, working under mild operating conditions, holding excellent ability to recognize regio and stereo selectivity and needing low energy input [6]. Holding these advantages, enzymatic polymerization is considered to be a method of polymer synthesis with potential to perform precise control of polymer structures. Is an energy saving and clean process (without by-products) allowing to design polymers with a new structure and biodegradable properties [7]. Previous studies have been evidencing the poor stability of the enzyme, the slow reaction rates and the low molecular weight of the products. These problems remain to limit large-scale commercial use of lipases [8]. To overcome these limitations, attempts have been made to improve the performance of lipases by the application of several new techniques. The use of ultrasound holds a considerable potential for improving the performance of enzyme catalysis processes [9].

* Corresponding author at: Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal and International Joint Research Laboratory for Textile and Fiber Bioprocesses, Jiangnan University, Wuxi 214122, China.

E-mail address: artur@deb.uminho.pt (A. Cavaco-Paulo).

¹ Both authors equally contributed to this work.

Ultrasonic irradiation has been recently observed as a novel approach for the synthesis of polyester compounds. As an acoustic pressure wave from a transducer propagates through the aqueous medium, changes in pressure causes the formation of cavitation bubbles to form oscillate and subsequently collapse phenomenon known as cavitation. Cavitation events are described as either stable or transient, and in both cases generate highly localized temperature [10]. During collapse of cavitation bubbles, the generation of large amount of energy causes dissociation of the reactant compounds into highly active radicals. The energy of the event is dependent on the intensity of cavitation which in turn depends on the amplitude and frequency of the acoustic wave among other factors [11]. The application of sonochemical reactions can favor the reaction chemistry and propagation by enhancing mass transfer and interphase mixing [12]. The use of ultrasound may enable to operate at milder operating parameters, at solvent free conditions decreasing therefore the overall reaction steps [13]. Considering the large amount of industrially polyesters and polymers synthesized via step-growth reactions (including interesterification reactions) there have been relatively few publications dealing with the use of ultrasound in this area.

Herein, we aim to compare the conventional process of polyester synthesis with the newly developed process including the use of ultrasounds and PET polymeric beads [14]. The polyesters produced were analyzed by MALDI-TOF (Matrix assisted laser desorption/ionization time of flight mass spectrometry) for polymerization degree quantification.

2. Materials and methods

2.1. Materials

Fermase CALB™ 10,000, a commercial *Candida 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 cm³/g, bulk density of 0.54 g/cm³ and an activity of 8000 propyl laurate units) was received as a gift sample from Fermenta Biotech Ltd., Mumbai, India. Ethylene glycol diacetate (purity 99%) was purchased from Acros Organics BVBA, Geel, Belgium. Diethyl glutarate (purity 99%+) was obtained from Adamas Reagent Co., Ltd., Shanghai, China. Dibenzyl malonate (purity 95%), and di-*n*-octyl phthalate (purity 98%) were bought from Alfa Aesar Chemical Co., Ltd., Shanghai, China. 2-Hydroxyterephthalic acid (HTA) (purity 98%) was got from J&K Scientific Ltd., Beijing, China. Poly (ethylene terephthalate) (PET) in granular was obtained from Sigma-Aldrich Co., Shanghai, China. Trichloromethane (Chloroform) (AR, 99%+), petroleum ether (AR, 30 °C–60 °C), Tetrahydrofuran (AR, 99%+), Sodium sulfate anhydrous (AR, 99%+), *p*-Phthalic acid (purity 99%), Sodium phosphate dibasic anhydrous (AR, 99%+) and sodium phosphate monobasic anhydrous (AR, 99%+) were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Whatman® Filter paper was obtained from Whatman Wohua Co., Ltd., Hangzhou, China. All the chemicals and enzymes were used directly without any further modification.

2.2. Experimental setup

The experimental setup involved the use of a water bath (WB) (model STD-134L, Standard Groups Co., Ltd., Shanghai, China) with orbital shaking with temperature controller and of an ultrasonic bath (US) (SK5210HP, Kudos Ultrasonic Instrument Co., Ltd., Shanghai, China) provided with 53 kHz frequency and 100 W power.

2.3. Characterization of the sonochemical reactor

2.3.1. Calorimetric characterization

The calorimetric characterization of the ultrasonic bath was based

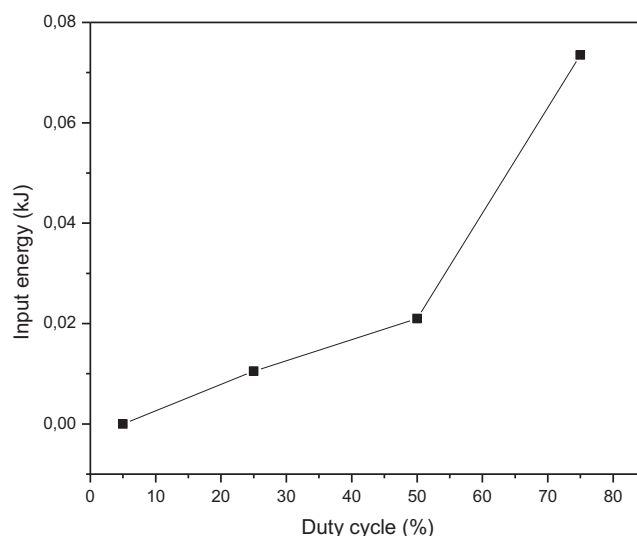


Fig. 1. Input energy (kJ) vs duty cycle (%) of ultrasonic bath (53 kHz, 100 W).

on the input energy measurement during a certain exposure time of ultrasonic irradiation varying the duty cycles of ultrasonic treatment [15]. The changes in temperature were measured by using a thermometer which was positioned at the center of the vials after completion of reaction. The energy profile was followed using distilled water for 30 min. and the corresponding calorific power was determined by following equation:

$$E = (\Delta T \times m \times C_p)$$

where, E is the calculated input energy (kJ) to raise the water temperature; ΔT is equal to the difference of the final and initial temperature (°C); m is the mass of the water (kg); C_p is the heat capacity of water (4200 J kg⁻¹ °C⁻¹).

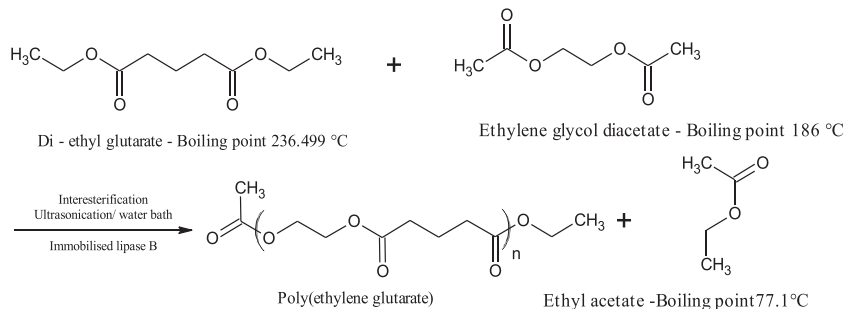
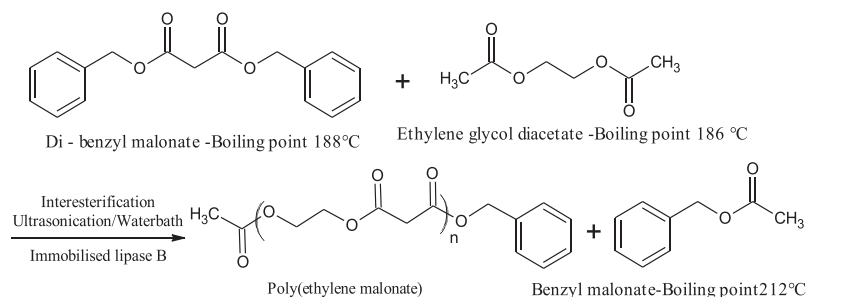
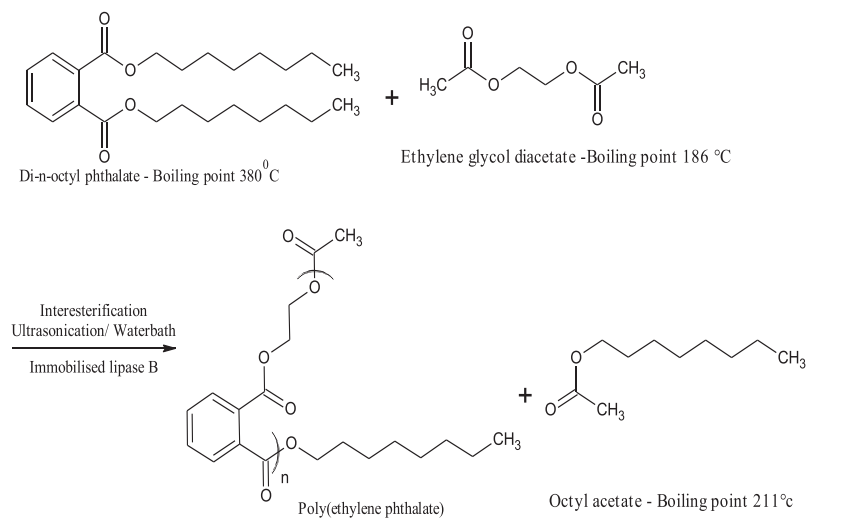
This characterization provide us to design the most favorable working conditions where the input of energy was highly enough to promote catalysis without compromising the catalytic behavior of the enzyme [16]. The calorimetric procedures were performed at 53 kHz using different duty cycles, namely 25%, 50%, 75%, 100% (Fig. 1). The experimental results show the effect of duty cycle on the input energy with the variable duty cycles. From Fig. 1 one can observe a proportional increase of input energy with the increase of duty cycle. To proceed with the following synthesis experiments we select the 50% duty cycle. We consider these conditions the best compromise between energy input and catalysts protection. High energy inputs would favor the synthesis reactions however detrimentally affect enzyme activity and stability.

2.4. Synthesis of polyesters

Three different schemes were designed for the synthesis of the different polyesters namely, poly (ethylene glutarate), poly (ethylene malonate) and poly (ethylene phthalate). The reaction mechanisms for their synthesis are depicted in Table 1. The reaction mixture for each scheme consisted of: equimolar ratio of ethylene glycol diacetate and diethyl glutarate for the synthesis of poly (ethylene glutarate); equimolar ratio of ethylene glycol diacetate and di-benzyl malonate for the synthesis of poly (ethylene malonate), and equimolar ratio of ethylene glycol diacetate and di-*n*-octyl phthalate for synthesis of poly (ethylene phthalate). The total reaction volume was 5 mL without solvents addition.

In the **conventional approach**, the reaction mixture containing the initial reagents and 1% (w/v) lipase was placed in a water bath at 100 rpm for 8 h at 40 °C. The **ultrasound assisted approach** was performed by alternating the use of water bath with the ultrasonic bath.

Table 1
Reaction mechanisms of polyesters synthesis and average polymerization degree of synthesized polyesters

Schemes	Synthesized polyesters	Reaction mechanism
A	Poly (ethylene glutarate)	 <p>Di - ethyl glutarate - Boiling point 236.499 °C</p> <p>Ethylene glycol diacetate - Boiling point 186 °C</p> <p>Poly(ethylene glutarate)</p> <p>Ethyl acetate - Boiling point 77.1 °C</p>
B	Poly (ethylene malonate)	 <p>Di - benzyl malonate - Boiling point 188 °C</p> <p>Ethylene glycol diacetate - Boiling point 186 °C</p> <p>Poly(ethylene malonate)</p> <p>Benzyl malonate - Boiling point 212 °C</p>
C	Poly (ethylene phthalate)	 <p>Di-n-octyl phthalate - Boiling point 380 °C</p> <p>Ethylene glycol diacetate - Boiling point 186 °C</p> <p>Poly(ethylene phthalate)</p> <p>Octyl acetate - Boiling point 211 °C</p>

Polymerization degree

Reaction conditions	Scheme A	Scheme B	Scheme C
Without PET in WB ¹	$n_{CA} = 3.6$	$n_{CA} = 3.6$	$n_{CA} = 2.6$
Without PET in WB + US ¹	$n_{CA} = 4.1$	$n_{CA} = 3.6$	$n_{CA} = 3.0$
With PET in WB	$n_{CA} = 3.3$	$n_{CA} = 3.4$	$n_{CA} = 2.9$
With PET in WB + US	$n_{CA} = 3.7$	$n_{CA} = 3.9$	$n_{CA} = 3.2$

¹ “In water bath (WB)” means that the reaction mixture was kept in water bath for 8 h at 40 °C with 100 rpm. “In ultrasound bath (US)” means that the reaction mixture was kept in water bath for 3 h, followed by ultrasound bath for 0.5 h, then in water bath for 3 h, followed by ultrasound bath for 0.5 h finally, then in water bath for 1 h at 40 °C, 100 rpm. n_{CA} represents carboxylic acid and alcohol as end groups of polyester; n_{CC} represents carboxylic acid as end groups of polyester; n_{AA} represents alcohols as end groups of polyester; n_{CA} represents carboxylic acid and alcohol as end groups of polyester.

The overall reaction was performed as follows: the reaction mixture containing the initial reagents and enzyme was placed in the water bath for 3 h, then it was transferred to the ultrasonic bath for 0.5 h, followed by water bath for 3 h, then was transferred again to the ultrasonic bath for 0.5 h, and finally to the water bath for 1 h. The reaction temperature was kept constant at 40 °C throughout the reaction. The total time of ultrasound application was 1 h at 53 kHz, 100 W and duty cycle 50%.

The effect of adsorbent PET polymeric beads on the polymerization reaction was also evaluated. For this, a set of reactions were completed with addition of PET polymeric beads (1%, w/v) in the reaction mixtures under the same reaction conditions describe above.

2.5. Purification of synthesized polyesters

For the purification of the obtained polyester, 3 mL of chloroform were added to the reaction mixture to dissolve the residual reagents and synthesized polyester. This solution was filtered out by using Whatman filter paper and chloroform washing was given to the separated enzyme to filter out all the reaction product. After evaporating out all the chloroform 15 mL of petroleum ether were added to precipitate the polyester. The solution was filtered out and the petroleum ether was evaporated. Finally, a mixture of tetrahydrofuran and petroleum ether in the proportion of 1:14 was added. The mixture was kept at –20 °C for overnight to allow the synthesized polyester to precipitate out. The mixture of polyester and residual reagents was then collected after evaporation the petroleum ether and tetrahydrofuran and samples were collected in chloroform and analyzed [5].

2.6. MALDI-TOF characterization

MALDI-TOF mass spectra of the reaction products were acquired using a microflex LT MALDI-TOF mass spectrometry (Bruker Daltonics GmbH, Germany) equipped with a 337-nm nitrogen laser. The matrix 2,5-dihydroxy-benzoic acid (DHB) at 20 mg/mL, was prepared in a solution of 30% acetonitrile and 0.1% Trifluoroacetic acid (TFA) in super pure water, and then mixed with samples (v/v, 1:1). A volume of 2 µL of each sample/matrix mixture was deposited on a ground steel target plate (Bruker part n° 8280800) and then allowed to dry at room temperature in air. The dried sample spots were analyzed by the

positive-ion method of RP300-4000 in the reflective mode.

2.7. Calculation of average polymerization degree of synthesized polyesters

The average degree of polymerization of synthesized polyesters was determined using the data obtained by LT MALDI-TOF mass spectrometry (Bruker Daltonics GmbH, Germany).

Fig. 2 shows the final possible chemical structures of the polyesters and the calculation of average degree of polymerization using the shown equation; the sum of the relative intensities of all the peaks in a samples mass spectrum should be equal to one.

3. Results and discussion

3.1. Average polymerization degree of polyesters

MALDI-TOF was used to analyze the molecular weight distribution of polyesters at different stages of the polymerization, allowing to scrutinize the changes in polyester functionalities occurred during reaction synthesis [17].

The enzymatically synthesized polyesters consist mainly on chains comprising one carboxylic acid end-group and one hydroxyl end-group, two hydroxyl end-groups or two carboxylic acid end-groups [18]. Herein, the solvent-free ester-ester exchange reaction of ethylene glycol diacetate with three different esters such as di-ethyl glutarate (Scheme A), di-benzyl malonate (Scheme B) and di-*n*-octyl phthalate (Scheme C) respectively, were performed in the presence of the biocatalyst immobilized lipase B from *Candida antarctica* (CALB). To push the reaction in forward direction, PET polymeric beads were used to adsorb the formed products [19]. The polymerization degrees obtained under the conventional method using a water bath were compared with the values obtained under the combined form using water bath and ultrasound in an alternated way.

Table 1 depicts the average polymerization degree of synthesized polyesters with different reaction conditions analyzed with MALDI-TOF MS. One can observe that in the case of Scheme A the highest average polymerization degree ($n_{CA} = 4.1$) was obtained under ultrasonic irradiation in the absence of PET polymeric beads. It seems that ultrasound helps to build up the polymeric chain but the formed

Degree of polymerization in average = $SUM \left(\frac{\text{Intensity of each peak}}{\text{Sum of intensity of peak}} \times \text{Number of repeat units} \right)$			
Reaction schemes	Possible end groups		
	Carboxylic acid and alcohol (n_{CA})	Carboxylic acids (n_{CC})	Alcohols (n_{AA})
A			
B			
C			
Note: <ul style="list-style-type: none"> The sum of relative intensities should be one. n_{CA} represents carboxylic acid and alcohol as end groups of polyester. n_{CC} represents carboxylic acids as end groups of polyester. n_{AA} represents alcohols as end groups of polyester. 			

Fig. 2. Possible structures of end groups and determination of the average polymerization degree.

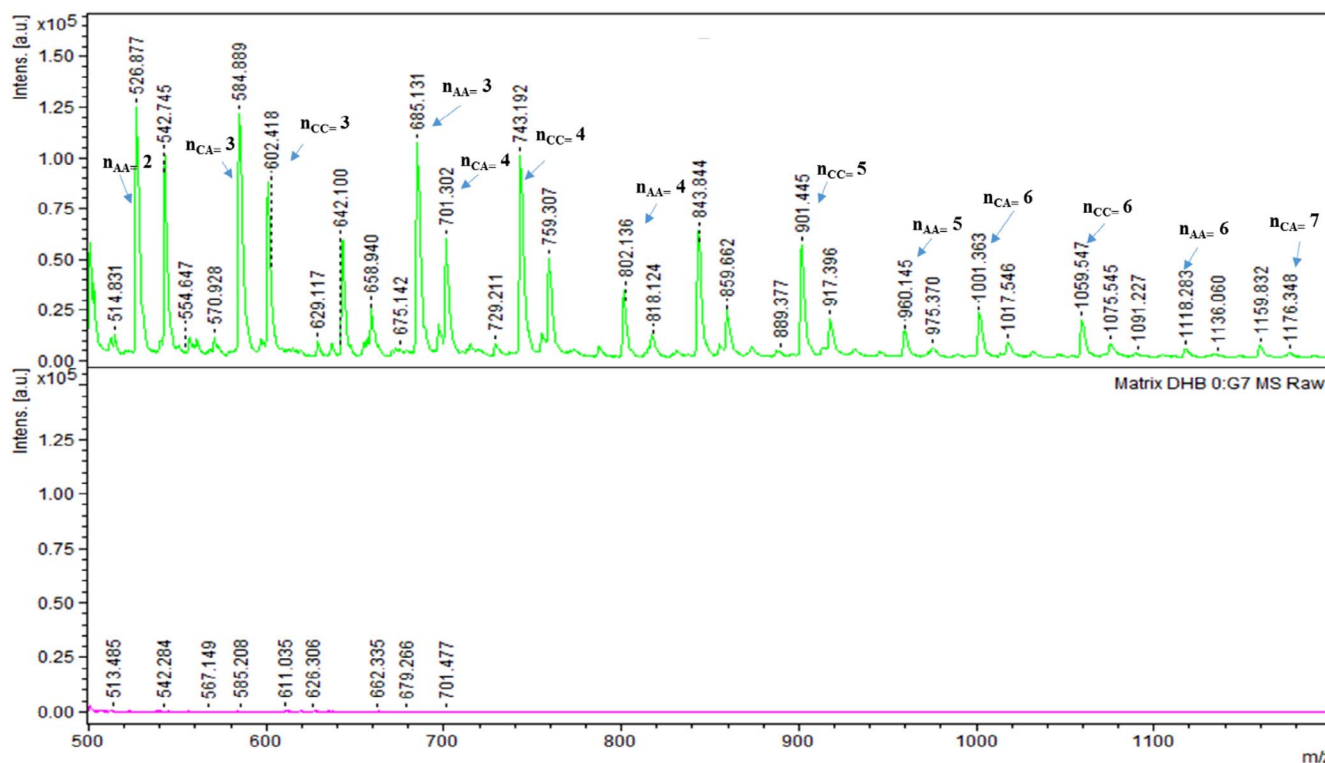


Fig. 3. MALDI-TOF mass spectra of poly(ethylene glutarate). nCA represents carboxylic acid and alcohol as end groups of polyester; nCC represents carboxylic acids as end groups of polyester; nAA represents alcohols as end groups of polyester.

polymer poly(ethylene glutarate) is less viable to adsorb on PET polymeric beads. In the case of Scheme B the highest average polymerization degree ($n_{CA} = 3.9$) was observed in the presence of ultrasonic irradiation using PET as adsorbent. Scheme C was synthesized more efficiently in the water bath in the presence of PET beads ($n_{CA} = 3.2$).

MALDI-TOF MS offers the possibility to determine the precise molar mass of polyesters. The polymeric structure (molecular weight) observed in the spectra resulted from different degrees of polymerization [20]. Table 3 of SI presents the list of molecular weight for oligomers for the synthesized polyesters such as poly(ethylene glutarate), poly(ethylene malonate), poly(ethylene phthalate) depending on the possible end groups. On the basis of the molecular weight of oligomers with different possible end groups, the average degree of polymerization was calculated according to the corresponding peaks in MALDI-TOF mass spectra.

One of the randomly selected MALDI-TOF mass spectra out of three schemes is shown in Fig. 3. All other spectra are provided as Supplementary data (Figs. S1–S9). In Fig. 2, the maximum degree of polymerization achieved for the synthesized poly(ethylene glutarate) was 7 ($n_{CA} = 7$), corresponding to a molecular weight of possible end group (Carboxylic acid + alcohol(n_{CA})) $C_2H_5O(C_7H_{10}O_4)_n C_2H_3O$, Molecular weight- $88 + 158n$; Two carboxylic acids(n_{CC}) $C_4H_7O_3(C_7H_{10}O_4)_n C_2H_3O$, Molecular weight $146 + 158n$; Two alcohols(n_{AA}) $C_2H_5O(C_7H_{10}O_4)_n C_7H_{11}O_3$, Molecular weight- $188 + 158n$.

3.2. Effect of ultrasound on polyesters synthesis

The application of sonication waves always brings about the activation of catalysis heterogeneous systems mainly because of the mass transfer effects caused by cavitation [21]. In the case of solid-liquid heterogeneous systems, the collapse of the cavitation bubbles produces significant structural and mechanical changes [22]. The effect of ultrasound on a heterogeneous system of ester-ester exchange reaction of polyester synthesis is related with the speed of agitation,

power, frequency, duty cycle and temperature. Herein, the heterogeneous system consisted of immobilized enzyme CALB with all other reagents present in the liquid form. The results from Table 1 indicate that ultrasound improved reaction synthesis when compared with the synthesis carried out in the water bath. It was observed that the reaction between ethylene glycol diacetate and di-esters was favored and interexchange of chemical group take place by mass transfer effect promoted by cavitation and proper mixing with agitation. Stirring in enzymatic reaction plays an important role to lower down external mass transfer boundaries [23]. We predict that ultrasound plays dual role in creating higher interfacial area of catalysis as well as in facilitating the process of interfacial transport to form polyester chain by breaking of covalent bonds of reagents. In addition, it is proved that ultrasound helps to substantial reduce the synthesis reaction time. In the conventional process the mass transfer is very slow as compared to ultrasonication requiring higher energy for activation, thus compromising the synthesis reaction rates [24]. The formation of liquid jet occurs around the boundaries of lipase during cavitation. Due to this liquid jet the less available enzyme molecules become more exposed to react with the substrates. Acyl group donated from diacetate will get interacted with carbonyl radical from other ester to design new structure of monomer molecule which results in polyester formation [25,26].

The total duration of time for which a certain power is applied to the reaction mixture is called duty cycle. The products formation depend on the amount of irradiated sonication waves which are related with the duty cycle. A continuous ultrasound exposure leads to continuous formation of cavitation bubbles without providing them enough time to collapse and causing the cushioning effect. According to the results obtained by calorimetric evaluation of the reactor, 50% duty cycle (5 min ON and 5 min OFF) is the feasible method for enzymatic synthesis because it will provide enough time to complete life cycle of cavitation bubbles which favors reaction to move in forward direction [27,28]. According to Fig. 1 it is not the point where the energy input is higher but is a compromise between the energy required to improve mass transfer without compromising enzyme activity. Herein, the

reactions were carried out in a water bath for 3 h followed by sonication for 0.5 h, then again in water bath for 3 h followed by 0.5 h of sonication and finally in water bath for 1 h, with the total reaction time of 8 h. The reaction process was performed in a cyclic manner always alternating both water bath and ultrasonic bath. This allowed to input twice the energy to the reaction mixture providing more exposure of the active site for the catalyst and at the same time control de temperature. The power applied for the reaction is around 100 W with 53 kHz frequency with 50% duty cycle. The energy generated due to cavitation collapse is high and may surpass the temperature of reaction. This would disturb the catalytic reaction and/or causing enzyme denaturation, hampering therefore the polymerization degree. The alternation between water bath and ultrasonic bath help to maintain stable the reaction temperature taking advantage of the positive effect of ultrasound [29].

3.3. Effect of PET polymer beads on polyesters synthesis

In order to improve the separation process of the synthesized resulting products, PET polymeric beads were included in the system during the reaction to act as adsorbents and their effect was evaluated [30]. The average polymerization degree of synthesized polyesters using water bath and ultrasound-assisted reactions with and without PET are shown in Table 1. The material adsorbed on the polymer beads was removed by continuous washing of the beads with chloroform and then analyzed by MALDI TOF MS (these results are provided in Supplementary data: Figs. S3, S6 and S9). The average degree of polymerization of Scheme B ($\eta_{AA} = 4.9$) obtained after washing of PET polymer beads is the highest compared with the other reaction schemes. It is well known that ultrasonication promotes the cleaning of porous surfaces due to formation of cavitation jets along the walls of the solid. The desorption of the material adsorbed may occur promoting the cleaning of the polymeric beads. To avoid this we have applied pulsed sonication with 50% duty cycle with cyclic processing for 3 h in the water bath followed by 0.5 h in the sonicator bath, then again 3 h in the water bath followed by 0.5 h in the sonicator bath followed by 1 h in the water bath. We believe that this alternation contributed to maintain an higher amount of material adsorbed [31]. PET polymeric beads are nothing but a porous material obtained from PET recycling which is meant to perform adsorption and are used in many processes [19]. Addition of PET in the reaction mixture acts as adsorbent and allow the reaction to proceed. The combination of ultrasound with PET beads was tested herein as a promising method of products separation of products during reaction. The adsorption of the resulting products can slightly shift the equilibrium of the reaction towards the synthesis of polyesters increasing therefore the polymerization degree [32]. In fact, the experimental results obtained from MALDI TOF MS show that the application of ultrasound and the use of PET beads can speed up reaction rates favoring the enzymatic polyesterification. In case of Scheme B and Scheme C, it was observed that average polymerization degree slightly increased with the addition of PET polymeric beads. But in case of Scheme A the average polymerization degree was lower when PET beads were added. This can be possible due to the lower viscosity of poly (ethylene glutarate) as compared to poly (ethylene malonate) and poly (ethylene phthalate). Another possibility relies on their different structural nature, both poly (ethylene malonate) and poly (ethylene phthalate) are aromatic polyesters while poly (ethylene glutarate) is an aliphatic polyester. Moreover, being PET polymeric beads made out of aromatic polyesters they can easily attract aromatic compounds.

3.4. Comparative study of synthesis of three different polyesters with ultrasound treatment

Lipase catalyzed solvent free synthesis of polyesters was successfully achieved under specific conditions: 40 °C under ultrasonic power of

50 W, 50% duty cycle and 53 kHz frequency with mechanical agitation of 100 rpm.

In a comparative study, combining the effect of ultrasound with the presence of PET polymeric beads as adsorbent material, the average degree of polymerization is given in Table 1. Results indicate the highest average degree of polymerization in the case of Scheme B (3.9) followed by Scheme A (3.7) and then Scheme C (3.2). The combined results proved that ultrasound (US) combined with WB accelerate the rate of reaction in all three schemes when compared with the conventional process. The mechanical effects of ultrasound improve the catalyst behavior, increasing the area of catalyst available, the mass transfer and speeding up the synthesis reaction [33].

Regarding the effect of PET polymeric beads, it was observed that scheme B (3.9) is more suitable towards separation of formed products by adsorption but in the case of scheme A (4.1) the average degree of polymerization is higher without use of PET polymer beads. It can be attributed to the observed viscosity of the product formed in Scheme B poly (ethylene malonate) which was higher than that observed for Scheme A poly (ethylene glutarate) and Scheme C poly (ethylene phthalate). On other hand all the polyesters are polar in nature and even micro porous surface of PET polymer beads used possess polarity. This combination of the polarity and the viscous nature of the liquid medium in the presence of aromatic poly esters results in adsorption phenomenon. Therefore, in case of Scheme B higher adsorption occurs due to higher polarity of molecule and viscous nature [34–36].

4. Conclusions

Three different polyesters, namely poly (ethylene glutarate), poly (ethylene malonate) and poly (ethylene phthalate) were successfully synthesized by lipase in solvent-free conditions. The effect of ultrasonic irradiation and the use of PET polymeric beads as adsorbent to separate out the synthesized polyester during reaction progress was studied in comparative manner. It was observed that ultrasound helps in increasing reaction rates corresponding to higher polymerization degree. We found the best operational conditions for the polymerization of the three polyesters. The reaction must be carried-out in cyclic manner: 3 h in water bath followed by 0.5 h in sonication; then 3 h in water bath followed by 0.5 h in sonication; followed by 1 h in water bath; with 50% duty cycle, 100 W power and 53 kHz frequency. We also could observe that the inclusion of PET beads did not favor all the reaction processes. It is clear that the success of these beads is dependent on the nature of the synthesis resulting products, being the aliphatic ones more easily adsorbed.

List of abbreviations

1. Di-ethyl glutarate – Scheme A
2. Di-benzyl malonate – Scheme B
3. Di-n-octyl phthalate – Scheme C
4. CALB – *Candida antarctica* lipase B
5. US – Ultrasonic bath
6. WB – Water bath
7. PET – Poly (ethylene terephthalate)
8. MALDI-TOF – Matrix assisted laser desorption/ionization time of flight mass spectrometry

Acknowledgements

All authors gratefully acknowledge the financial support provided by International Joint Research Laboratory for Textile and Fibre Bioprocesses at Jiangnan University. The authors are also thankful to the Department of Chemical Engineering, Institute of Chemical Technology, Matunga (E), Mumbai-400019, India and to the Bioprocess and Bio nanotechnology Research Group (BBRG) of University of Minho. Authors would like also to acknowledge the

Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte and to the Fundamental Research Funds for the Central Universities (No. JUSRP51622A) 2016, Jiangsu Province Scientific Research Innovation Project for Academic Graduate Students (No. KYLX16_0788).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ultsonch.2017.03.051>.

References

- J.N. Korley, S. Yazdi, K. McHugh, J. Kirk, J. Anderson, D. Putnam, One-step synthesis, biodegradation and biocompatibility of polyesters based on the metabolic synthesis, dihydroxyacetone, *Biomaterials* 98 (2016) 41–52.
- V. Delplace, J. Nicolas, Degradable vinyl polymers for biomedical applications, *Nat. Chem.* 7 (2015) 771–784.
- M.H. Bilal, M. Prehm, A.E. Njau, M.H. Samiullah, A. Meister, J. Kressler, Enzymatic synthesis and characterization of hydrophilic sugar based polyesters and their modification with stearic acid, *Polymers* 8 (2016) 1–11 (20734360).
- C.M. Thomas, Stereocontrolled ring-opening polymerization of cyclic esters: synthesis of new polyester microstructures, *Chem. Soc. Rev.* 39 (2010) 165–173.
- X. Zhao, S.R. Bansode, A. Ribeiro, A.S. Abreu, C. Oliveira, P. Parpot, P.R. Gogate, V.K. Rathod, A. Cavaco-Paulo, Ultrasound enhances lipase-catalyzed synthesis of poly (ethylene glutarate), *Ultrason. Sonochem.* 31 (2016) 506–511.
- K.M. Koeller, C.-H. Wong, Enzymes for chemical synthesis, *Nature* 409 (2001) 232–240.
- H. Uyama, S. Kobayashi, Enzymatic synthesis of polyesters via polycondensation, in: S. Kobayashi, H. Ritter, D. Kaplan (Eds.), *Enzyme-Catalyzed Synthesis of Polymers*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2006, pp. 133–158.
- L. Gustini, L. Finzel, C. Lavilla, B.A.J. Noordover, M.M.R.M. Hendrix, C. Gehrels, C.E. Koning, Understanding the limitations of the solvent-free enzymatic synthesis of sorbitol-containing polyesters, *ACS Sustainable Chem. Eng.* 4 (2016) 2259–2268.
- J.S. Alves, C. Garcia-Galan, M.F. Schein, A.M. Silva, O. Barbosa, M.A.Z. Ayub, R. Fernandez-Lafuente, R.C. Rodrigues, Combined effects of ultrasound and immobilization protocol on butyl acetate synthesis catalyzed by CALB, *Molecules* 19 (2014) 9562–9576.
- S. Shaik, S.H. Sonawane, S.S. Barkade, B.A. Bhanvase, Synthesis of inorganic, polymer and hybrid nanoparticles using ultrasound, in: M. Ashokkumar (Ed.), *Handbook of Ultrasonics and Sonochemistry*, Springer Singapore, Singapore, 2016, pp. 1–34.
- B. Xiang, J. Zhang, Using ultrasound-assisted dispersion and in situ emulsion polymerization to synthesize TiO₂/ASA (acrylonitrile-styrene-acrylate) nanocomposites, *Compos. B Eng.* 99 (2016) 196–202.
- K.C. Badgujar, B.M. Bhanage, The combine use of ultrasound and lipase immobilized on co-polymer matrix for efficient biocatalytic application studies, *J. Mol. Catal. B Enzym.* 122 (2015) 255–264.
- A.J. Jadhav, C.R. Holkar, A.D. Goswami, A.B. Pandit, D.V. Pinjari, Acoustic cavitation as a novel approach for extraction of oil from waste date seeds, *ACS Sustainable Chem. Eng.* 4 (2016) 4256–4263.
- L.P. Ramteke, P.R. Gogate, Improved treatment approach for the removal of aromatic compounds using polymeric beads in Fenton pretreatment and biological oxidation, *Environ. Sci. Pollut. Res.* (2016) 1–16.
- S. Perincek, A.E. Uzgur, K. Duran, A. Dogan, A.E. Korlu, İ.M. Bahtiyari, Design parameter investigation of industrial size ultrasound textile treatment bath, *Ultrason. Sonochem.* 16 (2009) 184–189.
- R. Silva, H. Ferreira, C. Little, A. Cavaco-Paulo, Effect of ultrasound parameters for unilamellar liposome preparation, *Ultrason. Sonochem.* 17 (2010) 628–632.
- S.R. Bansode, V.K. Rathod, Ultrasound assisted lipase catalysed synthesis of isoamyl butyrate, *Process Biochem.* 49 (2014) 1297–1303.
- M. Florczak, A. Michalski, A. Kacprzak, M. Brzeziński, T. Biedroń, A. Pająk, P. Kubisa, T. Biela, MALDI-TOF analysis of lactide oligomers with functional end groups, *React. Funct. Polym.* 104 (2016) 71–77.
- F. Monnaie, L. Verheyen, J. De Winter, P. Gerbaux, W. Brullot, T. Verbiest, G. Koeckelberghs, Influence of structure of end-group-functionalized poly(3-hexylthiophene) and poly(3-octylselenophene) anchored on Au nanoparticles, *Macromolecules* 48 (2015) 8752–8759.
- R. Mendoza-Carrasco, E.M. Cuerda-Correa, M.F. Alexandre-Franco, C. Fernández-González, V. Gómez-Serrano, Preparation of high-quality activated carbon from polyethyleneterephthalate (PET) bottle waste. Its use in the removal of pollutants in aqueous solution, *J. Environ. Manage.* 181 (2016) 522–535.
- K. Jiang, A. Aloor, J. Qu, C. Xiao, Z. Wu, C. Ma, L. Zhang, P.G. Wang, Rapid and sensitive MALDI MS analysis of oligosaccharides by using 2-hydrazinopyrimidine as a derivative reagent and co-matrix, *Anal. Bioanal. Chem.* (2016) 1–9.
- P.D. Tomke, V.K. Rathod, Enzyme as biocatalyst for synthesis of octyl ethanoate using acoustic cavitation: optimization and kinetic study, *Biocatal. Agric. Biotechnol.* 7 (2016) 145–153.
- G.V. Waghmare, M.D. Vetal, V.K. Rathod, Ultrasound assisted enzyme catalyzed synthesis of glycerol carbonate from glycerol and dimethyl carbonate, *Ultrason. Sonochem.* 22 (2015) 311–316.
- M. Daryabor, A. Ahmadi, H. Zilouei, Solvent extraction of cadmium and zinc from sulphate solutions: comparison of mechanical agitation and ultrasonic irradiation, *Ultrason. Sonochem.* 34 (2017) 931–937.
- N.A. Methrath Liyakathali, P.D. Muley, G. Aita, D. Boldor, Effect of frequency and reaction time in focused ultrasonic pretreatment of energy cane bagasse for bioethanol production, *Bioresour. Technol.* 200 (2016) 262–271.
- P.D. Tomke, V.K. Rathod, Ultrasound assisted lipase catalyzed synthesis of cinnamyl acetate via transesterification reaction in a solvent free medium, *Ultrason. Sonochem.* 27 (2015) 241–246.
- D. Pingret, A.-S. Fabiano-Tixier, F. Chemat, Degradation during application of ultrasound in food processing: a review, *Food Control* 31 (2013) 593–606.
- S. Dey, V.K. Rathod, Ultrasound assisted extraction of β -carotene from *Spirulina platensis*, *Ultrason. Sonochem.* 20 (2013) 271–276.
- X. Zhao, J. Fu, H. Wang, A. Riberio, A. Cavaco-Paulo, Enzymatic coating of cotton with poly (ethylene glutarate), *Process Biochem.* (2016). <http://dx.doi.org/10.1016/j.procbio.2016.07.022>.
- J. Rodrigues, A. Canet, I. Rivera, N.M. Osório, G. Sandoval, F. Valero, S. Ferreira-Dias, Biodiesel production from crude *Jatropha* oil catalyzed by non-commercial immobilized heterologous *Rhizopus oryzae* and *Carica papaya* lipases, *Bioresour. Technol.* 213 (2016) 88–95.
- N. Ngadi, M. Jusoh, R.A. Rahman, Z. Mohamad, Synthesis and characterization of carbon cryogel beads using coconut husks, *Appl. Mech. Mater.* 169 (2015) 183–186.
- J. Wang, L. Xu, C. Cheng, Y. Meng, A. Li, Preparation of new chelating fiber with waste PET as adsorbent for fast removal of Cu²⁺ and Ni²⁺ from water: kinetic and equilibrium adsorption studies, *Chem. Eng. J.* 193–194 (2012) 31–38.
- S. Saikia, N.K. Mahnot, C.L. Mahanta, A comparative study on the effect of conventional thermal pasteurisation, microwave and ultrasound treatments on the antioxidant activity of five fruit juices, *Food Sci. Technol. Int.* 22 (2016) 288–301.
- N.S.M. Yusof, B. Babgi, Y. Alghamdi, M. Aksu, J. Madhavan, M. Ashokkumar, Physical and chemical effects of acoustic cavitation in selected ultrasonic cleaning applications, *Ultrason. Sonochem.* 29 (2016) 568–576.
- F. Hippauf, W. Nickel, G.-P. Hao, K. Schwedtmann, L. Giebeler, S. Oswald, L. Borchardt, S. Doerfler, J.J. Weigand, S. Kaskel, The importance of pore size and surface polarity for polysulfide adsorption in lithium sulfur batteries, *Adv. Mater. Interfaces* (2016) n/a-n/a.
- A.R. Bagheri, M. Ghaedi, A. Asfaram, A.A. Bazrafshan, R. Jannesar, Comparative study on ultrasonic assisted adsorption of dyes from single system onto Fe₃O₄ magnetite nanoparticles loaded on activated carbon: experimental design methodology, *Ultrason. Sonochem.* 34 (2017) 294–304.