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Article

Surfactant-Free Peroxidase-Mediated Enzymatic Polymerization of a Biorenewable Butyrolactone Monomer via a Green Approach: Synthesis of Sustainable Biobased Latexes

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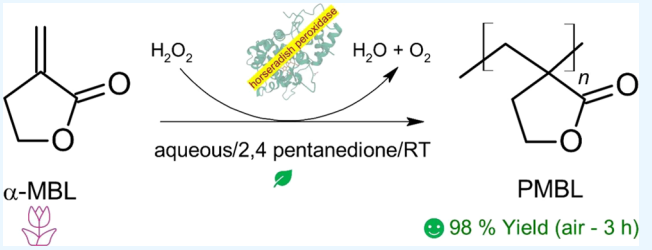
ABSTRACT: A green surfactant-free one-pot horseradish peroxidase-mediated enzymatic polymerization is successfully applied to produce a sustainable and thermally stable biobased high average molar mass poly(α-methylene-γ-butyrolactone) (PMBL) at ambient conditions in water for the first time. The initiation step required only very low concentrations of hydrogen peroxide and 2,4-pentanedione water-soluble initiator to generate the keto-enoxy radicals responsible for forming the primary latex particles. The polymer nanoparticles can be seen as monodisperse, and the biobased latexes are colloidally stable and likely stabilized by the adsorption of 2,4-pentanedione moieties on the particle surfaces. Polymerizations in air produced a 98% yield of PMBL after only 3 h, highlighting the relevance of molecular oxygen. An array of characterization techniques such as dynamic light scattering (DLS), Fourier transform infrared (FTIR), ^{1}H, ^{13}C, and HSQC two-dimensional (2D) nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and size-exclusion chromatography (SEC) are used to confirm the properties of the synthesized latexes. The PMBL exhibited high thermal stability, with only a 5% weight loss at 340 °C and a glass-transition temperature of 200 °C, which is double that of polymethyl methacrylate (PMMA). This research provides an interesting pathway for the synthesis of sustainable biobased latexes via enzymes in a green environment using just water at ambient conditions and the potential use of the polymer in high-temperature applications.



KEYWORDS: *green, sustainability, enzymatic polymerization, butyrolactones, biobased latexes*

1. INTRODUCTION

The interest in creating sustainable, biobased materials has grown in the past few decades to overcome issues of conventional plastics.[1−8] Those plastics have a significant carbon footprint and can build up in landfills and oceans, where they can linger for centuries or even longer.[4,5] Present environmental constraints, coupled with the depletion of fossil fuels, make it thus imperative to utilize greener materials in our society.[1−7]

The synthesis of polymers using biobased/renewable monomers via environmentally friendly and safe water-borne processes can be seen as the way to truly realize "green" polymeric materials.[1,2] Moreover, significant aspirations have driven extensive research interest toward the area of biocatalysis, particularly employing enzymes to produce such polymers using a highly selective and sustainable method in a more benign environment.[9−12] Biotechnology has indeed successfully reduced resource consumption and waste generation.[12−15] However, it has mainly been focused on the synthesis of small, chiral molecules that are predominantly used in the pharmaceutical industry, which has a small global impact on the chemical industry as a whole.[13] The polymer and plastic market is growing continuously and tremendously, where bulk polymers are produced annually in huge quantities and find potential applications in our daily lives.[8,16] To ensure such growth in a sustainable manner, a transition to greener synthesis routes is highly recommended.

Interestingly, biorenewable monomers based on a five-membered γ-butyrolactone ring (Figure 1a) represent natural and potential alternatives to fossil fuel monomers.[17−28] Those monomers are exocyclic analogues of methyl methacrylate (MMA) and exhibit interesting and unique properties as they have dual functionality (possess a vinyl group and a lactone

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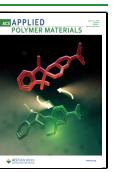

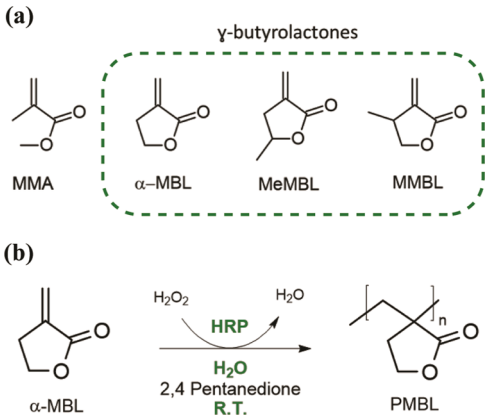

Figure 1. (a) Biobased five-membered ring γ-butyrolactones as natural alternatives to fossil-fuel-based MMA monomer. (b) HRP-mediated free-radical polymerization of α-MBL implemented in this study.

ring), where the vinyl moiety can be polymerized via free-radical polymerization, while the lactone ring can be polymerized via ring opening. Significantly, the associated polymers demonstrate a glass-transition temperature (T_g) that is twice that of PMMA (i.e., $T_g \sim 100$ °C), thereby rendering them suitable for potential applications at elevated temperatures.[17]

Alongside butyrolactones being attractive candidates in replacing monomers of fossil fuel origin, from a synthetic standpoint, they have been synthesized via a cost-effective method from itaconic acid (IA), which is a biomass intermediate produced from the fermentation of corn or rice.[17−21] One of those monomers (MMBL) is currently being produced commercially at a large scale from levulinic acid (a biomass intermediate) via a two-step catalytic process.[22] The biobased/sustainable polymers produced from γ-butyrolactone monomers have in turn been synthesized via free-radical polymerization techniques.[17,23−34] Nevertheless, such studies are scarce and are constrained by limitations. One of the drawbacks is that the majority utilize toxic organic solvents and provide low polymer yields.[17] Consequently, the process is vulnerable to residual monomer being present in the final polymer product, posing an environmental hazard.[17] Furthermore, to attain the desired mechanical and thermal characteristics, the average molar masses exceeding 20,000 g/mol need to be targeted, enabling the materials to exhibit high T_g and improved mechanical strengths. Most of the prior studies documented in the literature have exclusively been able to create polymers with low average molar masses (<22,000 g/mol) and have been mainly conducted in toxic organic solvents.[17,29] Moreover, polymerizations mentioned in the literature occurred at elevated temperatures (i.e., 50−80 °C), rendering the process energy-intensive, environmentally hostile, and unfavorable. All of these challenges curtail the large-scale production of sustainable butyrolactone polymers.

The present study deals with an increased sustainability potential for vinyl polymerization of α-methylene-γ-butyrolactone (α-MBL, Tulipalin A),[17,29,30,34] which is one of the most notable types of monomers in the butyrolactone family.[17] It can be directly obtained from tulips (*Tulipa gesneriana* L) and is widespread in plants.[34,35] α-MBL features a five-membered lactone ring (Figure 1a), which can be utilized for hydrolyzable drug bonding and copolymerization by ring-opening polymerization (ROP).[30,34] Furthermore, α-MBL has a nearly planar structure and is more polar in comparison to MMA, while its vinyl group is more reactive owing to the weaker steric interactions with neighboring groups.[30,34] Typically, α-MBL is polymerized using free-radical methods at high temperatures, resulting in sustainable polymers with unique and advanced properties,[35−38] such as high optical transparency, high mechanical strength (making them ideal for dental resins),[38] improved scratch resistance, and tunable antimicrobial properties.[30,34]

The sustainability increase is aimed at enzymatic free-radical polymerization, which is one of the promising techniques used in the synthesis of a diverse range of vinyl polymers. Enzyme-mediated conversions have high selectivity and biocatalytic activity, and low energy consumption (Figure 1b).[39−46] Peroxidases make up a major class of the oxidoreductase family of enzymes. They are heme (protoporphyrin IX)-containing enzymes that catalyze the oxidation of various substrates utilizing hydrogen peroxide as an electron acceptor to form free radicals in the process.[47−50]

The catalytic mechanism by which these enzymes operate is composed of several steps.[47−50] The first step involves a water ligand (**1**) being substituted by hydrogen peroxide (or other organic hydroperoxide) to form a peroxo complex (**2**). The second step, the heterolytic cleavage of the O−O bond, occurs to form compound **I**. Compound **I** then returns to the resting state by two hydrogen abstractions from the reducing substrates (In−H) forming two free radicals in the process (In$^{\cdot}$), as depicted in Figure 2b. The polymerization process then commences. By this, peroxidases play a role as "electron relays" by coupling a two-electron-transfer step to two single-electron-transfer steps.[47]

One of the most popular peroxidases is horseradish peroxidase (HRP) (Figure 2a).[47,48] HRP is found in horseradish, known in Latin as *Armoracia rusticana*, which is a perennial herb cultivated in temperate regions. This plant is rich in peroxidases, which are concentrated in its roots. However, the root contains a range of peroxidase isoenzymes of which HRP C isoenzyme is the most common. HRP isoenzymes are responsible for many physiological roles including lignification, cross-linking of cell wall polymers and resistance to infections.[47−50] Recently, a study shows that the power of HRP was explored in a one-pot two-enzyme cascade, coupling a recombinant oxidase with HRP to form free radicals that polymerize to form lignin oligomers.[50]

Various monomers, including methyl methacrylate,[45] styrene,[51] 4-methylstyrene,[51] 2-vinyl naphthalene,[51] and acrylamide,[52] have been polymerized using the HRP/H_2O_2/2,4-pentanedione system; however, no study has been reported on biobased butyrolactone monomers. Furthermore, most research involving HRP as a catalyst has been conducted under homogeneous conditions such as solution and bulk polymerization. Understandably, considerable efforts are currently being made to implement enzymatic polymerization in various aqueous heterogeneous systems. This has been driven by specific applications, industrial requirements for commercialization, and environmental concerns, as heterogeneous polymerization processes can be applied to a wider range of monomers and scales.[53−55]

Specifically, the aqueous heterogeneous radical polymerization technique is one of the most significant industrial processes for producing synthetic polymeric materials, with numerous applications for polymers prepared in aqueous

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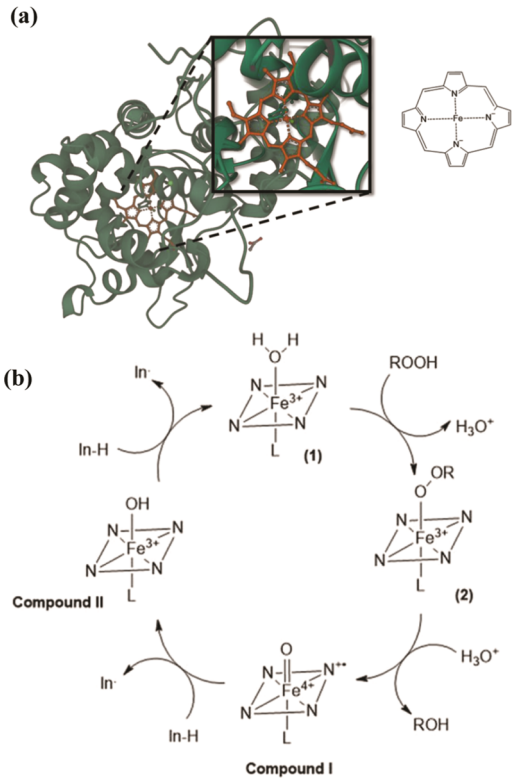

Figure 2. (a) 3D structure of horseradish peroxidase (PDB ID 1HCH) showing the heme (protoporphyrin IX) (inset) and a diagrammatic layout of the positioning of the iron atom (Fe) in the heme center. (b) The catalytic mechanism by which peroxidases form free radicals in the presence of a hydrogen or other organic hydroperoxide.[50] L represents the ligand, which, in most cases, is a histidine residue.

heterogeneous systems.[53,54] The number of such applications is expected to increase in the future owing to environmental regulations aimed at limiting the use of organic solvents. Consequently, the development of enzyme-mediated polymerization in aqueous heterogeneous systems is an important issue as enzymatic polymerization provides an environmentally friendly alternative route. Nevertheless, there have been only a few reports that have investigated HRP-mediated enzymatic polymerization under heterogeneous conditions.[43]

Moreover, emulsifier-free emulsion polymerization is a valuable technique for creating polymer particles in the absence of surfactants. This method has been extensively studied using various types of initiators, but there is a shortage of research investigating the use of enzyme-mediated reactions as initiating systems.

In the present study, we investigate a surfactant-free polymerization using the HRP enzyme to catalyze the synthesis of sustainable and thermally stable ($T_g \sim 200$ °C) poly(α-methylene-γ-butyrolactone) (PMBL) from the α-MBL monomer at room temperature (RT). This we did in the open air and in an environmentally friendly aqueous medium. The initiator used in this study (2,4-pentanedione) is expected to behave as a surfactant and plays the role of stabilizing the latex particles. This new synthetic technique allows the production of high molar mass PMBL nanoparticles in the form of a polymer latex for the first time, using a green and highly selective enzymatic reaction (as mentioned earlier in Figure 1b).

We thus overcome the use of surfactants in heterogeneous polymerizations, which limits the use of the final polymer particles in biomedical applications, and we target close to 100% final polymer yields in a timely manner. In our case, the initiator behaves as a surfactant to stabilize the latex particles. The synthesized biobased polymer colloid can find potential applications in various parts of the industry.

2. EXPERIMENTAL PART

2.1. Materials. Horseradish peroxidase (CAS: 9003−99−0, type VI, activity 295 purpullogallin units/mg, 44,000 g/mol) was purchased from Sigma-Aldrich. Methanol, 2,4-pentanedione, and hydrogen peroxide were all analytical grade and were used as received. α-Methylene-γ-butyrolactone (α-MBL/Tulipalin A > 95% purity, CAS: 547−65−9) was obtained from Tokyo Chemical Industries Co. (TCI) and used without further purification. Milli-Q Water (18.2 MΩ cm^{-1}) was generated using a Millipore Milli-Q-Academic Water Purification System. DMSO-d_6 (CAS: 2206−27−1) and chloroform-d (CAS: 865−49−6) were supplied from Sigma-Aldrich for ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and HSQC two-dimensional (2D) NMR analysis at 600 MHz. Dimethylformamide (DMF) with 0.01 mol L^{-1} LiBr was used as the size-exclusion chromatography (SEC) solvent for molecular weight (molar mass) determination.

2.2. Synthesis Protocols and Characterization Methods. *2.2.1. HRP Enzyme-Catalyzed Polymerization of Tulipalin A in Water under Air or N_2 Atmosphere.* 55 mg of α-MBL (5.6 mmol) was added to a solution of distilled water (2 mL) in a Schlenk tube at room temperature under a nitrogen atmosphere. Successive additions under a nitrogen atmosphere of 16 mg of HRP, 12 μL of 2,4-pentanedione (0.136 mmol), and 6 μL of hydrogen peroxide (0.092 mmol) were carried out. The reaction mixture was maintained under nitrogen with stirring (300 rpm) at RT for different reaction times (i.e., 6 and 24 h). After the reaction, the polymer latex was dried under a vacuum to remove the water, and then the solid product was collected and washed 3 times with methanol to remove any residual monomer and leftover HRP enzyme. However, in theory, washing with only water is possible, followed by vacuum oven drying to remove the α-MBL monomer, which has a boiling point of 204.4 °C. The final product was dried under vacuum, and a fluffy white powder was obtained. The same protocol was used when air was used instead of N_2. Each experiment was repeated 3 times for a reproducibility check, and the molar mass and yield results were reproducible within a ±5% experimental error. The replicate experiments are provided in Table S1 of the Supporting Information Section.

2.2.2. Fourier Transform Infrared Spectroscopy. Fourier transform infrared spectroscopy (FTIR) was utilized in transmittance mode on a Shimadzu IRTracer-100 to verify the successful polymerization of the α-MBL monomer by examining the disappearance of the vinyl group and analyzing the polymer characteristic functional groups.[30,34] Measurements were recorded in the range of 600−3500 cm^{-1}, using 64 scans at a resolution of 4 cm^{-1}. All functional groups of the α-MBL monomer and PMBL polymer were designated, as will be shown later in Figure 6c.

2.2.3. Nuclear Magnetic Resonance Measurements. ^{1}H, ^{13}C, and HSQC 2D NMR experiments were performed on a 600 MHz Bruker Advance Neo spectrometer equipped with a cryogenic probe at room temperature. The NMR samples were prepared by dilution of 15 mg of α-MBL monomer in 1 mL of $CDCl_3$ and 10 mg of PMBL in 1 mL of deuterated DMSO-d_6. Proton one-dimensional (1D) single pulse experiments were performed using a 4 s repetition delay and 16 scans; the ^{13}C 1D single pulse experiments were performed using a 2 s repetition delay and 1024 scans; and the HSQC 2D experiments were performed using a 2 s repetition delay and 2 scans. All spectra were processed by MestReNova 12.0 software. ^{1}H NMR (DMSO-d_6, 600 MHz) of PMBL: δ 4.2 (br, 2H, CH_2), δ 2.00 (br, 4H, CH_2, CH_2).[29,30,34]

2.2.4. Size-Exclusion Chromatography (SEC) Analysis. SEC was used to measure molecular weights of the polymer using a GPCMax system from Viscotek equipped with a 302 TDA detector array as well

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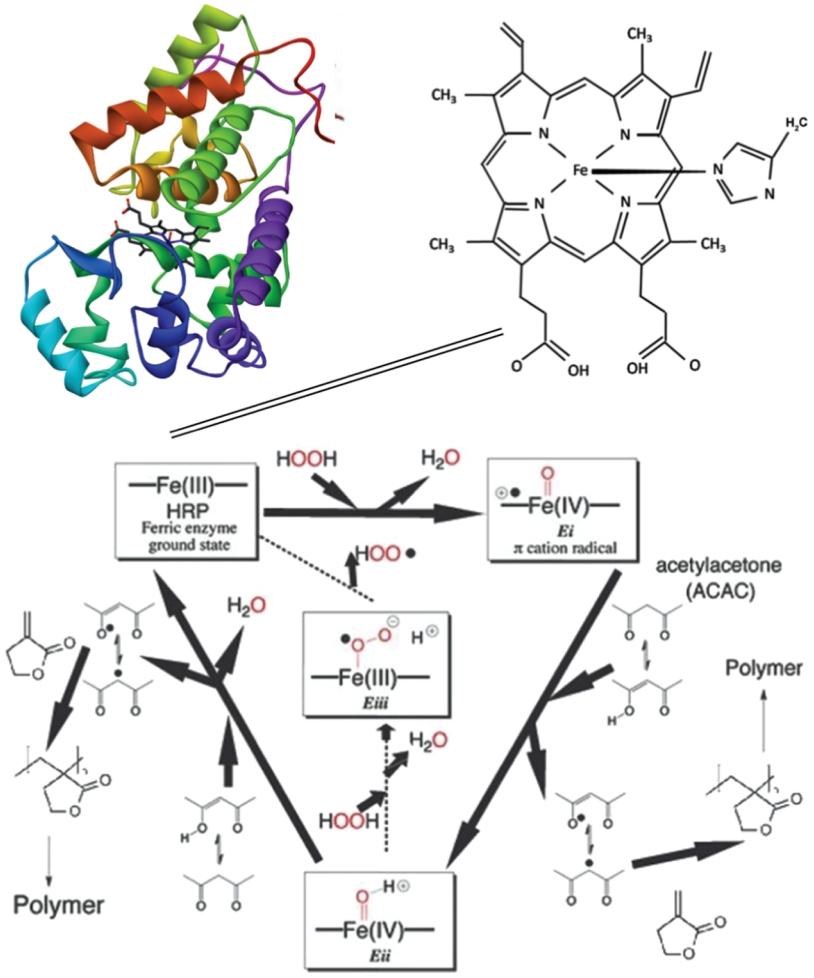

Figure 3. Proposed mechanism of the surfactant-free HRP-mediated polymerization of α-MBL in water.[43]

as two columns in series (Column Set: Polargel L + M, both 8 μm x 30 cm) from Agilent Technologies. The detectors and columns were kept at 50 °C, and the solvent containing DMF with 0.01 mol L^{-1} of LiBr was used as an eluent (flow rate = 1 mL min^{-1}). All PMBL polymer samples were previously washed with methanol to remove any residual monomer or HRP enzyme, as stated earlier in Section 2.2.1, prior to SEC analysis. For polymer sample preparation, the obtained PMBL dry powder was dissolved in DMF with 0.01 mol L^{-1} of LiBr at a (mass) concentration of ~5 mg mL^{-1} and run through a glass syringe equipped with a 0.2 μm nylon filter prior to injection. For calibration, near-monodisperse PMMA standards from Polymer Standard Services were used, and the resulting data were processed using the OmniSEC software to calculate the number-average molecular weight (or molar mass) (M_n), the weight-average molecular weight (M_w), and the molecular weight distribution ($Đ = M_w/M_n$) from the refractive-index (RI) detector.

2.2.5. Thermogravimetric Analysis. The thermal stability was determined by thermal gravimetric analysis (TGA). The degradation temperature was established on a TA-instrumental D2500 TGA. The sample was heated under an inert N_2 atmosphere from 20 to 600 °C at a rate of 10 °C·min^{-1}. The resulting data were processed using the TRIOS software (TA Instruments).

2.2.6. Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) was performed to study the thermal behavior of the polymer. The heat flow describes the amount of heat provided per unit time. The sample (~10 mg) was prepared in Tzero aluminum pans and placed in a DSC2A-01460 TA Instruments of the DSC25 type equipped with a cooler and an autosampler. The specimen was exposed to heating and cooling under a 50 mL·min^{-1} flow of nitrogen gas. The procedure started by heating the sample to 240 °C at a rate of 10 °C·min^{-1}, equilibrated at this temperature for 5 min, cooled to −70 °C followed by another 5 min isotherm, and then heated to 240 °C. The T_g value was obtained from the second heating cycle and analyzed by taking the midpoint of the slope of the peak via TRIOS software (v5.1.1.46572).

2.2.7. Dynamic Light Scattering Measurements. Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer 3000HS. The sample refractive index (RI) was set at 1.52 for PMBL.[17] The dispersant viscosity and RI were set to 0.89 Ns/m^2 and 1.33, respectively. The latex concentration used for DLS measurements was 0.1 mg/mL. The number-average hydrodynamic particle diameter was measured for each sample 5 times, and the average value of the five measurements was recorded. The polydispersity index for the particle size distribution was calculated from the standard deviation of the hypothetical Gaussian distribution (i.e., = σ^2/Z_D^2), where σ^2 is the standard deviation and Z_D is the Z-average mean size.

2.2.8. Transmission Electron Microscopy (TEM). A customary TEM grid preparation proceeded as follows: a particle solution was diluted with Milli-Q water to around 0.05 wt %. Subsequently, a 10 μL portion of the solution was permitted to naturally dry on a Formvar precoated copper TEM grid. The particles were examined by utilizing a JEOL-1010 transmission electron microscope at an accelerating voltage of 80 kV, employing a spot size of 6 at room temperature.

3. RESULTS AND DISCUSSION

We highlight via several experimental techniques, including SEC, NMR, FTIR, DLS, TEM, TGA, and DSC, a prosperous

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Table 1. Experimental Data of the Surfactant-Free HRP-Enzymatic Polymerization of α-MBL in Water

sample	reaction conditions	PMBL yield (%)	M_n (g/mol) (RI detector)	($Đ = M_w/M_n$)	particle diameter (nm)	polydispersity index (PDI)	refs
sample 1-air-0.5 h	RT, air, and 0.5 h	43	39,000	2.7	62 ± 8	0.21	
sample 2-air-2 h	RT, air, and 2 h	89	57,400	4.8	101 ± 10	0.14	
sample 3-air-3 h	RT, air, and 3 h	98	73,400	2.4	131 ± 8	0.09	
sample 4-N_2-6 h	RT, N_2, and 6 h	52	34,800	2.2	78 ± 7	0.16	
sample 5-N_2-24 h	RT, N_2, and 24 h	91	71,000	4.0	203 ± 10	0.18	
RAFT-MBL-Benz[a]	80 °C, benzene, 20 h (gelation after 30 min)	50	22,000	1.20			[17]
ATRP-MBL-DMF[b]	50 °C, 8 h, DMF, CuBr/2,2′ bipyridine, bromopropionitrile	77	15,800	1.14			[29]

[a]Reversible-addition−fragmentation chain-transfer (RAFT) polymerization of α-MBL in benzene solvent.[17] [b]Atom-transfer radical polymerization (ATRP) of α-MBL in DMF solvent.[29]

enzymatic, one-pot surfactant-free emulsion polymerization process for α-MBL, using HRP as a catalyst and 2,4-pentanedione as a water-soluble initiator. We have chosen 2,4-pentanedione as the initiator and no other cyclic β-diketones because HRP displays a greater level of specificity for 2,4-pentanedione as compared to other cyclic β-diketones, which exhibit less recognition. This is in line with the work of Rodrigues et al., who have suggested that it is difficult for cyclic β-diketones to access the active center of HRP, thereby leading to only a small amount of recognition by HRP.[56]

While the detailed mechanism of the HRP-mediated redox reaction remains rather unknown, it can be expected that β-diketone radicals created by the HRP-catalyzed oxidation of β-diketone (2,4-pentanedione in this study) initiate the radical polymerization of vinyl monomers with H_2O_2, as shown in Figure 3.[43,45] This figure illustrates that HRP is oxidized by H_2O_2 and undergoes two catalytically active states, HRP(*Ei*) and HRP(*Eii*), before returning to its native state. Each active form oxidizes the β-diketone, where it has been put forward that the keto-enoxy radicals from 2,4-pentanedione are the first radical species generated and play the main role in monomer initiation.[43,45] Excess H_2O_2 causes HRP to become an inactive form, HRP(*Eiii*), which spontaneously returns to the native enzyme state.[43−45] Therefore, the concentration of H_2O_2 plays a crucial role in regulating the HRP-mediated polymerization reaction.[43,45]

In this study, the surfactant- or emulsifier-free one-pot HRP-mediated polymerization of α-MBL has been executed in an uncomplicated arrangement under ambient reaction conditions (RT), in contrast to preceding studies conducted at 80 °C and expeditiously in the existence of low concentrations of hydrogen peroxide and 2,4-pentanedione in an aqueous medium.[17]

Table 1 presents a summary of the enzymatic polymerizations performed with α-MBL at various time intervals and under different atmospheric conditions, including air and N_2. It also includes a comparison with other solution polymerizations reported in the literature.[17,29] The PMBL yield (%) of all enzymatic polymerizations increased over time, as expected. Interestingly, our data demonstrate that the kinetics of polymerization are much faster in air than under a N_2 atmosphere. This confirms the significant role that O_2 plays during enzymatic polymerization cycles, which might increase the rate of oxidation of the β-diketone and consecutively increase the rate of monomer initiation and thus the overall rate of polymerization. Polymerizations with O_2 almost reached completion after 3 h in air, whereas it required 24 h to achieve a 91% PMBL yield under a N_2 atmosphere. These intriguing results provide motivation for polymer chemists to conduct enzymatic polymerizations using this system in the open air. Free-radical polymerizations are known to require expensive noble gases for purging (e.g., argon) as the inert atmosphere. In this work, we can produce a biobased polymer latex in 3 h in open air and at RT in an environmentally friendly manner. Moreover, the enzymatic reactions carried out in this study were repeated 3 times and the data were reproducible, as shown in Table S1.

Figure 4 displays the color of the polymerization medium at the beginning of the reaction (0 h), which is red in color

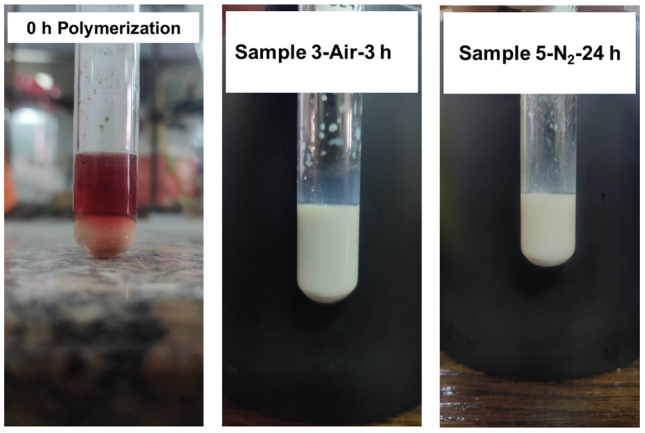

Figure 4. Reaction media color: (a) start of reaction (0 h), (b) 3 h in air, and (c) 24 h in N_2. The white region in panel (a) is the white stir bar located at the bottom of the Schlenk tube.

(representative of heme Fe^{3+}), after 3 h in the presence of air and after 24 h under a nitrogen (N_2) atmosphere. In air, after 0.5 h, the reaction color changed to white without any precipitation, indicating the formation of a stable PMBL latex.

The latex particles in this study are within the nanoscale range (60−300 nm as demonstrated in Table 1), and no precipitation was detected in the reaction medium. Additionally, the polymer particles were monodisperse, as indicated by the polydispersity index values in Table 1 that range from 0.09 to 0.21, which indicate that we have a narrow particle size distribution in the latex. The latexes have been additionally checked after 1 month and found to be stable with no signs of precipitation or colloidal instability. For polymerizations performed in dispersed media, the growth of the particles follows a nucleation growth process. It has been reported that

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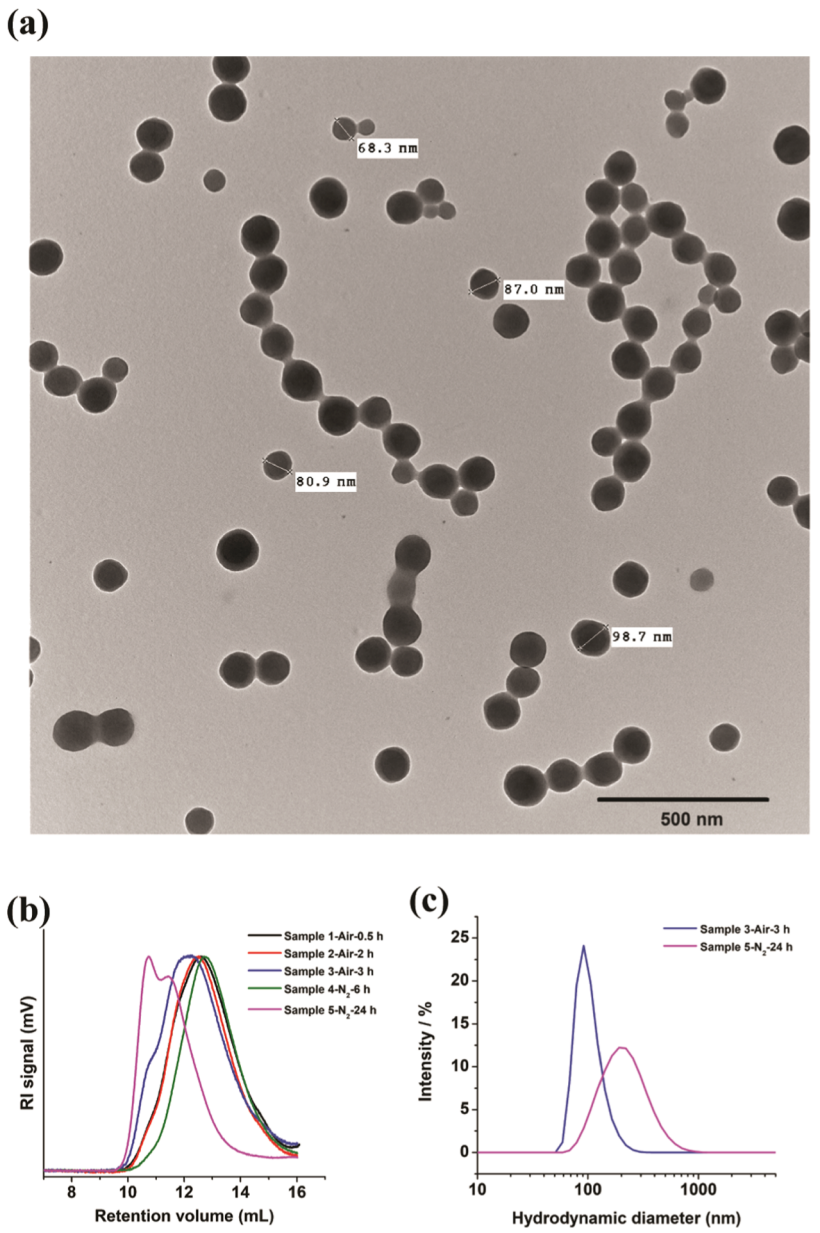

Figure 5. (a) TEM image of the PMBL latex prepared under a N_2 atmosphere (Sample 4-N_2-6 h). (b) Chromatograms obtained from the SEC-RI detector displaying the number-average molar masses (M_n) of PMBL prepared by surfactant-free HRP-enzymatic polymerization in air and N_2 atmospheres. (c) Particle size distributions of PMBL latexes prepared under both air and N_2 atmospheres.

the final average hydrodynamic diameters are highly dependent on the speed of the nucleation step and on the colloidal stability of the growing particles. In the case of the polymerization performed in air, the enzyme activity is high (a faster monomer conversion is obtained), which leads to a fast nucleation process and to an earlier stabilization of the growing particles by the 2,4-pentanedione initiator. This efficient coverage of the particle surface ensures the formation of relatively small particles (131 ± 8 nm) with a limited polydispersity index (0.09) after quasi-full conversion of the monomer. In the case of polymerization under N_2, the lower speed of polymerization forms particles that may not be efficiently stabilized by the 2,4-pentanedione initiator, which leads to a possible high extent of particle coagulation during the growing process. This is commonly known to increase not only the average particle sizes but also the polydispersity, as observed in this case (203 ± 10 nm and PDI = 0.18).

Figure 5a shows the spherical morphology of the latex particles as obtained by TEM. Moreover, Figure 5c depicts the narrow hydrodynamic diameters of the latexes that were prepared in both air and N_2 atmospheres. The particle size values obtained from DLS and TEM are in reasonable agreement. Additional TEM images for other reactions are provided in Figure S1 of the Supporting Information.

The dry white PMBL polymer was further characterized by SEC, and the number-average molar masses (M_n) are depicted in Figure 5b. Previous attempts in the literature to synthesize PMBL with a high average molar mass were unsuccessful, producing a maximum molar mass of 22,000 g mol^{-1}.[17] The lower average molar mass polymers synthesized in previous studies had therefore a lower T_g and poorer mechanical properties than those expected for PMBL.[17,29] Additionally, polymerizations were carried out in highly toxic organic solvents and reached low monomer conversions.[17]

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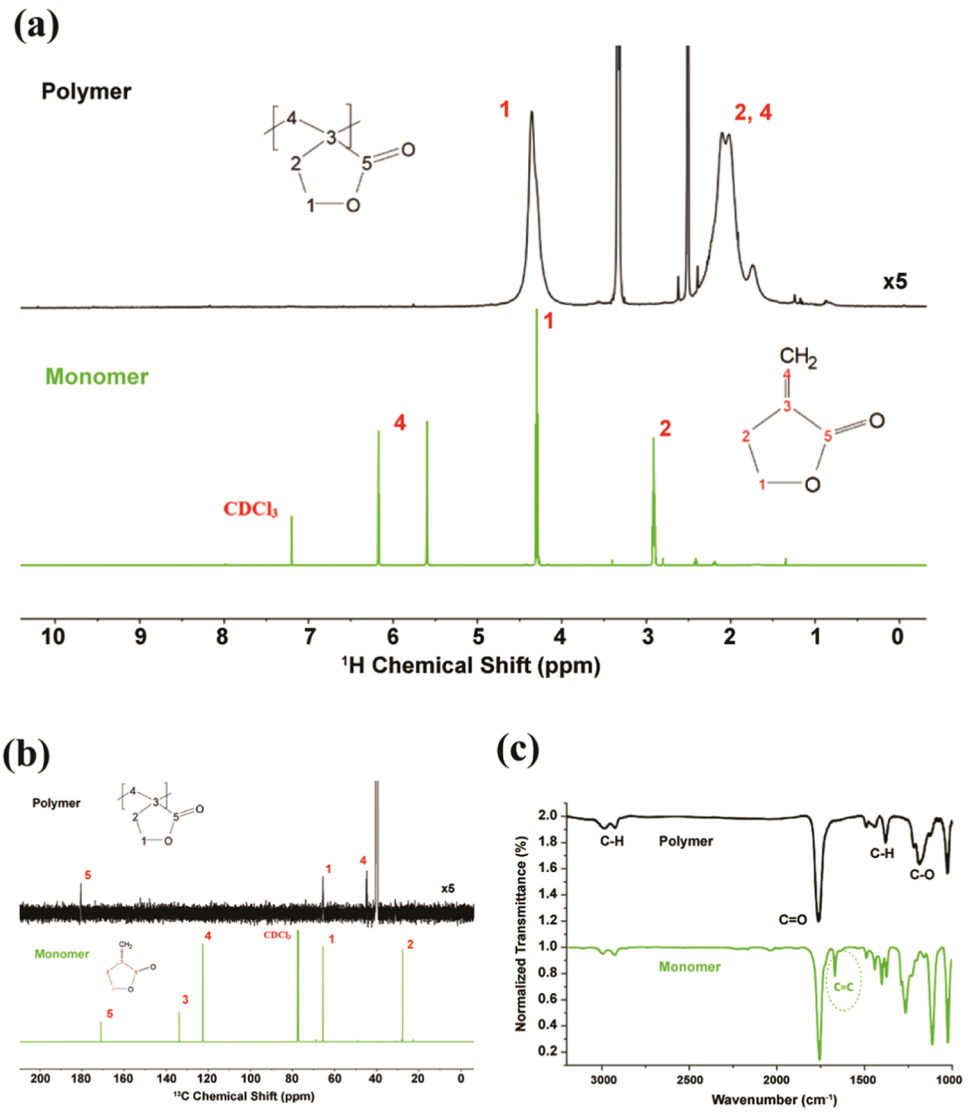

Figure 6. (a) ^{1}H NMR spectra for α-MBL in $CDCl_3$-*d* (7.26 ppm) and PMBL (Sample 5-N_2-24 h) in DMSO-d_6 (2.5 and 3.3 ppm) produced from enzymatic polymerization of α-MBL. The measurement was done at room temperature. α-MBL proton peak assignment label 2 (2.91 ppm, t, 6 Hz, 2), label 1 (4.3 ppm, t, 6 Hz, 2), label 4 (5.5 ppm, t, 3 Hz, 1), and (6.1 ppm, t, 3 Hz, 1). PMBL proton peak assignment labels 2 and 4 (1.5−2.5 ppm, 2) and label 1 (4.0−4.6 ppm, 1). (b) ^{13}C NMR spectra for α-MBL in $CDCl_3$-*d* (77.16 ppm) and PMBL in DMSO-d_6 (39.5 ppm) produced from enzymatic polymerization of α-MBL. The measurement was done at room temperature. α-MBL ^{13}C peak assignment label 2 (27.6 ppm), label 1 (65.6 ppm), label 4 (122.5 ppm), label 3 (133.8 ppm), and label 5 (171. ppm). PMBL ^{13}C peak assignment label 5 (180.5 ppm, s, 1), label 1 (65.6 ppm, s, 2), and labels 3 and 4 (44.7 ppm, t, 2). (c) FTIR spectrum of PMBL produced from enzymatic polymerization of the α-MBL monomer and polymer Sample 4-N_2-6 h.

The results in Figure 5b,c thus highlight the existence of a nucleation stage. In agreement with conventional emulsion polymerization data, it can be postulated that particle nucleation, which results from this enzymatic-surfactant-free emulsion polymerization, can be divided into two stages. In the first stage, a small quantity of monomer molecules, which are soluble in the aqueous phase, are activated by the 2,4-pentanedione radicals and polymerized. During this stage, the conversion of the monomer is typically low and most of the monomer is found in large monomer reservoirs (i.e., 1−10 mm droplet diameter). Oligomeric radicals that formed in the aqueous phase develop through precipitation and aggregation, resulting in the formation of primary latex particles. Polymerization of the monomer continues inside the swollen primary particles in a second stage with the monomer supplied by diffusion from the large monomer droplets.

The resulting polymer particles are stabilized by 2,4-pentanedione moieties located on the surface of the particles. Despite ketones not having a charge, the hydrophilic nature of 2,4-pentanedione provides stability through steric interactions with the formed particles. Pich et al. made a similar observation during the production of polystyrene particles that were functionalized with β-diketone groups via the surfactant-free emulsion copolymerization of styrene and acetoacetoxyethyl methacrylate.[57] Additionally, it can be put forward that some HRPs have adsorbed onto the surface of the particles, acting as a steric stabilizer and enhancing the colloidal stability of the resulting particles.[56,57] Therefore, it can be stated that water-soluble 2,4-pentanedione is responsible for the stability of our latex particles and is the initiating species for the polymerization process, and hence, its amount can control the final molar mass of the polymer.

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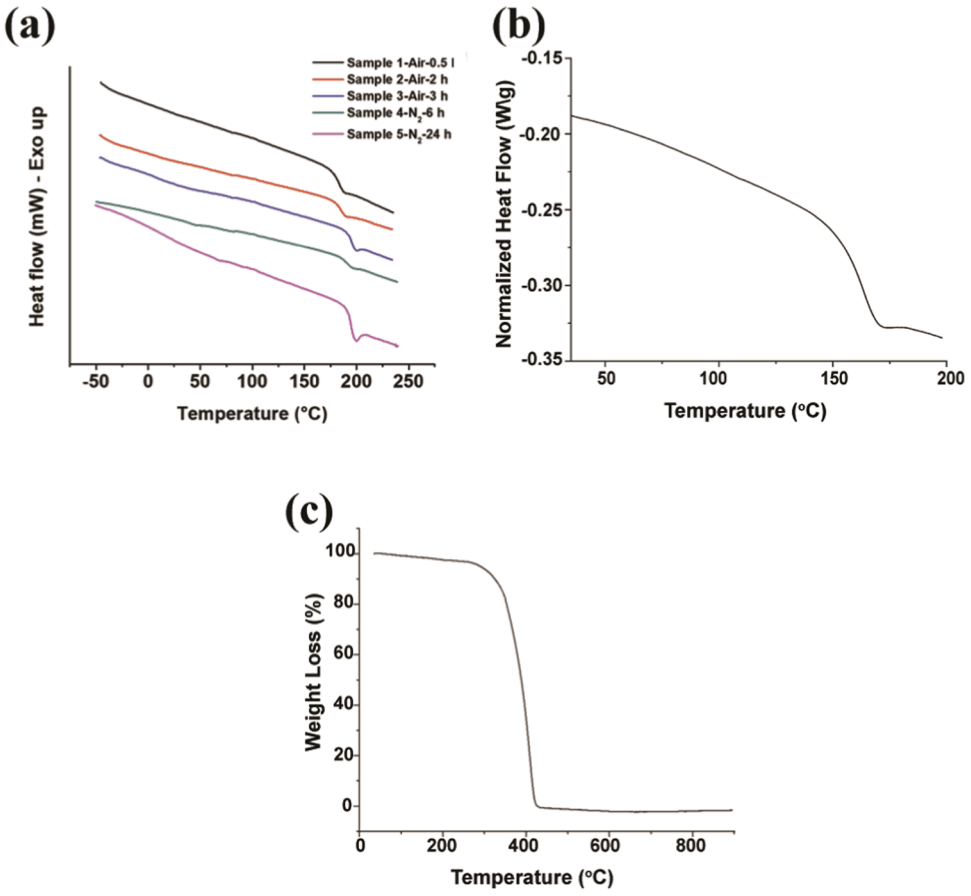

Figure 7. Characterization of the thermal properties of PMBL produced by enzymatic polymerization: (a) DSC from this study: T_g obtained from the exothermic peak of the second heating cycle (T_g = 200 °C); (b) DSC from our previous study: T_g obtained from the exothermic peak of the second heating cycle (T_g = 160 °C)[30] and (c) TGA of Sample 1-air-0.5 h.

FTIR spectroscopy has additionally been used as an analytical tool to confirm the structure of PMBL produced, as depicted in Figure 6c. The disappearance of the characteristic monomer peak at 1664 cm^{-1} in the PMBL spectrum, which is attributed to the C═C stretching of the vinyl group, proved the successful free-radical polymerization of the α-MBL monomer.[17,30,34] The peak between 2840 and 3000 cm^{-1} was assigned to the C−H stretching.[30,34] The most intense and sharp peak located at around 1760 cm^{-1} was attributed to the C═O lactone stretching, as previously reported in the literature, indicating that no ROP took place.[30] An additional peak corresponding to the C−O group is observed between 1100 and 1200 cm^{-1}, in line with prior reports.[17] A detailed description of the peaks is summarized in Table S2 in the Supporting Information.

The chemical structure of the PMBL has been further confirmed by solution-state ^{1}H NMR, as shown in Figure 6a in agreement with literature data.[29,30,34] The spectrum shows three different regions, labeled 1, 2, and 4 in Figure 6a, with a broad line shape and low intensity related to the polymeric backbone and ring structure and two peaks with high intensity at 2.5 and 3.35 ppm assigned for d_6-DMSO and water traces in the solvent. The peak intensity of regions 2 and 4 in the PMBL spectrum matches double the ratio of region 1, consistent with the number of protons measured. Furthermore, other ^{13}C NMR spectra are shown in Figure 6b for the monomer and polymer. Furthermore, additional data have been included in the Supporting Information section, differentiating between the HSQC 2D NMR spectrum of α-MBL (Figure S2), a contour plot for the HSQC 2D NMR spectrum of PMBL (Figure S3), a Stack plot for proton NMR spectra of PMBL polymers prepared under an air atmosphere (Figure S4), and a Stack plot for proton NMR spectra of PMBL (Figure S5).

The thermal properties of PMBL were first assessed using DSC, as depicted in Figure 7a. The T_g of PMBL has been determined from the second DSC heating cycle and found to have a high value of ∼200 °C, which is double that of PMMA (T_g of PMMA ∼ 100 °C). In our previous work (Figure 7b), the T_g of PMBL was found to be ∼160 °C, which is lower than that of the PMBL prepared in this study.[30,34] The M_n values of the polymers prepared in our previous study were in the range between 5000 and 10,000 g mol^{-1}, which is significantly lower than our current study, explaining this deviation. Consistently, the T_g dependence on molar mass has been previously explained by the Flory−Fox equation.[58,59]

To further analyze the polymer from a thermal point of view, Figure 7c shows the thermogram of PMBL obtained by TGA analysis for Sample 1-Air-0.5 h. PMBL has a high thermal stability, which is evident by the decomposition of only 5 wt % of the polymer at 340 °C. The complete degradation of the polymer takes place at 430 °C due to the decomposition of the lactone ring. This high thermal stability of PMBL is attributed to this ring, which provides the polymer chain with extra rigidity and thus enhances its thermal stability.[17,30,34,57] This thermal behavior is also in good agreement with previous reports.[17,30,34,57]

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4. CONCLUSIONS

A green, one-pot surfactant-free HRP-mediated enzymatic polymerization allowed the synthesis of sustainable polymers[60−65] based on α-MBL with a high average molar mass and thermal stability. The polymer latexes produced are colloidally stable and exhibited a narrow particle size distribution. The polymerization route as conducted in water under ambient conditions is selective and only requires a simple experimental setup. The study demonstrates the potential use of enzymes in producing sustainable polymer latexes for high-temperature applications.

The work is a milestone achievement for the enzymatic-mediated polymerization of vinyl monomers in heterogeneous systems to produce green latexes. Future work will involve optimizing the reaction conditions, testing the current system with other butyrolactone monomers, such as MeMBL and MMBL, and scaling up the reaction. This research thus paves the way for exploring the enzymatic polymerization of other intriguing butyrolactone monomers. Furthermore, controlled radical polymerizations could be applied in conjunction with the HRP enzyme to obtain polymers with low dispersity molar mass distributions (MMDs) in dispersed media. Model-based design is a needed next step for scale-up, as the field of enzymatic polymerization is less mature compared to conventional polymerization. Once this is taken up, we can evaluate other promising properties of the polymers such as optical, antimicrobial, and mechanical properties.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsapm.3c01740.

TEM image for biobased latex of Sample 2-Air-2 h (Figure S1); contour plot for the HSQC 2D NMR spectrum of α-MBL in $CDCl_3$-*d* (Figure S2); contour plot for the HSQC 2D NMR spectrum of PMBL in DMSO-d_6 produced from the enzymatic polymerization of α-MBL (Figure S3); stack plot for proton NMR spectra of PMBL in DMSO-d_6 (39.5 ppm) produced from enzymatic polymerization of α-MBL under air atmosphere (Figure S4); stack plot for proton NMR spectra of PMBL in DMSO-d_6 (39.5 ppm) produced from enzymatic polymerization of α-MBL under nitrogen conditions (Figure S5); replicate polymer yield values for all enzymatic polymerizations (Table S1); and peak assignments for the FTIR spectrum of PMBL (Table S2) (PDF)

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Conceptualization, E.A., E.S.J., and S.K.O.; methodology, E.A.; formal analysis, H.M.H; investigation, A.Y.A; resources, A.M.M. and E.A.; writing—original draft preparation, S.K.O. and H.M.H.; visualization, V.S.P.H.M. and D'h.D.R.; and supervision, S.K.O., E.S.J., and H.M.H. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

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