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Enzyme-Catalyzed Polymerization of End-Functionalized Polymers in a Microreactor

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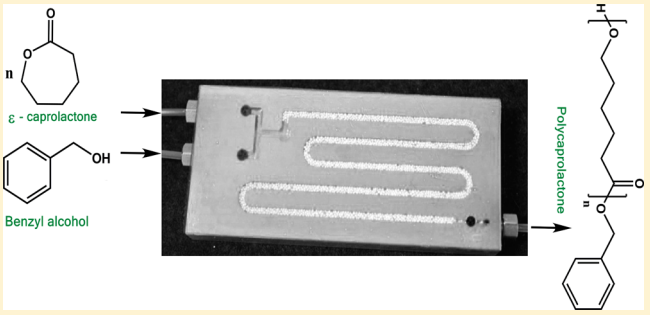
Supporting Information

ABSTRACT: An enzyme packed microreactor was compared with a batch reactor system to determine effects of reaction water content on immobilized *Candida antarctica* Lipase B (Novozyme 435, N435) reusability, efficiency of end-group functionalization, apparent monomer conversion rate constants (k_{app}), average molar mass (M_n), and leaching of *Candida antarctica* Lipase B (CALB) from Lewatit macroporous beads. Conversion of ε-caprolactone to poly(ε-caprolactone), PCL, using benzyl alcohol for end-functionalization, was the model system for studies conducted herein. The apparent rate constant in microreactor (k_{app} = 0.027 s^{-1}) was 27 times larger than in batch reactor (k_{app} = 0.001 s^{-1}). Furthermore, in microreactor, the M_n vs conversion plots for "dry" and "water saturated" conditions were similar. In contrast, under "water saturated" conditions in a batch reactor, M_n is much lower. Moreover, at both high and low water content, higher end-group functionalization was achieved for polymerizations in microreactor (0.75 to ≥0.98) as compared to batch reactor (0.2). Also, microreactors run for 30 cycles under "dry" or "water saturated" conditions gave product where the fraction of benzyl ester groups on chains remains high, between 80 and 90%. In contrast, in the "water saturated" batch system, the fraction of benzyl ester terminal groups remains at about 0.30 throughout all 11 reaction cycles. These results led to the conclusion that the microreactor design results in effectively "dry" conditions even when reactants are "water saturated". CALB leaching during 7, 18, and 25 reaction cycles in microreactor steadily increased from 10 to 15 and 22%. However, at 30 cycles, CALB leaching disproportionaly increased to 76% without apparent physical deterioration of beads. Comparative results in batch reactors for all experiments above are reported and discussed.



INTRODUCTION

Immobilized enzymes have been developed that catalyze a wide array of reactions. These reactions include an expanding number of examples in which immobilized enzymes are used for biotransformations conducted on an industrial scale.[1,2] Most enzymes are active at mild operational conditions of pH and temperatures (e.g., 30−50 °C). Furthermore, enzymes are produced from renewable resources and, in many cases, are free of heavy metals. These properties are attractive for the development of "sustainable processes" conducted with low-energy input, thereby resulting in safer processes.

Use of enzymes in industry has been limited due to their high cost, instability, and availability in small amounts.[3] However, by identifying the proper support, enzyme immobilization can stabilize the enzyme's tertiary structure and confirmation, thereby improving its operational stability.[4] Furthermore, enzyme immobilization on solid supports allows their facile recovery from products and subsequent reuse, a key issue in developing economical processes.[5] Novozyme 435 (N435),[6] the immobilized catalyst used in this study, consists of *Candida antarctica* Lipase B (CALB) physically immobilized on a macroporous acrylate resin. Indeed, CALB has attracted broad interest from academia and industry due to its ability to catalyze a wide range of reactions including amidation,[7] transesterification,[8] ring-opening of various cyclic compounds like carbonates,[9] phosphonates,[10] and cyclic depsipeptides.[11]

Due to the high cost of immobilized enzyme preparations such as N435, it is imperative that catalysts and processes are designed that improve catalyst reusability. The majority of academic studies on N435 or other immobilized CALB systems are focused on elucidating catalyst activity under various conditions or with a diverse range of substrates, and as a result, N435 recycling is not addressed. When N435 reusability has been addressed, loss in catalytic activity over multiple recycles is attributed to enzyme deactivation,[12] although CALB physical desorption from the solid support contributes or may even be the dominant cause of decreased N435 activity upon reuse. This lack of information on enzyme reusability and enzyme

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leaching is not specific to N435 but, instead, is a general characteristic of the immobilized biocatalyst literature.

Researchers recognizing the potential for enzyme desorption have studied strategies to increase catalyst reusability by post immobilization modification reactions. For example, enzymes physically immobilized on bead surfaces have been cross-linked[13] or entrapped by coating the bead with a silicone outer layer.[14] However, when cross-linking enzymes, a compromise must be reached between high cross-link density, perhaps causing reduced catalyst activity due to lost flexibility, and lower cross-linking density, which may not sufficiently reduce enzyme leaching.

In batch reactors, it is well-known that water plays a key role in N435-catalyzed ring-opening polymerization (ROP) of ε-caprolactone (ε-CL).[15] Free water, on the surface of CALB, acts as a lubricant maintaining its flexibility while undergoing reversible conformational changes upon reactions with substrates.[16] Furthermore, in N435-catalyzed ROP of ε-CL, water can act as an initiator such that the amount of free water bound to the lipase determines the number of chains initiated, and hence, the molecular weight averages of synthesized poly(ε-caprolactone), PCL.[17] Moreover, water concentration for enzyme-catalyzed reactions in organic media is well-known to affect enzyme activity such that there is an optimal water content leading to maximum enzyme activity.[17] In a recent publication by us, we demonstrated the feasibility of conducting N435-catalyzed ROP of ε-CL in a microreactor, and the results were compared to the identical reaction conducted in batch mode.[18]

There is a need to create new platforms to perform reactions that enable (i) conducting several reactions simultaneously, (ii) decreased reaction times, (iii) superior control of reaction conditions, and (iv) decreased generation of hazardous chemical waste. Relative to conventional batch reactors, microreactors allow vast improvements in energy efficiency, reaction speed, yield, safety, reliability, scalability, on-site/on-demand production and provide a finer degree of process control.[20] Further, to overcome limitations due to the high viscosity in the solvent-free esterification of polyglycerol-3 and related polyols, such as poly(ethylene glycol)s, an alternative reactor concept comprising of a bubble column reactor has been reported to prevent mechanical erosion of Novozym 435, caused by mechanical stirring of the reaction mixture. Further this reactor configuration was reported to outperform conventional methods such as stirred tank or fixed bed reactors.[19] These reasons have stimulated researchers to develop and study microreactor systems.

Work has begun to explore the benefits of enzymes immobilized on solid supports packed within microchannels with special interest in the development of biotransformations for the synthesis of molecules for therapeutic applications.[21,22] Examples include PikC hydroxylase immobilized on Ni-NTA agarose beads for hydroxylation of methylmycin to neo-methylmycin and the synthesis and functionalization of polyketides using two microchannel reactors in series.[23]

Polymerizations in microchannel flow systems conducted using traditional catalysts show considerable promise to provide benefits over batch reactor systems. For example, due to improved temperature control in microreactor systems, free radical polymerizations gave products with lower PDIs than these polymers synthesized in batch reactions.[24] Homopolymerizations of NCA−amino acid monomers conducted in microreactors gave higher molecular weights with lower polydispersity than identical reactions conducted in batch mode.[25] Furthermore, microfluidic methods were developed to carry out controlled radical polymerizations.[26]

In this study, the synthesis of end-functionalized oligomers of PCL was studied using a microreactor system consisting of high catalyst surface area due to microchannels packed with macroporous immobilized N435 beads. The model initiator, benzyl alcohol, was used to probe initiator reaction efficiency. Results from this work can guide other studies where end groups consist of bioactive molecules at chain termini that endow corresponding materials processed into various forms to have important therapeutic,[27−29] antimicrobial,[30] and anti-biofouling[31] properties.

The present work uses a microreactor system with N435 bead packed channels as a platform to investigate effects of reaction water content and number of microchannel reactor reuses on the efficiency of end-group functionalization, monomer conversion, chain molecular weight averages, and leaching/desorption of lipase from N435. Results in microchannels were compared with a batch reactor system. Intriguing differences between the batch and microchannel reactor system underscore fundamental dissimilarities between these two systems. New insights were gained into the different effects of water content in batch and microchannel reactor systems.

EXPERIMENTAL SECTION

Chemicals. All chemicals used were obtained from Sigma-Aldrich Inc. Novozym N435, a commercially available immobilized lipase preparation with *Candida antartica* Lipase (CALB) physically immobilized on a mesoporous poly(methyl methacrylate) support (Lewatit VP OC 1600), was obtained from Novozymes. The molecular mass of CALB is 33 000 g/mol. Based on supplier specifications, N435 beads contain 10 mass % CALB. This was confirmed herein by elemental analysis of N435 bead nitrogen content. Furthermore, N435 beads were sieved to obtain the bead fraction with diameter 400 ± 50 μm. Since water in reactions can participate in chain initiation and hydrolysis, care was taken to dry and store reactants and N435 beads. Sieved N435 beads were stored over desiccants in a vacuum desiccator. ε-CL was fractionally distilled over CaH_2 under nitrogen and stored in a glovebox. Toluene-d_8 was distilled over sodium metal under nitrogen and stored over 4 Å activated molecular sieves in a glovebox. Water content was measured using a Mettler 275 KF coulometric Karl Fischer titrator using Hydranal coulomat AG and Hydranal coulomat *CG* (Sigma). A hydranal water standard (0.1% w/w) was used to calibrate the Karl Fischer (KF) titrator. Water content in N435 beads was determined by extracting the beads with methanol overnight and analyzing the filtrate relative to a methanol control. Water content of distilled toluene-d_8, ε-CL, benzyl alcohol, and dried N435 was 0.001, 0.012, 0.001, and 0.40 mass %, respectively. Thus, the major source of water in reactions is N435 beads.

Batch Reactors. For kinetic studies, dry toluene-d_8 (2 mL) and benzyl alcohol (0.05 mL) were transferred via syringe under dry N_2(g) into a flask containing N435 (100 mg). This suspension, as well as a separate flask containing ε-CL, was equilibrated for 15 min to the reaction temperature (70 °C). Thereafter, ε-CL (3 mL) was transferred to the reaction flask via syringe under dry N_2(g) to start the polymerization. For reusability studies in batch, ε-CL ROP reactions were performed in a 100 mL two-neck round-bottom flask equipped with an overhead stirrer and maintained under nitrogen environment. The dimensions of the stir blade were such that the blades allow a clearance of 1.5 cm from the vessel walls to ensure that beads are not crushed due to confinement. Reaction flasks were placed in a heated oil bath with a feedback temperature controller. Dry toluene-d_8 (6 mL) and benzyl alcohol (0.15 mL) were transferred via syringe under dry N_2(g) into a flask containing N435 (300 mg). After this suspension and ε-CL equilibrated to the desired reaction

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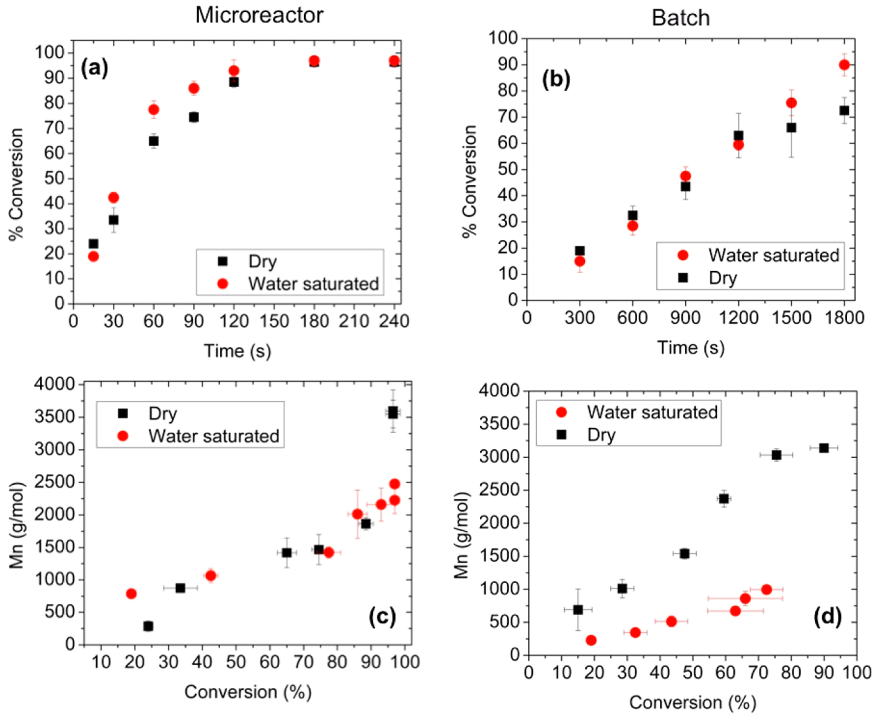

Figure 1. Conversion of ε-caprolactone as a function of residence or reaction time at 70 °C using benzyl alcohol as a C-terminal end-functionalization agent in (a) microreactor and (b) batch mode. Number-average molecular mass (M_n) as a function of conversion at 70 °C in (c) microreactor and (d) batch mode, at 70 °C, for 90 s in microreactor and 1800 s in batch reactor. The term "dry" represents experiments conducted using dried toluene-d_8 (water content ~0.001% w/w). The term "water saturated" denotes experiments conducted in toluene-d_8 saturated with water (~0.033% w/w). Error bars indicate one standard uncertainty based on measurements on at least three different sample.

temperature, ε-CL (3 mL) was transferred to the reaction flask via syringe under dry N_2(g). Here, the mass ratio of CALB to ε-CL and ε-CL to benzyl alcohol was maintained at 1:100 and 20:1 by mass, respectively. The overhead stirrer to mix the reaction contents was set at 60 rad/s. The reaction temperature was monitored by a thermocouple inserted in the reaction mixture. Aliquots were removed via syringe at predetermined time intervals from the reaction mixture to monitor values of % ε-CL conversion and PCL molecular mass. To study N435 reusability, at the end of polymerizations reaction products were transferred via syringe from used N435 beads. Thereafter, fresh reactants were transferred to recovered beads, and the reaction was performed again exactly as described above. All experiments were performed at least two times to determine experimental variability. All the reagents, toluene, benzyl alcohol, and ε-CL, were distilled and stored under anhydrous conditions in a glovebox. For experiments conducted under "dry conditions" all the reactants were used in dry condition, while to establish "water saturated" conditions, water saturated toluene (0.033% w/w water) was used instead of dry toluene.

Microreactor Platform. The microreactor used was identical to that reported previously by our laboratories.[18] Microchannels with dimensions of 2 mm width, 1 mm depth, and 260 mm length were milled on a 10 mm thick aluminum block. The channels were covered with Kapton film using ResinLab epoxy adhesive (E950g), and the epoxy was treated at 120 °C to obtain sufficient adhesive strength. The channels were filled with 100 mg N435 beads of diameter 400 ± 50 μm by applying vacuum at one end of the reactor and introducing beads at the other end. The extra space at both ends of the microchannel was equally filled with blank beads without enzyme on it (Lewatit VP OC 1600). In other words, the first 65 mm of the microchannel reactor contained blank beads (25 mg, diameter 400 ± 50 μm), the central 130 mm in the microchannel contained N435,and the later 65 mm contained blank beads (25 mg, diameter 400 ± 50 μm). The packing fraction (ε) of beads was estimated as ≈0.5 by flowing toluene-d_8 through the reactor. We assumed that reactions in the microchannel reactor occur by uniform plug flow, and the residence time (t) is estimated as $V(1-\varepsilon)/Q$, where V ($W \times D \times L$) is the total volume of the reactor and Q is the volumetric flow rate, where W is the width, D is the depth, and L is the length of the microchannels.

The microreactor was placed on a uniform heating stage to control the experimental temperature with a variability of ±0.5 °C. To ensure temperature uniformity, the microchannel interior temperature was intermittently measured by inserting a thermocouple. Through two separate inlets on the microreactor, the toluene-d_8/benzyl alcohol mixture and ε-CL, both pre-equilibrated to the desired reaction temperature, were introduced at fixed flow rates to obtain a 2:1 ratio of toluene-d_8 and ε-CL inside the microreactor. For experiments to determine ε-CL ROP kinetics, 3 mL of the ε-CL/toluene-d_8 mixture was passed through the microreactor at different flow rates, indicating different reaction times. For N435 reusability studies the reaction mixture was continuously passed through the microreactor, and 3 mL aliquots were collected for every reaction cycle. All microreactor experiments were performed at least three times to determine experimental variability. As used for batch reactions, for experiments conducted under "dry conditions" all the reactants were transferred to syringes in glovebox, while to establish "water saturated" conditions, water saturated toluene (0.033% w/w water) was used instead of dry toluene.

Characterization. Proton (^{1}H) NMR was used to determine (i) monomer conversion, (ii) number-average molar mass (M_n), and (iii) the fraction of chains initiated by benzyl alcohol. ^{1}H NMR spectra were recorded on a Bruker NMR spectrometer (model DPX300) at 300 MHz. Assignment of signals was based on that published elsewhere.[15] A representative ^{1}H NMR spectrum (300 MHz, toluene-d) of PCL with benzyl ester/carboxylic acid and hydroxyl terminal groups at the C- and O-terminal chain end positions is given in the Supporting Information (Figure S1).

Signals for structural analysis included those at 3.78 (t, J 6.5 Hz, −[C═O]−OCH$\underline{H}_2$CH$_2$−), due to protons of ε-CL monomer; resonances at 3.99 (t, 6.5 Hz, −[C═O]−OCH$\underline{H}_2$CH$_2$−), due to PCL repeat units along chains; and signals at 3.53 (t, 6.5 Hz,

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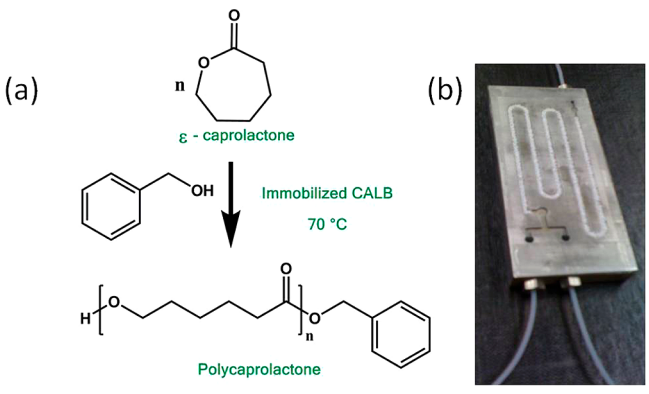
HOC$\underline{H_2}$CH$_2$−), due to chain-end units.[15] The ratios of signals at 3.99 to 3.78 and 3.99 to 3.53 were used to calculate the monomer conversion and M_n, respectively. The signal at 4.99 was assigned to the methylene (*CH*2) proton of polycaprolactone (PCL) end-capped with benzyl alcohol and the ratio of 4.99 to 3.53 used to calculate fraction of chains initiated by benzyl alcohol. The signal at 4.62 was assigned to the methylene (*CH*2) protons of unreacted benzyl alcohol. (Representative NMR spectra are provided in the Supporting Information.)

RESULTS AND DISCUSSION

Ring-opening polymerization (ROP) of ε-CL was conducted in a microreactor and batch reactor for reaction times of 15−240 and 300−2700 s, respectively, at 70 °C. In microreactor, different reaction times were obtained by changing the flow rate, where lower flow rates give longer reaction times and vice versa. In batch mode, samples were taken at predetermined intervals. Reactions were conducted in either dried toluene-d_8, designated the "dry" system, or with water saturated (0.033% w/w) toluene-d_8, referred to herein as the "water saturated" system. Values of % ε-CL conversion and M_n were determined by ^{1}H NMR analysis following previously published methods (see Mei et al. (2003) and Supporting Information Figure S1). As observed in Figures 1a and 1b, both "water saturated" and "dry" systems followed similar trends of % ε-CL versus time curves, just in different time intervals. In microreactor, maximum monomer conversion (∼90%) was achieved in 120 s, while in the batch reactor, both "water saturated" and "dry" systems reached ∼60% monomer conversion in 1200 s. Thereafter, the rate of monomer conversion in batch for the "dry" system increased more slowly relative to the "water saturated" system. This is attributed to the gradual depletion of water molecules from N435 beads, which can lower enzyme activity for propagation reactions. These results are consistent with that observed previously by Mei et al.,[18] where step-condensation reactions were favored with decreased water content from 1.15 (% w/w) to 0.60 (% w/w).[15,17]

Scheme 1. (a) Reaction Scheme for the Ring-Opening Polymerization of ε-Caprolactone Using Benzyl Alcohol to End-Cap Poly(ε-caprolactone); (b) Picture of the Microreactor Setup



Monomer conversion data were fit in the first-order reaction equation

$$-\ln(1 - X_t) = k_{app}t$$

where X_t is fractional monomer conversion for a residence time of t and k_{app} is the apparent rate constant. A semilogarithmic plot of ε-CL conversion as a function of time was constructed and is displayed in Figure S2. The apparent rate constant in microreactor (k_{app} = 0.027 s^{-1}) was 27 times larger than the batch reactor (k_{app} = 0.001 s^{-1}) due to the higher catalyst surface area (SA) to reaction volume ratio and the shorter diffusional path length[32] in microreactor than batch reactors. The microreactor contains 100 mg of N435 within a reactor volume of 130 mm^3. Hence, the catalyst loading density in the microreactor is 100 mg/130 mm^3 (0.77 mg/mm^3). In batch, the reaction contains 100 mg of N435 suspended in 3 mL such that the catalyst loading density is 0.03 mg/mm^3. Therefore, the catalyst loading is ∼25 times higher in the microreactor. The SA of N435 beads is reported as 80 m^2/g.[33] Thus, 100 mg beads will have a SA of 8 m^2/100 mg. Hence, the SA to volume ratio in the microreactor is 8 m^2/130 mm^3 (0.06 m^2/mm^3), whereas in batch reactors, the SA to volume is 8 m^2/3000 mm^3 (0.0026 m^2/mm^3). The above analysis shows that the SA to volume ratio in the microreactor is 23 times higher than in the batch reactor.

Values of PCL M_n in microreactor polymerizations run under "dry" and "water saturated" conditions were similar up to about 90% conversion (Figure 1c). Under "dry" conditions, as conversion progressed from 90% to 96%, M_n almost doubled (1800 to 3500), whereas under "water saturated" conditions, further increase in conversion to 96% gave M_n ∼ 2300, consistent with the general trend of the ε-CL vs conversion plot. Figure 1d shows the identical experiment conducted in Figure 1c but using the batch reactor. Trends of M_n vs conversion for polymerizations in batch were dramatically different from those in microreactor. First, in the microreactor, trends under "dry" and "water saturated" conditions were similar other than above 90% conversion. However, in the batch system, "dry" and "water saturated" conditions resulted in very different M_n vs conversion plots. Under "dry conditions", M_n values increased linearly with conversion up to about 80%. Relative to the microreactor system under "dry conditions", at 75% conversion, M_n was 2 times larger (3000 vs 1500) in the batch system. Under "water saturated" conditions in batch, M_n increased slowly from ∼250 to 1000 as % conversion increased from 19% to 73%. Such large effects of reaction water content on M_n values in batch systems is consistent with previously published results.[34] In summary, water functions as an initiator along with benzyl alcohol such that, at higher water content, chain initiation occurs to a greater extent for the same quantity of monomer, resulting in a larger number of chains and, therefore, lower M_n. Furthermore, as reactions progress under "dry" conditions, a deviation from linearity of M_n versus conversion plots occurs as events of step-condensation reactions increase in frequency (see also Figure S2). It is well-known that step-condensation propagation reactions result in exponential increases in molecular weight.[15]

The results above demonstrate that, based on the experimental design of the microreactor, the environment of the "wet" reaction is effectively that of "dry" conditions. In other words, under "water saturated" reaction conditions, the water content of CALB within Lewatit beads in the microreactor must be less than that in the batch system. This behavior can be explained since the microreactor is a dynamic system. Even under "water saturated" conditions, the majority of the water resides in the beads. In the initial volumes of monomer and solvent to flow past the beads, substantial amounts of water can partition from the beads to the solution phase, where chains are initiated at high rates, continuing to

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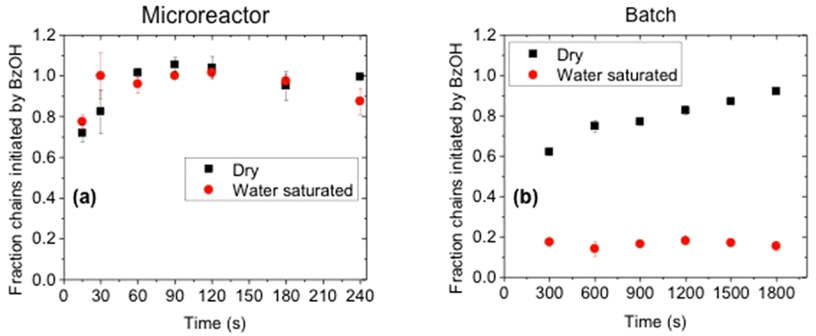

Figure 2. Fraction of chains initiated by benzyl alcohol (BzOH) in (a) microreactor and (b) batch mode as a function of reaction time. Each data point is obtained by using fresh (never used) N435 and the reactants in both microreactor and batch. The errors indicate one standard uncertainty based on measurements on at least three different samples.

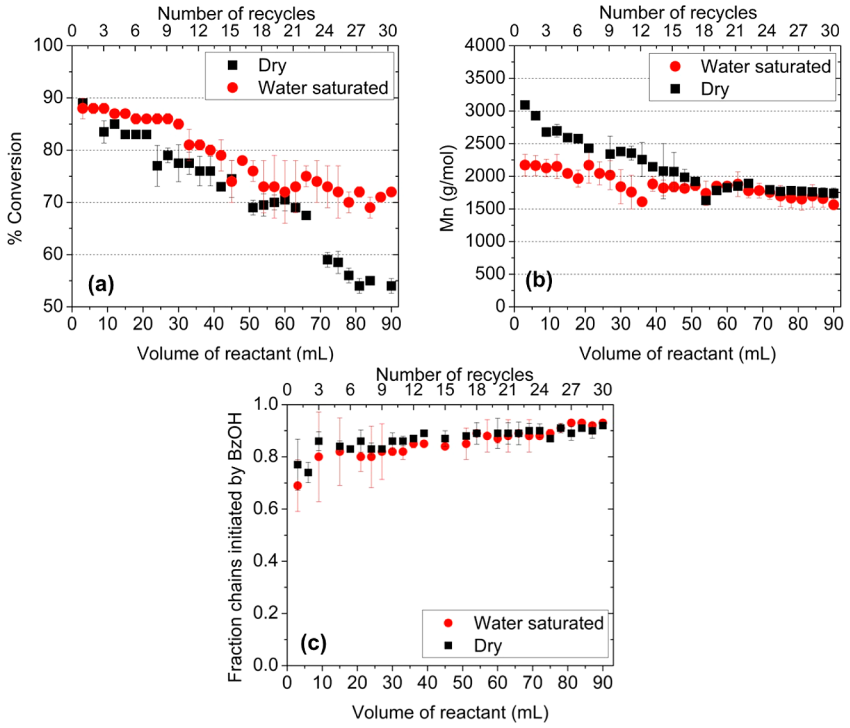

Figure 3. N435 reusability as a function of the number of reuse cycles during continuous PCL synthesis at 70 °C in microreactor. As indicators of N435 reusability, plots were constructed of % ε-CL conversion (a), M_n (b), and fractional content of benzyl ester groups at PCL chain ends (c) as a function of the number of recycles or volume of reactant. Each data point indicates 3 mL of reactant processed through the microreactor, and data is presented for a total of 30 cycles or 90 mL. Error bars indicate one standard uncertainty based on measurements of at least three different samples.

deplete the system of water. After some brief period of maximum partitioning, the total water in the system will never reach the initially high concentrations, as the beads become effectively dry. This occurs since "water saturated" toluene entering microchannels transfers a fraction of the water it carries to CALB within N435 beads while toluene exiting microchannel reactor provides a route for water to leave the system. In contrast, for batch systems, water does not dynamically enter and exit the reactor but, instead, remains during the course of the reaction, as do the total number of chains. As a consequence, the actual water content associated or neighboring CALB molecules is greater in the batch reactor, resulting in a higher rate of chain initiation and chain hydrolysis where water acts as an acyl acceptor.

The relative efficiency of PCL end-functionalization at the C-terminus by reaction with BzOH, where the ratio of ε-CL to BzOH in the monomer feed is fixed at 20:1 mol/mol, was compared using microreactor and batch systems. The fraction of C-terminal benzyl ester moieties at PCL chain ends was determined by ^{1}H NMR analysis following previously published methods (see Figure S1 and accompanying discussion in the Supporting Information). Figure 2a shows that, in microreactor, high fractions of chains are end-functionalized by benzyl alcohol for both "water saturated" and "dry" systems. As the reaction time increased from 15 to 90 s, the fraction of BzOH esterified PCL chain-ends increased from about 0.75 to ≥0.98. The extent of end-functionalization for "dry" and "water saturated" systems is nearly identical. This similarity is consistent with M_n versus conversion results for "dry" and "water saturated" microreactor systems discussed above. In contrast to the microreactor, in batch under "dry" conditions a regular increase in the fraction of chains with benzyl ester C-terminal groups occurs with increasing reaction time (Figure 2b). That is, from 300 to 1800 s, the fraction of BzOH

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esterified PCL chain ends increased from about 0.60 to 0.90. This increase in BzOH chain ends follows the increase in % ε-CL conversion over this same time period (Figure 1b). Mechanistically, the formation of 60% benzyl ester chain ends with 40% carboxyl C-terminal moieties is explained by the competition between water and BzOH for initiation of enzyme-activated monomer. Increased benzyl ester end-group content at chain ends as propagation continues is attributed to the following three pathways: (i) additional chain-initiation reactions between BzOH and enzyme-activated monomer, (ii) activation of carboxyl terminal chain ends by reaction with lipase (chain-end activation) with subsequent chain-end esterification with BzOH, and (iii) intrachain activation with subsequent chain-end esterification with BzOH. Detailed discussions of these reaction steps that occur between CALB, ε-CL, initiator (e.g., water or benzyl alcohol), and PCL chains are given in publications by us[15,34,35] and others.[35]

Comparing the fraction of chain ends with benzyl ester C-terminal groups for "dry" and "water saturated" reactions in microreactor and batch systems demonstrates the very different environments of these two reaction systems. In batch, C-terminal chain ends predominantly consist of carboxylic acid moieties. In other words, the fraction of benzyl ester chain ends remains below 0.2 throughout the reaction (to 1800 s). In the microreactor, under "water saturated" conditions, by 90 s, chain ends consist of $\geq$0.98 benzyl ester groups. Hence, as discussed above, the microreactor design results in an environment such that "water saturated" reactions behave similarly to "dry" conditions where the content of water associated with CALB is relatively low.

N435 reusability for BzOH end-functionalized PCL synthesis was evaluated in the microreactor system. 90 mL of reaction mixture consisting of ε-CL and BzOH (20:1 mol/mol) in toluene-d_8 was continuously processed through the microreactor, and 3 mL aliquots for each reaction cycle were collected. Based on the results in Figure 1a that showed maximum % ε-CL conversion was reached within a 120 s reaction time, the flow rate was adjusted to 65 μL/s.

Aliquots from each reaction cycle were analyzed as above by ^{1}H NMR to simultaneously ascertain values of % ε-CL conversion, M_n, and end-capping efficiency. Results in Figure 3a show that, for the "dry" system over 30 reaction cycles, % ε-CL conversion decreased gradually from 90 to 55%. In contrast, for the "water saturated" system over the first 20 cycles, conversion dropped from 90 to 70% (~22% decrease). Thereafter, over the following 10 cycles, no further decrease in % ε-CL conversion was observed. The larger decrease in % conversion for the "dry" system over the 30 cycles is consistent with water depletion, resulting in decreased enzyme activity. Water depletion is expected under "dry" conditions since, over repeated reaction cycles, water bound to the enzyme is gradually consumed due to reactions in which water acts as chain initiator generating carboxyl terminal groups found on about 10−20% of total chains formed (Figure 3c).

For "dry" and "water saturated" microreactor systems, PCL M_n values gradually decreased over the first 18−20 cycles from about 3000 to 1750 g/mol and 2250 to 1750 g/mol, respectively. Since % conversion over the same number of cycles decreased by 22%, this accounts perfectly for the decrease in M_n under "water saturated" conditions. However, regardless of similar decreases in % conversion under "dry" and "water saturated" conditions over the first 10 reaction cycles (30 mL reaction volume), M_n values under "dry" conditions are larger. From cycles 20 to 30, M_n values in both "dry" and "water saturated" conditions show no substantial change. For "water saturated" conditions these results are consistent with the relative constancy of % conversion values, with values that fluctuate from 75 to 69%. However, under "dry" conditions, % conversion from cycle 20 to 30 decreases from 70 to 55% while M_n shows no significant change. The general phenomena seen above where M_n values are greater than those predicted based solely on chain-end growth from % conversion values is explained by condensation reactions that occur at a greater frequency under "dry" rather than "water saturated" conditions. Figure 3c shows that, regardless of whether microreactor reactions are conducted for 30 cycles under "dry" or "water saturated" conditions, the fraction of benzyl ester groups on chains remains high, between 80 and 90% of chains. Similar results were found above (see Figure 2a) when comparing the fraction of benzyl ester terminal groups on PCL chains during the course of one reaction cycle under "dry" and "water saturated" reaction conditions. The fraction of chain ends with benzyl ester end groups will depend on competition between BzOH and water for chain initiation reactions with enzyme-activated monomer as well as other chain-end functionalization reactions that occur during chain-end activation or intrachain activation reactions.[36] Important here is that the occurrence of high end-group contents of benzyl ester chain ends found during the course of one reaction cycle was found to extend to 30 reaction cycles. We believe this result is a consequence of the microreactor experimental design—an environment that creates "dry" conditions nearby CALB molecules even when the solvent is "water saturated" as discussed above.

Water content measurements of N435 beads at zero time and after the 30th cycle were measured for both "water saturated" and "dry" systems. Values were obtained by removing N435 beads from the microreactor in a glovebox, extracting beads with dry methanol overnight and measuring water content of the methanol extract by Karl Fischer titration. Water content values were adjusted relative to a methanol control. Results of N435 bead water content (wt %) under "water saturated" conditions at time 0 (WS 0) and after the 30th recycle (WS 30) are 0.12 $\pm$ 0.01 and 0.02 $\pm$ 0.001. Thus, even though N435 beads are continuously provided with water saturated toluene solution, there is a net loss in bead water content over the 30 runs. This result is consistent with the above experimental findings that, even when water saturated toluene is used as the carrier solution, the microreactor design results in "dry" conditions nearby CALB molecules. Water content of N435 beads under "dry" conditions at time 0 (NW 0) and after the 30th recycle (NW 30) are 0.06 $\pm$ 0.01 and 0.006 $\pm$ 0.001, respectively. For "dry" conditions, pre-equilibration of the microreactor with dry reactant led to depletion of total water content of the beads from 0.40 to 0.06%. In batch reactions, water content of N435 beads under "dry" conditions at time 0 (NW 0) and after the seventh recycle (NW 7) is 0.09 $\pm$ 0.001 and 0.06 $\pm$ 0.011, respectively. N435 bead water content under "water saturated" batch conditions at time 0 (WS 0) and after the seventh recycle (WS 7) are 0.18 $\pm$ 0.02 and 0.16 $\pm$ 0.01, respectively. The similarity of N435 water content values at time 0 and after the seventh reaction cycle for both "dry" and "water saturated" conditions is consistent with that. Unlike the microreactor which is a dynamic system where "water saturated" or "dry" toluene exits the reactor providing a route for water to leave the system, in batch systems changes in water content require water consumption or evolution which is

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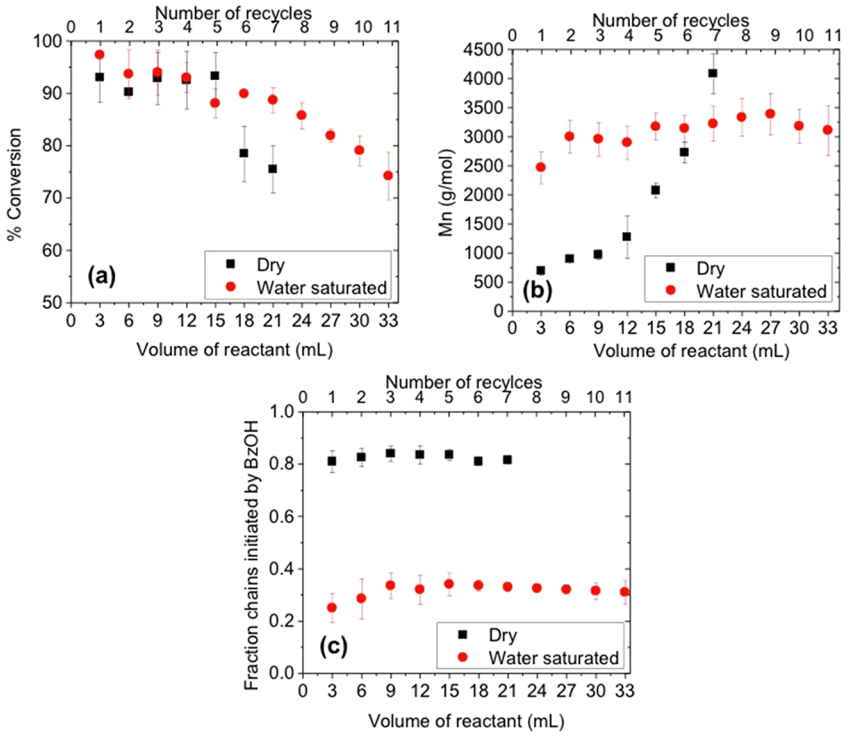

Figure 4. N435 reusability as a function of the number of reuse cycles during the semi-continuous synthesis of PCL at 70 °C in batch where the reaction time for each run is 1800 s. As an indicator of N435 reusability, plots were constructed of % ε-CL conversion (a), M_n (b), and fractional content of benzyl ester groups at PCL chain ends (c) as a function of the number of recycles or volume of reactant. Each data point or reaction run indicates 3 mL of reactant processed through the batch reactor. Error bars indicate one standard uncertainty based on measurements of at least three different samples.

a relatively small affect herein since the major reaction during PCL synthesis is transesterification between the chain end hydroxyl group and enzyme-activated monomer.

Reusability of N435 in the batch reactor system was also studied. Figure 4a shows that, over seven reaction cycles, % ε-CL conversion for the "water saturated" system remained at about 90%. Additional reaction cycles up to 11 resulted in a continuous and slow decrease to 75% conversion. Under "dry" reaction conditions, % ε-CL conversion remained at about 90% over the first five reaction cycles and, thereafter, decreased so that by the seventh run ε-CL conversion was 75%. A similar decrease in % ε-CL conversion under "dry" conditions in microreactor occurred after about 12 cycles (Figure 3a). Values of M_n as a function of the number of reaction cycles are displayed in Figure 4b. Under "water saturated" conditions in the batch reactor, M_n values over 11 reaction cycles remained at about 3000. This is curious in that, over the same series of reactions, conversion values decreased after the seventh reaction run. In the microreactor, M_n remained invariable over the first nine reaction cycles with values that were slightly lower (about 2200) and which thereafter slowly decreased (Figure 3b). Conversely, under "dry" conditions, differences between M_n values over multiple cycles conducted in microreactor and batch systems are dramatic. In batch, M_n values increased dramatically over the seven reaction cycles. After the first, fourth, and seventh cycles M_n was 700, 1200, and 4000, respectively. This is consistent with "dry" conditions where, after each reaction cycle, water content in reactions decreases due to water consumption by chain initiation, resulting in an increased propensity for propagation via step-condensation reactions between chain segments. Study of Figure 3b over the first seven microreactor cycles under "dry" conditions shows a relatively small change in M_n from about 3100 in the first cycle to 2400 in the seventh cycle. While these results are insufficient to make quantitative conclusions over the relative frequency of condensation reactions under "dry" conditions in batch versus microreactor, it is evident that under "dry" conditions propagation via condensation is occurring in both batch and microreactor systems and plays an important role in determining end-functionalized PCL molecular weight.

The fraction of benzyl ester terminated chains as a function of N435 reuse cycles is displayed in Figure 4c. Under "water saturated" conditions, the fraction of benzyl ester terminal groups remains at about 0.30 throughout all 11 reaction cycles. Under "dry" reaction conditions, the fraction of benzyl ester terminal groups remained at about 0.8 over the seven reaction cycles. This result reflects the divergent effects of decreased enzyme activity with increased N435 reuse evident by decreased conversion values (Figure 4a) that would tend to decrease the fraction of benzyl ester terminal groups and an increase in the frequency of step-condensation reactions that would tend towards an increase in benzyl ester terminal groups. Results above showed that, regardless of whether microreactor reactions were conducted for 30 cycles under "dry" or "water saturated" conditions, the fraction of benzyl ester groups on chains remains between 0.80 and 0.90 (Figure 3c). Hence, in contrast to batch reactors, microreactor conditions are less sensitive to reaction water content, enabling high end-group functionalization over 30 reaction cycles.

Another factor to be considered when interrogating N435 reuse is the potential that CALB becomes physically desorbed from beads resulting in a change in catalyst concentration during reuse cycles. Figure 5 displays the results of enzyme leaching (e.g., physical desorption) in batch and microreactor

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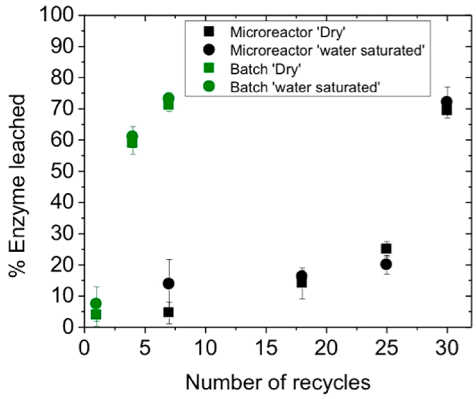

Figure 5. Comparison of CALB leaching (% w/w) determined by elemental analysis of reused N435 during ε-CL polymerizations as a function of number of recycles/reuse in batch and microreactor mode under "dry" and "water saturated" conditions.

under "dry" and "water saturated" conditions. First, there is no significant difference between enzyme leaching under "dry" and "water saturated" conditions. In other words, at least for these two sets of experimental conditions, reaction water content does not affect enzyme leaching from beads. However, enzyme leaching differs greatly dependent on whether reactions are conducted in microreactor or in batch. In microreactor, enzyme leaching increased from mean values of about 10, 15 and 22% from cycles 7, 18, and 25, respectively. From cycle 25 to 30, CALB leaching increased exponentially to about 76%. The occurrence of enzyme desorption in itself is not surprising given the enzyme is only physically bound to the support. However, it would be anticipated that the gradual increase in enzyme leaching observed through cycle 25 would continue from cycle 25 to 30. However, instead, leaching increased to an extent that would only be explained by a significant change in the physical structure or other conditions affecting the adsorption equilibria of N435 beads. This possibility is explored below. For batch reactions, enzyme leaching increased from about 5% after 1 cycle to about 60 and 71% after 4 and 7 cycles, respectively. Again, as was seen in microreactor, high extents of leaching occurred. However, in batch, leaching occurred more rapidly over fewer cycles. In contrast to the microreactor, batch reactor systems do not contain enzyme-free Lewatit beads. Thus, the catastrophic loss in enzyme from beads by the fourth reaction cycle should result in corresponding large decreases in enzyme activity. However, the plot of ε-CL conversion as a function of reaction cycle in Figure 4a shows a loss in enzyme activity that is small over seven cycles. Nevertheless, the relationship between enzyme content in N435 beads and activity for PCL polymerizations has not been established.

Indeed, this is new territory where systematic studies are needed to understand how enzyme removal from beads and corresponding affects on activity are correlated. It is intuitively obvious that in such an immobilized enzyme−catalyst system enzyme molecules residing on catalyst beads will have differing activities due to their local environments. Furthermore, enzyme desorption will be a function of many factors that, in addition to the reaction conditions employed, will also depend on the reactants, products, and solvent system. Gaining a fundamental understanding of factors affecting desorption of enzymes from immobilized beads is critically important, especially in such systems as this where the enzyme is physically adsorbed. Studies are underway in our laboratory to systematically address this question.

Results indicate about 70% of CALB leached after the seventh recycle in batch and 30th recycle in microreactor (Figure 5). Regardless of the reactor configuration used (microreactor or batch), scanning electron microscopy (SEM) images of beads from microreactor and batch shows no physical/mechanical damage to the beads over repeated use (Figure S4). This eliminates the possibility that a catastrophic physical deterioration of beads in microreactor or batch systems caused this extensive leaching of CALB from beads.

■ SUMMARY OF RESULTS

Reaction water content and number of microchannel reactor reuses were variables investigated for the synthesis of benzyl ester end-functionalized PCL. Results in the microreactor system with N435 bead packed channels were compared to those for a batch reactor system. The apparent rate constant in microreactor (k_{app} = 0.027 s^{-1}) was 27 times larger than the batch reactor (k_{app} = 0.001 s^{-1}). The faster rate in microreactor is attributed to the 23 times higher catalyst surface area (SA) to reaction volume ratio as well as the shorter diffusional path length[37] in microreactor than batch reactors. Remarkably, in microreactor, the M_n vs conversion plot for "dry" and "water saturated" conditions were similar. In contrast, under "water saturated" conditions in batch reactor, M_n is much lower. We explain this behavior as being due to the dynamic system of the microreactor where "water saturated" toluene entering microchannels transfers a fraction of its water content to CALB while the remaining water in toluene exits the system. Batch systems differ in that water does not dynamically enter and exit the reactor but, instead, remains during the course of the reaction. Hence, the experimental design of the microreactor is such that the environment of the "wet" reaction results in effectively "dry" conditions. An important consequence of this behavior is that, in microreactor, a high fraction of chains are end-functionalized by benzyl alcohol for both "water saturated" and "dry" systems. Indeed, the fraction of benzyl alcohol esterified PCL chain ends increased from about 0.75 to ≥0.98 as reaction time progressed from 15 to 90 s. Furthermore, microreactors run for 30 cycles under "dry" or "water saturated" conditions gave product where the fraction of benzyl ester groups on chains remains high, between 80 and 90%. In contrast, in the "water saturated" batch system, the fraction of benzyl ester terminal groups remains at about 0.30 throughout all 11 reaction cycles. Hence, the microreactor design that results in effectively "dry" conditions even when the reactants are "water saturated" enables consistently high end-group functionalization over 30 reaction cycles.

Other aspects of catalyst reuse in microreactor and batch systems were studied that provided new insights. With respect to changes in conversion and M_n values, reuse results in microreactor under "water saturated" conditions are promising. Percent conversion over the first 20 cycles dropped from 90% to 70% (~22% decrease), and over the following 10 cycles, no further decrease in % ε-CL conversion was observed. Furthermore, PCL M_n values gradually decreased over the first 18−20 cycles from about 2250 to 1750 g/mol. Taken together with the retention over 30 cycles of a high fraction of benzyl ester groups (above), the reuse results appear promising. The loss in % conversion was substantially greater under "dry" conditions in microreactor. However, what was striking given the results above is CALB leaching that increased from mean

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values of about 10, 15, and 22 and 76% from cycles 7, 18, and 25 and 30, respectively. That enzyme desorption occurs is not surprising since CALB is physically bound to the support. However, instead of a gradual increase in enzyme leaching as occurred through the first 25 cycles, leaching increased to an extent that one might expect in the event of a catastrophic change in the physical structure of N435 beads. However, based on scanning electron microscopy (SEM) images of beads from microreactor, physical/mechanical damage was not observed over the full 30 cycles.

Based on this work, new research has been initiated in both microreactor and batch systems to better understand factors that influence leaching with the aim of minimizing its occurrence.

■ ASSOCIATED CONTENT

Supporting Information

Estimation of conversion, M_n and end-group modification from ^{1}H NMR, semilogarithmic plot of monomer conversion fitted with first-order reaction kinetics, number-average molar mass (M_n) as a function of time, SEM image, elemental analysis method to determine enzyme leaching. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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