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Chemoenzymatic lignin valorization: Production of epoxidized pre-polymers using *Candida antarctica* lipase B

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ABSTRACT

An innovative chemoenzymatic catalytic system for functionalizing lignin from *Organosolv* and *Kraft* pulping processes to obtain oxirane rings was investigated. Novozym435 (immobilized *C. antarctica* lipase B: CalB) was used to catalyze the peroxidation of caprylic acid to peroxycaprylic acid, which in turn reacted with unsaturated C—C bonds to form the oxirane ring. The conversion of OH groups to oxirane rings (epoxides) reached 90% and 55% after 12 h for the two processes, respectively. The residual enzyme activity over the time course of the reactions indicated transient denaturing due to association with the lignin substrate (10–50%) as well as irreversible denaturation due to exposure to hydrogen peroxide. Functionalized lignin has potential applications in the production of epoxy adhesive resins, and chemoenzymatic synthesis represents a “greener” pathway to this synthesis.

1. Introduction

The economic viability of biorefineries to produce fuels from biomass is greatly enhanced by the conversion of biomass fractions that are not involved in the biofuel production, such as lignin, into value-added co-products [1,2]. Several strategies for the generation of valuable co-products have been evaluated [3–5]. The results indicate that producing ethanol as a single product is not economically feasible, whereas when furfural is a co-product, the process becomes viable economically [1], highlighting the importance of co-product valorization for biorefinery sustainability. The most effective way to achieve optimal co-product synthesis is to incorporate co-product processing requirements into the facility design process. In that way, economic and environmental criteria for fuel and co-product synthesis can be applied directly to biorefinery design [2,4].

Lignin offers a significant product opportunity. It can be converted into high-value products including carbon fiber, engineering plastics and thermoplastic elastomers, polymeric foams and membranes, epoxy resins, and a variety of fuels and chemicals that are currently being made by petroleum derivatives [2,6–9]. Lignin depolymerization, the first step in many of these conversion scenarios, is challenging, given the different bond strengths of the ether (C—O—C) and carbon–carbon (C—C) linkages, and the tendency for low-molecular-weight species to undergo condensation reactions. Because of the heterogeneity of low molecular weight species and the diversity of functional lignin, degradation products are difficult to upgrade [6,7].

One potential product from low molecular weight lignin fractions is epoxy resin [2,8–11]. The functional epoxide group contains an oxirane ring, which is important in the synthesis of adhesive resins, polyurethane foams, glycols, carbonyls, alcohols and amine derivatives [8,9,11–13]. Approximately 75% of epoxy resins contain the diglycidyl ether of bisphenol A (DGEBA), prepared by the coupling of bisphenol A (BPA) and epichlorohydrin (EP) [11,12,14]. Since reactions rarely go to completion, resins and polymers usually contain trace amounts of the original reactants. BPA exposure is problematic for humans due to its estrogen-like structure that can disrupt the endocrine system [11,14–16]. These recent findings have prompted efforts to develop commercial epoxides that are BPA-free, which is one objective of the present research.

Synthesis of oxirane rings using lignocellulosic and phenolic compounds, specifically vanillic acid and gallic acid, have been studied [8,9,11,14,17,18]. Given that epichlorohydrin and other phenolic substrates used in epoxy synthesis are generally derived from petroleum, the approach taken in this work represents a green and clean technology. Other green technologies proposed to incorporate polyphenolic compounds into epoxides include a chemical alkylation step and a subsequent enzymatic alkene epoxidation. This has not been commercialized because of the attendant safety issues and the risk of

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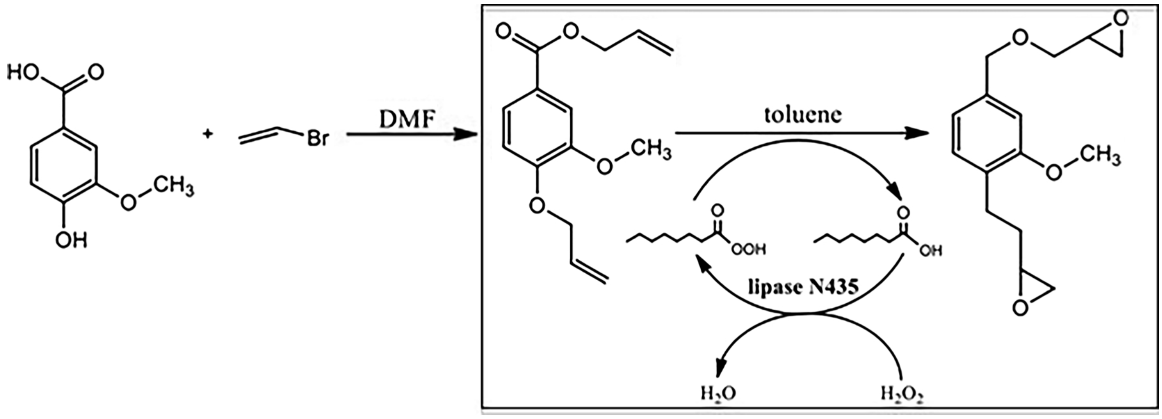

Fig. 1. Chemoenzymatic epoxidation of vanillic acid. The functionalization of the two OH groups is indicated, followed (in box) by the lipase-catalyzed chemo-enzymatic reaction cycle generating peroxy acids from carboxylic acid and subsequent Prilezhaev epoxidation.

defunctionalizing the epoxide [18]. Björkling [19] described an alternative method to produce epoxides from alkenes (Fig. 1; in box). Small quantities of fatty acids were used to study the epoxidation reaction cascade catalyzed by lipase [14,20]. Enzymatic formation of peroxycarboxylic acid from hydrogen peroxide and carboxylic acid is the first step in the cascade [21]. The peroxy acid subsequently reacts with the unsaturated C—C double bond in the fatty acid, forming the epoxide and regenerating the original carboxylic acid [13,21,22].

Table 1 presents different chemoenzymatic and chemical mechanisms for epoxidation, including systems using immobilized *Candida antarctica* lipase B. The enzymatic systems were evaluated between 25 °C and 50 °C for reaction times up to 48 h. The ultimate conversion achieved appears to depend on the specific reactions evaluated, and generally are comparable to results achieved using a chemical reagent such as *m*-chloroperoxybenzoic acid (*m*CPBA).

CalB's ability as part of a chemoenzymatic system to catalyze diverse reactions, such as epoxidation, lactonization and desymmetrization, has been investigated extensively [21,27,28]. Some results for example chemoenzymatic epoxidation of alkenes using CalB compared to the chemoenzymatic by a *Rhizomucor miehei* lipase to form epoxides using limonene and (s)-carvone was studied, finding optimal parameters to condition a good yield in the epoxide production in both cases [21].

It is proposed that this technology could be effectively adapted to lignin functionalization through the conversion of hydroxyl groups (phenolic, aliphatic and carboxyl) to oxirane ring structures, creating an epoxy resin precursor. Allylation chemistry effectively activates the hydroxyl structures, creating the carbon–carbon double bonds necessary for oxirane ring formation. It is important to obtain lignin fractions (from Organosolv or Kraft processes) containing the largest number of available hydroxyl groups, and to understand the relationship between pretreatment technologies and hydroxy content. It is also hypothesized that lower lignin molecular weight would make these hydroxyls more accessible for the allylation reaction [14], and the subsequent epoxidation. This work characterizes the activity of the proposed chemo-enzymatic catalytic system towards fractions of Organosolv and Kraft lignin. The model system chosen involves functionalized (allylated) lignin, immobilized *Candida antarctica* lipase B (CalB; commercial preparation is Novozym 435 (N435)), hydrogen peroxide and caprylic acid. Of particular interest is the reactivity of different lignin fractions (Organosolv and Kraft) that could affect the rate and degree of epoxidation achievable.

2. Materials and methods

2.1. Reagents

The Organosolv pretreatment of *Eucalyptus globulus* woodchips was performed in a 5-gallon reactor (Parr reactor 4889) in the presence of a 50/50 (v/v) ethanol/water mixture at a temperature of 200 °C for 6 min. Lignin was recovered through precipitation with 3% H_2SO_4 and dried in an oven at 40 °C for 72 h prior to fractionation. Fractionated lignin was analyzed by GPC and used in epoxidation studies. The Kraft lignin was obtained from the Domtar process (BioChoice lignin) [29]. The standard epoxidation reaction mixture contained a phenolic source of hydroxyl groups (vanillic acid (> 98%), Organosolv lignin or Kraft

Table 1
Comparison between chemoenzymatic and chemical mechanisms reported using CalB, *m*CPBA and N435.

Catalyst	Peroxyacid	Solvent	Product	Conversion %	Conditions	Source
CalB		EA	α-cyano epoxides	85	3 h, 50° C	[23]
		EA	Azoxybenzenes	74	1 h, 25° C	[24]
		Toluene	Oleic acid epoxides	20	0.5 h, 50° C	[25]
CalB Nanoflower		Toluene	Oleic acid epoxides	97	0.5 h, 50° C	[25]
None	*m*CPBA	DCM	Vanillic acid epoxides	62	24 h, 25° C	[14]
	*m*CPBA	DCM	Gallic acid epoxides	68	24 h, 25° C	[14]
	*m*CPBA	EA	Azoxybenzenes	66	1 h, 25° C	[24]
N435		EA	α-cyano epoxides	91	3 h, 50° C	[23]
		EA	Azoxybenzenes	94	1 h, 25° C	[24]
		Toluene	Methyl oleate epoxides	69	6 h, 37° C	[26]
		Toluene	Vanillic acid epoxides	87	48 h, 40° C	[14]
		Toluene	Gallic acid epoxides	89	48 h, 40° C	[14]
		Toluene	Oleic acid epoxides	95	0.5 h, 50° C	[25]
	Caprylic	Toluene	Vanillic acid epoxides	60	12 h, 40° C	This work
	Caprylic	Toluene	Organosolv lignin epoxides	90	12 h, 40° C	This work
	Caprylic	Toluene	Kraft lignin epoxides	55	12 h, 40° C	This work