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Wood mimetic hydrogel beads for enzyme immobilization

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ABSTRACT

Wood component-based composite hydrogels have potential applications in biomedical fields owing to their low cost, biodegradability, and biocompatibility. The controllable properties of wood mimetic composites containing three major wood components are useful for enzyme immobilization. Here, lipase from *Candida rugosa* was entrapped in wood mimetic beads containing cellulose, xylan, and lignin by dissolving wood components with lipase in [Emim][Ac], followed by reconstitution. Lipase entrapped in cellulose/xylan/lignin beads in a 5:3:2 ratio showed the highest activity; this ratio is very similar to that in natural wood. The lipase entrapped in various wood mimetic beads showed increased thermal and pH stability. The half-life times of lipase entrapped in cellulose/alkali lignin hydrogel were 31- and 82-times higher than those of free lipase during incubation under denaturing conditions of high temperature and low pH, respectively. Owing to their biocompatibility, biodegradability, and controllable properties, wood mimetic hydrogel beads can be used to immobilize various enzymes for applications in the biomedical, bioelectronic, and biocatalytic fields.

1. Introduction

Composites are engineered materials that have two or more constituents with different physical or chemical properties (Šimkovic, 2008). Recently, biopolymer-based composites have received considerable attention for applications in biomedical fields, as they are biocompatible, biodegradable, and possess a high degree of functionality (Kim, An, Won, Kim, & Lee, 2012; Lee, Miyauchi, Dordick, & Linhardt, 2010). Biopolymers are naturally obtainable macromolecules including polysaccharides, polyphenols, polyesters, polyamides, and proteins. The inherent biodegradability of these materials makes biopolymers particularly promising for developing environmentally friendly materials (Simmons et al., 2011). However, the preparation of such biopolymer-based composite materials remains a challenge because of the low solubility of many biopolymers in conventional hydrophobic or hydrophilic solvents such as hexane, acetone, dimethylsulfoxide, and *N*,*N*-dimethylacetamide.

Lignocellulosic biomass, such as agricultural residues, forestry wastes, waste paper, and energy crops, has long been recognized for its potential value as a sustainable source of biopolymer composites (Himmel et al., 2007; Li, Wang, & Zhao, 2008). Lignocellulose consists of three major biopolymers, that is, cellulose, hemicellulose, and lignin, which have distinct chemical, physical, and structural properties. Cellulose, a linear polysaccharide of D-glucose residues linked by β-(1 → 4)-glycosidic bonds, is the most abundant renewable biopolymer on earth and its wood content ranges from 41% to 50% (Brown, 2004). It has excellent thermal and mechanical properties and biocompatibility and is a promising material for biochemical engineering because of economic and scientific reasons (Gemeiner, Stefuca, & Bales, 1993). Hemicelluloses are heterogeneous, branched polymers of pentoses, hexoses, and acetylated sugars, with xylans being the most plentiful of the hemicelluloses. They are as commonly found as cellulose, but their molecular weight is lower than that of cellulose and their wood content ranges from 25% to 35% (Saha, 2003). Lignin is an aromatic network polymer composed of phenylpropanoid units and helps to bind cellulose and hemicelluloses together. Its wood content ranges from 18% to 35% (Chandra et al., 2007; Pettersen, 1984). Lignin is more hydrophobic than cellulose and hemicelluloses, and its properties are dependent on the extraction methods used. Although lignin is usually recognized as waste material, in recent years, many studies on the application of lignin have been reported (Chung & Washburn, 2012; Lora & Glasser, 2002; Stewart, 2008). To prepare

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lignocellulose-based materials, the development of solvents with high dissolving power for lignocellulose is very important, because lignocelluloses are highly difficult to handle due to their crystalline structure.

Recently, we reported the preparation of lignocellulose-based materials and biomimetic synthetic wood composites containing cellulose, hemicellulose, and lignin by using ionic liquids (ILs) (Ahn et al., 2012; Kang et al., 2013; Lee, Kim, Lee, Lee, & Park, 2011; Park et al., 2011; Simmons et al., 2011). Cellulose/starch/lignin film, lignocellulose aerogel, and all-wood composites were also prepared by using ILs (Wu, Wang, Li, Li, & Wang, 2009; Li et al., 2011; Lu et al., 2012; Shibata, Yamazoe, Kuribayashi, & Okuyama, 2013). ILs are organic salts that usually melt below 100 °C. The interest in ILs stems from their potential application as "green solvents" due to their high thermal stability and non-volatility (Sheldon, Lau, Sorgedrager, & van Rantwijk, 2002). In chemical processes, ILs exhibit excellent physical characteristics, including the ability to dissolve polar and non-polar organic, inorganic, and polymeric compounds (Lee, Doherty, Linhardt, & Dordick, 2009). ILs have also been shown to be capable of dissolving lignocellulosic biomasses. In our previous work, three major components of wood were fully dissolved in 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]) and successfully reconstituted to various forms such as hydrogel, thin film, and microfiber by molding, film casting, and electrospinning (Lee et al., 2011; Park et al., 2011; Simmons et al., 2011). Fabrication of homogeneous composites from cellulose/hemicellulose/lignin was achieved via an uncomplicated process by using [Emim][Ac]. Biomimetic synthetic wood films containing cellulose, xylan, and lignin showed smoother surface textures, higher water resistance, and higher tensile strengths than cellulose films. In particular, tailor-made synthetic wood composites with controllable properties, including hydrophilicity/hydrophobicity, dielectric constant, cohesiveness, and light protection, could be prepared by changing the ratio of cellulose, xylan, and lignin (Simmons et al., 2011). A variety of additives, such as polyethylene glycol, multi-walled carbon nanotube, chitosan, porphyrin, and poly(3-octylthiophene), could also be entrapped in the synthetic wood composites by simple dissolution or by dispersion of the additives to the wood solution containing the three wood components (Lee et al., 2011; Park et al., 2011; Simmons et al., 2011). Biomimetic synthetic wood composites can provide great opportunities to develop environmentally friendly and biocompatible materials.

Enzymes have been recognized as efficient and environmentally friendly catalysts because of their high specificity and catalytic activity under mild conditions. However, the industrial applications of enzymes have been limited due to their low stability and difficult recovery for subsequent use. Enzyme immobilization is the most commonly used strategy for overcoming these drawbacks (Sheldon, 2007). Entrapment, an immobilization technique, can be defined as the physical restriction of an enzyme within a confined polymer network such as a hydrogel. Hydrogels are three-dimensional polymer networks that are swollen because of the presence of large amounts of water. On the basis of the source, hydrogels can be divided into natural polymer-based gels and synthetic polymer-based gels. On the basis of the cross-linking method, hydrogels can be divided into chemical gels and physical gels. Recently, biopolymer-based hydrogels have received considerable attention in biomedical fields, including tissue engineering, drug delivery systems, contact lenses, and biosensors because of their safety, hydrophilicity, biocompatibility, and biodegradability (Chang & Zhang, 2011; Vlierberghe, Dubruel, & Schacht, 2011). Various polysaccharide hydrogels, such as alginate, chitosan, agarose, carrageenan, and gelatin have been employed for the entrapment of a number of enzymes such as lipases, lactases, invertases, and peroxidases (Betigeri & Neau, 2002; Jegannathan, Chan, & Ravindra, 2009; Cheirsilp, Jeamjounkhaw, & H-Kittikun, 2009; Matto & Husain, 2009; Won, Kim, Kim, Park, & Moon, 2005; Pulat & Akalin, 2013). However, only a few studies have been performed on the entrapment of enzymes in non-derivatized cellulose hydrogels. Recently, Turner, Spear, Holbrey, and Rogers (2004) attempted to use [Bmim][Cl] to entrap laccase in a cellulose membrane. The enzyme was entrapped in cellulose but showed low residual activity because of IL-induced denaturation. More recently, we reported the successful entrapment of lipase in cellulose/chitosan, cellulose/agarose, and cellulose/carrageenan composites by using biocompatible [Emim][Ac] (Kim et al., 2012). Cellulose/biopolymer composite hydrogels proved to be good supports for the entrapment of lipase and have many potential applications.

In this work, lipase from *Candida rugosa*, which has many industrial applications in the fields of pharmaceuticals, cosmetics, food, perfumery, and bioremediation, was entrapped in wood mimetic composite hydrogels for the first time. Knowledge regarding which properties of synthetic wood composites can be modified may be beneficial for increasing the activity and stability of the immobilized lipase. The effects of the source and composition of each component in the wood mimetic composite hydrogel on the activity and stability of the entrapped lipase were also investigated in the present study.

2. Experimental

2.1. Materials

Cellulose (microcrystalline cellulose; cotton linter with medium fiber form; cotton linter with long fiber form), xylan from birchwood, alkali lignin, organosolv lignin, [Emim][Ac], *p*-nitrophenyl butyrate, and lipase from *C. rugosa* were purchased from Sigma-Aldrich (St. Louis, MO, USA). Isopropanol, acetic acid, sodium acetate trihydrate, and acetonitrile were purchased from Samchun Pure Chemical (Gyeonggi-do, South Korea). *p*-Nitrophenol was obtained from Kanto Chemical (Tokyo, Japan). Milled wood lignins (MWLs) from *Pinus rigida* and *Populus albaglandulosa* were prepared according to the Bjorkman method (Bjorkman, 1956). All other chemicals used in this study were of analytical grade and were used without further purification.

2.2. Preparation of wood component-based composite hydrogel beads containing lipase

To prepare wood component-based composite hydrogel beads containing lipase, we used our previously reported method with slight modifications (Kim et al., 2012). Various contents of cellulose, xylan, and lignins were dissolved in 5 mL of [Emim][Ac] while stirring at 80 °C for 1 h. Transparent solutions containing wood components were dried under vacuum at 60 °C to remove air bubbles. One milliliter of each wood component solution was mixed with 50 mg of lipase powder at room temperature. The resultant mixture was then added drop-wise into 1 L of distilled water at a rate of 50 μL/min with vigorous stirring by using a 1-mL plastic syringe with a 26-gauge needle and a syringe pump (LSP01-2A; Longer Pump; China). The hydrogel beads were cured in distilled water for 30 min. Washing was conducted three times, and the absence of [Emim][Ac] was confirmed by measuring the optical density of the washing solution at 211 nm.

2.3. Determination of lipase activity

The hydrolytic activity of the entrapped lipase was determined by conducting a spectrophotometric assay. A hydrogel bead containing lipase was placed in a 50-mL Falcon tube containing 9.5 mL of 0.1 M phosphate buffer (pH 7.0). The reaction was

started by adding 0.5 mL of substrate solution prepared by dissolving 10 mM *p*-nitrophenyl butyrate in isopropanol and was carried out at 25 °C in a water bath with shaking at 120 rpm. Periodically, 300 μL aliquots were removed, diluted with 300 μL of acetonitrile, and then centrifuged to obtain the supernatant. The activity was expressed as the initial rate and determined by measuring the increase in absorbance at 400 nm by the *p*-nitrophenol produced during the lipase-catalyzed hydrolysis of *p*-nitrophenyl butyrate. The initial rate measurements were carried out in triplicate (Lee, Doan, Ha, Chang, & Koo, 2007).

2.4. Thermal stability and pH stability of the entrapped lipase

The thermal stability and pH stability of free and entrapped lipase were measured based on the residual activity of lipase after treatment. To measure the thermal stability of the entrapped lipase, a hydrogel bead containing lipase was placed in a 50-mL Falcon tube containing 1 mL of 0.1 M phosphate buffer (pH 7.0). The beads were incubated at 60 °C for a determined period, and then cooled down in cold water for 10 min. Subsequently, the remaining activity of lipase was determined by measuring the hydrolysis of *p*-nitrophenyl butyrate. To measure the pH stability of the entrapped lipase, the beads were incubated in 0.1 M acetate buffer (pH 3.0) at 45 °C. Ten micrograms of free lipase was used for analysis of the thermal and pH stability of free lipase.

2.5. Bead characterization

The size of the hydrogel beads was measured using a digital caliper (Fuso; Japan). The diameter of each bead was measured at three different angles and averaged. Ten beads were used to calculate an average bead size. The dry weight of the beads was measured after drying at 60 °C for 12 h. For analysis of the surface of the beads, hydrogel beads were frozen overnight at −70 °C and then dried at −80 °C under vacuum for 12 h. All freeze-dried samples were sputter-coated with gold prior to observation. The surface of the samples was studied using a scanning electron microscope (JSM 6308; JEOL; Japan).

3. Results and discussion

3.1. Entrapment of lipase into wood component-based hydrogel beads

Cotton linter cellulose with long fiber form (LFC) was used as the major component of the wood mimetic hydrogel bead for entrapping lipase, because the rigidity of the hydrogel bead prepared from microcrystalline cellulose (MCC) with low degree of polymerization (DP) was not appropriate for repeated use. Cellulose hydrogel beads prepared with 4% MCC were easily breakable, whereas the rigidity of the cellulose hydrogel bead prepared with 4% LFC was suitable for repeated use. Xylan from birchwood and alkali lignin was added to the 4% LFC solution to prepare the wood solution. Lipase from *C. rugosa* was mixed with this solution and then the biopolymers were reconstituted with distilled water in order to prepare wood component-based hydrogel beads containing lipase. Fig. 1 shows the photographic images and scanning electron microscopy (SEM) images of various wood component-based hydrogel beads containing lipase. All of the hydrogel beads prepared had a regular spherical shape. Addition of lignin to cellulose changed the color of the hydrogel bead to dark brown, while the color of the cellulose/xylan hydrogel bead was pale yellow. Cellulose and cellulose/lignin beads had a smoother surface than the xylan-containing beads. The surface of the freeze-dried beads containing xylan was contracted because xylan is more hydrophilic than cellulose and lignin. However, none of the hydrogel beads shrunk to a great extent during freeze-drying. These results indicate that the degree of swelling and surface characteristics of wood component-based hydrogel beads can be controlled by changing the ratio of cellulose, xylan, and lignin.

Various lipase-entrapped wood component-based hydrogel beads were prepared with LFC, xylan, and various lignins, including alkali lignin, organosolv lignin, and MWLs from *P. rigida* and *P. albaglandulosa* (Table 1). The activity of lipase entrapped in cellulose bead (sample no. 1) prepared with 4% cellulose was 10.2×10^{-3} μmol/(min·bead). Cellulose, xylan, or lignin (2%) was added to the 4% cellulose solution to study the effect of additives on the activity of the entrapped lipase. When additional cellulose (sample no. 2) or xylan (sample no. 3) was added, the activity of lipase entrapped in cellulose or the cellulose/xylan bead was enhanced by ~24%. The addition of various lignins to the cellulose solution (samples no. 4–7) also increased the activity of the entrapped lipase. The highest activity of entrapped lipase was obtained with the solution containing 4% cellulose and 2% MWL from *P. albaglandulosa*. Lipase entrapped in cellulose beads prepared with 6% cellulose had higher activity than the beads prepared with 4% cellulose; this difference may be caused by higher protein loading due to the denser structure (Kim et al., 2012). The dry weights of cellulose beads prepared with 4% and 6% cellulose were 0.31 and 0.55 mg, respectively, although the wet bead sizes were similar. When additional cellulose, xylan, or lignin was added to the 4% cellulose solution, the dry weight of the bead increased without change in the wet bead size. Alkali lignin, organosolv lignin, and MWL from *P. albaglandulosa* were more efficient additives than additional cellulose with respect to increasing the activity. Lipase entrapped in the cellulose/lignin bead had higher activity than that the bead containing only cellulose; this difference might have been caused by higher protein loading and lipase activation due to the more hydrophobic nature. The hydrophobicity of lignin may induce the conformational change in *C. rugosa* lipase to its active open form (Palomo et al., 2002). However, accurate determination of the amount of loaded protein was difficult because of the interference of lignin in the protein assay. Additional studies may be needed to understand the effect of lignin on the activity of the entrapped lipase. To understand the synergistic effects of xylan and lignin on the activity of the entrapped lipase, 2% xylan (sample no. 3), 2% lignin (sample no. 4), and 1% xylan and 1% lignin (sample no. 8) were added to fixed 4% cellulose solution to prepare wood solution. The activities of lipase entrapped in the cellulose/xylan bead, cellulose/lignin bead, and cellulose/xylan/lignin bead were 23%, 40%, and 54% higher, respectively, than the activity of lipase entrapped in cellulose (sample no. 1). These results suggest that xylan and lignin can synergistically increase the activity of the lipase entrapped in cellulose composite beads.

3.2. Stability of lipase entrapped in wood component-based hydrogel beads

The thermal stability of lipase entrapped in various wood component-based hydrogel beads was measured at 60 °C (Table 1 and Fig. 2). The residual activity of lipase entrapped with 4% cellulose and 2% alkali lignin was 87% after 2 h incubation, whereas free lipase retained only 10% of the initial activity. The immobilized lipase prepared with 4% cellulose and 2% alkali lignin retained 40% of the initial activity after 24 h incubation at 60 °C. Highly enhanced thermal stability of lipase could be obtained by adding alkali lignin to the cellulose solution, whereas addition of xylan could not enhance the thermal stability of lipase. The half-life time of immobilized lipases was measured to obtain in-depth information regarding the effect of each component on thermal stability. The half-life time of free lipase was 0.3 h. The entrapped lipase showed significantly enhanced thermal stability. The half-life time

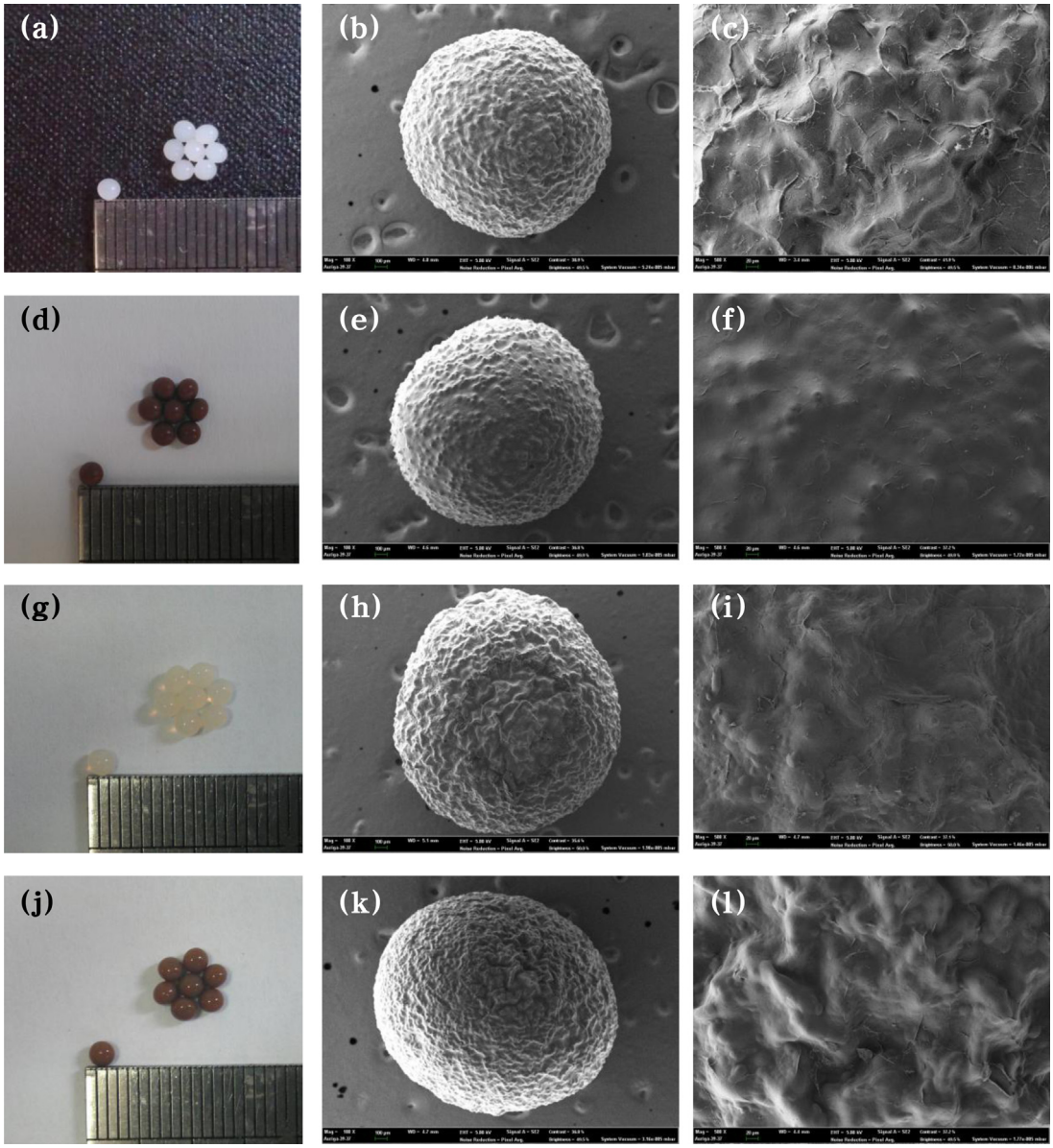

Fig. 1. Photo images and scanning electron microscopy (SEM) images of various wood mimetic hydrogel beads containing lipase. (a)–(c): cellulose bead, (d)–(f): cellulose/alkali lignin bead, (g)–(i): cellulose/xylan bead, (j)–(l): cellulose/xylan/alkali lignin bead.

of the lipase entrapped with 4% cellulose was 3.7 h, which is 12 times higher than that of free lipase. The addition of 2% alkali lignin (sample no. 4), 2% organosolv lignin (sample no. 5), or 1% xylan and 1% alkali lignin (sample no. 8) to the 4% cellulose solution enhanced the stability of the entrapped lipase; this entrapped lipase was even stable than the entrapped lipase prepared by using 4% cellulose solution. The increase in the thermal stability of the entrapped lipase due to lignin could be explained by the

Table 1
The characteristics of various wood mimetic hydrogel beads containing lipase.

Sample No.	Concentration of each component in solution (%)			Activity ($\times 10^{-3}$ μmol/min/bead)	Wet bead size (mm)	Dried bead weight (mg)	Half-life time (h)	
	Cellulose	Xylan	Lignin				Incubation at 60 °C	Incubation at pH 3
1	4	0	0	10.2 ± 0.4	2.40	0.31	3.7	58.2
2	6	0	0	12.8 ± 0.9	2.49	0.55	3.1	–
3	4	2	0	12.5 ± 0.4	2.51	0.45	2.7	–
4	4	0	2 (Alkali)	14.3 ± 0.8	2.44	0.42	9.2	81.8
5	4	0	2 (Organosolv)	14.8 ± 1.2	2.45	0.50	4.6	–
6	4	0	2 (*P. rigida*)	12.4 ± 0.4	2.45	0.58	3.9	–
7	4	0	2 (*P. albaglandulosa*)	15.2 ± 1.2	2.45	0.50	2.3	–
8	4	1	1 (Alkali)	15.7 ± 0.8	2.46	0.43	6.5	66.2

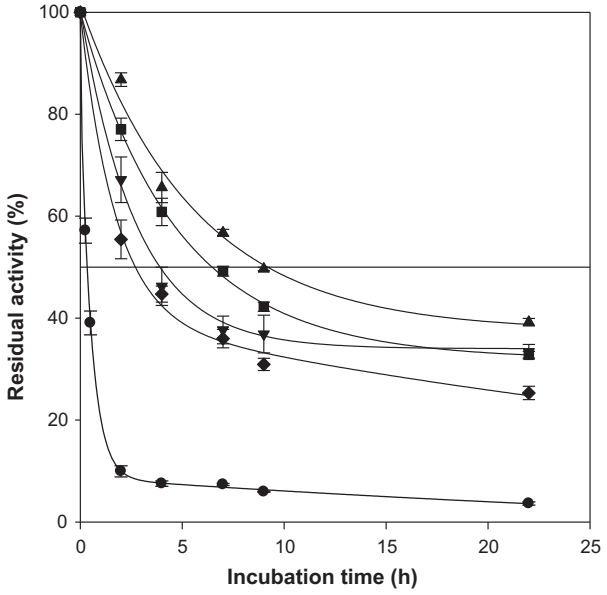

Fig. 2. The thermal stability of free lipase (●) and lipases entrapped in various wood mimetic hydrogel beads at 60 °C: bead prepared with 4% cellulose (▼); bead prepared with 4% cellulose and 2% alkali lignin (▲); bead prepared with 4% cellulose and 2% xylan (♦); bead prepared with 4% cellulose, 1% xylan, and 1% alkali lignin (■).

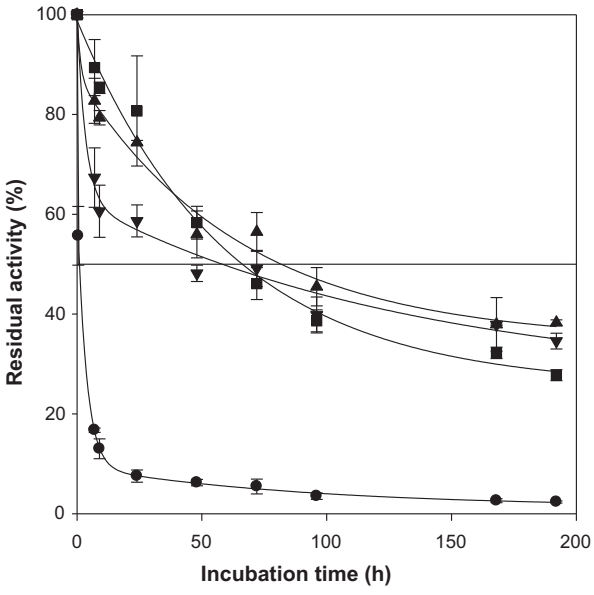

Fig. 3. The pH stability of free lipase (●) and lipases entrapped in various wood mimetic hydrogel beads at pH 3.0 and 45 °C: bead prepared with 4% cellulose (▼); bead prepared with 4% cellulose and 2% alkali lignin (▲); bead prepared with 4% cellulose, 1% xylan, and 1% alkali lignin (■).

stabilization of the active, open form of *C. rugosa* lipase due to the hydrophobic nature of lignin (Palomo et al., 2002). Interestingly, the half-life time of lipase entrapped with 4% cellulose and 2% alkali lignin was 9.2 h and 31-times higher, respectively, than that of free lipase. The half-life time of this entrapped lipase was also 2.5-fold higher than that of lipase entrapped with 4% cellulose. Alkali lignin had a higher stabilization effect than that of the other lignin types; this difference may be caused by enhanced interaction with lipase due to the presence of thiol groups in the structure of alkali lignin. Yiu, Wright, and Botting (2001) also reported that the trypsin immobilized on a thiol-functionalized molecular sieve (SBA-15) showed the highest stability among various functionalized supports because of enhanced interaction between —S—S— bonds on the surface of trypsin and the thiol groups on the SBA-15 surface. The lipase from *C. rugosa* used in the current study has two disulfide bridges (Benjamin & Pandey, 1998). The thermal stability of wood component-based beads increased in the following order: cellulose/MWL from *P. albaglandulosa* < cellulose/xylan < cellulose (6%) < cellulose (4%) ≈ cellulose/MWL from *P. rigida* < cellulose/organosolv lignin < cellulose/xylan/lignin < cellulose/alkali lignin. The trend for stability was very different from that of activity, which increased in the following order: cellulose (4%) < cellulose/MWL from *P. rigida* ≈ cellulose/xylan < cellulose (6%) < cellulose/alkali lignin < cellulose/organosolv lignin < cellulose/MWL from *P. albaglandulosa* < cellulose/xylan/alkali lignin. When both the activity and stability of the entrapped lipase were considered, cellulose/alkali lignin and cellulose/xylan/alkali lignin beads were found to be the most efficient support for entrapping lipase.

The pH stability of lipase entrapped in various wood component-based hydrogel beads was also measured at pH 3.0 (Table 1 and Fig. 3). The residual activity of lipase entrapped with 4% cellulose, 1% xylan, and 1% alkali lignin was 90% after 7 h incubation, whereas free lipase retained only 17% of the initial activity. The immobilized lipase prepared with 4% cellulose and 2% alkali lignin retained 38% of the initial activity after 192 h incubation. The half-life time of free lipase was 1.0 h. The half-life time of the lipase entrapped with 4% cellulose was 58-fold higher than that of the free lipase. Surprisingly, the half-life times of lipase entrapped in cellulose/alkali lignin and cellulose/xylan/alkali lignin were 82- and 66-times higher, respectively, than that of the free lipase. The activity of lipase entrapped in cellulose significantly decreased during the initial incubation, while the activity of lipase entrapped in cellulose/alkali lignin and cellulose/xylan/alkali lignin was well retained. These results show that cellulose is a very efficient matrix for increasing the pH stability of lipase from *C. rugosa*, and that the addition of alkali lignin to the cellulose composites can enhance the stabilization effect.

3.3. Entrapment of lipase into wood mimetic beads containing all three of the wood components

The cellulose/xylan/lignin composite containing the three major components of natural wood can be referred to as a wood mimetic composite and might be the most efficient support for stabilizing lipase with high activity. The kinds and compositions of the three wood components were changed to study the effect of each of them on the activity of the entrapped lipase (Table 2). The total content of the three components was fixed at 7%. The effect of cellulose on the activity of the entrapped lipase was investigated by using MCC, cotton linter cellulose of medium fiber form (MFC), and LFC (samples no. 9–11). The wood mimetic bead prepared with LFC showed the highest activity, probably due to the dense internal structure of the LFC hydrogel with a high DP. The cellulose/xylan/alkali lignin ratio was changed while maintaining the fixed total content (sample no. 11, 15–17). The highest activity was obtained with a cellulose/xylan/lignin ratio of 5:3:2. Interestingly, this ratio is very similar to that of natural wood. In a previous study, synthetic wood film prepared with cellulose/xylan/lignin in a 5:3:2 ratio showed the highest tensile strength and smooth surface (Simmons et al., 2011). At the cellulose/xylan/lignin ratio of 5:3:2, various lignins were used to study the effect of lignin on the activity of the entrapped lipase (samples no. 11–14). The highest activity was obtained with MWL from *P. albaglandulosa*. The effect of lignin in the wood mimetic beads on the activity of the entrapped lipase was very similar to that in the cellulose/lignin composite (samples no. 4–7). These results show that the activity of lipase entrapped in

Table 2
The activity of lipase entrapped in various wood mimetic hydrogel beads containing all three wood components.

Sample no.	Concentration of each component in wood solution (%)			Activity ($\times 10^{-3}$ μmol/min/bead)
	Cellulose	Xylan	Lignin	
9	3.5 (MCC)	2.1	1.4 (Alkali)	17.8 ± 1.4
10	3.5 (MFC)	2.1	1.4 (Alkali)	15.5 ± 1.7
11	3.5 (LFC)	2.1	1.4 (Alkali)	19.4 ± 1.9
12	3.5 (LFC)	2.1	1.4 (Organosolv)	21.2 ± 1.7
13	3.5 (LFC)	2.1	1.4 (*P. rigida*)	18.7 ± 0.3
14	3.5 (LFC)	2.1	1.4 (*P. albaglandulosa*)	21.9 ± 2.2
15	4.5 (LFC)	1.6	0.9 (Alkali)	13.9 ± 0.9
16	3.0 (LFC)	3.1	0.9 (Alkali)	14.6 ± 0.6
17	3.0 (LFC)	1.6	2.4 (Alkali)	16.0 ± 1.0

wood mimetic hydrogel beads can be controlled and optimized by changing the kinds and content of each wood component.

4. Conclusions

In this work, lipase from *C. rugosa* was successfully entrapped in wood mimetic hydrogel beads containing cellulose, xylan, and lignin by using [Emim][Ac]. The activity of the entrapped lipase could be controlled by changing the source and amount of each wood component. Interestingly, the highest activity of the entrapped lipase was obtained by using a ratio of wood components similar to that of natural wood. Lignin was the most important component in enhancing the thermal and pH stability of the entrapped lipase. These results suggest the potential application of lignin, which is currently recognized as waste material. Wood mimetic hydrogel beads proved to be good supports for enzyme immobilization and have many applications in the biomedical, bioelectronic, and biocatalytic fields owing to their biocompatibility, biodegradability, and controllable properties.

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