Facile Construction of Synergistic $\beta$-Glucosidase and Cellulase Sequential Co-immobilization System for Enhanced Biomass Conversion

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Abstract

Converting renewable cellulose into glucose via cellulase catalysis for further production of biofuel has been recognized as one of the most promising ways for solving energy crisis. However, the hydrolysis performance of immobilized cellulase was not satisfactory for practical application due to the reduced catalytic efficiency and lack of $\beta$-glucosidase (BG) component in cellulase. Here, a facile method was developed to sequentially co-immobilize BG and cellulase by polymeric microparticles with hierarchical structure. In this strategy, BG was firstly entrapped into the cross-linked poly(ethylene glycol) (PEG) microparticles via inverse emulsion polymerization initiated by isopropyl thioxanthone (ITX) under the irradiation of visible light, leaving the formed ITX semi-pinacol (ITXSP) dormant groups on surface of BG-loaded microparticles, which could further be activated by visible light irradiation and initiated a graft polymerization to introduce poly(acrylic acid) (PAA) brush on the PEG core. After that, cellulase was covalently bonded on the PAA chains via carbodiimide reaction. The synergic effect of BG and cellulase was verified in the dual enzyme immobilization system, which led to a better stability at a wide range of temperature and pH than free enzymes. The dual enzymes system exhibited excellent reusability, which could retain 75% and 57% of the initial activity after 10 cycles of hydrolysis of carboxyl methyl cellulose and 5 cycles of hydrolysis of filter paper, respectively, indicative of the potential in biofuel areas in a cost-effective manner.

Keywords

Visible light; Grafting polymerization; Enzyme immobilization; Cellulase

INTRODUCTION

The conversion of cellulose into fermentable reducing sugars to produce bioethanol is taken as an alternative energy source to replace fossil fuels.$^{[1,2]}$ Compared to conventionally hydrolysis route using acid or alkaline, enzymatic hydrolysis of cellulose by cellulase is a promising way due to its moderate reaction condition and sustainable technology.$^{[3-5]}$ However, high cost of cellulase has significantly hindered its commercial application.$^{[6,7]}$ Significant efforts have been expended to decrease the cost of sugar production, including genetic-engineering technology and cellulase immobilization.$^{[8,9]}$ Meanwhile, recycling of cellulase by immobilizing it on suitable matrix becomes a common solution for cellulase hydrolysis in a cost-effective manner.

The commonly used enzyme immobilization methods include entrapment, cross-linking, physical absorption, and covalent bonding.$^{[10]}$ Unlike generally used enzymes, the immobilization of cellulase should be specially arranged, for its catalytical substrate, cellulose, is not soluble in water. To successfully perform the hydrolysis of cellulose, cellulase must be absorbed on the backbone of cellulose first.$^{[11-13]}$ Thus, it is important to maintain the mobility of cellulase when it was immobilized on the support. Although plenty of efforts has been made to immobilize cellulase on various particles recently,$^{[14-17]}$ little attention was paid to increasing the mobility of cellulase. Fixing cellulase on flexible polymer brush, which is grafted on particle carriers, is a promising solution for improving the absorption capability of cellulase during enzymatic hydrolysis of cellulose. The immobilized cellulase could move with polymer brushes, adjusting itself to optimally bond with cellulose chain. The most comprehensively studied cellulase, which is mainly obtained from fungus, consists of three main components called endo-1,4-$\beta$-glucanase (EG), cellobiohydrolase...
For cellulose hydrolysis, EG randomly disrupts accessible regions of cellulose to provide more hydrolysable end sites. CBH catalyzes end site of cellulose to release cellobiose, while only a minor fraction is hydrolyzed to glucose, leading to the accumulation of cellobiose. However, the accumulation of cellobiose would aggressively deteriorate the activities of cellulase, which further limits the enzymatic hydrolysis rate of cellulose.[21,22] Due to the low proportion of BG in cellulase, the hydrolysis rate of cellobiose is restricted, impairing the catalytic efficiency of the whole hydrolysis reaction.[23,24] By adding extra BG, large amounts of glucose are produced from cellobiose, hence greatly improving the rate of hydrolysis of cellulose to glucose.[20] This synergistic effect of BG and cellulase has drawn wide attentions and been employed to co-immobilize the two enzymes.[25–28] However, in previous studies, enzymes were indiscriminately co-immobilized together or the matrices employed were limited in bulk materials, such as phase-forming copolymers, polyurethane foam, and low density polyethylene (LDPE) film, leading to the low enzyme loading, weak accessibility of enzymes to substrate, and limitation of mass transfer processes during hydrolysis of insoluble cellulose, which would not satisfy the real world applications.

Aiming to improve the efficiency of cellulose-to-glucose conversion catalyzed by cellulase, here we developed a strategy to sequentially co-immobilize BG and cellulase on carrier with novel hierarchical microparticle structure. By this hierarchical microparticle, BG and cellulase were separately immobilized in the inner and outer of the hierarchical microparticle to optimize enzyme loading distinct from that of immobilized in the inner and outer of the hierarchical microparticle to optimize enzyme loading distinct from that of in-discriminate co-immobilization system, and miniaturized enzyme immobilization matrices benefited the accessibility of substrate to enzymes, especially for the insoluble hydrolysis substrate cellulose. Here, visible light induced graft polymerization was firstly employed to construct the hierarchical microparticle carrier in water phase, demonstrating its superiority in enzyme immobilization. In this co-immobilization system, BG was encapsulated into cross-linked poly(ethylene glycol) (PEG) microparticles via visible light induced inverse emulsion polymerization, and cellulase was covalently bonded onto the flexible poly(acrylic acid) (PAA) brush grafted on PEG microparticle via visible light induced graft polymerization in a mild reaction condition. As cellulase was covalently bonded on the PAA brush grafted on PEG microparticles, the flexibility of chains of PAA provided cellulase with enough mobility space for the adsorption onto the cellulose. In this way, cellulose could be hydrolysed effectively by the synergistic effect of BG and cellulase.[19,29–31]

**EXPERIMENTAL**

**Materials**

Isopropylthioxanthone (ITX) and ethyl 4-dimethylaminobenzoyl (EDAB) were purchased from TH-UNIS Insight Co., Ltd. Acrylic acid (AA), 4-nitrophenyl β-D-glucopyranoside, Span 80, Tween 80, liquid paraffin, and carboxyl methyl cellulose (CMC) salt were obtained from Alfa Aesar (China) Chemical Co., Ltd. N-hydroxysulfosuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and 4-nitrophenyl-β-D-glucopyranoside were provided by Tokyo Chemical Industry Co., Ltd. Whatman No.1 filter paper was obtained from GE Ltd. (Shanghai, China). Cellulase from Trichoderma reesei (EC 3.2.1.4), β-glucosidase (BG) from almonds (EC 3.2.1.21), and poly(ethylene glycol) diacrylate (PEGDA, Mn = 575 g/mol) were purchased from Sigma Aldrich Chemical Co., Ltd. Acrylic acid was vacuum distilled to remove inhibitors, and all other materials were used as received.

**Entrapment of BG**

The entrapment of BG was similar to our previous works for papain entrapment by visible light induced inverse emulsion polymerization.[32] Briefly, 0.1 g of BG was dissolved in 3.6 g phosphate buffer saline (PBS) with 0.25% glutaraldehyde inside, and then 2.4 g of PEGDA was added. The mixture was dropped into organic phase (0.15 g of ITX, 0.10 g of EDAB, 0.18 g of Span 80, 0.42 g of Tween 80, and 60 g of liquid paraffin), and irradiated under visible light (6000 μW/cm², λ = 420 nm) for 2 h. The BG immobilized microparticles were obtained after washing with petroleum ether for one time and deionized water for 3 times.

**Photografting Polymerization of PAA**

Introduction of PAA on microparticles was achieved by visible light induced graft polymerization. Typically, the PEG microparticles were suspended in 10 wt% AA solution, and pH of the AA solution was adjusted to 5.0 by adding 1.0 mol/L NaHCO₃. Then the suspension system was irradiated by visible light (6000 μW/cm², λ = 420 nm) for 6 h. The graft yield was determined by acid-base titration with phenolphthalein as the indicator and calculated by the following equation.

\[
\text{Graft yield (\%) = } \frac{V_{\text{NaOH}} \times 0.01 \times 72.06}{W_{\text{AA}}}
\]

where \(V_{\text{NaOH}}\) is the volume of NaOH (0.01 mol/L) consumed in the acid-base titration, 72.06 is the molar molecular mass of AA, and \(W_{\text{AA}}\) is the initial weight of AA in the grafting polymerization.

In order to observe the grafted PAA, it was stained by rhodamine B by electrostatic interaction. The microparticles were dispersed into the rhodamine B solution (10⁻⁵ g/L) for 30 min at room temperature in the dark. After that, the stained microparticles were thoroughly washed with PBS buffer for several times for fluorescence observation.

**Conjugation of Cellulase to Poly(PEGDA)-g-PAA Particles**

Cellulase was conjugated to poly(PEGDA)-g-PAA particles using EDC/NHS coupling chemistry.[33] Here, 0.5 g of poly(PEGDA)-g-PAA particles were suspended in 20 mL of 0.10 mol/L MES buffer at pH 5, after which EDC (0.6 g) and NHS (0.36 g) (molar ratio is 1:1) were added and the solution was incubated at room temperature for 1 h. The activated particles were separated from the solution by centrifugation, and washed with PBS buffer (0.01 mol/L, pH 7.5) for three times. Then the activated particles and 500 μL of cellulase solution were mixed into 50 mL of citrate buffer solution (0.01 mol/L, pH 5.0), and the mixture was incubated at 4 °C for 5 h. In order to remove the adsorbed cellulase, the obtained precipitations were washed three times with 0.02 mol/L acetic acid (containing 1.0 mol/L NaCl and CaCl₂).[34]

**Enzyme Activity Assay**

The activity of BG was determined based on the reported
methods with 4-nitrophenyl β-D-glucopyranoside used as the substrate, and one unit of BG activity was defined as the amount of enzyme that made 1 μmol of p-nitrophenol released per minute under standard conditions. In this study, a certain amount of free or immobilized BG was dissolved in 1 mL of citrate buffer solution (0.01 mol/L, pH 5.0) in a 50 mL centrifuge tube, then 1 mL of 4-nitrophenyl (β-D-glucopyranoside solution (10 mg/mL) was added to the mixture at 50 °C and incubated for 10 min with gentle stirring. The reaction was terminated by adding 2 mL of 1.0 mol/L sodium carbonate, and the released p-nitrophenol was measured by the absorbance at 405 nm, and the residual activity of free and immobilized BG was calculated from the following equation:

\[ \text{Residual activity} \% = \frac{A}{A_{\text{max}}} \times 100 \]  

(2)

where \( A \) is the activity of free or immobilized BG at a certain pH or temperature, and \( A_{\text{max}} \) is the maximum activity of free or immobilized BG.

The effect of temperature on the BG activity was examined over a temperature range from 10 °C to 90 °C at pH 5.0 with a series of temperature gradients. The effect of pH on the enzyme activity was studied over a pH range from 3 to 9 with 0.01 mol/L citrate buffer solution (pH 3–5), PBS buffer (pH 6–8), and sodium carbonate buffer (pH 9) at 50 °C, respectively. Reusability of immobilized enzyme was also evaluated by the enzymatic reaction of 10 min, and then the immobilized BG was washed with citrate buffer solution (0.01 mol/L, pH 5.0) three times before starting a new cycle.

Cellulase activity was determined based on the standard procedure of IUPAC with some modifications, and one unit of cellulase activity was defined as the amount of cellulase that hydrolyzes CMC to produce 1 μmol of glucose per minute. 100 μL of free cellulase or equivalent immobilized cellulase was mixed with 10 mL of 1.0 wt% CMC solution (dissolved in 0.01 mol/L citrate buffer solution, pH 5.0) to perform the hydrolysis. The reaction was carried out at 50 °C for 1 h, and the amount of glucose produced during the reaction was determined by a biosensor (SBA-40D from Biology Institute of Shandong Academy of Sciences, China).

The effect of temperature and pH on the cellulase activity was examined with the same parameters of BG activity assay. Reusability of immobilized cellulase was studied under the same enzymatic reaction condition for 60 min. The recovered immobilized cellulase was washed with 0.01 mol/L citrate buffer solution (pH 5.0) for three times before starting a new cycle.

Hydrolysis of Cellulose

Whatman No.1 filter paper was used as the substrate for hydrolysis catalyzed by the dual enzymes system. In this process, 10 mg of filter paper and 1.0 g of dual enzymes immobilized microparticles were dispersed into 10 mL of 0.01 mol/L citrate buffer solution (pH 5.0) and incubated at 50 °C for 24 h. The amount of glucose produced during the reaction was determined by a biosensor (SBA-40D). The glucose yield was calculated by the following equation:

\[ \text{Glucose yield} \% = \frac{c_{\text{glc}} \times V_{\text{glc}} \times 0.9}{W} \]  

(3)

where \( c_{\text{glc}} \) is the concentration of glucose determined by the biosensor, \( V_{\text{glc}} \) is the volume of hydrolysis reaction solution, and \( W \) is the initial weight of filter paper added for hydrolysis. For the recycling experiment, the microparticles were separated by centrifugation and washed by citrate buffer solution for three times after each 24 h reaction. The concentration of released glucose was determined by a biosensor (SBA-40D).

**Instruments**

Dynamic light scattering and Zeta PALS (Brookhaven Instruments Corp., USA) were used to detect the diameter and the zeta potential of the microparticles. Scanning electron microscopy (SEM, JSM-7500F from JEOL Japan Electronics Co., Ltd., Japan) was used to determine the morphology of the microparticles. Fourier transform IR (FTIR) spectra of the microparticles were recorded on a Nicolet NEXUS 670 Fourier-transform infrared spectrometer (Thermo Nicolet Co., Ltd., USA). The elementary composition was probed by XPS (ESCALAB 250 from Thermo Fisher Scientific Co., Ltd., USA) with a monochromator. A UV-Vis spectrophotometer (H3900, HITACHI, Japan) was used to determine the activity of free and immobilized enzymes. The fluorescence images were recorded on a confocal laser scanning microscope (CLSM, Leica SP8 from Leica, Germany) at an excitation wavelength of 490 nm. The concentration of glucose was determined with a biosensor (SBA-40D from Biology Institute of Shandong Academy of Sciences, China).

**RESULTS AND DISCUSSION**

**Immobilization of BG and Cellulase on Poly(PEGDA)-g-PAA Particles**

Considering the delicate nature of enzymes, a facile and mild technique, visible light-induced living radical polymerization, was introduced to achieve this design. Scheme 1 shows the synthetic routes of sequentially co-immobilizing BG and cellulase. BG was firstly in situ encapsulated into the cross-linked PEG microparticles by visible light induced inverse emulsion polymerization initiated by ITX and EDAB as described in our previous work. Under the irradiation of visible light, ITX could be excited from singlet state (ITXS) to triplet state (ITXT) through intersystem crossing. Then ITXT abstracted the hydrogen from EDAB to form the EDAB initiating radical and ITX semipinacol (ITXSP) radicals. The as-formed EDAB radicals initiated the polymerization of PEGDA to form cross-linked PEG network, while ITXSP radicals had no initiation ability and coupled with propagation radical to form dormant groups onto the surface of PEG microparticles. As BG was dissolved together with PEGDA, it could be in situ entrapped after polymerization. The ITXSP dormant group could be re-dissociated under irradiation of visible light to generate surface radicals, which would still initiate the polymerization of acrylic acid. Therefore, the PAA brush could be introduced on the cross-linked PEG microparticles. Although the pKa of PAA was 4.3, carboxyl still remained on PAA chain at pH 5.0. Hence, cellulase could be covalently bonded on the grafted PAA chains by the amide bond formation via carbodimide activation.

To verify the feasibility of our strategy, cross-linked PEG microparticles without BG were firstly prepared by visible light induced inverse emulsion polymerization. The surface chemical composition of PEG microparticles was detected by XPS. Fig. 1(a) shows the C 1s core-level spectra of poly(PEGDA), which could be curve-fitted into three com-
ponents with binding energies at about 284.6, 286.2, and 288.7 eV, attributed to the C−C/C−H, C−O/C−S, and C=O species, respectively.\textsuperscript{[42]} In addition, the S 2p core-level spectrum appeared at the binding energy of about 168 eV (Fig. S1 in the electronic supplementary information, ESI),\textsuperscript{[43]} indicating the presence of ITXSP on the surface of the microparticles, which acted as dormant groups for the followed graft polymerization.

The introduction of PAA brushes on microparticles was verified by zeta potential measurements with pH varied from

\[ \text{Fig. 1} \quad (a) \text{XPS core-level spectrum of C 1s of the PEG microparticles. (b) Zeta potential of PEG microparticles (□) and hairy microparticles (○) as a function of pH. CLSM images of hairy microparticles: (c) bright, (d) fluorescence, and (e) merged. The scale bars are 10 μm.} \]
2.0 to 8.0. In Fig. 1(b), the isoelectric point of the microparticles was found at about pH = 2.4, and PAA grafted microparticles became negatively charged as the pH increased. The zeta potential became steady at ~60 mV as pH > 4.0, which is in good accordance with the pKₐ of PAA. In contrast, the PEG microparticles without PAA brushes were electroneutral in pH 2.0–8.0, which proved that the negative charge of hairy particles came from the PAA brushes. The grafting of PAA onto the PEG microparticles was also confirmed by FTIR analysis by determining the increase of the absorption of C=O stretching vibration of PAA at 1730 cm⁻¹, which has been normalized to the absorption of O=C—O stretching vibration (1110 cm⁻¹) derived from the main chain of PEG (Fig. S2a in ESI). With the increase of visible light irradiation time, the absorption ratio increased from 0.9 to 1.4 (Fig. S2b in ESI), indicating the grafting amount of PAA increased accordingly. For an accurate investigation, the grafting yield of PAA was measured by acid-base titration. In Fig. S3 (in ESI), the grafting yield increased almost linearly with the irradiation time, which is in accordance with the results in Fig. S2 (in ESI). Furthermore, increasing irradiation intensity or monomer concentrations would also lead to an increase in grafting yield of PAA (Fig. S3b in ESI). To visualize the polymer brushes, rhodamine B was used to stain the charged PAA by electrostatic interaction. As observed by CLSM (Figs. 1c−1e), the PEG microparticles could not be stained by rhodamine B and showed no fluorescence. In comparison, red fluorescence surrounding the PEG microparticles was observed, verifying that PAA was successfully grafted on the microparticles with the size about 10 μm.

**Activity and Reusability of Enzyme Immobilization Systems**

For the immobilization of dual enzymes, BG was added to the aqueous phase of the inverse emulsion and in situ encapsulated into the cross-linked PEG microparticles after polymerization. And the CLSM images demonstrated the uniform distribution of BG in the microparticles (Fig. S4 in ESI). As BG was entrapped into the microspheric cross-linked PEG network, miniaturized enzyme immobilization matrices would benefit the accessibility of cellobiose to BG, which could consequently enhance the mass transfer processes.

After introducing PAA chain, cellulase was covalently conjugated with the grafted brush by the formation of amide bond. The immobilization of cellulase on the surface of microparticles could be determined by XPS, where no BG was encapsulated into the microparticles. As shown in Fig. 2(a), the C 1s core-level spectrum of hairy microparticles attached with cellulase could be curve-fitted into five peaks. The peaks with binding energies at about 284.6, 286.2, and 288.7 eV were attributed to the C—C, C—O, and C=O species, respectively. Another two peaks, 285.9 eV for C—N species and 287.4 eV for O=C—N species, were associated with the amino and peptide bonds in cellulase. In addition, the N 1s core-level spectrum at the binding energy of about 399 eV also appeared for the cellulase immobilized microparticles (Fig. 2b). From the analysis of XPS as well as FTIR in Fig. S5 (in ESI), it can be concluded that cellulase was successfully conjugated to the PAA brushes, and the immobilization yield of cellulase was determined to be around 85%, exhibiting the highest activity of 3.5 U/mg when the concentration of EDC was 30 mg/mL (Fig. S6 in ESI).

Because PAA was grafted onto the PEG microparticles in the presence of BG, the effect of graft polymerization process on the activity of the encapsulated BG was studied. The results are shown in Fig. 3. It is found that 87% of the initial activity was retained after the graft polymerization, which suggests that the moderate reaction condition of visible light induced graft polymerization had limited impairment on the activity of immobilized BG. The reusability of immobilized BG

![Fig. 2](https://doi.org/10.1007/s10118-020-2437-3)

**Fig. 2** The XPS core-level spectra of (a) C 1s and (b) N 1s of the hairy microparticles immobilized with cellulase.

![Fig. 3](https://doi.org/10.1007/s10118-020-2437-3)

**Fig. 3** The residual activity of entrapped BG during the visible light induced graft polymerization.
and cellulase was evaluated individually in Fig. S7 (in ESI), especially for immobilized BG with 91% activities remained after 10 cycles, indicating the satisfactory protection of BG from PEG networks. Besides, after covalently binding onto PAA chains, cellulase exhibited a good reusability as well. From the results, the superiority of the visible light induced polymerization was demonstrated in the field of bioactive molecules immobilization.

The effects of temperature, pH, and visible light irradiation on the activity of BG and cellulase after co-immobilization were investigated. When BG and cellulase were immobilized individually by PEG microparticles or hairy microparticles, both of them exhibited a better temperature and pH stability than free enzymes (Fig. S8 in ESI).

Similarly, as they were co-immobilized on hairy microparticles, the results presented in Fig. 4(a) reveal that the optimum temperature of dual enzymes system was 50 °C, which is similar to that of free enzyme. However, the activity of free cellulase decreased at temperatures above 60 °C and reduced to 65% at 70 °C. In comparison, the activity of the immobilized dual enzymes dropped down slowly and retained 80% relative activity at 70 °C, which clearly indicates that the immobilization of dual enzymes improved their stability at high temperature. The optimum pH of the immobilized dual enzymes and free cellulase was found at 5.0, and the residual activity of immobilized enzymes showed the identical trend of free cellulase with variation of pH from 3.0 to 8.0 (Fig. 4b).

These results suggest that our strategy to immobilize dual enzymes by the hairy microparticles structure is favorable for their activity retention.

Since the individually immobilized BG and cellulase exhibited a good reusability, the dual-enzyme co-immobilization system should keep its reusability for cycles as well. As expected, excellent recycling performance was demonstrated. As a result, dual-enzyme co-immobilization system retained >75% original activity after 10 cycles with CMC as the substrate (Fig. 5), which exhibited a better reusability than reported works,[46–49] implying its potential for catalytically hydrolyzing the insoluble cellulose.

![Fig. 4](https://doi.org/10.1007/s10118-020-2437-3)

**Fig. 4** Effect of (a) temperature and (b) pH on the activity of free cellulase (in dash) and immobilized dual enzymes (in solid).

![Fig. 5](https://doi.org/10.1007/s10118-020-2437-3)

**Fig. 5** Reusability of immobilized dual enzymes for hydrolysis of CMC.

### Hydrolysis Performance of Dual-enzyme Co-immobilization System on Converting Solid Cellulose

Although the sequentially co-immobilized dual-enzyme system had superior reusability for hydrolysis of CMC, conditions may not be favorable in bioethanol industry due to the bulkiness of insoluble cellulose, presence of inhibitors, and nonproductive enzyme absorption. Therefore, it is necessary to investigate the application of immobilized dual enzymes in the mimicked industrial conditions.

Here we used filter paper as substrate for the evaluation. As cellulase was immobilized on the flexible PAA brushes on the microparticles, it could provide sufficient mobility for cellulase to absorb on the insoluble cellulose of filter paper (Fig. 6a), which will be beneficial to the hydrolysis of bulk cellulose. As shown in Fig. 6(b), cellulase immobilized directly on the surface of the PEGDA/PAA copolymer microparticles showed the slowest hydrolysis rate to filter paper, of which the glucose yield was only 15% after 48 h of hydrolysis (50 °C, pH 5.0). However, the glucose yield could reach 40% after 48 h of hydrolysis for cellulase immobilized on hairy microparticles, which indicated that the mobility of cellulase on PAA brush was significantly improved, demonstrating the enhanced mass transfer of cellulose to immobilized cellulase. As BG was co-immobilized on the core of hairy microparticles, the glucose yield increased to 55% and 71% for the ratio of immobilized BG and cellulase of 0.25 and 0.5, respectively. These results verify the synergic effect of BG and cellulase, and demonstrate our expectation of enhanced biomass conversion.
The reusability of immobilized enzymes was also investigated with 1.0 mg/mL filter paper as the substrate and reacted at 50 °C, pH 5.0 for 24 h. The relative activity in each cycle was determined and the result is shown in Fig. 6(c). It shows that the dual-enzyme immobilization system could retain 57% of original activity after five cycles, implying its potential in the industrial production in a cost-effective way. Moreover, in the hydrolysis of filter paper (Fig. 6c), the co-immobilization system was in the certain conditions of pH 5.0 and 50 °C for 48 h with 9% loss of its original activities, and 120 h with 43% loss of its original activities, demonstrating its superior pH and thermal stability, which would benefit the long time usage of the dual-enzyme co-immobilization system in the real world applications.

CONCLUSIONS

In this study, a rationally designed strategy to sequentially co-immobilize BG and cellulase for enhanced conversion of cellulose to glucose was developed. Activities of both enzymes after co-immobilization were effectively retained by the mild reaction condition offered by visible light induced polymerization. The superiority of immobilizing cellulase on flexible polymer brushes has been demonstrated, and the synergistic effect of BG and cellulase after co-immobilization was verified by the hydrolysis of filter paper, which provided a promising technique for enhanced biomass conversion and lowered cost for biofuels as the enzymes recycled.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at https://doi.org/10.1007/s10118-020-2437-3.

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