Korean J. Chem. Eng., **27**(2), 469-473 (2010)
DOI: 10.1007/s11814-010-0096-8

Recovery of cellulases by adsorption/desorption using cation exchange resins

Jin Chuan Wu†, Kiat Rui Ng, Joanne Chong, Kai Jie Yang, Xiao Ping Lam, Chai Teck Nam, and Aninto Jati Nugroho

Institute of Chemical & Engineering Sciences, Agency for Science, Technology and Research (A*Star), 1 Pesek Road, Jurong Island, Singapore 627833
(*Received 6 February 2009 • accepted 29 September 2009*)

Abstract−Cellulases from *Trichoderma reesei* were recovered by adsorption in sodium acetate buffer at lower pH using cation exchange resins followed by desorption at higher pH. The weakly acidic ion exchange resin WK10 was found to be the best among the six resins tested in terms of the enzyme activity recovery. The optimal pH values for the adsorption and desorption were 4.0 and 8.0, respectively, and the optimal adsorption and desorption times were both 5 h. Almost 100% of the initial cellulase activity was recovered under the optimal conditions with the supplement of β-glucosidase, which was unable to be efficiently recovered due to its strong adsorption (95.7%) but poor desorption (1.9%).

Key words: Cellulase, β-Glucosidase, Adsorption, Desorption, Ion Exchange Resin, Activity Recovery

INTRODUCTION

Lignocellulose is the most abundant renewable resource on earth. Large-scale production of biofuels and chemicals from this low-cost sustainable resource would provide huge environmental, economical and social benefits [1,2]. Lignocellulose, however, cannot be directly digested by microbes of commercial interest and needs to be pretreated into its three major components: cellulose, hemicellulose and lignin [3,4]. During pretreatment, hemicellulose is usually effectively degraded to oligosaccharides and fermentable sugars (D-xylose, L-arabinose and D-glucose), but cellulose is hardly degraded and needs to be further subjected to acid or enzymatic hydrolysis to convert it into glucose before it can be utilized by microbes [5-7]. Compared to acid hydrolysis, enzymatic hydrolysis is more promising as it eliminates the use of a large amount of chemicals, achieves high glucose yield and avoids the formation of inhibitory byproducts [8-10,20]. Cellulase, the enzyme that catalyzes cellulose hydrolysis to glucose, is actually a complex mixture of at least 3 key enzymes: endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21). Endoglucanase randomly attacks the internal bonds in cellulose chain and acts mainly on the amorphous cellulose. Exoglucanase hydrolyzes from the chain ends and produces predominantly cellobiose, which is cleaved to two glucose molecules by β-glucosidase [11,12]. A key factor that hinders the commercialization of enzymatic cellulose hydrolysis is the high cost of cellulase enzymes. Attempts have been made to reduce the cost of the enzymatic hydrolysis step by either producing cellulase enzymes by microbial cultivation [13,14] or by recycling the free enzymes after hydrolysis [15,16]. Wen et al. [13] reported the production of cellulase/β-glucosidase by the mixed fungi culture of *Trichoderma reesei* and *Aspergillus phoenicis* on dairy manure and got a cellulase product suitable for hydrolyzing manure cellulose with a ratio of β-glucosidase activity to filter paper activity of 0.41. Adsul et al. [14] produced cellulases by cultivating *Penicillium janthinellum* and its mutants for lactic acid production from bagasse cellulose by simultaneous saccharification and fermentation. Steele et al. [15] recovered cellulase enzymes by ultrafiltration following the hydrolysis of ammonium fiber explosion-treated corn stover and achieved 60-66.6% recovery for cellulase and 76.4-88% for cellobiase. Ramos et al. [16] recycled Celluclast cellulases based on their re-adsorption on the substrate after hydrolysis and succeeded in recycling the enzyme mixture for five consecutive steps with the supplement of β-glucosidase, which was not expected to adsorb efficiently on the cellulosic residue. Cation exchange chromatography has been extensively utilized for separation and purification of cellulase complexes by NaCl gradient elution for characterizing the individual enzymes [21-23]. In terms of the recycle and reuse of cellulases for cellulose hydrolysis, recovery of all the three key enzymes as a mixture with minimized salt contamination is more beneficial for industrial applications.

Here we report the recovery of cellulase enzymes as a complex mixture using commercially available acidic ion exchange resins by simply adjusting the pH of the solutions for adsorption and desorption of the cellulases.

MATERIALS AND METHODS

1. Materials

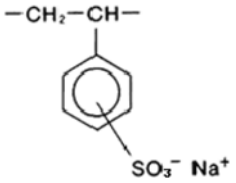
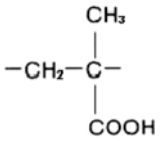
Cellulase from *Trichoderma reesei* ATCC 26921, β-glucosidase from almond, cellulose, cellobiose and 3,5-dinitrosalysilic acid were purchased from Sigma. Diaion ion exchange resins WK10, HPK25, PK-216, PK212, SK112 and SK1B were from Mitsubishi Chemicals, Japan (Table 1). All other chemicals were of an analytical grade and obtained commercially.

2. Adsorption/Desorption Experiments

A typical adsorption/desorption experiment was as follows. To 40 ml sodium acetate buffer (10 mM, pH 4.0) containing cellulases

†To whom correspondence should be addressed.
E-mail: wu_jinchuan@ices.a-star.edu.sg

Table 1. Properties of the Diaion ion exchange resins used

Grade name	HPK25	PK212	PK216	SK1B	SK112	WK10
Chemical structure						
Shipping density (g/*l*-R)	790	765	780	825	855	615
Total exchange capacity (meq/ml-R)	1.7 min	1.5 min	1.75 min	2.0 min	2.1 min	2.5 min
Water content (%)	37-47	52-58	46-52	43-50	32-42	53-59
Particle size on 1,180 μm				5% max		
through 300 μm				1% max		
Effective size (mm)				0.40		
Uniformity coefficient				1.6 max		

[0.38 mg/ml] was added 700 mg WK10 resin followed by shaking (350 rpm) for 5 h at 25 °C. The resin was collected by filtration, put into 40 ml sodium acetate buffer (10 mM, pH 8.0) and shaken (350 rpm) at 25 °C for 5 h. The amounts of proteins adsorbed and desorbed were calculated according to the differences of protein amounts in the solutions before and after the adsorption/desorption. Protein concentration was determined based on the UV absorption at 280 nm. The cellulases desorbed were defined as a percentage of the total proteins released in the desorption solution to the total proteins adsorbed on the resin before the desorption.

3. Determination of Cellulase Activity

Cellulase solution (1 ml) from the adsorption/desorption experiments was mixed with 39 ml of sodium acetate buffer (10 mM, pH 5.0) followed by addition of cellulose (2.5 mg/ml) with or without the supplement of β-glucosidase (0.31 mg/ml). The mixture was incubated at 37 °C under shaking (180 rpm) for 20 min to 2 h followed by removal of cellulose by centrifugation at 10,000 rpm for 3 min. The glucose concentration in the supernatant was determined using the 3,5-dinitrosalisylic acid (DNS) method. The glucose contained in the enzyme preparations was subtracted from the total glucose. Specific activity of the cellulase enzyme was expressed as mg glucose h^{-1} mg^{-1} proteins. Activity recovery was defined as the percentage of the total activity of the cellulases desorbed from the adsorbents to that of the cellulases before the adsorption.

RESULTS AND DISCUSSION

1. Effect of pH on Cellulase Adsorption

Two resins, WK10 and HPK25, were chosen as the representatives of weakly and strongly acidic resins, respectively, to investigate the effect of buffer pH on cellulase adsorption (Fig. 1). For both resins, the optimal pH was found to be 4.0. It is well known that buffer pH affects the charge density of enzyme surface, leading to the variation of electrostatic interaction between enzyme and resins. When buffer pH is below the isoelectric point (pI) of an enzyme, the enzyme surface is positively charged, favoring its combination with the acidic resins. As cellulase is actually a mixture of at least three key enzymes, endoglucanase, exoglucanase and β-glucosidase [11,12], the optimal pH 4.0 should be a compromise of the three key enzymes in terms of the highest adsorption of the total enzymes. Cellulase enzymes are mostly slightly acid proteins (pI= 3-6) and moderately stable below pH 3 and above pH 10 [19]. The optimal pH (4.0) for adsorption is very near to the optimal pH (5.0) for the cellulolytic activity. The buffer pH for adsorption was fixed at 4.0 in the subsequent studies.

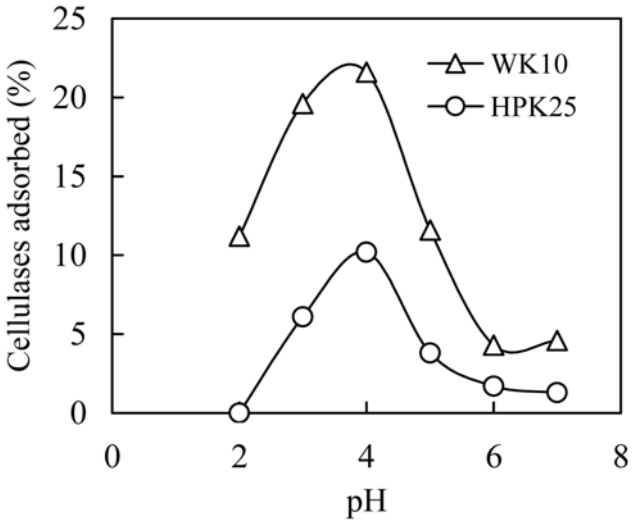


Fig. 1. Effect of pH on cellulase adsorption. Adsorption conditions: 10 mM sodium acetate buffer 40 ml, cellulases 0.38 mg/ml, adsorbents 200 mg, time 3 h, temperature 25 °C, shaking speed 350 rpm.

2. Effect of Adsorbent Type on Cellulase Adsorption

Fig. 2 shows that among the six resins tested, WK10 showed the highest cellulase adsorption and HPK25 was the second. Other resins showed much lower adsorption compared to these two. The highest adsorption capacity of WK10 might be ascribed to its lowest shipping density and highest total exchange capacity (Table 1). Although all the other resins except WK10 have the same chemical structure, their adsorption capacities were quite different, indicating that there existed other mechanisms such as the simple physical adsorption for the cellulase uptake in addition to the ion exchange.

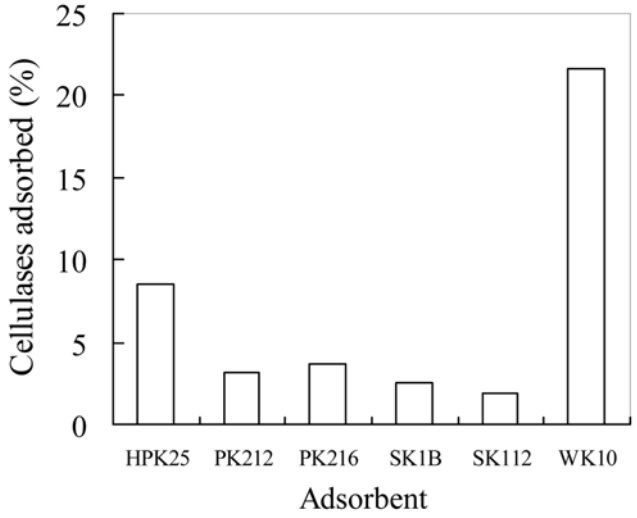


Fig. 2. Effect of adsorbent type on cellulase adsorption. Adsorption conditions: 10 mM sodium acetate buffer 40 ml, pH 4.0, cellulases 0.38 mg/ml, adsorbents 200 mg, time 3 h, temperature 25 °C, shaking speed 350 rpm.

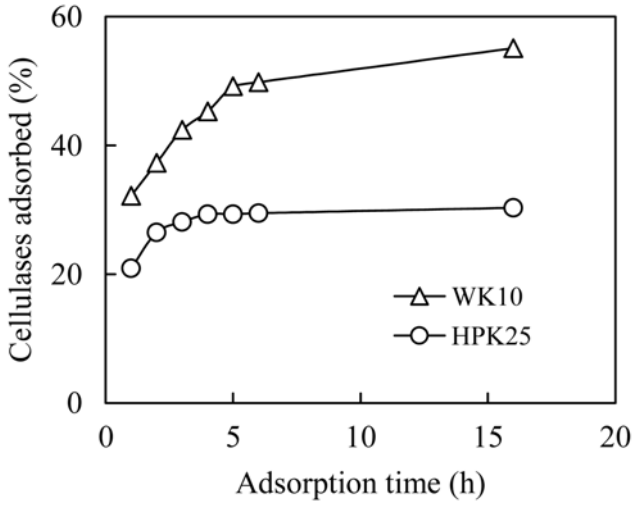


Fig. 4. Effect of time on cellulase adsorption. Adsorption conditions: 10 mM sodium acetate buffer 40 ml, pH 4.0, cellulases 0.38 mg/ml, adsorbents 700 mg, temperature 25 °C, shaking speed 350 rpm.

This presumption is further intensified by the high adsorption capacity of WK 10, which is a weakly acidic resin and has a different chemical structure from others. In terms of recovering more enzymes through adsorption/desorption, stronger adsorption of the enzymes is required. However, too strong adsorption may lead to a difficulty in desorption, resulting in lower total enzyme recovery. We selected both WK10 and HPK25 for the subsequent studies.

3. Effect of Adsorbent Amount on Cellulase Adsorption

As shown in Fig. 3, the enzyme adsorption obviously increased with increasing adsorbent amount. In comparison, the enzyme adsorption on WK10 was more significantly affected by the adsorbent amount than that on HPK25, which might be ascribed to the higher total exchange capacity of WK10 than HPK25 (Table 1). The enzyme adsorption became less changed when the HPK25 amount was over 400 mg, but it rapidly increased with increasing WK10 amount until 700 mg. For convenience of comparison, the amount of 700 mg was selected for both resins in the subsequent studies.

4. Effect of Time on Cellulase Adsorption

Fig. 4 shows that the cellulase adsorption on WK10 rapidly increased with increasing adsorption time in the first 5 h and became less changed afterwards. In the case of HPK25, the cellulase adsorption rapidly increased in the first 2 h and became less varied after

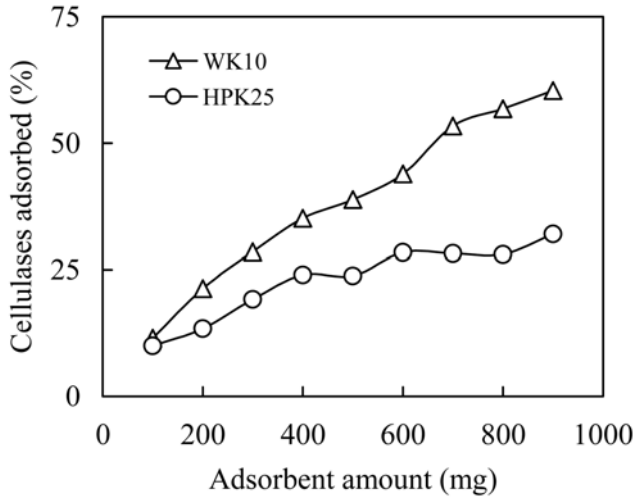


Fig. 3. Effect of adsorbent amount on cellulase adsorption. Adsorption conditions: 10 mM sodium acetate buffer 40 ml, pH 4.0, cellulases 0.38 mg/ml, time 3 h, temperature 25 °C, shaking speed 350 rpm.

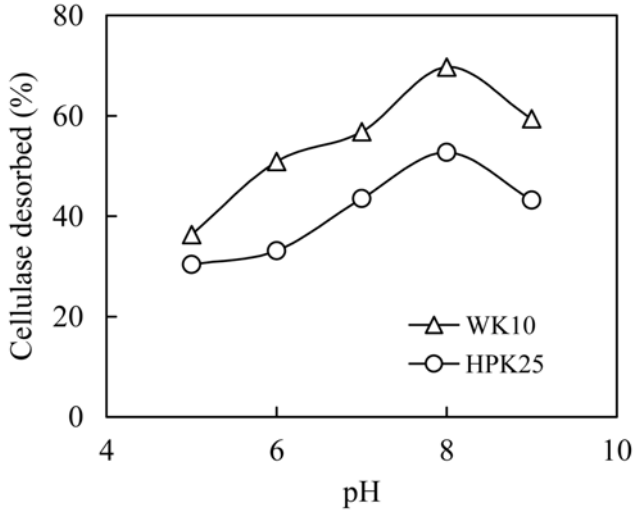


Fig. 5. Effect of buffer pH on cellulase desorption. Adsorption was conducted in 40 ml sodium acetate buffer (10 mM) containing 0.38 mg/ml cellulases and 700 mg adsorbents with shaking at 350 rpm and 25 °C for 5 h. Desorption conditions: 10 mM sodium acetate buffer 40 ml, time 2 h, shaking speed 350 rpm, temperature 25 °C. The pH 8.0 solution was prepared by adjusting the pH of 10 mM sodium acetate with 2 M HCl or NaOH.

that. The capacity of cellulase adsorption on WK10 was about twice that on HPK25. The adsorption time was fixed at 5 h for both resins in the subsequent studies.

5. Effect of pH on Cellulase Desorption

The adsorbed cellulases were desorbed by altering the buffer pH. As mentioned earlier, when the buffer pH is higher than the pI of an enzyme, the enzyme surface becomes negatively charged, leading to the desorption of the adsorbed enzymes from the resins as a result of the repulsion between the enzyme and resins. The optimal desorption pH was found to be 8.0 (Fig. 5), regardless of the type of the resins. Further increasing the aqueous pH led to a decrease in cellulase desorption, which might have been caused by the deformation or even precipitation of cellulases at higher pHs. We did observe protein precipitation at higher pHs. The desorption experiments were conducted at pH 8.0 in the subsequent studies.

6. Effect of Time on Cellulase Desorption

Fig. 6 shows that the optimal cellulase desorption was observed after 5 h, regardless of the type of resins. Further increasing desorption time resulted in a decrease in cellulase desorption, possibly due to the enzyme deactivation and precipitation. The desorption of cellulases from WK10 was obviously higher than that from HPK25. Considering that the adsorption of cellulases on WK10 was also higher than that on HPK25 (Fig. 1), WK10 was selected as the best resin for the subsequent studies on activity recovery.

7. Effect of Desorption Time on Cellulase Activity Recovery

The recovery of cellulase activity was tested with or without the supplement of β-glucosidase to the cellulase mixture after the desorption (Fig. 7). When no β-glucosidase was supplemented, the activity recovery was only 20-30%. However, when β-glucosidase was supplemented to the desorbed cellulase mixtures, the activity recovery was significantly increased. After desorption for 4-5 h, the activity recovery reached almost 100% with the supplement of β-glucosidase. The lower activity recovery without the supplement of β-glucosidase indicates that β-glucosidase was not effectively recovered by the adsorption/desorption.

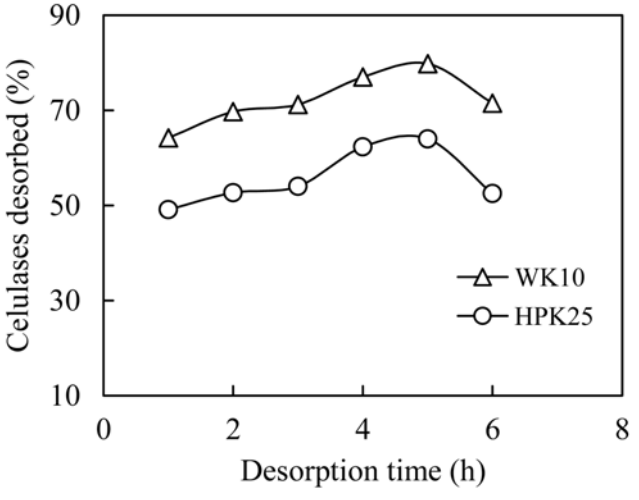


Fig. 6. Effect of time on cellulase desorption. Adsorption was conducted in 40 ml sodium acetate buffer (10 mM, pH 4.0) containing 0.38 mg/ml cellulases and 700 mg adsorbents with shaking at 350 rpm and 25 °C for 5 h. Desorption conditions: 10 mM sodium acetate buffer 40 ml, pH 8.0, shaking speed 350 rpm, temperature 25 °C.

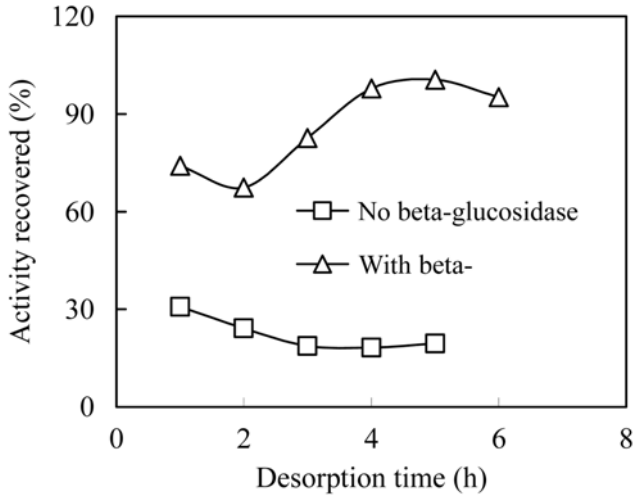


Fig. 7. Effect of desorption time on recovery of cellulase activity. Adsorption was conducted in 40 ml sodium acetate buffer (10 mM, pH 4.0) containing 0.38 mg/ml cellulases and 700 mg WK10 with shaking at 350 rpm and 25 °C for 5 h. Desorption conditions: 10 mM sodium acetate buffer 40 ml, pH 8.0, shaking speed 350 rpm, temperature 25 °C. Enzyme activity was assayed by incubating the diluted enzyme solutions in 10 mM sodium acetate buffer (pH 5) containing cellulose (2.5 mg/ml) at 37 °C with shaking (180 rpm) followed by detection of the produced glucose using 3,5-dinitrosalysilic acid method.

To investigate the exact reason leading to the inefficient recovery of β-glucosidase, we tested the adsorption/desorption of cellulases supplemented with β-glucosidase in the beginning and the adsorption/desorption of β-glucosidase alone (Table 2). Clearly, β-

Table 2. Recovery of β-glucosidase by adsorption/desorption using WK10

Operation mode	Adsorption (%)	Desorption (%)	Protein recovery (%)	Activity recovery (%)
β-Glucosidase supplemented with cellulases[a]	58.0	59.5	34.5	100
β-Glucosidase only[b]	95.7	1.9	1.8	-

[a]Adsorption was conducted in 40 ml sodium acetate buffer (10 mM, pH 4.0) containing cellulases (0.38 mg/ml) supplemented with β-glucosidase (0.31 mg/ml) and 700 mg WK10 for 5 h. Desorption was performed in 40 ml sodium acetate buffer (10 mM, pH 8.0). The shaking speed and temperature were, respectively, 350 rpm and 25 °C for both adsorption and desorption. Enzyme activity was assayed by incubating diluted enzyme solutions in sodium acetate buffer (10 mM, pH 5) containing cellulose (2.5 mg/ml) at 37 °C with shaking (180 rpm) followed by detection of the produced glucose using 3,5-dinitrosalysilic acid method

[b]Adsorption and desorption were conducted following the same procedures as described above except that the adsorption buffer contained only β-glucosidase (0.31 mg/ml)

glucosidase was strongly adsorbed (95.7%) by WK10 but poorly desorbed (1.9%) from it, leading to the low enzyme activity recovery. In the case of mixed cellulase enzymes, the high protein recovery was thus primarily contributed by the recovery of exoglucanase and endoglucanase. The poor desorption of β-glucosidases might be ascribed to their higher pI (up to 9-10) [17], which may lead to a positively charged enzyme surface at the desorption pH of 8.0, unfavorable for the stripping of the enzymes from the adsorbent. The strongly bound β-glucosidase on the resins may interfere with the adsorption of cellulases at the next step. A possible way of removing the β-glucosidase is to immerse the resins in solutions of a higher pH, which may help release the β-glucosidase but also may completely denature it due to the extreme condition. It is worth mentioning that even under the optimal conditions, only about 35-40% of the total proteins were recovered, but the total activity recovery of cellulases was almost 100% with the supplement of β-glucosidase. It is unlikely that all the exoglucanase and endoglucanase were recovered. A possible reason of the complete activity recovery is that the ratio of the three cellulase enzymes became more reasonable after the adsorption/desorption operations improving their synergistic effect [11,12]. Another reason might be the insufficient amount of β-glucosidase available in the original cellulase preparation. It has been reported that β-glucosidase only comprises as little as 1% of the total mass of cellulolytic enzymes in *T. reesei* [17], and supplement of β-glucosidase into *T. reesei* cellulases is often required to improve the total activity of the cellulolytic enzymes [4,18].

CONCLUSIONS

Cellulases from *Trichoderma reesei* were successfully recovered by simple adsorption and desorption using commercially available weakly acidic ion exchange resin WK10. Under the optimal conditions, the activity recovery reached almost 100% with the supplement of β-glucosidase. However, the cellulase activity recovery was only 20-30% without the supplement of β-glucosidase, which was proved to be caused by the strong adsorption (95.7%) of β-glucosidase on WK10 but poor desorption (1.9%) from it. The high activity recovery is ascribed to the enhanced synergistic effect of the cellulase enzymes after the adsorption/desorption or the insufficient amount of β-glucosidase available in the original cellulase preparation.

ACKNOWLEDGMENTS

This work was supported by the Agency for Science, Technology and Research (A*Star) of Singapore (ICES/07-113002).

REFERENCES

1. Y. Lin and S. Tanaka, *Appl. Microbiol. Biotechnol*., **69**, 627 (2006).
2. J. D. McMillan, *Renew. Energy*, **10**, 295 (1997).
3. B. C. Saha, L. B. Iten, M. A. Cotta and Y. V. Wu, *Process Biochem*., **40**, 3693 (2005).
4. B. H. Um, M. N. Karim and L. L. Henk, *Appl. Biochem. Biotechnol*., **105-108**, 115 (2003).
5. Y. C. Chung, A. Bakalinsky and M. H. Penner, *Appl. Biochem. Biotechnol*., **121-124**, 947 (2003).
6. Y. Nakamura, T. Sawada and E. Inoue, *J. Chem. Technol. Biotechnol*., **76**, 879 (2001).
7. B. Palmarola-Adrados, M. Calbe and G. Zacchi, *Appl. Biochem. Biotechnol*., **113-116**, 989 (2004).
8. J. B. Kristensen, J. Borjesson, M. H. Bruun, F. Tjerneld and H. Jorgensen, *Enzyme Microb. Technol*., **40**, 888 (2007).
9. S. B. Kim and J. W. Chun, *Appl. Biochem. Biotechnol*., **113-116**, 1023 (2003).
10. X. Pan, D. Xie, N. Gilkes, D. J. Gregg and J. N. Saddler, *Appl. Biochem. Biotechnol.*, **121-124**, 1069 (2005).
11. J. Lee, *J. Biotechnol*., **56**, 1 (1997).
12. J. Szczodrak and J. Fiedurex, *Biomass Energy*, **10**, 367 (1996).
13. Z. Wen, W. Liao and S. Chen, *Appl. Biochem. Biotechnol*., **121-124**, 93 (2005).
14. M. G. Adsul, A. J. Varma and D. V. Gokhale, *Green Chem*., **9**, 58 (2007).
15. L. P. Ramos, C. Breuil and J. N. Saddler, *Enzyme Microb. Technol*., **15**, 19 (1993).
16. B. Steele, S. Rai, J. Nghiem and M. Stowers, *Appl. Biochem. Biotechnol*., **121-124**, 901 (2005).
17. C. S. Evans, *Appl. Microbiol. Biotechnol*., **22**, 128 (1985).
18. M. J. Negro, P. Manzanares, I. Ballesteros, J. M. Oliva, A. Cabanas and M. Ballesteros, *Appl. Biochem. Biotechnol*., **105-108**, 87 (2003).
19. S. Hayashida, K. Mo and A. Hosoda, *Appl. Environ. Microbiol*., **54**, 1523 (1988).
20. K. D. Oh and C. Kim, *Korean J. Chem. Eng*., **4**, 105 (1987).
21. J. Singh, N. Batra and R. C. Sobti, *J. Ind. Microbiol. Biotechnol*., **31**, 51 (2004).
22. C. Y. Hu and L. P. Lin, *World J. Microbiol. Biotechnol*., **19**, 515 (2003).
23. Y. H. Li, M. Ding, J. Wang, G. J. Xu and F. Zhao, *Appl. Microbiol. Biotechnol*., **70**, 430 (2006).