Bioprocess Biosyst Eng (2016) 39:133–140
DOI 10.1007/s00449-015-1497-1

ORIGINAL PAPER

Cost evaluation of cellulase enzyme for industrial-scale cellulosic ethanol production based on rigorous Aspen Plus modeling

Gang Liu[1] · Jian Zhang[1] · Jie Bao[1]

Received: 16 May 2015 / Accepted: 26 October 2015 / Published online: 5 November 2015

Abstract Cost reduction on cellulase enzyme usage has been the central effort in the commercialization of fuel ethanol production from lignocellulose biomass. Therefore, establishing an accurate evaluation method on cellulase enzyme cost is crucially important to support the health development of the future biorefinery industry. Currently, the cellulase cost evaluation methods were complicated and various controversial or even conflict results were presented. To give a reliable evaluation on this important topic, a rigorous analysis based on the Aspen Plus flowsheet simulation in the commercial scale ethanol plant was proposed in this study. The minimum ethanol selling price (MESP) was used as the indicator to show the impacts of varying enzyme supply modes, enzyme prices, process parameters, as well as enzyme loading on the enzyme cost. The results reveal that the enzyme cost drives the cellulosic ethanol price below the minimum profit point when the enzyme is purchased from the current industrial enzyme market. An innovative production of cellulase enzyme such as on-site enzyme production should be explored and tested in the industrial scale to yield an economically sound enzyme supply for the future cellulosic ethanol production.

Keywords Cost evaluation · Cellulase · Ethanol · Lignocellulose · Aspen Plus modeling

✉ Jie Bao
jbao@ecust.edu.cn

[1] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China

Introduction

High cost of cellulase enzyme is one of the major barriers for commercialization of bioethanol production from lignocellulose biomass [1, 2]. Currently, the cellulase cost evaluation methods were complicated and various controversial or even conflict results were presented [3]. Some claimed that the cellulase enzyme cost was only varied from $0.1 to $0.4/gal of ethanol, supporting that the current technology was already economically sound [4–6]. On the other hand, others pointed out that the cellulase enzyme cost was still as high as up $0.69/gal ethanol [7] or even $1.47/gal ethanol [1] by quoting the current market prices of cellulase products in detergent, textile and animal feed industries [8]. Such as high cellulase cost certainly gave a negative evaluation on the feasibility of commercial cellulosic ethanol production using the available cellulase technology.

On the other hand, on-site or near-site production of cellulase enzyme is proposed as a promising way to the significant reduction of enzyme cost below $0.3/gal ethanol, because of its simplified purification and logistics, as well as the potential cheap carbon source utilization from lignocellulose material [9]. Hong et al. [10] compared the two enzyme production methods and found that the on-site mode reduced up to 30 % of the cellulase enzyme cost compared to the off-site mode. Takimura et al. [11] found that up to 70 % reduction of cellulase cost was achieved using the on-site produced enzyme than that using the purchased enzyme.

Due to the importance of cellulase enzyme cost evaluation, it is definitely necessary to make the evaluation on the uniform platform. In this study, the Aspen Plus model developed by NREL [12] on the scale of daily 2000 tons of dry corn stover capacity was selected for evaluating the minimum ethanol selling price (MESP) under the varying enzyme supply modes, enzyme prices, process parameters, as well as enzyme loading. The analysis of cellulase enzyme cost on overall cellulosic ethanol and impact of the commercialization were discussed based on the calculation.

Materials and methods

Process model

The process model used in this study is based on the design report about process design and economics for biochemical conversion of lignocellulosic biomass to ethanol which issued by NREL in 2011 [12]. The process simulation is developed using Aspen Plus software (AspenTech Co., Cambridge, MA, USA) by NREL with the changes on cellulase enzyme supply mode. The physical property data and the process parameters of the Aspen Plus process model from the NREL report [13]. The plant size is 2000 metric tons dry corn stover each day with an 8410 operating hours per year. Figure 1 shows the schematic flowsheet of cellulose ethanol production, in which the corn stover is pre-processed and delivered for dilute sulfuric acid pretreatment. The hydrolysis is carried out using on-site produced or purchased enzymes. The monosaccharides (both glucose and xylose) obtained are converted to ethanol. The fermentation broth is distillated and then purified to 99.5 % (v/v) by the molecular sieve column. The liquid is treated by anaerobic and aerobic digestions and then the treated water is recycled. The solid residue, biogas and the digestion sludge are combusted to generate steam for electricity generation and process heating.

The Aspen Plus model of the ethanol production from corn stover is shown in Fig. 2, including nine areas of feedstock handing, pretreatment and conditioning, enzymatic hydrolysis and fermentation, cellulase enzyme production, product purification, wastewater treatment, residue combustion, storage, and utilities system. Figure 2a shows that the process design with the cellulase enzyme is provided by the on-site production unit in the process flowsheet. Figure 2b shows the same as Fig. 2a, except the cellulase enzyme is provided by purchasing from the outside industrial enzyme makers instead of the production inside this process flowsheet; therefore, only eight areas are included in Fig. 2b with no enzyme production unit. The area A100 is the feed handling, in which the corn stover is temporarily stored, crushed and dust removed by a uniform-format feedstock supply system. The area A200 is pretreatment and conditioning, in which the feedstock is treated by dilute acid at high temperature for a short time at 30 % solids content. The area A300 is enzymatic hydrolysis and fermentation, in which the corn stover is completely hydrolyzed by the cellulase enzyme provided by either the on-site produced cellulase enzyme or the purchased cellulase enzyme, and then co-fermented by *Zymomonas mobilis*. The cellulase enzyme dosage in this study is set to 20 mg protein/g cellulose, same to the NREL report [12] unless mentioned elsewhere. The area A400 is cellulase enzyme supply unit, in which the enzyme is produced by *Trichoderma reesei* in the process flowsheet in Fig. 2a, or provided by purchasing from the outside markers. The area A500 is product recovery, in which the ethanol fermentation broth is purified into the 99.5 % fuel ethanol product by solid–liquid separation, distillation and molecular sieve adsorption. The area A600 is wastewater treatment, in which the process wastewater is treated by anaerobic and aerobic digestion, and then recycled. The area A700 is storage unit for chemicals consumed and produced in the process. The area A800 is residues' combustion, in which the solid residue and biogas are combusted to produce high-pressure steam for process and heat electricity generation. The area A900 is utilities, in which the cooling water, chilled water, process water and power are provided.

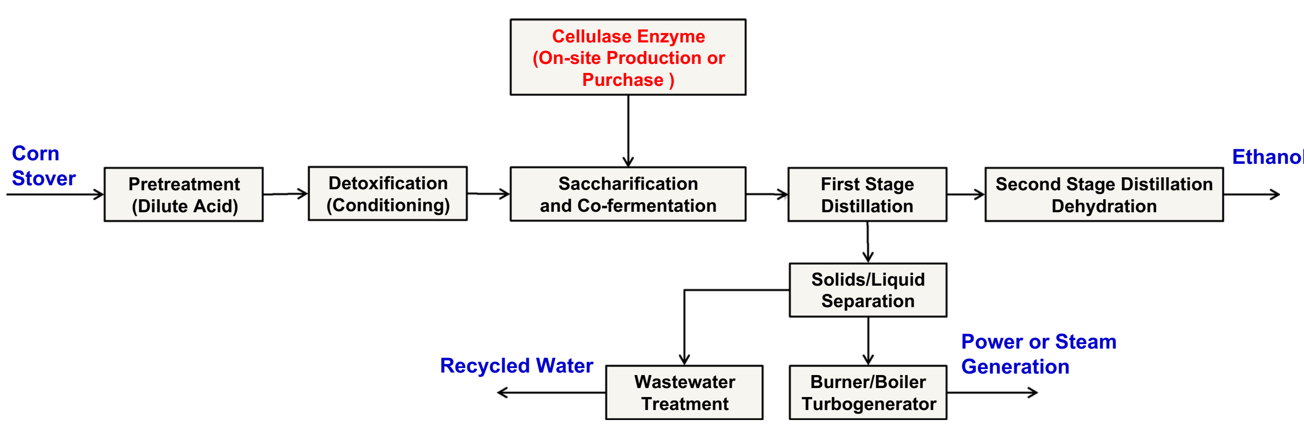

Fig. 1 Conceptual diagram of cellulosic ethanol production process from corn stover feedstock

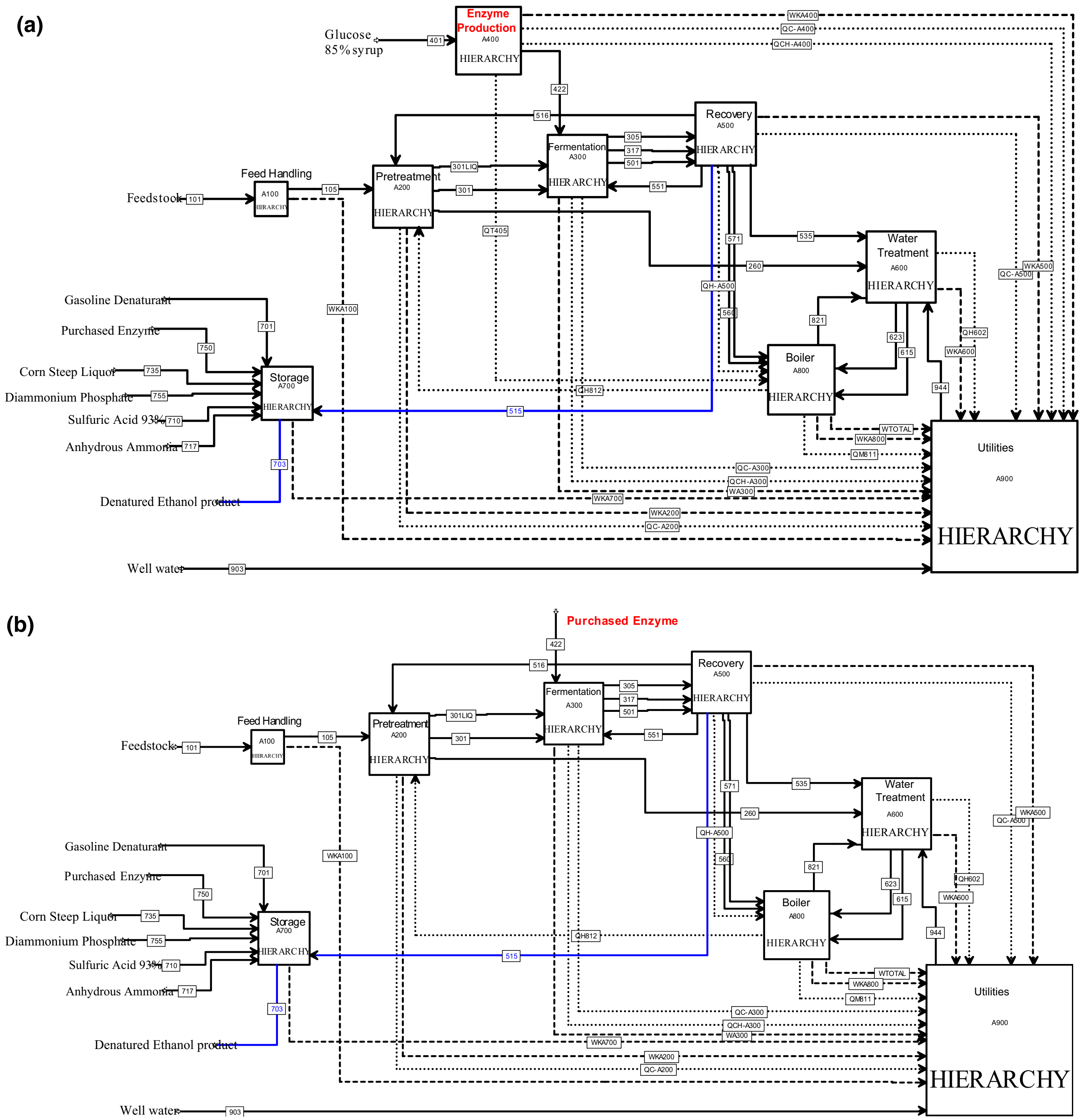

Fig. 2 Flowchart of cellulosic ethanol production process from corn stover feedstock on Aspen Plus platform. **a** Using the on-site produced cellulase enzyme; **b** using the purchased cellulase enzyme

Economic evaluation method

The material and energy balance data from the Aspen Plus simulation are used to assist in determining the number and size of equipment and chemical usage. 2007 is used as the reference year and all the costs were adjusted to 2007 using Eq. (1). The number and size of equipment items, the equipment quotes, the installation factors, the chemical cost, the employee salaries and cost index are directly cited from the original document on economic calculation model "Economic model to accompany the 2011 NREL Biochem design report" available in http://www.nrel.gov/extranet/biorefinery/aspen_models/downloads/DW1102A/DW1102A.xlsm, in which the capital costs were adjusted using the plant cost index from Chemical Engineering magazine to a common basis year of 2007, the chemical costs were cited from the industrial inorganic chemical index based on SRI Consulting, the employee salaries were calculated using the labor indices by the United States Department of Labor Bureau of Labor Statistics [12].

$$\text{Cost} = (\text{base cost})\left(\frac{\text{2007 cost index}}{\text{base year index}}\right) \quad (1)$$

Equipment cost, warehouse cost, site development cost, additional piping cost, and indirect costs which include prorateable costs, field expenses, project contingency, etc. make up the total capital investment (TCI). Variable operating costs are only occurring in the process of production; it includes the cost of raw materials, waste treatment cost and by-products credits. Fixed operating costs are normally occurring in the whole production process. These costs include labor and all kinds of other costs. Once TCI, variable and fixed operating costs are determined, a discounted cash flow rate of return analysis to determine the minimum ethanol selling price (MESP, $/gal) required to obtain a net present value of zero for a 10 % internal rate of return after taxes. The assumptive parameters used in the discounted cash flow analysis are also followed the NREL model [12].

Results and discussion

Impact of enzyme supply mode on the enzyme cost and MESP

Cellulase enzyme can be supplied by either purchased from enzyme makers, or on-site production. When the enzyme is purchased for the use of the proposed cellulosic ethanol plant, five different cellulase prices are cited from the industrial enzyme market and the published reports. For uniform citation of enzyme quality from different makers and sources, the price per kg enzyme protein ($/kg protein) is used as the indicator.

Enzyme case 1

Assume that the cellulase enzyme protein with the same value to soy protein, the cheapest protein on the market [1]: $1.25/kg protein (2011$).

Enzyme case 2

Assume that the cellulase enzyme protein is purchased under the price estimated by Kazi et al. [7]: $507 per metric ton of the enzyme broth containing 10 % proteins, equivalent to $5.07/kg (2007$).

Enzyme case 3

Assume that the cellulase enzyme is purchased from Novozymes with the claimed enzyme cost of $0.50/gal ethanol (http://novozymes.com/en/news/news-archive/Pages/45713.aspx). In the proposed process [12], the ethanol production is 21,672.41 kg/h and the enzyme usage is 579 kg protein/h, which are equivalent to 7256.35 gal ethanol/h (21,672.41 kg/h is divided by the ethanol density 0.789, then transformed to gal). The enzyme protein price is equivalent to 7256.35 × 0.50/579 = $6.27/kg (2010$).

Enzyme case 4

Assume that the cellulase enzyme protein is purchased under the price estimated by Klein-Marcuschamer et al. [1] on the fungal cellulase production: $10.14/kg (2007$).

Enzyme case 5

Assume that the cellulase enzyme is Youtell #6 purchased from Chinese enzyme market at 13 Chinese Yuan (RMB)/kg enzyme with the protein content of 9 % [14], equivalent to 13/6.2 (the present exchange rate of dollar to RMB)/0.09 = $23.30/kg (2013$).

The cases study of cellulase enzymes are conducted on Aspen Plus model and Microsoft Excel to give the enzyme cost for producing one gallon of ethanol and the minimum ethanol selling price (MESP) from the proposed plant as shown in Table 1.

Table 1 shows when the enzyme purchase method is used, the enzyme price is crucially important to the MESP and even drives the MESP from cost effective to a non-competitive situation at all. Currently, the ethanol price is averaged $2.31/gal from 2005 until 2014 on the industrial fuel market (http://www.tradingeconomics.com/commodity/ethanol). Therefore, when the enzyme costs are in enzyme case 1 ($1.25/kg protein), case 2 ($5.07/kg protein), and case 3 ($6.27/kg protein), the overall fuel ethanol costs indicated by MESP for the proposed plant are $1.89/gal, $2.20/gal, and $2.24/gal, respectively. In these three cases, the ethanol cost is below the average market price, thus the commercialization feasibility exists. When the enzyme costs are in enzyme case 4 ($10.14/kg protein) and case 5 ($23.30/kg protein), the MESPs are $2.61/gal and $3.46/gal, respectively, indicating a negative profit for the proposed plant, thus the commercialization feasibility is lost.

At the lowest cellulase price in enzyme case 1 (same to soybean protein), the enzyme cost is $0.09/gal ethanol and the MESP is only $1.89/gal ethanol, but this case has little possibility because the protein from microbial fermentation

Table 1 Impact of the cellulase enzyme supply modes on MESP

	Purchased mode					On-site mode[a]
	Enzyme case 1	Enzyme case 2	Enzyme case 3	Enzyme case 4	Enzyme case 5	Enzyme case 6
Enzyme unit price ($/kg protein)	1.25	5.07	6.27	10.14	23.30	4.24[c]
Enzyme cost ($/gal ethanol)	0.09	0.40	0.44	0.81	1.66	0.34
Feedstock cost ($/gal ethanol)	0.74	0.74	0.74	0.74	0.74	0.74
Conversion cost ($/gal ethanol)	1.06	1.06	1.06	1.06	1.06	1.07
MESP ($/gal ethanol)[b]	1.89	2.20	2.24	2.61	3.46	2.15

[a] The on-site production cost of the cellulase enzyme used in NREL design [12]

[b] MESP is the summary of the three items, enzyme cost, feedstock cost, and conversion cost

certainly is more expensive than the cheapest soybean protein. On the other extreme, the highest cellulase price in enzyme case 5 is a made-in-China product purchased from the industrial enzyme market with the open price tag. The enzyme cost is as much as $1.65/gal and the MESP is up to $3.46/gal, accounting for 48 % of the total ethanol cost. The price is approximately the same with that of textile and animal feed cellulases. This may reflect the most realistic case of the current enzyme cost when the enzyme is purchased from the market, although it may contain certain profit margin between the selling price and the real production cost.

Enzyme case 6 represents the on-site enzyme production cost cited from the NREL design [12] using filamentous fungus as fermenting strain and glucose as the primary carbon source. As shown in Table 1, the enzyme cost is only $4.24/kg protein and $0.34/gal ethanol, and the MESP is $2.15/gal. The cost is close to the purchase enzyme case 2 and below the average fuel ethanol price on the market ($2.31/gal), indicating a great promising way for effective supply of cellulase enzymes in the future. Although the total installed equipment cost increases, the on-site mode provides obvious advantages of no fungus cell removal (cells die in the anaerobic ethanol fermentation), enzyme concentrating and purifying steps, as well as logistic transportation and storage [15]. However, these advantages still require the validation of industrial-scale operations.

Enzyme cost under different process parameters

Enzyme cost is highly dependent on the process technology level such as hydrolysis yield, fermentation productivity, and overall ethanol yield [2]. In this analysis, five process cases are summarized based on the previous designs or publications, then the enzyme cost and the MESP are calculated on the Aspen Plus model for the proposed plant. The base case (process case 1) is the NREL design [12], in which 90 % of cellulose is converted to glucose with the cellulase dosage of 20 mg protein/g cellulose at 20 % solids content, and the fermenting microorganism *Z. mobilis* converts 95 % of glucose, 85 % of xylose, and 85 % of arabinose to ethanol:

Process case 1

2011 NREL design as described above [12].

Process case 2

Process case 1 with no arabinose conversion to ethanol, based on the fermenting strain *Z. mobilis* with relatively poor utilization of arabinose [16].

Process case 3

Process case 2 with 40 % of xylose conversion to ethanol, instead of 85 %, based on the fact that available strains for xylose utilization commonly are moderate around 40 % of conversion efficiency, instead of 85 % [17].

Process case 4

Process case 2 with 0 % of xylose conversion to ethanol, representing the processes using ethanol fermenting strains without xylose utilization capacity [18].

Process case 5

Process case 4 with 85 % of cellulose conversion to glucose, 90 % of glucose conversion to ethanol, based on the most conventional biorefinery processes with moderate conversion yield [19].

Table 2 shows the changes of cellulase enzyme cost and MESP with varied processing parameters when the cellulase is provided by on-site and purchase. With the declining overall ethanol yield from process case 1–5, the cellulase enzyme cost and the corresponding MESP at both enzyme supply methods increase sharply. When the enzyme is provided by on-site production, the enzyme cost increases from $0.34/gal ethanol to $0.56/gal ethanol from

Table 2 Impact of the varying process parameters on the enzyme cost and MESP

	Process case 1	Process case 2	Process case 3	Process case 4	Process case 5
Cellulose to glucose (%)	90	90	90	90	85
Glucose to ethanol (%)	95	95	95	95	90
Xylose to ethanol (%)	85	85	40	0	0
Arabinose to ethanol (%)	85	0	0	0	0
Ethanol yield (gal/ton dry CS)	87.08	83.66	70.22	58.31	52.91
Ethanol production (million gal/year)	61.0	58.6	49.2	40.8	37.1
On-site enzyme production at the cost of the enzyme case 6					
Enzyme cost ($/gal ethanol)	0.34	0.35	0.42	0.51	0.56
Feedstock cost ($/gal ethanol)	0.74	0.77	0.92	1.11	1.22
Conversion cost ($/gal ethanol)	1.07	1.10	1.27	1.50	1.60
MESP ($/gal ethanol)	2.15	2.23	2.61	3.12	3.38
Purchased enzyme at the price of the enzyme case 5					
Enzyme cost ($/gal ethanol)	1.66	1.72	2.05	2.47	2.71
Feedstock cost ($/gal ethanol)	0.74	0.77	0.92	1.11	1.22
Conversion cost ($/gal ethanol)	1.06	1.11	1.32	1.59	1.76
MESP ($/galethanol)	3.46	3.60	4.29	5.17	5.69

process case 1 to 5, and the corresponding MESP increases from the $2.15 to $3.38/gal. Compared to the average market price of fuel ethanol ($2.31/gal), the profit switch point turns to be negative from process case 3 ($2.61/gal), in which the xylose conversion to ethanol is reduced to 40 %. When the enzyme is provided by purchase under enzyme case 5 in which the enzyme is purchased from industrial market with open price tag ($23.30/kg protein), all the process cases are in negative profit, indicating the awesome situation of the enzyme cost: even the most optimistic process case could not meet the profit point on industrial plant, if the cellulase enzyme is purchased from the current industrial enzyme market at the open price.

The results indicate that even under the same enzyme dosage, the low process performance leads to the reduced ethanol production, then inevitably increased enzyme cost per ton of ethanol product. An innovative way of cellulase enzyme supply different from the conventional purchase way should be explored and tested in the industrial scale to yield an economically sound enzyme supply for the future cellulosic ethanol industry.

Impact of enzyme loading on the enzyme cost and MESP

In theory, the cellulase is not consumed in the hydrolysis process of biomass. However, over time cellulase may become inactivated by denatured or irreversibly combination with solid particles, especially when the presence of lignin [20]. The cellulase enzyme loading is very high in current cellulosic ethanol production process. If the enzyme loading can be reduced, the cost of enzyme will reduce naturally. The cellulase enzyme loading can be decreased by cellulase recycle using, improving cellulase performance or improving the hydrolysis condition [9]. With the continuous improvement of pretreatment technologies and enzyme preparations, the enzyme amount consumed in hydrolysis process will be decreased. Assume that cellulosic ethanol production at a high conversion rate of saccharification and fermentation process (process case 1), MESP and enzyme cost are calculated using different cellulase enzyme loading, as shown in Table 3.

Table 3 shows that cellulose ethanol and enzyme cost linear decrease when cellulase enzyme loading reduced from 35 to 5 mg/g cellulose. Compared to the average market selling price ($2.13/gal) in recent years, when on-site production enzyme loading more than 30 mg/g cellulose, and purchased enzyme loading more than 10 mg/g cellulose, the cellulose ethanol will have not market competitiveness. When on-site production enzyme loading as low as 5 mg/g cellulose (case 1), MESP reduced to $1.89/gal, and cellulase enzyme cost is $0.08/gal ethanol. When purchased enzyme is consumed only 5 mg/g cellulose during ethanol production, MESP is $2.21/gal, and enzyme cost is $0.41/gal ethanol. However, amylase, one of the enzymes that degrading corn starch for fermentation, costs only $0.02–0.04/gal ethanol [21]. By comparison, cellulase enzyme cost is very high. Enzyme loading still needs to be further reduced as much as possible.

When on-site enzyme production mode is used, ethanol conversion cost increases slightly because the on-site production of enzyme leads to the cost increase on wastewater treatment and utility cost. When purchased enzyme mode is used, ethanol conversion cost does not change as long as

Table 3 Impact of the enzyme loading on the enzyme cost and MESP

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Enzyme loading (mg/g cellulose)	5	10	15	20	25	30	35
On-site enzyme production at the cost of the enzyme case 6							
Enzyme cost ($/gal ethanol)	0.08	0.18	0.26	0.34	0.42	0.51	0.59
Feedstock cost ($/gal ethanol)	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Conversion cost ($/gal ethanol)	1.07	1.07	1.07	1.07	1.08	1.08	1.08
MESP ($/gal ethanol)	1.89	1.99	2.07	2.15	2.24	2.33	2.41
Purchased enzyme at the price of the enzyme case 5							
Enzyme cost ($/gal ethanol)	0.41	0.83	1.25	1.66	2.08	2.49	2.91
Feedstock cost ($/gal ethanol)	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Conversion cost ($/gal ethanol)	1.06	1.06	1.06	1.06	1.06	1.06	1.06
MESP ($/gal ethanol)	2.21	2.63	3.05	3.46	3.88	4.29	4.71

Only the cellulase enzyme dosage is changed, but all the other operation data kept same to show a simple case analysis on the MESP increased by increased enzyme dosage. The ethanol yield change with the increased cellulase enzyme dosage is not considered

the same enzyme dosage is used, although the overall MESP changes significantly because of the enzyme prices change when different enzyme production mode and enzymes from different makers are used.

Conclusion

The dosage of enzyme and the ethanol yield are the main factors affecting the enzyme cost. Based on actual purchased price of cellulase enzyme in industrial enzyme market and the conventional ethanol yield, enzyme cost is up to $2.71/gal ethanol, accounting for 48 % of the MESP. On-site enzyme production can significantly reduce enzyme cost, providing a promising option for the large-scale cellulose ethanol production. Reducing the enzyme loading is also an important way to decrease the cellulose ethanol cost. The enzyme cost for commercial-scale cellulosic ethanol is still expensive and the cost reduction strategies announced in the previous reports are still waiting for the industrial data verification.

Acknowledgments This research was supported by the National High-Tech Program of China (2012AA022301, 2014AA021901) and the Natural Science Foundation of China (21306048).

References

1. Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA, Blanch HW (2012) The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnol Bioeng 109:1083–1087
2. Chovau S, Degrauwe D, Bruggen BV (2013) Critical analysis of techno-economic estimates for the production cost of lignocellulosic bio-ethanol. Renew Sust Energ Rev 26:307–321
3. Geddes CC, Nieves IU, Ingram LO (2011) Advances in ethanol production. Curr Opin Biotechnol 22:312–319
4. Sassner P, Galbe M, Zacchi G (2008) Techno-economic evaluation of bioethanol production from three different lignocellulosic materials. Biomass Bioenerg 32:422–430
5. Aden A, Ruth M, Ibsen K, Jechura J, Neeves K, Sheehan J, Wallace B (2002) Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. National Renewable Energy Laboratory, Golden
6. Wingren A, Galbe M, Roslander C, Rudolf A, Zacchi G (2005) Effect of reduction in yeast and enzyme concentrations in a simultaneous-saccharification-and-fermentation-based bioethanol process: technical and economic evaluation. Appl Biochem Biotech 122:485–499
7. Kazi FK, Fortman J, Anex R, Kothandaraman G, Hsu D, Aden A, Dutta A (2010) Techno-economic analysis of biochemical scenarios for production of cellulosic ethanol. National Renewable Energy Laboratory, Golden
8. Phitsuwan P, Laohakunjit N, Kerdchoechuen O, Kyu KL, Ratanakanokchai K (2013) Present and potential applications of cellulases in agriculture, biotechnology, and bioenergy. Folia Microbiol 58:163–176
9. Merino ST, Cherry J (2007) Progress and challenges in enzyme development for biomass utilization. Adv Biochem Engin Biotechnol 108:95–120
10. Hong Y, Nizami AS, Pourbafrani M, Saville BA, MacLean HL (2013) Impact of cellulase production on environmental and financial metrics for lignocellulosic ethanol. Biofuels Bioprod Bioref 7:303–313
11. Takimura O, Yanagida T, Fujimoto S, Minowa T (2013) Estimation of bioethanol production cost from rice straw by on-site enzyme production. J JPA Petrol Inst 56:150–155
12. Humbird D, David R, Tao L, Kinchin C, Hsu D, Aden A (2011) Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: dilute-acid pretreatment and enzymatic hydrolysis of corn stover. National Renewable Energy Laboratory, Golden
13. Wooley R, Putsche V (1996) Development of an ASPEN PLUS physical property database for biofuels components. National Renewable Energy Laboratory, Golden
14. Fang ZH, Zhang J, Lu QM, Bao J (2013) Process development of short-chain polyols synthesis from corn stover by combination of enzymatic hydrolysis and catalytic hydrogenolysis. Biotechnol Rep 3:15–20

15. Kovacs K, Szakacs G, Zacchi G (2009) Comparative enzymatic hydrolysis of pretreated spruce by supernatants, whole fermentation broths and washed mycelia of *Trichoderma reesei* and *Trichoderma atroviride*. Bioresour Technol 100:1350–1357
16. Deanda K, Zhang M, Eddy C, Picataggio S (1996) Development of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering. Appl Environ Microb 62:4465–4470
17. Rudolf A, Baudel H, Zacchi G, Hahn-Hagerdal B (2007) Simultaneous saccharification and fermentation of steam-pretreated bagasse using *Saccharomyces cerevisiae* TMB3400 and Pichia stipitis CBS6054. Biotechnol Bioeng 99:783–790
18. Wyman CE, Spindler DD, Grohmann K (1992) Simultaneous saccharification and fermentation of several lignocellulosic feedstocks to fuel ethanol. Biomass Bioenerg 3:301–307
19. Olofsson K, Bertilsson M, Liden G (2008) A short review on SSF—an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnol Biofuels 1:1–14
20. Kumar L, Arantes V, Chandra R, Saddler J (2012) The lignin present in steam pretreated softwood binds enzymes and limits cellulose accessibility. Bioresour Technol 103:201–208
21. Schubert C (2006) Can biofuels finally take center stage? Nat Biotechnol 24:777–784