Process Optimization of *β*-glucosidase Production by a Mutant Strain, *Aspergillus niger* C112

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Enzymatic saccharification is a key step in the green conversion of lignocellulose to biofuels and other products. A key deficiency in common biocatalytic systems, such as *Trichoderma reesei*, is the insufficient presence of *β*-glucosidase (BGL). This study intended to develop an efficient process of BGL production as an enhancement to the *T. reesei* system. The authors investigated the process optimization of BGL by the mutant strain *Aspergillus niger* C112, which was previously developed in the authors' laboratory. The culture medium and process (carbon, nitrogen, temperature, and pH) were optimized for cost-effective BGL production, which led to a maximum BGL activity of 8.91 ± 0.35 U/mL. In addition, the dynamics of the physio-chemical parameters (zeta potential and dissolved organic matter) of the process were studied and showed good correlations to the yield of BGL. Furthermore, a three-dimensional excitation-emission matrix fluorescence spectroscopy was successfully applied for analyzing the component, origin, and dynamics of dissolved organic matter, which contributed to a further understanding and optimization of BGL production.

Keywords: β-glucosidase; Aspergillus niger; Process optimization; Fermentation; Biorefinery; Excitation-emission matrix fluorescence spectroscopy

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INTRODUCTION

The enzymatic saccharification of sugar-based macromolecules (cellulose and hemicellulose) of lignocellulose biomass is crucial for the production of biofuels (*e.g.* ethanol and butanol) and other products (*e.g.* lactic acid and polyhydroxyalkanoates) (Hasunuma *et al*. 2013; Maity 2015). Under the synergistic actions of at least three cellulase families (endoglucanase, exocellobiohydrolase, *β*-glucosidase, *etc*.), cellulose is hydrolyzed and saccharified smoothly and orderly into glucose, and then converted into targeted products (Fujita *et al*. 2004; Jeoh *et al*. 2017). Currently, industrially and commercially used cellulase preparations have been mostly produced by the fungi *Trichoderma* spp. (such as *Trichoderma reesei*), which hold sufficient and robust cellulase such as endoglucanase and exocellobiohydrolase (Barati and Sadegh Amiri 2015). Nevertheless, because of the insufficient presence of *β*-glucosidase (BGL) in *T. reesei*, cellulase preparations improvement should be carried out for cost-effective saccharification (Escamilla-Alvarado *et al*. 2016). The supplementation and enhancement of BGL produced by other microbes in cellulase preparations is a promising method (Vijaya Rani *et al*. 2014).

Many natural microbial sources (*Aspergillus*, *Penicillium*, *Trichoderma*, *Saccharomyces*, and *Pseudomonas*, *etc.*) have been exploited for BGL production, wherein *Aspergillus niger* is one of the most robust and promising candidates (Narasimha *et al*. 2016). Currently, much effort has been focused on strain improvement, culture medium, process optimization, *etc.*, to enhance the efficient production of BGL. For example, chemical reagents such as ethyl methanesulfonate, acridine orange, and N-methyl N'-nitro-N-nitrosoguanidine, (Pal and Das 2005; Lotfy *et al*. 2007; Wang *et al*. 2016), ultraviolet irradiation (Mahalakshmi *et al*. 2009), gamma radiation (Ottenheim *et al*. 2015), genome shuffling (Li *et al*. 2014), protoplast fusion (Khattab and Bazaraa 2005), and regulation target encoding genes (Stricker *et al*. 2008) were used for *A. niger* improvement. The feed stock (*e.g.* corn stover, wheat straw, sugar cane bagasse, wheat bran, and glycerol) (Delabona *et al*. 2013; Abdella *et al*. 2014), promoters, inducers (*e.g.* easily metabolizable sugars, monoterpene glycoside, xylose, maltose, and trace elements) (Shoseyov *et al*. 1988; Lu *et al*. 2010), and nutrient complementation (*e.g.* organic-inorganic nitrogen complementation) (Wang *et al*. 2012) were applied for culture medium optimization. The fermentation strategies (*e.g.* submerged and solid-state fermentation) and process parameters (*e.g.* inoculum volume, stirring rate, temperature, oxygen, pH, and time) (Park *et al*. 2002) were involved in the process optimization. However, when aiming at cost-effective BGL production and industrialization, a greater emphasis should be focused on the screening of the robust biocatalyst (fermentation microbe), low-cost culture medium (mainly influenced by substrates and nutrients), process optimization, *etc.* Moreover, the process dynamics should be characterized for cost-effective BGL production.

In this experiment, efforts were made to optimize the medium and process parameters using Response Surface Methodology (RSM) for cost-effective BGL production with a mutant strain *A. niger* C112. The process physio-chemical properties, such as zeta potential and dissolved organic matter (DOM) in the fermentation broth, were studied and shown to have strong correlations to the BGL yield. Further, the dynamics of DOMs were characterized by a three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy.

EXPERIMENTAL

Materials

The authors' own lab BGL production strains were treated by ultraviolet irradiation for strain optimization. The most vigorous one (holding the biggest BGL production capability) *A. niger* C112 was stored in the China Center for Type Culture Collection (CCTCC) (Wuhan, China), holding the strain culture preservation number M2012129 (Shi 2011). One copy of the stored *A. niger* C112 was used in this paper.

Methods

Media and culture

The glycerol-preserved *A. niger* C112 stored in a -80 °C refrigerator was first activated on a potato dextrose agar (PDA) medium (300 g/L potato, 20 g/L glucose, and 20 g/L agar) for 6 days at 28 °C. Two loops of spores on the PDA medium were inoculated into 250-mL flasks holding 50 mL of seed medium (300 g/L potato and 20 g/L glucose) and activated for 48 h in preparation for inoculation. The well-grown spores (mycelial pellet-shaped ribbon) were inoculated into 250-mL flasks with 50 mL of fermentation

medium (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) that contained 6 g/L potassium dihydrogen phosphate (KH_2PO_4), 0.9 g/L anhydrous calcium chloride ($CaCl_2$), 0.9 g/L magnesium sulfate ($MgSO_4$), 1 mL/L Tween-80, 1 mL/L Mandels trace elements (1.4 g/L zinc sulfate ($ZnSO_4$), 1.6 g/L manganese sulfate ($MnSO_4$), 5 g/L ferrous sulfate ($FeSO_4$), 3.7 g/L cobalt chloride ($CoCl_2$)), and 100 mL/L citrate buffer, at a pH of 4.8. The doses of carbon source and nitrogen source were 30 g/L and 7 g/L, respectively. The culture pH was not adjusted.

Medium optimization

Previous studies from our lab (none reported) has indicated that the corn cob, straw powder, wheat bran, bagasse, rice bran, industrial cellulose, corn stalk, and starch (Changsha Minghui Biotech Co., Ltd., Changsha, China) were tested for the effect of carbon source on BGL production. The strategies of inorganic-inorganic nitrogen and organic-inorganic nitrogen complementation were performed for nitrogen source improvement using yeast extract powder, peptone, $(NH_4)_2SO_4$, corn syrup, and $NaNO_3$. In addition, the RSM test of four factors at three different levels using Box-Behnken design was applied by the software Design-Expert 8.0.6 (Stat-Ease, Inc., Minneapolis, USA) for optimization of carbon, nitrogen, temperature, and pH, with three replicates. The data analysis and graphs were conducted by SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, USA).

Process dynamic characterization

The zeta potential and DOM particle size were both measured using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK) according to previous literature (Elanthikkal *et al*. 2010).

The information involved in the component, origin, and dynamics of DOMs was obtained by a Hitachi Spectrophotometer F-4600 (Hitachi Ltd., Tokyo, Japan), according to previous literature (He *et al*. 2014). The scanning emission (Em) and excitation (Ex) wavelength were 200 nm to 700 nm and 350 nm to 800 nm, respectively, coupled by a scanning speed of 2400 nm/min. The Rayleigh light scattering was eliminated by a 290 nm emission cut-off filter. Deionized water was used as a control. The EEM values were analyzed by fluorescence regional integration (FRI) (Chen *et al*. 2003). The ratio of fluorescence intensity of Em 450 nm to Em 500 nm at Ex 370 nm ($f_{450/500}$) was measured to determine the origin of humic acid-like matter (McKnight *et al*. 2001).

Analytical methods

The sample was pretreated by an Eppendorf 5804 R (Eppendorf, Hamburg, Germany) centrifuge with a 8000 round/min (6829 (×g)) centrifugation. The supernatant was then filtered with a 0.45 μm filter membrane. The filtrate was prepared for analysis.

The BGL activity was measured by the *p*-nitrophenyl-*β*-D-galactopyranoside (*p*-NPG) method (Abdella *et al.* 2014). One unit of BGL activity was defined as the μmol of *p*-Nitrophenyl released per milliliter of enzyme per minute. The pH was determined by a pH meter Delta320 (Mettler Toledo Instruments (Shanghai) Ltd., Shanghai, China). The pH was determined by a pH meter Delta320 (Mettler Toledo Instruments (Shanghai) Ltd., Shanghai, China).

RESULTS AND DISCUSSION

Carbon Source *vs.* BGL Production

As shown in Fig. 1, the relatively highest BGL activity of 5.19 ± 0.25 U/mL was obtained with corn cob as the carbon source, followed by straw powder, wheat bran, bagasse, rice bran, industrial cellulose, corn stalk, and starch. This result was similar to previous research (Relwani *et al*. 2008). However, the BGL activity in this paper was substantially lower than that of the authors' lab's previous study (7.22 U/mL) under similar conditions (Shi 2011), which might have been due to the degeneration of the mutant for undiscovered reasons.

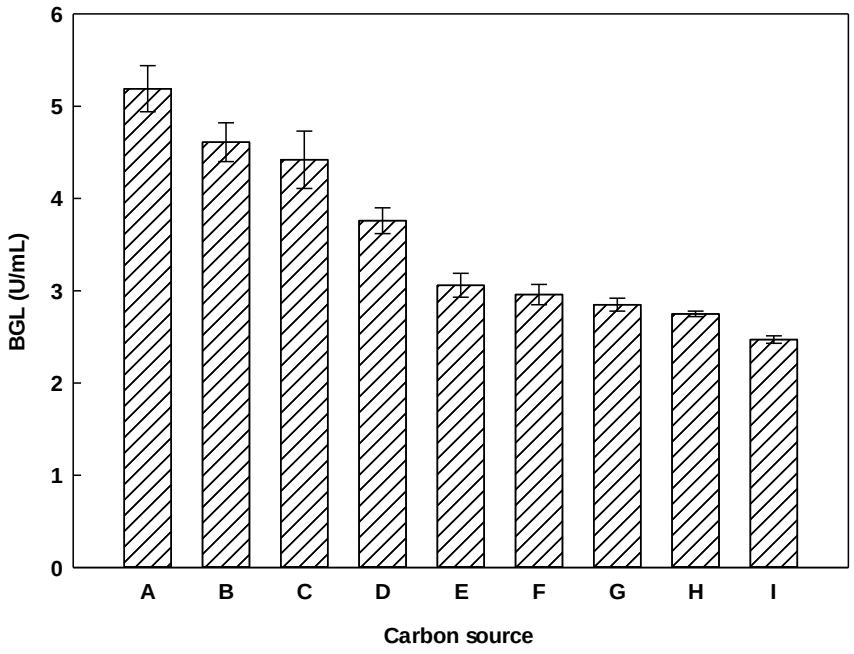

Fig. 1. Effect of single carbon source on BGL production; A through I represents corn cob, straw powder, wheat bran, bagasse, rice bran, industrial cellulose, corn stalk, and starch, respectively

Table 1. Particle Size of Carbon Source on BGL Production*

BGL (U/mL)	Corn Cob (Untreated)	Corn Cob (30 Mesh)	Corn Cob (50 Mesh)
Straw Powder (Untreated)	5.19 ± 0.24	3.11 ± 0.16	7.48 ± 0.21
Straw Powder (30 Mesh)	6.16 ± 0.33	4.29 ± 0.27	6.72 ± 0.32
Straw Powder (50 Mesh)	5.49 ± 0.25	4.33 ± 0.34	6.06 ± 0.46

*The straw powder and corn cob were treated using a grinder and screened by different mesh sieves, orderly; the total dose of carbon source was 30 g/L; the ratio of straw to corn was 1.0

The substrate particle size, which affects the mass and heat transfer, attachment of microbes to substrate, accessibilities of nutrition, physio-chemical properties of fluent, *etc.*, is usually studied in a solid-state fermentation (Thomas *et al*. 2013), rather than in a submerged fermentation (Izumi *et al*. 2010). As shown in Table 1, a smaller substrate particle size noticeably boosted BGL production, holding the relatively highest BGL

activity of 7.48 ± 0.21 U/mL (untreated straw powder + 50 mesh corn cob), which might be due to the combined effect of more oligomers (*e.g.* cello-oligosaccharides) being released and moderate culture conditions (Thomas *et al*. 2013).

In Figs. 1 and 2, a carbon source cocktail was superior to a single source for BGL production. The activities of BGL decreased with increased ratios of corn cob/straw powder from 0.3 to 0.5, and then increased from 1.0 to 2.0, with a maximum BGL activity of 8.81 ± 0.19 U/mL (corn cob/straw powder ratio of 0.8, corresponded to 13.3 g/L corn cob and 16.7 g/L straw powder), which was approximately double of that using corn cob as the carbon source. Commonly, agricultural residues (corn cob, straw powder, wheat-straw, *etc.*) are suitable substrates for BGL production. Moreover, a substrate cocktail has been proven to be better than a single substrate for BGL production, because more promoters and inducers (carbohydrates) are produced during the fermentation process (Delabona *et al*. 2013). Likewise, the diversity of substrates contributes to reduced costs in BGL production. Thus, for higher cost-effective BGL production, a carbon source cocktail (corn cob and straw powder with the ratio of 0.8) was selected for BGL production.

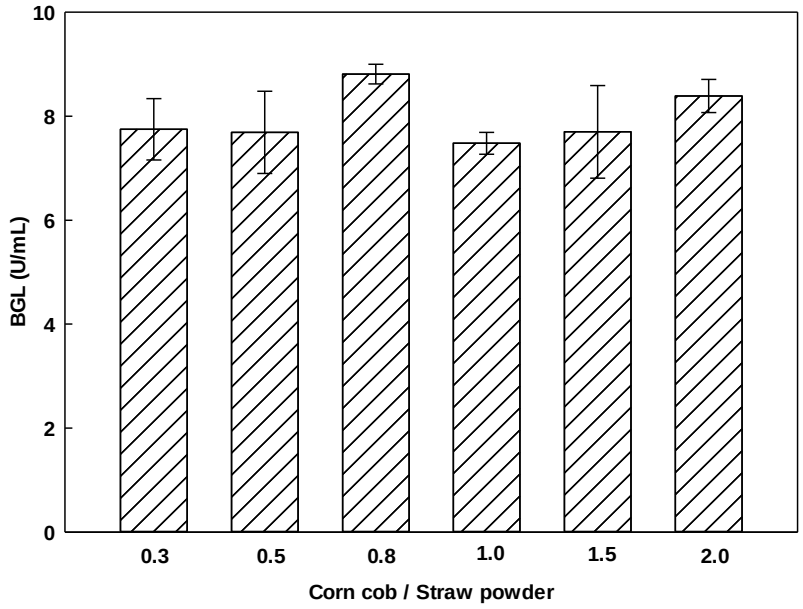

Fig. 2. Effect of single carbon source cocktail on BGL production

Nitrogen Source *vs.* BGL Production

In Fig. 3, the nitrogen source that influenced BGL secretion was ordered by $(NH_4)_2SO_4$ > NH_4NO_3 > NH_4NO_3 + $(NH_4)_2SO_4$ > $(NH_4)_2SO_4$ + Urea > peptone > peptone + $(NH_4)_2SO_4$ > urea, holding a relatively high BGL activity of 6.41 ± 0.24 U/mL. Generally, the inorganic nitrogen sources ($(NH_4)_2SO_4$ and NH_4NO_3) performed somewhat superior to the organic ones (peptone and urea) for BGL production, which was not in accordance with some previous studies (Narasimha *et al*. 2006). It was reported that the effect of the nitrogen source on cellulase production was variable, relying on strains, nature, and the dose of nitrogen (Kachlishvili *et al*. 2006). Typically, inorganic nitrogen (*e.g.* ammonium) is more easily assimilated by fungal strains than organic nitrogen (*e.g.* urea). However, some of the amino acids in organic nitrogen sources (*e.g.* beef extract, yeast extract, and peptone) can be assimilated and directly incorporated into proteins, having the advantage of enzyme synthesis over inorganic sources (Gottschalk *et al*. 2013).

Furthermore, the strategies of organic-inorganic (yeast extract + $(NH_4)_2SO_4$) (Wang *et al*. 2012) and inorganic-inorganic nitrogen (NH_4Cl + $(NH_4)_2SO_4$) (Kumar and Singh 2001) complementation were successfully applied for BGL production. However in this paper, unsatisfactory results were obtained using both organic-inorganic ($(NH_4)_2SO_4$ + Urea and peptone + $(NH_4)_2SO_4$) and inorganic-inorganic (NH_4NO_3 + $(NH_4)_2SO_4$) nitrogen compounds, which might be caused by the inhibitory effect on BGL production (Joo *et al*. 2010). Therefore, $(NH_4)_2SO_4$ was selected as the nitrogen source for BGL production.

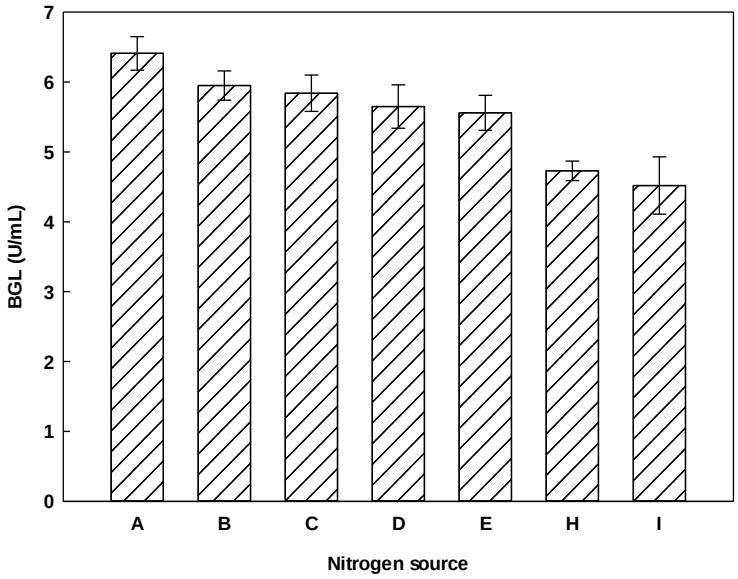

Fig. 3. Nitrogen source on BGL production, A through I represent nitrogen source: $NH_4)_2SO_4$, NH_4NO_3, NH_4NO_3 + $(NH_4)_2SO_4$, $(NH_4)_2SO_4$ + urea, peptone, peptone + $(NH_4)_2SO_4$, and urea, respectively; all the complex nitrogen sources held the mass ratios of 1:1

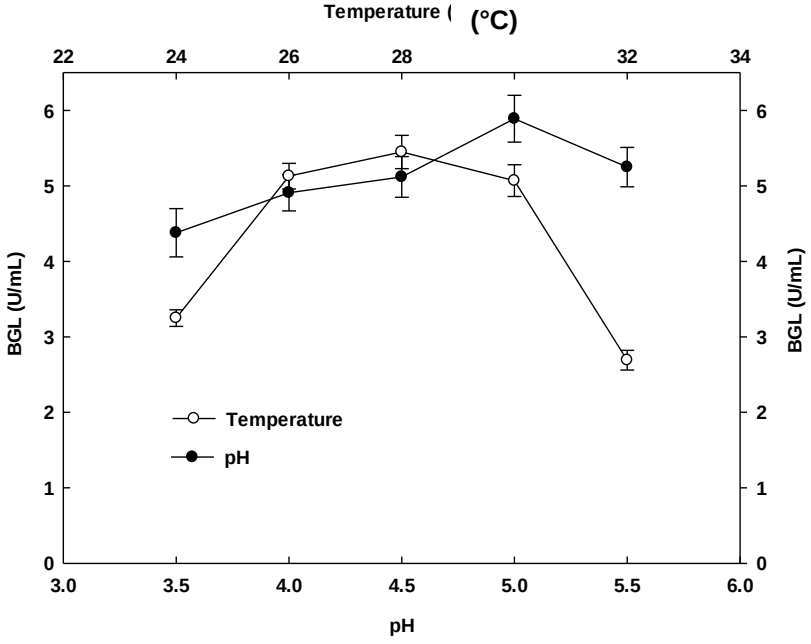

Fig. 4. Changes of temperature and pH in the BGL production process

Temperature and pH *vs.* BGL Production

Generally, the culture pH and temperature greatly influence microbial growth, metabolic abilities (*e.g.* enzyme secreting), product properties (*e.g.* enzyme stability), *etc.* As shown in Fig. 4, the BGL activities increased during the initial temperature of 24.0 °C to 28.0 °C and then dropped notably, with a maximum BGL activity of 5.45 U/mL ± 0.22 U/mL (28 °C). Meanwhile, the BGL activities increased the initial pH from 3.5 to 5.0 and then dropped, holding a maximum BGL activity of 5.89 ± 0.31 U/mL (pH 5.0). The effects of pH and temperature on BGL production were similar to those of previous studies (Sohail *et al.* 2009).

Table 2. Results of Un-coded Process Variables and Observed, Predicted Responses

Run	Factors				BGL (U/mL)	
	A: Carbon (g/L)	*B*: Nitrogen (g/L)	*C*: Temperature (°C)	*D*: pH	Observed	Predicted
1	30.0	7.5	28.0	5.0	8.80±0.28	8.82
2	30.0	7.5	28.0	5.0	8.83±0.30	8.84
3	40.0	7.5	28.0	4.5	8.08±0.19	8.09
4	30.0	10.0	25.0	5.0	7.31±0.20	7.33
5	30.0	10.0	28.0	4.5	7.38±0.24	7.39
6	20.0	7.5	25.0	5.0	6.94±0.17	6.94
7	30.0	7.5	30.0	4.5	7.22±0.31	7.24
8	30.0	5.0	25.0	5.0	7.24±0.25	7.24
9	20.0	5.0	28.0	5.0	6.99±0.28	7.04
10	40.0	7.5	25.0	5.0	7.97±0.31	7.95
11	40.0	7.5	30.0	5.0	8.14±0.23	8.10
12	20.0	7.5	30.0	5.0	6.93±0.30	6.90
13	40.0	7.5	28.0	5.5	8.12±0.35	8.13
14	30.0	5.0	28.0	4.5	7.36±0.31	7.32
15	30.0	7.5	25.0	4.5	7.17±0.28	7.17
16	40.0	10.0	28.0	5.0	8.24±0.29	8.23
17	20.0	7.5	28.0	5.5	7.06±0.17	7.06
18	30.0	7.5	25.0	5.5	7.26±0.24	7.26
19	30.0	7.5	28.0	5.0	8.84±0.33	8.82
20	40.0	5.0	28.0	5.0	8.08±0.31	8.13
21	30.0	10.0	30.0	5.0	7.39±0.28	7.39
22	30.0	5.0	30.0	5.0	7.30±0.33	7.29
23	30.0	5.0	28.0	5.5	7.43±0.26	7.38
24	30.0	7.5	30.0	5.5	7.26±0.19	7.30
25	20.0	7.5	28.0	4.5	6.96±0.28	6.95
26	30.0	10.0	28.0	5.5	7.48±0.34	7.48
27	20.0	10.0	28.0	5.0	7.13±0.36	7.12

RSM Optimization

The design and results of RSM are shown in Table 2, wherein, a model ($R^2 = 0.991$) in terms of coded factors (carbon, nitrogen, temperature, and pH) was simulated as follows,

$$Y= 8.82 + 0.55A + 0.044B - 0.029C + 0.037D + 0.005AB - 0.045AC-0.015AD - 0.005BC + 0.0075BD + 0.013CD - 0.52A^2 - 0.68B^2 - 0.83C^2 - 0.75D^2$$

where *Y* is BGL (U/mL), *A* is carbon (mg/L), *B* is nitrogen (mg/L), *C* is temperature (°C), and *D* is pH.

The four factors influenced BGL production significantly ($P < 0.0001$) (Table 3). While the interaction of carbon and temperature influenced BGL production significantly ($P < 0.05$), the others did not ($P > 0.05$). Moreover, a maximum BGL (8.97 U/mL) was obtained by optimizing the RSM model, holding the corresponding carbon, nitrogen, temperature, and pH of 35.35 g/L, 7.59 g/L, 27.94 °C, and 5.03, respectively. Three replicated experiments were performed to verify the validity of the optimized conditions, holding a maximum BGL (8.91 ± 0.35 U/mL). The model was effective and accurate for predicting the production of BGL.

Table 3. Analysis of Variance (ANOVA) for Observed Factors

Sources	Square Sum	Freedom	Mean Square	F value	P value
Model	9.28	14	0.66	531.65	< 0.0001
A	3.65	1	3.65	2929.76	< 0.0001
B	0.023	1	0.023	18.780	0.001
C	0.01	1	0.01	8.19	0.0143
D	0.016	1	0.016	12.940	0.0037
AB	1.00E-04	1	1.00E-04	0.08	0.7818
AC	8.10E-03	1	8.10E-03	6.50	0.0255
AD	9.00E-04	1	9.00E-04	0.72	0.4121
BC	1.00E-04	1	1.00E-04	0.08	0.7818
BD	2.25E-04	1	2.25E-04	0.18	0.6785
CD	6.25E-04	1	6.25E-04	0.50	0.4924
A^2	1.42	1	1.42	1142.14	< 0.0001
B^2	2.47	1	2.47	1980.83	< 0.0001
C^2	3.68	1	3.68	2950.45	< 0.0001
D^2	2.99	1	2.99	2401.34	< 0.0001
Residual Error	0.015	12	1.25E-03		
Lack of Fit Value	0.014	10	1.41E-03	3.250	0.258
Pure Error	8.67E-04	2	4.33E-04		
Sum	9.29	26			

Process Dynamic Characteristics

As an indicator of particle electrical charge properties (*e.g.* positively or negatively charged) of amphoteric matters (*e.g.* protein, enzyme and cell membrane), zeta potential has usually been measured in submerged fermentation (Jeon *et al*. 2013; Singh *et al*. 2014). Generally, the zeta potential presents negative or positive value when the amphoteric matter isoelectric point (pI) is less or more than the solution pH, respectively (Bowen *et al*. 1998).

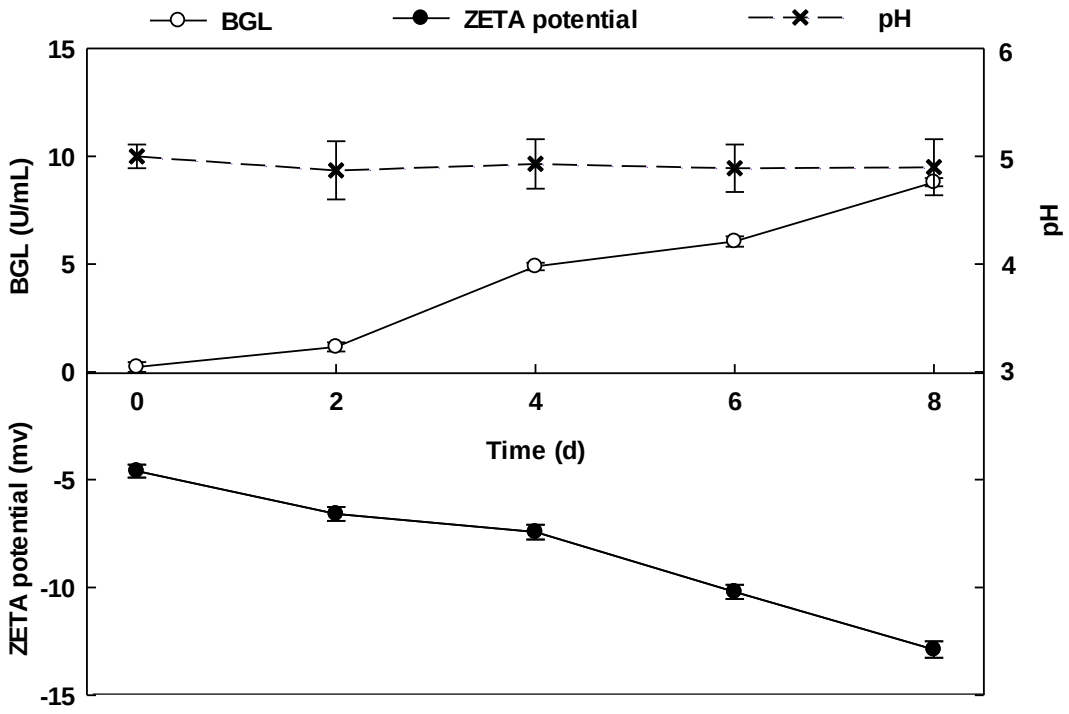

Fig. 5. Dynamic of zeta potential, BGL, and pH over time

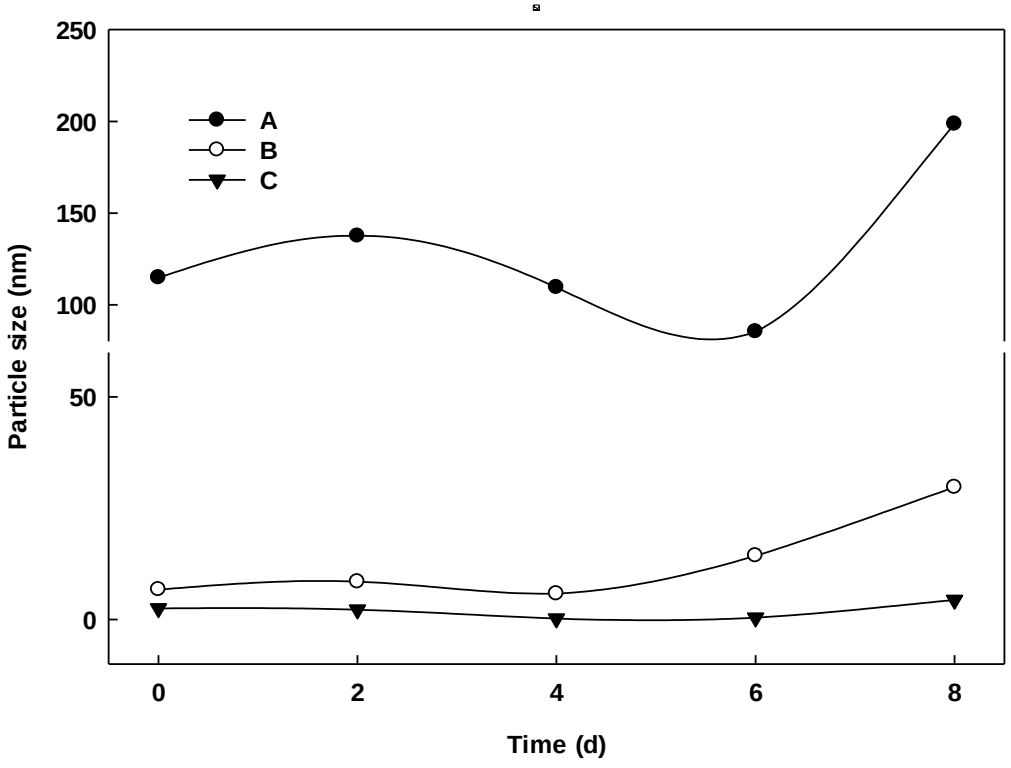

Fig. 6. Dynamic of DOMs particle size over time; A, B, C represent the highest amount of unknown particles exited in three ranges: 80 nm to 200 nm, 5 nm to 80 nm, and < 5 nm, respectively

It was reported that the pI of BGL secreted by *A. niger* strains was from 3.20 to 4.05 (McCleary and Harrington 1988; Shoseyov *et al.* 1988; Watanabe *et al.* 1992; Unno *et al*. 1993; Yan and Lin 1997; Lambert *et al.* 2003), which was less than the pH of the broth used in this work (which fluctuated around 4.5) (Fig. 5), leading to the BGL surface being negatively charged.

Besides, it is known that the microbial cellulases (*e.g.* BGL) are expressed only under the conditions in which induced cello-oligosaccharides (*e.g.* sophorose) were present, which are mainly enzymatic hydrolyzed from the lignocellulose carbon sources. Accompanied by the releasing of cello-oligosaccharides, plenty of carboxyl groups are exposed and attached to the cellulosic surfaces, causing the lignocellulose carbon sources negatively charged (Peri *et al*. 2012). It was described from Fig. 5, possibly due to the combined effects of negatively charged BGL and cellulosic particle, the zeta potential of broth increased with the yield of BGL over the whole fermentation process (-4.6 mV to -12.88 mV). The DOMs particle sizes (8 nm to 200 nm and 5 nm to 80 nm) increased over time despite some drops at some time for unknown reasons (Fig. 6). The dynamics of zeta potential and DOMs particle size might be related to the production of enzymes and degradation of carbon substrates, and ultimately lead to effects on process operations like product separation and extraction.

The EEM method has recently been widely used for characterizing DOMs in liquid media (wastewater, activated sludge, fermentation broth, *etc.*), primarily for the advantage of cheap and rapid determination of DOMs according to their unique fluorescence spectra information (Wan *et al*. 2012). In addition, it was reported that there are commonly five components of DOMs – aromatic protein, aromatic protein II, fulvic acid-like, soluble microbial byproduct-like, and humic acid-like – with definitive Ex/Em values, (220 to 250)/(280 to 330) nm, (220 to 250)/(330 to 380) nm, (220 to 250)/(380 to 500) nm, (250 to 400)/(280 to 380) nm, and (250 to 400)/(380 to 500) nm, respectively (Chen *et al*. 2003). For a deeper understanding of the dynamics of DOMs in fermentation broth, the EEM method was applied in this work. In Fig. 7, four components of DOMs (aromatic protein II, fulvic acid-like, soluble microbial byproduct-like, and humic acid-like matters) were determined in the samples. The humic acid-like matters were the main components, mostly accounting for half of the total fluorescence relative intensities (Fig. 10), with the peak Ex/Em values of 390/485 nm (0 d), 380/458 nm (2 d), 390/467 nm (4 d), 400/494 nm (6 d), and 400/494 nm (8 d), respectively. Moreover, the $f_{450/500}$ values of the samples decreased from the initial 1.36 (0 d) to 0.85 (8 d), indicating that humic acid-like matter were mainly derived from the carbon source cocktail (Mcknight *et al*. 2001) and were consequently influenced by *A. niger* C112 to some extent. In addition, the increases of aromatic protein II and soluble microbial byproduct-like matters over time (not during the period of 0 d to 2 d) were probably due in part to enzyme secretion of *A. niger* C112 (Fig. 8), leaving room for further research to be conducted. Furthermore, the ratios of fluorescence intensities of fulvic acid-like matters to humic acid-like matter were small (0.28 to 0.46, calculated by the values in Fig. 7), meaning high degrees of humification of the substrates (Wei *et al*. 2014).

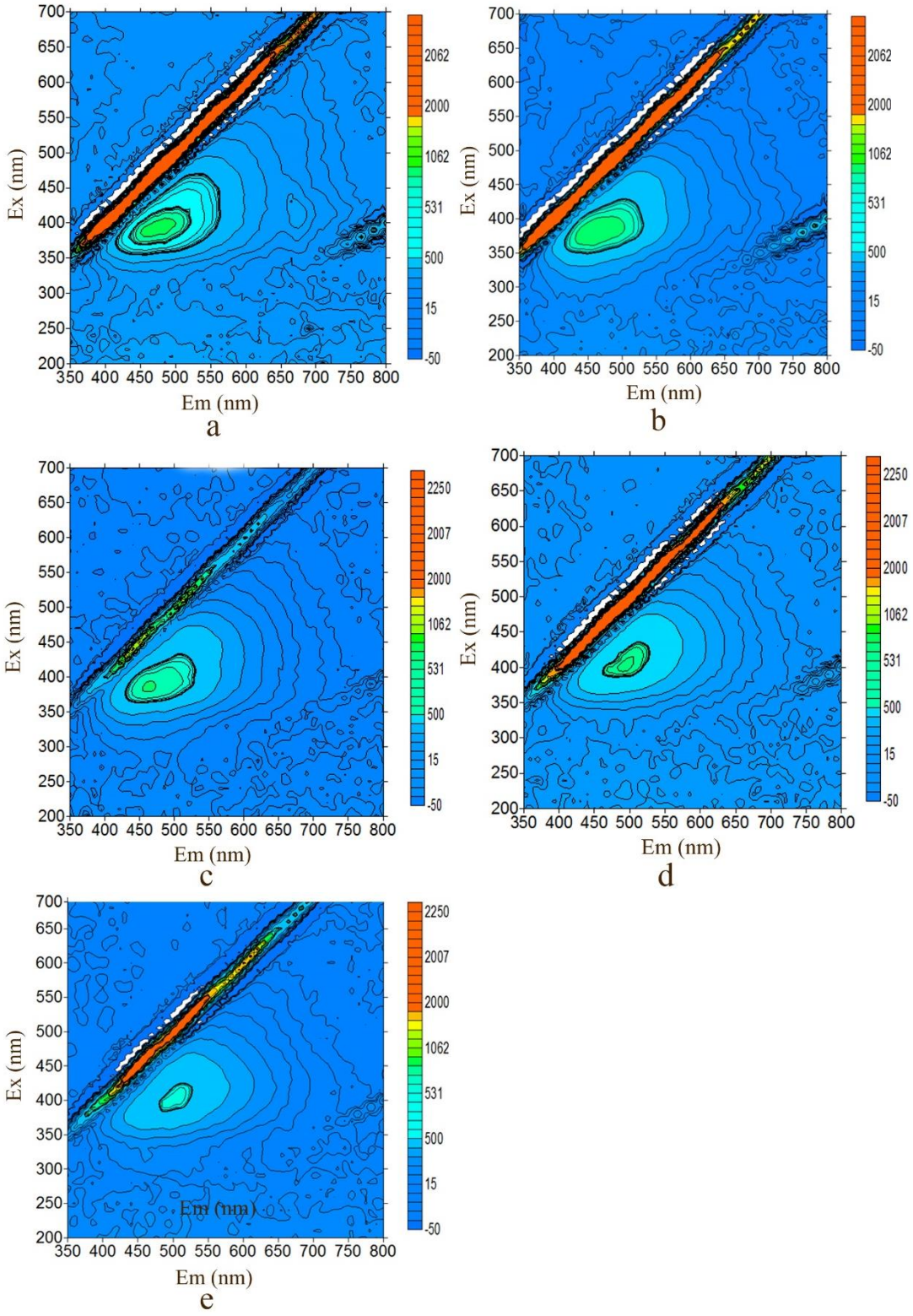

Fig. 7. EEM fluorescence spectra of samples; a, b, c, d, and e represent the sample: 0 d, 2 d, 4 d, 6 d, and 8 d, respectively

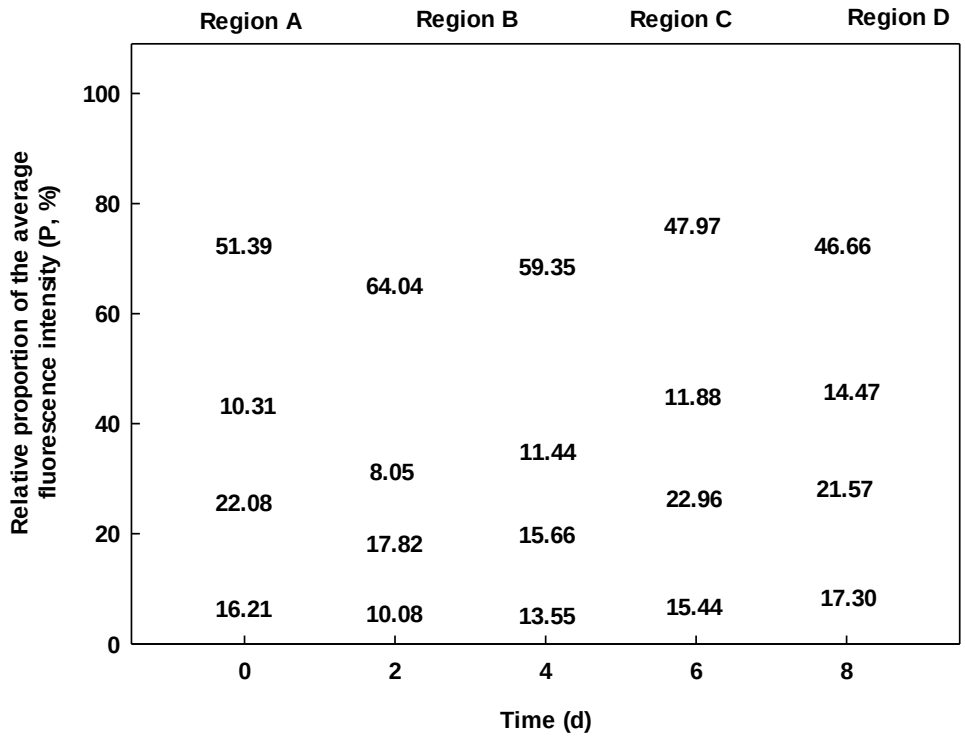

Fig. 8. EEM fluorescence spectra of samples; region A through D represent aromatic protein II, fulvic acid-like, soluble microbial by-product-like, and humic acid-like, respectively

CONCLUSIONS

1. The mutant strain *A. niger* C112 was successfully used for cost-effective β-glucosidase (BGL) production. A maximum BGL activity of 8.91 ± 0.35 U/mL was obtained by medium and process optimization, with the carbon cocktail (straw powder + corn cob), nitrogen ($(NH_4)_2SO_4$), temperature, and pH of 35.35 g/L, 7.59 g/L, 27.94 °C, and 5.03, respectively.
2. Possibly due to the combined effect of more oligomers released and moderate culture conditions, the carbon source cocktail performed significantly superior to the single substrate.
3. This is the first report on the application of the excitation-emission matrix (EEM) fluorescence spectroscopy method for understanding and characterizing the components, origin, and dynamics of dissolved organic matter (DOMs) involved in BGL production, contributing to further process optimization.

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