Combined Ferric Chloride and Phanerochaete Chrysosporium Pretreatment on Corn Stalk Degradation

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Abstract: In order to shorten the pretreatment cycle of microorganisms and enhance the pretreatment effect, the corn stalk was pretreated by means of metal ions and microorganisms, the effects of combined pretreatment of ferric chloride and white rot fungi on the degradation of 40 and 80 mesh corn stalks were studied. The content of cellulose, hemicellulose and lignin changes and the yield of reducing sugar in the fermentation broth were analyzed. The results showed that when the concentration of ferric chloride solution was 0.4mol/L, the solid-liquid ratio was 1/20g/ml, and the 80 mesh corn stalk material was treated at 30 min at 100~108℃, the cellulose content increased by 56.08%, the hemicellulose decreased by 13.06% and the lignin increased by 37.80%. When ferric chloride treated corn stalks were inoculated with *Phanerochaete chrysosporium*, the reducing sugar content reached 2.52 mg/ml on the twelfth day, and the yield of reducing sugar was the highest in the fermentation broth. The study opens up a new way for high efficiency, clean and low energy consumption of biomass pretreatment technology of crop straw.

1 INTRODUCTION

China is rich in biomass raw materials, with an annual output of approximately 700 million tons of crop stalk, of which corn stalk accounts for approximately 35%. This represents a tremendous resource for energy and environmental solutions in China. The main components of crop stalk are cellulose, hemicellulose, and lignin. Of these, hemicellulose can be readily degraded into monosaccharides (mainly xylose) and a small amount of arabinose, mannose and galactose. Cellulose accounts for approximately 40% of the total stalk weight; it mainly consists of glucose, and its crystal structure is difficult to degrade. Lignin is a phenolic polymer that bonds cellulose to hemicellulose. To convert the cellulose in corn stalk into fermentable sugars, the stalk must be pretreated using a specialized method. The lignin bound to the cellulose can only be broken after the pretreatment, thus exposing the cellulose to facilitate the subsequent enzymatic hydrolysis and fermentation (Lissens, 2004; Palonen, 2004). Pretreatment with metal ions is conducted under normal conditions and is thus environmentally friendly (Galbe, 2002; Bailey, 1996; Saricks, 1999), with zero vapor pressure, high thermal stability and catalytic function, and the pretreatment waste can be recycled. The metal ion pretreatment reduces the cellulose's crystallinity, thus increasing its accessibility and benefitting its subsequent hydrolysis (Solomon, 2007). Lopez-Linares and Romero of the University of Jaén, Spain, treated olive tree biomass with metal ions at 0.265 mol/L for 30 min at 152.6 °C and achieved high rates of hemicellulose removal and hydrolysis (López-Linares, 2013). Zeng of Chongqing University, China, treated biomass with 0.6 mol/L ferric chloride solution for 15 min at 170 °C at a solid to liquid ratio of 10:1 mL/g, increasing the relative cellulose content by 72.19% and the reducing sugar yield by 131.6% (Zeng, 2013).

The biological treatment method has the advantages of mild reaction conditions, low energy consumption, environmental friendliness, and high target product yield; thus, it has great potential for development (Saha, 2016; Kim, 2008; Ranganathan, 2008; Henriksson, 2000). The degradation of lignin by white rot fungi mainly uses peroxidase secreted by white rot fungi to catalyze lignin oxidation. Lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase (Lac) are the three main enzymes for lignin degradation by white rot fungi (Wan,

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2012). White rot fungi not only have unique lignin degradation ability, but also, like most other fungi, have the ability to degrade cellulose and hemicellulose to provide carbon source for their own growth. The degradation of cellulose by white rot fungi depends on the cellulase secreted by white rot fungi, and the degradation of hemicellulose depends on the hemicellulase secreted by white rot fungi. Cellulase from white rot fungi is also composed of Endoglucanase (EG, EC 3.2.1.4), Cellobiohydrolase (CBH, EC 3.2.1.91) and beta-glucosidase (EC 3.2.1.21) (Kirk, 1997). Cellulose endonuclease hydrolyzes the amorphous region of cellulose microfibers and releases new end of cellulose chain. Cellulose endonuclease hydrolyzes the next cellobiose unit from end of cellulose chain in turn, and finally hydrolyzes to glucose under the action of beta-glucosidase. Xu et al. pretreated corn stalk with Irpex lacteus CD2 at a cellulase dosage of 20 filter paper units (FPU)/g dry matter for 25 days to achieve hydrolysis and reached a saccharification rate of 66.4% (Xu, 2010). Sun et al. pretreated corn stalk with Trametes hirsuta yj9 to increase total sugar conversion from enzymatic hydrolysis (Sun, 2011).

However, due to the long period of microbial pretreatment, it is difficult to apply in large-scale industrial production, which restricts the further application and development of biological pretreatment. Ferric chloride solution achieves the purpose of pretreatment mainly by destroying hemicellulose of straw. The treatment cycle of straw treated with ferric chloride and inoculated with white rot fungi will be shortened and straw will be further degraded to enhance the effect of pretreatment. In this study, we investigated the effect of the process and parameters of the pretreatment using ferric chloride combined with Phanerochaete chrysosporium on the reducing sugar content of corn stalk after enzymatic hydrolysis. The pretreatment ability was determined by the content change of cellulose, hemicellulose and lignin and the yield of reducing sugar. It opens up a new way for high efficiency, clean and low energy consumption of biomass pretreatment technology of crop straw.

2 MATERIALS AND METHODS

2.1 Corn Stalk Raw Material

Corn stalk was collected from the experimental site of the College of Mechanical and Electrical Engineering of Henan Agricultural University, air-dried, pulverized with a pulverizer, and sieved through 40-mesh (0.425 mm) and 80-mesh (0.175 mm) standard sieves, and the siftage was dried at 75 °C to a constant weight. The main composition of corn stalk was showed in Table 1.

Table 1. Main composition of corn stalk.

Raw material	Cellulose (g)	Hemicellulose (g)	Lignin (g)	Other (g)
40-mesh	0.252	0.315	0.127	0.306
80-mesh	0.255	0.314	0.127	0.304

Note: The total raw material weight used in the determination was 1.000 g.

2.2 P. chrysosporium Culture

P. chrysosporium (GIMCC No: GIM3.393) was purchased from the Preservation Center of Microorganisms of the Research Institute of Microbiology of Guangdong Province. The lyophilized P. chrysosporium mycelia were dissolved in sterile water and revived. They were then inoculated onto potato dextrose agar (PDA) slant medium and cultured at 28 °C for 7 days, from which the second-generation subculture was inoculated, cultured for 7 days and then stored at 4 °C for later use.

2.3 Ferric Chloride Pretreatment

Five grams of dried corn stalk powder sieved through 40-mesh or 80-mesh filters was placed into a 500-mL Erlenmeyer flask, to which ferric chloride solution of different concentrations (0.1, 0.2, 0.3, 0.4, or 0.5 mol/L) was added at a solid to liquid ratio (g/mL) of 1/10 (a) or 1/20 (b). The sample was then mixed thoroughly and treated at 100–108 °C for 15 (c) or 30 min (d). The cellulose, hemicellulose and lignin contents were determined after the above pretreatment.

2.4 P. chrysosporium Pretreatment

After rinsing to neutral pH, corn stalk siftage after the ferric chloride pretreatments or 5 g of dried corn stalk siftage sieved through 40-mesh or 80-mesh filters was placed in a 300-mL Erlenmeyer flask, to which distilled water was added at a solid to liquid ratio of 1:5. The flask was sealed with a sterile membrane and treated at 121 °C for 1 h. Next, P. chrysosporium growing in the logarithmic phase was punched with a puncher, and several punch disks were inoculated into 6 flasks and cultured at 28 °C. On days 6, 12, 18, 24, and 30 after inoculation, the reducing sugar content was determined.

2.5 Determination Methods

The cellulose, lignin and hemicellulose contents in the corn stalk were determined per the method of Wang (Wang, 1987). The reducing sugar content in the hydrolysate was determined using the 3, 5-dinitrosalicylic acid colorimetric method on a Model 752 spectrophotometer (Ghose, 1987).

3 RESULTS AND DISCUSSION

3.1 Effects of Different Ferric Chloride Pretreatment Conditions on Cellulose and Hemicellulose Yields

The changes in cellulose, hemicellulose and lignin contents of the 40-mesh corn stalk siftage treated with ferric chloride are shown in Fig.1 After ferric chloride treatment, the cellulose content increased in all treatment groups, with little variation in increments among the groups. The highest content was 0.385 g, and the lowest content was 0.359 g, which was a significant increase compared with the original content of 0.252 g in the raw material. The hemicellulose content for all treatment groups decreased, while the lignin content increased because the ferric chloride pretreatment destroyed the scaffolding structure of the cellulose, lignin and hemicellulose in the corn stalk, partially releasing hemicellulose and increasing the cellulose content while releasing more lignin. The cellulose and lignin contents of the corn stalk treated with 0.5 mol/L ferric chloride solution at 100–108 °C for 30 min at a solid to liquid ratio of 1/20 g/mL increased significantly by 52.78% and 37.80%, respectively, relative to those of the raw material, while the hemicellulose content decreased by 12.06% compared with that of the raw material.

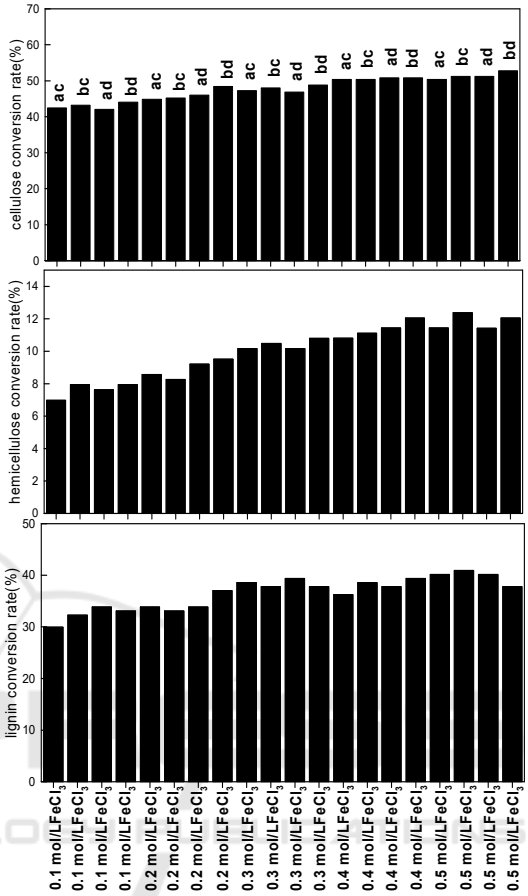


Figure 1: Conversion rate of each component of 40-mesh corn stalk siftage treated with ferric chloride.

The changes in cellulose, hemicellulose and lignin content of the 80-mesh corn stalk siftage treated with ferric chloride are shown in Fig. 2 After ferric chloride treatment, the cellulose content increased in all treatment groups, and the increments varied little among the groups. The hemicellulose content decreased in all treatment groups, while the lignin content increased. The cellulose and lignin contents of the corn stalk treated with 0.5 mol/L ferric chloride solution at 100–108 °C for 30 min at a solid to liquid ratio of 1/20 g/mL increased significantly by 56.08% and 37.80%, respectively, compared with those of the raw material, while the hemicellulose content decreased by 13.06% compared with that of the raw material. This treatment effect was the best.

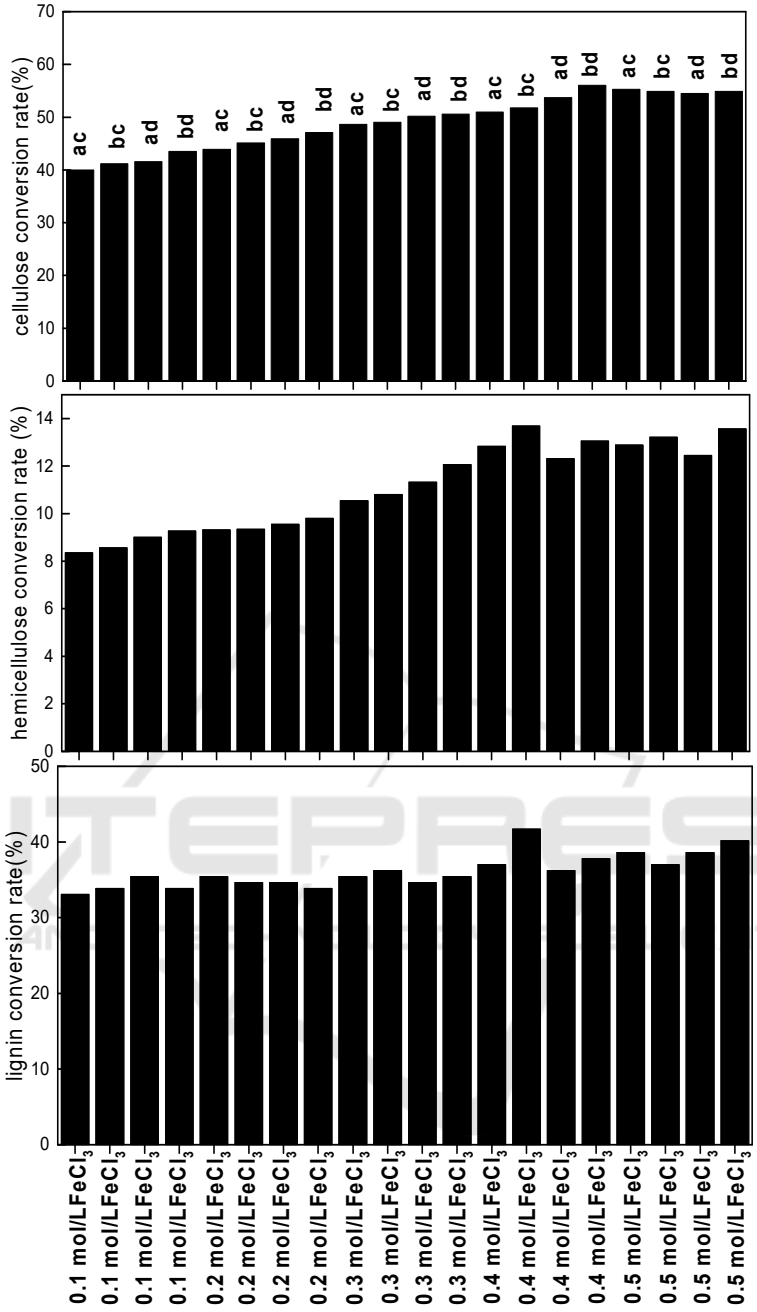
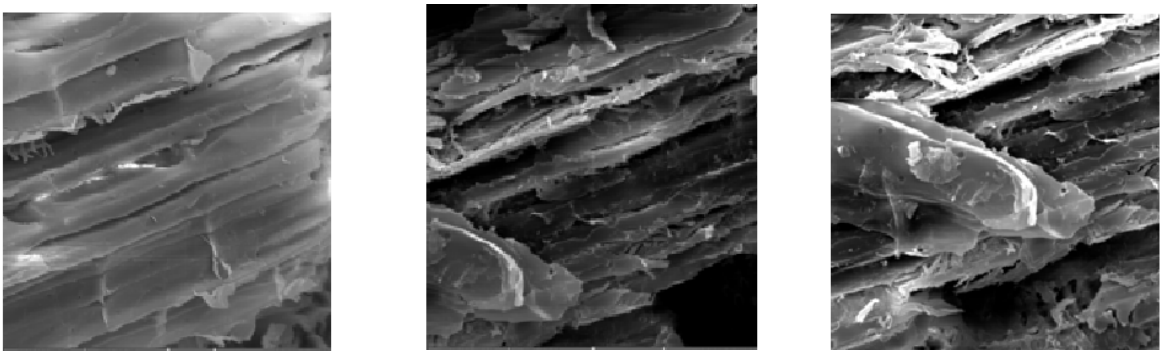


Figure 2: Conversion rate of each component of 80-mesh corn stalk siftage treated with ferric chloride.

After treatment with ferric chloride, the corn stalk material's morphology changed markedly (Fig.3). The untreated 40-mesh corn stalk siftage showed a smooth, flat, uniform, and undamaged surface, while the treated corn stalk siftage showed a rough surface, with disoriented surface structure texture, holes and longitudinal fiber breakage. The treated 80-mesh corn stalk siftage showed remarkable changes in surface structure, with highly disarrayed texture in its surface structure and holes in the partially fishnet-shaped skeleton. The ferric chloride pretreatment not only destroyed the surface structure of the corn stalk raw material but also enlarged the size of pores on the surface, thus increasing the specific surface area. Compared with the 40-mesh corn stalk siftage treated with ferric chloride, the 80-mesh corn stalk siftage treated with ferric chloride showed more complete and profound breakage, indicating that the lignin

component was destroyed more effectively for the smaller sized corn stalk.



(a) 40-mesh corn stalk Siftage

(b) 40-mesh corn stalk siftage treated with ferric chloride

(c) 80-mesh corn stalk siftage treated with ferric chloride

Figure 3: Electron scanning microscopy of the corn stalk raw material.

After determining the cellulose, hemicellulose and lignin contents of the 40- and 80-mesh corn stalk siftages treated with ferric chloride, the optimal combination of the treatment conditions was determined and used in combination with the subsequent P. chrysosporium treatment for corn stalk degradation. The cellulose, hemicellulose and lignin contents of the 40- and 80-mesh corn stalk siftages treated with ferric chloride under the optimal conditions are shown in Table 2.

Table 2: Optimal conditions for the ferric chloride treatment.

Stalk particle size	Ferric chloride concentration mol/L	Solid to liquid ratio g/mL	Temperature/°C	Time/min	Cellulose/g	Hemicellulose/g	Lignin/g
40-mesh	0.5	1/20	100–108	30	0.385	0.276	0.175
80-mesh	0.4	1/20	100–108	30	0.398	0.273	0.175

3.2 Effect of P. chrysosporium Pretreatment Conditions on the Reducing Sugar Yield

Fig.4 shows the changes in the reducing sugar content of the 40-mesh and 80-mesh corn stalk siftages treated with P. chrysosporium over time. The reducing sugar content first decreased, then increased, again decreased, and finally stabilized. This occurred because P. chrysosporium first consumed small molecular carbon sources in the substrate to propagate, thus decreasing the reducing sugar content. After the mycelia matured, the fungus began degrading the corn stalk, which increased the reducing sugar content. As shown in Fig.4, on day 6 after fungal inoculation, the reducing sugar content was high, peaking at 1.13 mg/mL for the fungus-treated 40-mesh corn stalk siftage and at 1.20 mg/mL for the fungus-treated 80-mesh corn stalk siftage, although at this time, the stalk degradation was incomplete. The reducing sugar content of the fungus-treated 40-mesh corn stalk siftage reached its lowest point at 0.39 mg/mL on day 12, while that of the fungus-treated 80-mesh corn stalk siftage reached its lowest point at 0.71 mg/mL on day 18. The reducing sugar content in the corn stalk then rose again, peaking at 0.97 mg/mL and 0.99 mg/mL for the fungus-treated 40-mesh and fungus-treated 80-mesh corn stalk siftages, respectively, on day 24, then declined and gradually stabilized over days 24–30.

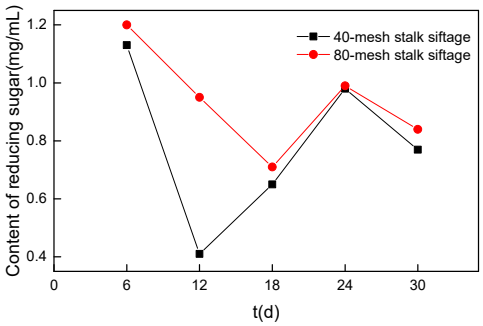


Figure 4: Changes in the reducing sugar content in corn stalks treated with P. chrysosporium over time.

Comparison of the fungus-treated 80-mesh and 40-mesh corn stalk siftages shows that on day 12, the reducing sugar contents of the fungus-treated 40-mesh and fungus-treated 80-mesh corn stalk siftages were 0.41 mg/mL and 0.95 mg/mL, respectively, with the largest gap at 0.54 mg/mL, likely because the 80-mesh corn stalk siftage was smaller than the 40-mesh corn stalk siftage, which is conducive to fungal growth. During days 6–12, the fungus grew rapidly. On day 12, it had already finished degrading the 80-mesh corn stalk siftage, yielding a higher reducing sugar content.

3.3 Effect of the Combined Ferric Chloride and P. chrysosporium Pretreatment on the Reducing Sugar Yield

Fig.5 shows that as the treatment time increased, the reducing sugar contents of the 40-mesh and 80-mesh corn stalk siftages treated with the combined P. chrysosporium and ferric chloride increased, then decreased, and finally stabilized. This occurred because on day 6, the fungus had already matured and begun to degrade the corn stalk during days 6–12, thus increasing the reducing sugar content in the substrate. On day 12, the reducing sugar contents of the 40-mesh and 80-mesh corn stalk siftages peaked at 1.92 mg/mL and 2.52 mg/mL, respectively, subsequently reaching their respective lowest points at 1.41 mg/mL and 1.69 mg/mL on day 18. On day 24, the reducing sugar content of the 40-mesh corn stalk siftage increased slightly and then stabilized, while that of the 80-mesh corn stalk siftage showed little change and remained stable during days 18–24.

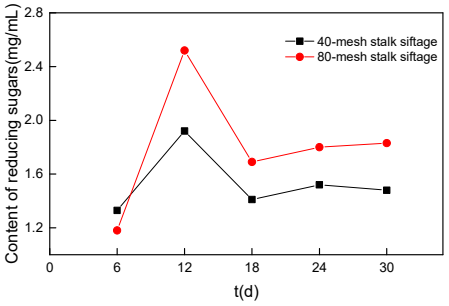


Figure 5: Changes in reducing sugar content of the 40-mesh and 80-mesh corn stalk siftage treated with the combined P. chrysosporium and ferric chloride pretreatment over time.

The reducing sugar contents of the 40-mesh and 80-mesh corn stalk siftages treated with combined P. chrysosporium and ferric chloride differed little on days 6, 12, 18, 24, and 30; however, on day 6, the reducing sugar contents of the 40-mesh and 80-mesh corn stalk siftages were 1.33 mg/mL and 1.18 mg/mL, respectively. The reducing sugar content of the 40-mesh corn stalk siftage was higher than that of the 80-mesh corn stalk siftage, likely because after the ferric chloride treatment, the cell wall of the 80-mesh corn stalk siftage was destroyed more completely than that of the 40-mesh corn stalk siftage, and the smaller particle size was more conducive to mycelial propagation and growth. On day 6, the fungus had already matured and begun to degrade the 80-mesh corn stalk siftage. In summary, after the combined pretreatment, the 80-mesh corn stalk siftage produced more reducing sugars than did the 40-mesh corn stalk siftage and showed the highest reducing sugar content on day 12 after fungal inoculation.

3.4 Effect of Different Treatments on the Reducing Sugar Yield of Corn Stalk

As shown in Fig.6, the reducing sugar contents of the corn stalk with different treatments exhibited different change trends. When treated with P. chrysosporium alone, the reducing sugar contents of the 40-mesh and 80-mesh corn stalk siftages first decreased, then increased, decreased again and finally stabilized. This occurred because shortly after being inoculated into the corn stalk, the fungus fed on the reducing sugars to grow while producing reducing sugars by degrading the corn stalk. In the combined treatment, the reducing sugar content of

the treated corn stalk increased first, peaked on day 12, then decreased and finally stabilized. This occurred because after being treated with ferric chloride, the corn stalk's cell wall structure was destroyed, allowing the fungus to directly and rapidly degrade the lignin and cellulose to form polysaccharides. Therefore, after being treated with ferric chloride, treating the corn stalk again with P. chrysosporium significantly improved the reducing sugar production yield and cycle.

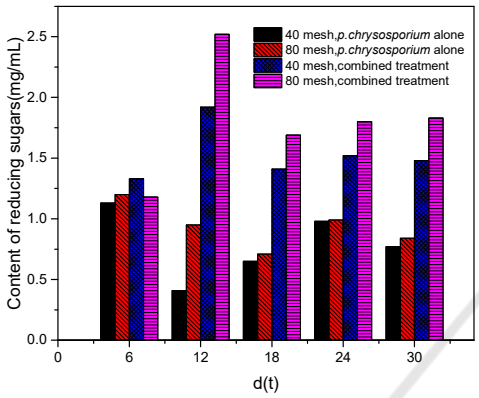


Figure 6: Comparison of the reducing sugar content of the corn stalk with different treatments

4 CONCLUSION

In this study, corn stalk was combined pretreated by ferric chloride and P. chrysosporium. The reducing sugar content of the P. chrysosporium-treated 80-mesh corn stalk siftage previously treated with ferric chloride was 2.52 mg/mL on day 12 after the fungal inoculation when the reducing sugar content in the fermentation broth was the highest.

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