

Research Article

Establishment of a Highly Efficient Corn Stock-Degrading Microbial Consortium and Its Degradation Effect

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Corn stalks are not easily degradable and thus have a low utilization rate. In this paper, a highly efficient corn stalk-degrading microbial consortium, designated as SDP, was established using the strains Z22 (*Bacillus subtilis*), Z15 (*Bacillus megaterium*), and Z08 (*Aspergillus tubingensis*). Moreover, the fermentation conditions for enzyme production by SDP were optimized through orthogonal experiments. The Van Soest method was used to determine the cellulose, hemicellulose, and lignin contents in the degraded corn stalks using the SDP consortium to evaluate its degrading effect. Scanning electron microscopy was used to analyze the micromorphological structure changes of the stalks to evaluate the degradation effects of SDP on corn stalks. The carboxymethyl cellulase activity of SDP reached 6.37 U/mL. Moreover, the enzyme production effect was optimal when the culture conditions were as follows: temperature, 30°C; time, 5 d; nitrogen source, NH₄NO₃; and initial pH, 7.0. The SDP consortium showed an improved ability to degrade corn stalks. The cellulose, hemicellulose, and lignin contents were reduced by 32.15%, 23.87%, and 7.98%, respectively, after culturing for 20 d. This study provides insights to guide further research and development of consortia for efficient corn stalk degradation.

1. Introduction

Crop stalks are the main by-product of food production. Although the total annual global stalk output is about 7 billion tons [1], only 30% is used for feed and industrial production. Most stalks are processed through incineration, with only a small proportion returned to the field [2]. Therefore, developing a safe and environmentally friendly stalk treatment method with low-energy consumption is necessary to effectively utilize stalk resources [3]. In recent years, microbial stalk degradation has attracted much attention; the production of biofuels and biological products from renewable biomass straw and other sources and the development of flexible and integrated biorefined products are the key links to achieve the transition from a petroleum-based economy to a new bio-economy seeking more efficient and sustainable global development [4, 5]. In the process of biorefinery conversion, the pretreatment of biomass straw by microbial fermentation and the separation of strains that can effectively degrade the straw are one of the methods to

improve the bioconversion efficiency and product yield. Different biorefinery pretreatments of biomass straw can achieve different effects, for example, the use of straw-degrading microorganisms can increase the accessibility of cellulose; mechanical treatment can increase the contact surface area and better enzymatic hydrolysis accessibility of substrates and is not sensitive to the content of lignin or hemicellulose. Chemical treatment can dissolve some of the hemicellulose, loosening the complex cross-linked structure of cellulose, hemicellulose, and lignin, and increasing the relative content of cellulose components, allowing microorganisms or enzymes to better degrade cellulose. Washing away the sugars produced by acid digestion is done to avoid producing product inhibition that would lead to a reduction in the cellulase-producing abilities of microorganisms. Therefore, the development of an appropriate pretreatment technology is a key step to realize the feasibility of the whole biorefinery conversion process [6, 7]. In this paper, corn stover was treated by mechanical cutting and dilute acid, and biomass stover was biorefined by efficient degradation of

stover microbial flora, which achieved the expected effect and provided a theoretical basis for the development of modern bio-economy.

Studies have shown that it is difficult for a single microbial species to efficiently degrade cellulosic substances due to environmental factors and the cellulose-degrading enzymes produced [8]. However, the enzymes produced by the microorganisms are diversified when multiple species of cellulose-degrading bacteria are mixed to form a microbial consortium. The synergistic effect of various enzymes improves the utilization rate of cellulosic materials [9]. Li et al. isolated and screened five strains from the soil in Gongga, a mountainous area in the Western Sichuan Plateau. The consortium had higher filter paper enzyme activity, indicating that they could efficiently degrade cellulose [10]. Jiang et al. screened strains from a field where corn stalks were returned, and they established a consortium termed F1 that had an excellent degradation effect on corn and high enzyme activity [11]. Therefore, the synergistic effect of multiple cellulose-degrading strains results in the secretion of multiple cellulase enzymes for effective cellulose degradation. As a result, research into microbial consortia deserves more attention than that of single degrading strains [12]. Three strains that can efficiently and stably degrade cellulose were identified in our previous work. Herein, the stability of a consortium containing these three strains (designated as SDP) was assessed, as was their relationship, optimal enzyme production conditions, and degradation effect, in order to obtain a highly efficient corn stalk-degrading consortium. Carboxymethyl cellulase activity was used as the evaluation index to establish the SDP consortium that is highly efficient in degrading corn stalks. Stalk degradation experiments were also conducted, providing a theoretical foundation for practical applications.

2. Materials and Methods

Corn stalks were obtained from the experimental cornfield of Shihezi University. The crushed corn straw powder is treated with 0.75% dilute sulfuric acid at 121°C for 1 h, washed to neutral, dried and cut into 2-3 cm, and set aside. Subsequently, the stalks were filtered through a 40-mesh sieve and put in a ventilated and dry place for later use. The three strains that were previously isolated, purified, and cryopreserved, namely, Z22 (*Bacillus subtilis*), Z15 (*Bacillus megaterium*), and Z08 (*Aspergillus tubingensis*), were used as the degrading strains. All the chemicals involved in the experiment were provided by Shanghai Puzhen Biotechnology Co LTD, and all the chemicals were analytically pure.

Carboxymethyl cellulose (CMC g/L) medium: 15 g CMC-Na, 1 g NH₄NO₃, 1 g yeast extract, 0.5 g MgSO₄·7H₂O, 1 g KH₂PO₄, and 20 g agar. The medium was sterilized at 121°C for 20 min. CMC-Na medium (activation medium) (g/L): 15 g CMC-Na, 1 g NH₄NO₃, 1 g yeast extract, 0.5 g MgSO₄·7H₂O, and 1 g KH₂PO₄; pH natural. The medium was sterilized at 121°C for 20 min. Liquid culture medium: 20 g stalk powder and 120 mL inorganic salt solution (1.0 g KH₂PO₄, 0.1 g NaCl, 0.3 g MgSO₄·7H₂O, 2.5 g NaNO₃, 0.01 g

FeCl₃, and 0.1 g CaCl₂) were mixed in a 250 ml flask. The medium was sterilized at 121°C for 20 min.

2.1. Antagonism Assays between Strains. The dominant isolated and purified strain was transferred from the slant medium to the liquid medium for propagation. Strain Z22 solution (0.5 mL) was spread on the carboxymethylcellulose plate medium. Strain Z15 and Z08 were spotted on the surface of the culture plate and then cultured at a constant temperature of 30°C for 2-5 d before observing whether there was antagonism and growth inhibition among the growing strains.

2.2. Establishment of Consortia. The isolated and purified strains were mixed at a volume ratio of 1:1 (v/v) and then inoculated in the liquid medium at 30°C and 120 r/min. The glucose standard curve was first plotted, followed by the DNS color development method. 10 ml of washed and dried cuvettes with stoppers were taken, numbered and preheated for 5-10 min in a 50°C water bath, then 2.0 ml of CMC-Na buffer solution and 0.5 ml of enzyme solution were added to each cuvette and mixed, and held for 30 min in a 50°C water bath. The optical density values of the blank control and the treated enzyme solution were measured at 540 nm, and the amount of glucose contained in the enzyme solution was derived from the glucose standard curve to calculate the enzyme activity. According to the activity of CMC enzyme, the strain combination with high performance was obtained.

2.2.1. Definition of CMC Enzyme Activity. The amount of enzyme that catalyzes the hydrolysis of cellulose to produce 1.0 μmol of glucose per minute is one enzyme activity unit (U) [13].

2.3. Optimization of the Cultivation Conditions of the Consortium. A 4-factor 5-level orthogonal experiment was conducted to optimize the culture condition parameters. The levels of each factor were as follows: temperature, 25°C, 30°C, 35°C, 40°C, and 45°C; time; 1, 2, 3, 4, and 5 d; nitrogen source, peptone, yeast extract, beef extract, peptone + beef extract, and NH₄NO₃; and pH value, 4.0, 5.0, 6.0, 7.0, and 8.0. Each treatment had three replicates. The enzyme activity of the consortium was measured after culturing. See Table 1 for level settings.

2.4. Determination of the Effect of the Consortium in Degrading Corn Stalks. Stalk powder (0.50 g) was dried to a constant weight under the optimal enzyme production conditions. The dried powder was put into a 50 mL liquid medium and then the SDP consortium was inoculated into the liquid medium. Culturing was performed at 30°C and 120 r/min with three replicates. The degraded products were removed every 5 d. The modified Van Soest's acid detergent fiber method was used to determine cellulose, hemicellulose, and lignin degradation rates in the corn stalks [14].

TABLE 1: Factors and levels of fermentation condition parameters of the consortium.

Factor level	Temperature (A)/°C	Culturing time (B)/d	Nitrogen source (C)/g	Initial pH (D)
1	20	5	Peptone	4.0
2	25	4	Yeast extract	5.0
3	30	3	Beef extract	6.0
4	35	2	Peptone + beef extract	7.0
5	40	1	NH ₄ NO ₃	8.0

2.4.1. Determination of Cellulose, Lignin, and Hemicellulose Content in Treated Straw by Differential Gravimetry

- (1) Dry the treated samples in an oven at 60°C to a constant weight and set aside in a desiccator.
- (2) Weigh two dried 0.5000 g samples and place them in a high type beaker, then add several drops of decahydronaphthalene and 100 mL of neutral detergent and 0.5 g of anhydrous sodium sulfite, cover with a condensation lid, place on an electric stove and boil for 5-10 min, allow the mixture to fully reflux for 1 h and then cool off the fire for 10 min, place a crucible of known constant weight on the extraction flask, remove all the residues, and rinse the residue with hot water. Rinse the slag until the neutral detergent present in the filtrate is cleanly rinsed and pH = 7.0 (verified with blue litmus paper), then wash the slag with 20 ml acetone twice until the flowing acetone solution is colorless, put it into the drying oven, and then carry out weighing of the filter hopper and straw powder as W1.
- (3) Similarly, two dried samples of 0.5000 g were taken and placed in a high-type beaker, several drops of decahydronaphthalene and 100 mL of acid detergent were added to the beaker, and the same process as neutral washing was performed. The residue was washed with 20 ml of acetone until the filtrate showed a neutral reaction (verified with blue litmus paper), then rinsed repeatedly until the acetone was drawn off, and the crucible was dried and weighed which was taken as W2.
- (4) The residue obtained from step (3) was added to 72% sulfuric acid, digested for 3 h at 20°C, filtered and rinsed to neutral, and the residue was dried and weighed as W3. Then the residue was first slowly cauterized in a crucible over medium heat and then moved to a 600°C Muffle furnace for 2 h. The crucible was removed and cooled to constant weight in a desiccator and weighed as W4.

Result calculation: W1–W2 = Hemicellulose.
W2–W3 = cellulose
W3–W4=Lignin

2.5. *Analysis of the Surface Structure of Corn Stalks after Degradation by the Consortium.* The degradation effect of the consortium on corn stalks was observed through the surface structure. Scanning electron microscopy (SHI-MADZU) was used to observe the structural changes of corn stalks before and after 30 d of degradation [15].

The conditions of the analysis are as follows: (1). Sampling: the untreated 40-mesh corn straw powder and single strains Z08, Z15, and Z22 and the straw powder after 8 d of fermentation and degradation of the constructed colony SDP, respectively, were sampled. (2). Fixation: add 2% glutaraldehyde fixative to the treated samples in the fume hood, and then put them into the vacuum pumping machine to draw off the vacuum, while shaking to make the samples lay flat. (3). Rinsing: rinse the samples 3 times (10 min each) with PBS buffer. (4). Dehydration: the samples were dehydrated by 30, 50, 70, 80, 90, and 100% ethanol solutions for 10 min each. (5). Displacement: after the sample was dehydrated for 15 min, the transition was made to isoamyl acetate for displacement 2 times, each time for 15 min. (6). Drying: make the sample as good as possible to maintain the fine structure of the surface, using CO₂ critical point dryer for drying. (7). Coating: take a small amount of dry straw sample, using tweezers to gently paste the sample on the copper sheet with conductive adhesive and then put into the ion sputtering instrument for gold plating. (8). Scanning electron microscope observation: adjust the focus to make clear, and then take images with good specificity.

2.6. *Statistical Analyses.* Minitab Statistical Software was used for level range analysis and analysis of variance on the 4-factor 5-level orthogonal experimental data.

3. Results

3.1. *Antagonism of Cellulose-Degrading Strains.* The dominant purified strains Z08, Z15, and Z22 were combined in pairs to test strain antagonism (Table 2). These strains showed no antagonism, and the hyphae could grow in an evenly mixed manner, thus showing high compatibility. Therefore, the abovementioned three strains can be mixed to establish a stalk-degrading consortium for exploring the degrading effect on corn stalks.

3.2. *Enzyme Activity of the Constructed Consortia.* Cellulose-degrading strains without antagonistic reactions were mixed at a volume ratio of 1 : 1 to prepare a microbial suspension. The microbial suspension was then inoculated into the liquid enzyme-producing medium ($v : v = 5 : 100$). The CMC activity of the fermentation broth in each group was measured every 24 h of culturing at 30°C and 120 r/min (Figure 1).

The enzyme activity of the consortia was highest on the 5th day, reaching 6.37 U/mL. Culturing two or more combined strains could increase the enzyme activity to a

TABLE 2: Antagonistic relationship between strains.

Strain	Z08	Z15	Z22
Z08		—	—
Z15	—		—
Z22	—	—	

Note: “—” indicates that there is no antagonism, and “+” indicates that there is antagonism.

certain extent since cellulase is composed of a complex enzyme system. The combination of Z08 + Z15 + Z22 had the most significant effect. The diversity of enzyme production from different strains in mixed culturing allows the enzyme systems at certain ratios achieve a coordinated effect, thereby improving the ability to degrade stalks and thus increasing enzyme activity of the mixed group. Different strains can coexist based on the theory of ecological niches because they avoid mutual competition between the populations through the synergy effect. In this study, the compatibility between the strains allowed the synergistic strain combination to degrade natural cellulose [16]. Moreover, the CMC enzyme activity of the natural stalk-degrading SDP consortium was better than the CMC enzyme activity (1.7 IU/mL) reported by Zeng [17] and that of the mixed strains selected from the soil by Feng et al. [18]. These results indicate that the SDP consortium (Z08 + Z15 + Z22) can efficiently degrade natural stalks.

3.3. Optimization of Enzyme Production Conditions of the Consortium. The 4-factor 5-level orthogonal design table L_{25} (45) was designed using the orthogonal experimental design method (Table 3). Treatments were set as shown in Table 1, and each treatment had three replicates (a total of 75 samples). In this experiment, the amount of inoculation and nitrogen source was 1 mL and 1 g/L, respectively. The optimal culture condition was determined based on a statistical analysis of the CMC enzyme activity of the substrate.

The variance of the orthogonal experiment results was analyzed using the Minitab software. The statistical analysis of the results is shown in Tables 3 and 4. The order of the primary and secondary factors affecting the enzyme activity of the consortium system was as follows: temperature > culturing time > initial pH > nitrogen source, based on range R -value (Table 3) and the T -value (Table 4).

The main effects of the four factors A, B, C, and D were also analyzed (Figure 2). In this experiment, by orthogonal test using Minitab software for ANOVA, it can be seen that the average of each level of A (temperature) factor, the best enzyme activity of the composite bacterial system is at A3, indicating that the best temperature for fermentation is 30°C; for B (time period) factor, it can be observed that the highest degradation enzyme activity of the composite bacterial system is at B1, so the best time period for fermentation is 5 d. From the analysis of the mean value of each level of C (nitrogen source) factor, it can be seen that the highest enzyme activity of the composite bacterial system is at C5, indicating that NH_4NO_3 is the best nitrogen source; from the analysis of

the mean level of each D (initial pH) factor, it can be seen that the best degradation enzyme activity of the composite bacterial system is at D3, indicating that the optimum initial pH is 7.0, inferring that the order of the primary and secondary effect factors on the enzyme activity of the consortium is as follows: temperature > time period > initial pH > nitrogen source.

3.4. Determination of the Degradation Effect of the Consortium on Corn Stalks. The consortium was inoculated into the liquid fermentation medium at a ratio of 5:100 (v/v). Each treatment had three replicates. The culture was incubated at 30°C and 120 r/min for 20 d. Samples were taken every 5 d to determine cellulose, hemicellulose, and lignin degradation rates in the corn stalks.

The corn stalks contained 36.26% cellulose, 27.60% hemicellulose, and 15.78% lignin before pretreatment. However, pretreatment with the SDP consortium decreased the content of the three components in the stalks (Figure 3). For instance, cellulose, hemicellulose, and lignin contents decreased by 32.15%, 23.87%, and 7.98%, respectively.

Cellulose and hemicellulose degradation process is more obvious, indicating that dilute sulfuric acid pretreatment increased the degradability of straw xylan; thus, the composite bacterial system can better degrade cellulose and hemicellulose, while lignin is poor, which proves that degrading lignin is difficult [6, 19]. Cellulose is a polymer compound formed via the polymerization of β -1,4-glycosidic bonds, which are easily degradable. Hemicellulose is composed of xylose, galactose, mannose, and other components, and it needs two or more hydrolysis reactions to convert the large molecule sugars into small molecules (e.g., glucose). Lignin is a three-dimensional polyphenol network aromatic polymer compound connected by ether bonds and C-C bonds, which are difficult to degrade [20].

3.5. Analysis of the Surface Structure of the Corn Stalks after Degradation by the Consortium. Scanning electron microscopy was used to image the fracture surface, degraded surface, and microscopic surface morphology of the corn stalk before and after the treatment. The fibrous tissue of the corn stalks had structural changes before and after the pretreatment. The physically treated stalks had broken fibers due to the external force, but the treatment did not destroy the integrity of its overall structure (Figure 4(a)). The waxy surface of the fiber on the primary wall was smooth, and the structure was neat and compact, with high crystallinity. It did not absorb water and swell and had a lot of lignin. The morphological characteristics of the stalk fiber and the overall appearance of the cell wall were altered (Figure 4(b)). The surface structure of the stalks was destroyed with many fractures and residues. The primary wall was damaged and had different degrees of deformation and displacement. The fracture could absorb water and expand, making the plasticity of the fiber stronger and softer. The inside diameter of the stalk was significantly reduced, while the surface area of the fiber increased. The secondary wall layers began to fall off

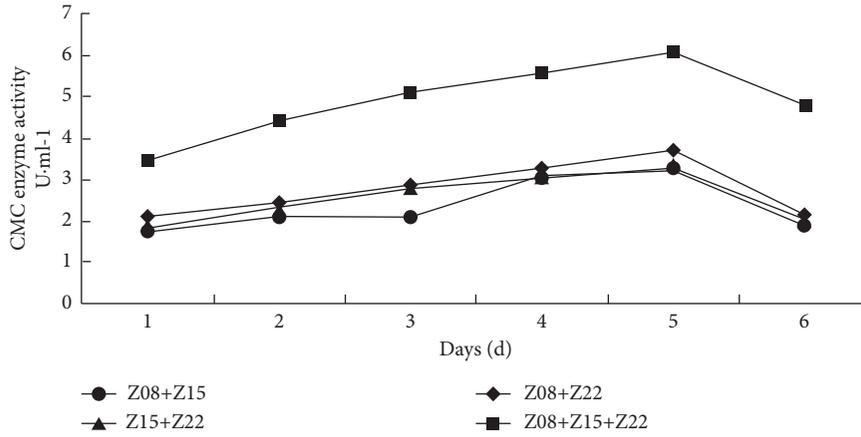


FIGURE 1: Curves for CMC enzyme activity of the consortium against culturing time.

TABLE 3: Orthogonal design table L25 (45) of the culture condition parameters of the consortium.

Factor exp#	Factor				CMC enzyme activity (U/mL)			Mean
	A	B	C	D	r1	r2	r3	
1	1 (20)	1 (5)	1 (P)	1 (4.0)	5.3785	6.4705	5.7799	5.8763
2	1 (20)	2 (4)	2 (Y)	2 (5.0)	4.5129	4.5409	4.5269	4.5269
3	1 (20)	3 (3)	3 (B)	3 (6.0)	4.9819	4.9492	4.9725	4.9679
4	1 (20)	4 (2)	4 (P + B)	4 (7.0)	5.9502	6.1485	6.1019	6.0669
5	1 (20)	5 (1)	5 (NH ₄ NO ₃)	5 (8.0)	5.5022	4.2422	4.9375	4.8940
6	2 (25)	1 (5)	2 (Y)	3 (6.0)	6.7342	5.2222	5.9525	5.9696
7	2 (25)	2 (4)	3 (B)	4 (7.0)	5.8942	6.1275	5.9619	5.9945
8	2 (25)	3 (3)	4 (P + B)	5 (8.0)	4.5339	5.0425	4.8792	4.8185
9	2 (25)	4 (2)	5 (NH ₄ NO ₃)	1 (4.0)	6.0319	5.1592	5.6025	5.5979
10	2 (25)	5 (1)	1 (P)	2 (5.0)	4.9632	6.1299	5.4812	5.5248
11	3 (30)	1 (5)	3 (B)	5 (8.0)	5.6865	6.0505	5.7262	5.8211
12	3 (30)	2 (4)	4 (P + B)	1 (4.0)	5.1895	5.8125	5.3762	5.4594
13	3 (30)	3 (3)	1 (P)	2 (5.0)	6.3352	6.1952	6.2512	6.2605
14	3 (30)	4 (5)	5 (NH ₄ NO ₃)	3 (7.0)	6.1509	6.4169	6.2179	6.2619
15	3 (30)	5 (1)	2 (Y)	4 (6.0)	5.3225	4.7649	5.1172	5.0682
16	4 (35)	1 (5)	4 (P + B)	2 (5.0)	6.0692	4.7299	5.3902	5.3964
17	4 (35)	2 (4)	5 (NH ₄ NO ₃)	3 (6.0)	6.3889	5.4509	5.8779	5.9059
18	4 (35)	3 (3)	1 (P)	4 (7.0)	6.5009	4.6155	5.3972	5.5045
19	4 (35)	4 (2)	2 (Y)	5 (8.0)	5.1102	5.0682	5.1032	5.0939
20	4 (35)	5 (1)	3 (B)	1 (4.0)	4.4919	4.5059	4.5012	4.4996
21	5 (40)	1 (5)	5 (NH ₄ NO ₃)	4 (7.0)	5.8919	4.7485	5.2152	5.2852
22	5(40)	2 (4)	1 (P)	5 (8.0)	4.6505	4.4965	4.5759	4.5743
23	5 (40)	3 (3)	2 (Y)	1 (4.0)	4.4919	5.4182	4.9352	4.9484
24	5 (40)	4 (2)	3 (B)	2 (5.0)	4.6785	4.5945	4.6249	4.6326
25	5 (40)	5 (1)	4 (P + B)	3 (6.0)	5.1989	4.7509	5.1125	5.0208
K1	5.266	5.670	5.548	5.276				
K2	5.581	5.292	5.121	5.268				
K3	5.774	5.300	5.183	5.625				
K4	5.280	5.531	5.352	5.584				
K5	4.892	5.001	5.589	5.040				
R	0.882	0.668	0.467	0.585				

and slide, with a significantly reduced internal diameter. The surface area of the fibers increased, indicating that strains had completely invaded the inside of the stalks, with several degrading enzymes secreted. Consequently, the internal lignocellulose was degraded.

4. Discussion

Bacteria and fungi have stalk-degrading properties. Fungi secrete many enzymes that can degrade lignin; *Bacillus* has the characteristics of fast growth and reproduction, strong

TABLE 4: Variance analysis table for orthogonal test results under consortium culture conditions.

Item	Coefficient	Coefficient standard error	<i>T</i>	<i>P</i>
Constant	4.8927	0.73364	6.669	0.001
A	0.7918	0.31709	2.497	0.023
B	-0.1098	0.06135	-1.790	0.091
C	-0.5834	0.31709	-1.840	0.083
D	0.6140	0.31709	1.936	0.070
A2	-0.1494	0.05185	-2.882	0.010
C2	0.1024	0.05185	1.975	0.065
D2	-0.1049	0.05185	-2.024	0.059
S	0.433810			

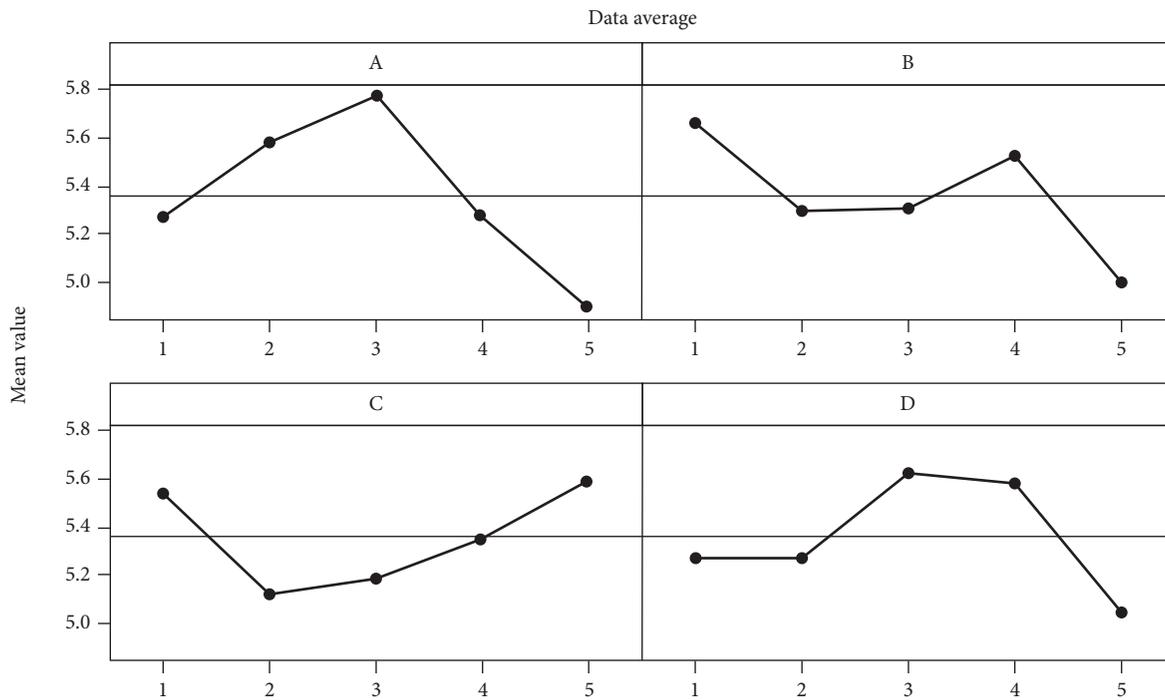


FIGURE 2: Main effects of the factors.

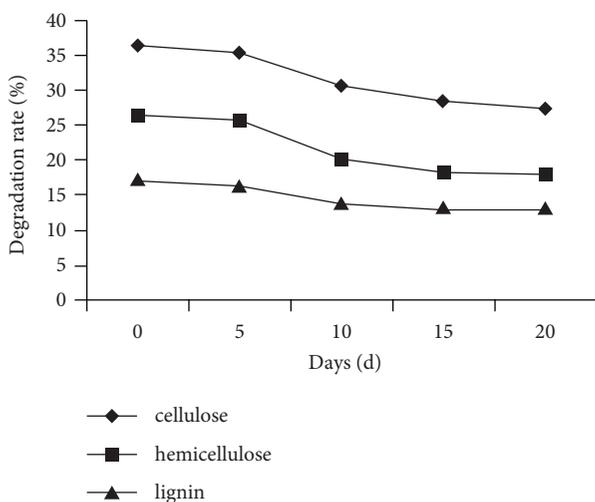


FIGURE 3: Changes in cellulose, hemicellulose, and lignin contents.

stress resistance, and multienzyme activity. Therefore, the combination of fungi and cellulase-producing *Bacillus* strains is essential for efficient cellulose degradation. Bacteria are represented by different species and have various enzymatic activities and thus can efficiently degrade cellulose substances through a synergistic effect [21, 22]. In this paper, antagonism analysis was conducted on the three dominant strains obtained in the preliminary screening. The nonantagonistic strains were mixed to determine the enzyme activity of each combination. Finally, a strain SDP consortium with higher enzyme activity and without antagonism was established and selected for further analysis. The optimization of the fermentation conditions showed that the enzyme activity of SDP was higher than the activity of other combinations. Moreover, cellulose, hemicellulose, and lignin in corn stalks were degraded by 32.15%, 23.87%, and 7.98%, respectively, indicating that the consortium can significantly degrade stalks. The developed consortium has

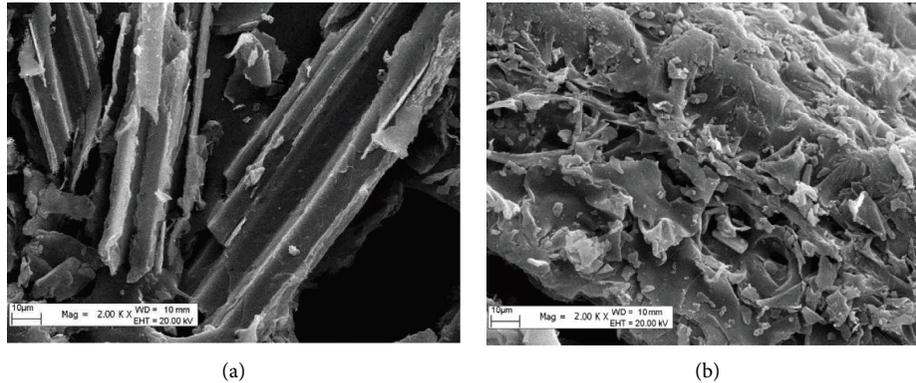


FIGURE 4: Scanning electron micrographs of stalk fibers before and after treatment.

not been previously reported, thus providing resources and a theoretical basis for stalk degradation. The high-efficiency degradation consortium, HD, established by Wang et al. showed an enzyme activity 1.9 times higher than that of single strains. Moreover, the corn stalk degradation rate of HD was significantly higher than that of a single strain (degradation rate, 47%) [23]. Liu et al. [24] developed a consortium, YM, with a higher degradation effect on the three cellulose components of corn stalks than any single strain. The YM consortium degraded cellulose, hemicellulose, and lignin by 39.02%, 57.69%, and 25.79%, respectively. Duan et al. [25] also established a highly efficient stalk-degrading consortium, with a higher stalk degradation rate, reaching 34.52%, than the effect of every single strain. Zhang et al. [26] developed a liquid-fermented consortium with a higher stalk degradation rate (53%) than every strain. The consortium degraded cellulose, hemicellulose, and lignin by 30%, 29%, and 32%, respectively. Hui et al. [27] used XDC-2 to degrade different kinds of straw, and the results showed that hemicellulose in each component had the best utilization effect. The maximum value of xylanase activity reached 335 U/mL. Qing et al. [28] screened strains from the soil, rotten stalks, and cattle and sheep dung and then obtained a group of strains with high-efficiency cellulase production via subculturing. The strains could stably degrade stalks under a low-temperature environment. Li et al. [29] showed that *Bacillus amyloliquefaciens* can degrade cellulose, hemicellulose, and lignin in corn stalks by 30.5%, 41.4%, and 48.4%, respectively, after fermentation for 24 d. The stalk degradation effect of the consortium was higher than that of the single strain, consistent with the above-mentioned research results. These results show that strain combination can be used to develop various consortia and a more diverse enzyme production system, which can degrade cellulose through a synergistic effect, thus improving the degradation effect in stalks.

5. Conclusions

In this study, the CMC enzyme activity of each combination of strains Z08, Z15, and Z22 was measured, and finally, a composite strain SDP with no antagonism and high enzyme

activity was constructed, and its enzyme activity reached 6.37 U/ml. The optimal culture conditions of the consortium were: temperature, 30°C; culturing time, 5 d; nitrogen source, NH_4NO_3 ; and initial pH, 7.0. SDP had a good degradability of corn stalks. The SDP degraded cellulose, hemicellulose, and lignin content by 32.15%, 23.87%, and 7.98%, respectively. The scanning electron micrographs of the stalks before and after the degradation showed that the internal and external structures of the stalks were damaged and that the stalks became loose and soft, with decreased fiber crystallinity. Therefore, the SDP consortium can help research the degradation and utilization of corn stalks and other organic matter. However, the consortium was obtained at room temperature, and thus further research at higher and lower temperatures is needed. Moreover, the results of this study should be translated into reagent samples in practical applications. Finally, further studies are needed to assess whether the same reagent can adapt to different environments since different management practices are used in various crop fields.

Data Availability

All data used for this study are available from the corresponding author upon request.

Conflicts of Interest

No potential conflicts of interest were reported by the authors.

Authors' Contributions

All authors contributed equally in the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version. Erhong Zhang and Meng Wang are co-first authors.

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References

- [1] O. J. Sanchez and C. A. Cardona, "Trends in biotechnological production of fuel ethanol from different feed stocks," *Bioresource Technology*, vol. 99, pp. 5270–5295, 2008.
- [2] X. Zeng, Y. Ma, and L. Ma, "Utilization of straw in biomass energy in China," *Renewable and Sustainable Energy Reviews*, vol. 11, pp. 976–987, 2007.
- [3] X. Q. Zhang, Z. F. Wang, M. Y. Cen, H. H. Bai, and N. Ta, "Analysis of yield and current comprehensive utilization of crop straws in China," *Journal of China Agricultural University*, vol. 26, pp. 30–41, 2021.
- [4] H. Guo, J. Chang, Q. Yin et al., "Effect of the combined physical and chemical treatments with microbial fermentation on corn straw degradation," *Bioresource Technology*, vol. 148, pp. 361–365, 2013.
- [5] P. Manzanera, "The role of biorefining research in the development of a modern bioeconomy," *Acta Innovations*, vol. 37, pp. 47–56, 2020.
- [6] T. A. Lloyd and C. E. Wyman, "Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids," *Bioresource Technology*, vol. 96, no. 18, pp. 1967–1977, 2005.
- [7] M. H. L. Silveira, A. R. C. Morais, A. M. Da Costa Lopes et al., "Current pretreatment technologies for the development of cellulosic ethanol and biorefineries," *ChemSusChem*, vol. 8, no. 20, pp. 3366–3390, 2015.
- [8] L. R. Lynd, P. J. Weimer, W. H. van Zyl, and I. S. Pretorius, "Microbial cellulose utilization: fundamentals and biotechnology," *Microbiology and Molecular Biology Reviews*, vol. 66, no. 4, p. 739, 2002.
- [9] M. Kolasa, B. K. Ahring, P. S. Lübeck, and M. Lubeck, "Co-cultivation of *Trichoderma reesei* Rut C30 with three black *Aspergillus* strains facilitates efficient hydrolysis of pretreated wheat straw and shows promises for on-site enzyme production," *Bioresource Technology*, vol. 169, pp. 143–148, 2014.
- [10] J. Li, H. N. Zhang, and C. Zhao, "Isolation and screening of high-efficiency cellulose-degrading bacteria, construction of complex bacteria and analysis of straw degradation effect," *Chinese Journal of Applied and Environmental Biology*, vol. 22, pp. 689–696, 2016.
- [11] G. F. Jiang, T. J. Yang, and H. P. Zheng, "Construction and degradation effect evaluation of corn stalk degradation fungi complex strain," *Journal of Plant Nutrition and Fertilizer*, vol. 27, pp. 284–292, 2021.
- [12] Y. Y. Wang, G. X. Li, M. H. Chi et al., "Effects of co-digestion of cucumber residues to corn stover and pig manure ratio on methane production in solid state anaerobic digestion," *Bioresource Technology*, vol. 250, pp. 328–336, 2018.
- [13] Y. S. Gong, Y. C. Li, W. B. Wu, and S. Y. Liu, "Screening and mixed fermentation of thermophilic cellulolytic bacteria," *Jiangsu Agricultural Sciences*, vol. 3, pp. 394–396, 2010.
- [14] Y. W. Wang and W. Y. Xu, "Procedure for the quantitative analysis of hemicellulose cellulose and lignin in lignocellulosic solid matrix ferments," *Microbiology Bulletin*, vol. 14, pp. 81–84, 1987.
- [15] X. Feng, Y. H. Pei, Z. X. Zhou, and Y. H. Xie, "Screening of stalk cellulose-degrading microorganisms and construction of a microbial community with high capacity," *Journal of Northwest A&F University*, vol. 19, pp. 35–37, 2004.
- [16] W. Song, X. Wang, J. Gu et al., "Effects of different swine manure to wheat straw ratios on antibiotic resistance genes and the microbial community structure during anaerobic digestion," *Bioresource Technology*, vol. 231, pp. 1–8, 2017.
- [17] Q. L. Zeng, "Isolation and identification of straw cellulose-biodegrading filamentous fungi," *Hubei Province Agricultural Science*, vol. 47, pp. 652–655, 2008.
- [18] X. Feng, Y. H. Pei, and X. F. Zhou, "Screening of stalk cellulose-degrading microorganism and construction of a microbial community with high capacity," *Journal of Northwest A&F University (Natural Science Edition)*, vol. 25, pp. 30–32, 2017.
- [19] W. Fang, J. Ye, P. Zhang, X. Zhu, and S. Zhou, "Solid-state anaerobic fermentation of spent mushroom compost for volatile fatty acids production by pH regulation," *International Journal of Hydrogen Energy*, vol. 42, no. 29, pp. 18295–18300, 2017.
- [20] C. X. Xie, J. Z. Sun, and C. L. Li, "Research progress on degradation of lignin by bacteria," *Microbiology Bulletin*, vol. 42, pp. 1122–1123, 2015.
- [21] G. Zhou, J. Zhang, L. Chen, C. Zhang, and Z. Yu, "Temperature and straw quality regulate the microbial phospholipid fatty acid composition associated with straw decomposition," *Pedosphere*, vol. 26, no. 3, pp. 386–398, 2016.
- [22] S. C. L'opez, B. Theelen, S. Manserra et al., "Functional diversity in *dichomitus squalens* monokaryons," *Ima Fungus*, vol. 8, no. 1, pp. 17–25, 2017.
- [23] X. G. Wang, L. Tian, and E. Z. Wang, "Construction and degradation effect evaluation of high efficient microbial complex strains for corn straw degradation," *Biotechnology Bulletin*, vol. 26, pp. 23–26, 2021.
- [24] X. Liu, D. X. Shan, and M. R. Sun, "Visualizing patterns of and differences in fine particulate matter (PM_{2.5}) research between the USA and China over the last 25 Years: a bibliometric analysis," *Journal of Northeast Agricultural University*, vol. 34, pp. 35–39, 2019.
- [25] J. Duan, *Screening of Cellulose-Degrading Bacteria from Straw in Northeast China and Construction of Efficient Degrading Bacteria*, Jilin Agricultural University, Jilin, China, 2015.
- [26] S. Zhang, D. Shan, X. Liu, and M. Sun, "Cellulose-degrading strains: their screening and application to corn straw in low-temperature environments: their screening and application to corn straw in low-temperature environments," *Polish Journal of Environmental Studies*, vol. 27, no. 5, pp. 2349–2355, 2018.
- [27] W. Hui, L. Jiajia, L. Yucai et al., "Bioconversion of un-pretreated lignocellulosic materials by a microbial consortium XDC-2," *Bioresource Technology*, vol. 136, pp. 481–487, 2013.
- [28] G. E. Qing, J. L. Gao, and X. F. Yu, "Species composition and degradation stability of the composite strain GF-20 for high efficiency degradation of corn straw at low temperature," *Agricultural Sciences in China*, vol. 49, pp. 443–454, 2016.
- [29] H. Y. Li, W. Li, and S. N. Li, "Degradation and characterization of compound agent of *Bacillus amyloliquefaciens* on corn straw," *Journal of grass industry*, vol. 26, pp. 153–167, 2017.