Analysis of Key Factors Affecting Microbial Proliferation During the Solid-State Fermentation of Corn Husk-based Protein

Yaping Yan,[a,b,c,d,e] Yinan Sun,[e] Xing Gao,[a,b,c,d] Jinna Cui,[a,b,c,d] Junjie Gao,[e] and Zhanying Liu [a,b,c,d,*]

Corn husk, a significant by-product of the corn deep-processing industry, is currently utilized as coarse feed, yielding very low economic benefits while consuming high amounts of energy and water. This study focused on the high-value utilization of corn husk resources. A two-step enzymatic hydrolysis and combined microbial fermentation was adopted to produce corn husk microbial protein feed. The true protein content was increased by 103% through yeast proliferation. To explore the key driving factors affecting yeast proliferation, a quantitative polymerase chain reaction (qPCR) was adopted to analyze the succession of yeast communities during the fermentation of corn husks. Redundancy analysis (RDA) and variance inflation factor (VIF) were applied to examine the relationship between physicochemical factors and yeast microbial community. The results revealed that, in terms of fermentation time, the uppermost driving factors influencing yeast abundance is moisture content; in terms of contribution, both cellulose content and moisture content serve as the most significant driving factors for yeast proliferation. This research revealed that microbial-enzyme synergy can significantly increase the true protein content of feed, and the key driving factors identified further provide theoretical references for the controllable yeast fermentation.

DOI: 10.15376/biores.20.3.6476-6489

Keywords: Corn husks; Microbial-enzyme synergy; Driving factors; Microbial protein

Contact information: a: Engineering Research Center of Inner Mongolia for Green Manufacturing in Bio-fermentation Industry, Hohhot, Inner Mongolia, 010051, China; b: Specialized Technology Research and Pilot Public Service Platform for Biological Fermentation in Inner Mongolia, Hohhot, Inner Mongolia, 010051, China; c: Center for Energy Conservation and Emission Reduction in Fermentation Industry in Inner Mongolia, Hohhot, Inner Mongolia, 010051, China; d: College of Chemical Engineering, Inner Mongolia University of Technology, Hohhot, Inner Mongolia, 010051, China; e: Inner Mongolia Ecological Environment Scientific Research Institute Limited, Hohhot, Inner Mongolia, 010051, China;
** Correspondence: hgxylzy2008@imut.edu.cn*

INTRODUCTION

The rapid development of animal husbandry has led to an increasing demand for protein feeds. Soybean meal, the primary protein feed ingredient, is projected to experience a 1.6% annual increase in demand for soybeans until 2027. However, concerns have arisen regarding the impact of expanded soybean cultivation on land use, which may lead to environmental consequences (Chaudhary and Kastner 2016; Spiller *et al*. 2020). In response to the rising demand for protein feeds and concerns about the environmental impact associated with increased soybean cultivation, efforts are being made to explore alternative sources of feed protein with a lower environmental impact compared to

traditional protein crops. China released the Three-year Action Plan for Soybean Meal Reduction and Substitution in 2023.

Corn is the most widely cultivated cereal globally, with production exceeding 1.1 billion tons, according to FAOStat statistics (Erenstein *et al*. 2022). In China, corn production totals 289 million tons, accounting for 25.4% of the global output, of which approximately 800 million tons of corn undergo deep processing, generating about 10 million tons of by-products, such as corn husks. Currently, there are two main ways to use corn husks. The first is as roughage for ruminant animals due to their high crude fiber content, though their low nutritional value limits the intestinal absorption by animals. The second involves mixing the corn husks with ammonium sulfate effluent produced by the corn processing to feed ruminant animals in the form of sprouting corn husks. However, this can cause digestive issues such as diarrhea, which has raised concerns among animal caretakers. Therefore, identifying high-value uses for corn husks is key to address the challenges in extended and supplemented chain in the corn deep processing industry but also to offering a new path for feed soybean meal reduction and replacement.

Current research on the production of microbial protein feeds from high-cellulosic raw materials primarily focuses on strain performance, process optimization, and succession patterns of microbial community (Qu *et al*. 2018; Bai *et al*. 2020; Han *et al*. 2020). In a study on the effects of *Bacillus subtilis GYB6*, *Saccharomyces cerevisiae NJ1*, and *Bacillus amyloliquefaciens* Y8 on antinutritional factors and nutrient composition of canola meal protein feed, Zhu *et al*. (2023) found that the co-fermentation of these three microbes significantly reduced the levels of glucosinolates, phytic acid, crude fiber, and tannins, while greatly increasing the contents of crude protein, amino acid, and peptide. Su *et al*. 2021) applied a two-step solid-state fermentation to improve the nutritional characteristics and microbial protein content of a corn germ flour and corn husk mixture. This improvement was achieved by optimizing the fermentation temperature, solid-to-liquid ratio, and fermentation time using a combination of three microorganisms and proteases (Su *et al*. 2021). With the principle of "functional complementation," Liu *et al*. (2023) utilized cellulolytic bacteria, such as *Trichoderma reesei*, *Aspergillus niger*, and *Penicillium*, to enzymatically degrade vinasse and produce hydrolysis products for yeast fermentation, which increased the true protein content by 53.5%. These studies demonstrate that producing protein feed from high-cellulose raw materials involves not only the degradation of cellulose and hemicellulose but also the synthesis of microbial proteins. As a result, this process is frequently used in mixed fermentation or microbial-enzyme synergistic fermentation. Most research on microbial-enzyme synergistic fermentation focuses on improving proteins, probiotics, and prebiotics. For example, Ma *et al*. (2024) used walnut meal to prepare antimicrobial peptides from walnut glutenin through co-fermentation with *Bacillus subtilis* and alkaline protease. Su *et al*. (2022) investigated a two-stage microbial-enzyme synergistic fermentation process to degrease rice husks, thereby increasing the levels of soluble proteins, amino acids, and organic acids, while reducing the activity of lipase and fat oxidase, and enhancing the activity of probiotics. However, there are fewer studies on the synergistic coupling process of cellulose degradation and protein synthesis and interaction mechanism of artificial combined bacterial community. In addition, the key driving factors behind the fermentation remain unclear.

Using a series of optimization experiments, this research investigated the feasibility and process conditions of microbial-enzyme synergistic fermentation of corn husks for microbial protein feed production. It explored how environmental factors such as pH,

fermentation temperature, moisture content, presence of cellulose and hemicellulose affect the fermentation process. The dynamic behavior of *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Candida utilis* was analyzed, with a particular focus on the key factors driving the rapid proliferation of yeast. The findings provide a new strategy for the adjustable production of corn husk mycelium protein feed.

EXPERIMENTS

Materials

Raw materials

The raw materials were mainly corn husks providing the carbon source and ammonium sulfates providing the non-protein nitrogen source during fermentation from the by-products of glutamic acid production of Inner Mongolia Fufeng Biotechnology. Tables 1 and 2 list the components of corn husks and ammonium sulfate by-products, respectively.

Table 1. Determination of Fermented Components of Corn Husks

Component (%)	Corn Husks
Crude protein	8.7±0.08
True protein	4.5±0.07
Cellulose	35.7±0.21
Hemicellulose	25.4±0.56
Moisture content	0.1±0.02
Note: Data are presented as the mean ± standard deviation	

Table 2. Determination of Ammonium Sulfate By-Product Components

Component (%)	Ammonium Sulfate By-Products
Ammonium sulfate	23.4±0.14
Calcium	17.8±0.36
Magnesia	0.6±0.02
Sulfur	0.8±0.04
Phosphorus	0.4±0.06
Potassium	1.2±0.07
Total nitrogen	7.2±0.16
Total amino acids	14.6±0.34
Note: Data are presented as the mean ± standard deviation	

Microorganisms

Candida utilis (*C. utilis* CGMCC 2.2878) was purchased from China General Microbiological Culture of Collection Center (CGMCC). *Saccharomyces cerevisiae* (*S. cerevisiae* CICC 32236) that can ferment glucose and sucrose and *Bacillus subtilis* (*B.subtilis* CICC 1009) that can produce xylanase and decompose hemicellulose were purchased from China Center of Industrial Culture Collection (CICC).

Enzyme preparation

Cellulase was purchased from Novozymes (China) Biotechnology Co., Ltd. with an enzyme activity of 700 EGU/g. Xylanase was purchased from Novozymes (China) Biotechnology Co., Ltd. with an enzyme activity of 100,000 U/g.

Culture medium

Yeast-extract peptone dextrose (YPD) medium containing 20.0 g of protein peptone, 20.0 g of glucose, 10.0 g of yeast extract powder, and 1 L of distilled water, with pH not adjusted, was added with 20.0 g of solid or agarslant culture medium for sterilization at 115 °C for 20 min.

Luria-Bertani (LB) medium containing 10.0 g of protein peptone, 10.0 g of sodium chloride, 5.0 g of yeast extract powder, and 1 L of distilled water, with natural pH, was added with solid or agarslant culture medium for sterilization at 121 °C.

Solid fermentation medium contained 1,000.0 g of corn husks, 20.0 g of ammonium sulfates, 2.0 g of potassium dihydrogen phosphates, 2.0 g of magnesium sulfates, 1.0 g of sodium chlorides, and 1 L of distilled water, with natural pH. The inorganic salts were dissolved in water and mixed with corn husks for sterilization at 120 °C for 20 min.

Yeast seed culture was cultivated using YPD medium at 180 rpm at 30 °C to 1×10^9 to 20×10^9 viable bacteria; *Bacillus subtilis* seed culture was cultivated using LB medium at 180 rpm at 37 °C to 1×10^9 to 20×10^9 viable bacteria.

Methods

Experimental methods

The experiment was conducted based on the optimal microbial-enzyme synergistic fermentation process determined in the previous stage, which was divided into enzymatic digestion stage and yeast proliferation stage (Fig. 1). The enzymatic digestion was completed in the sterilization pot, with the initial moisture content of 60% at a reduced temperature of 55 ± 1℃ following material sterilization. Then, 0.2% (w/w) cellulase and 0.02% (w/w) xylanase were added, followed by stirring and closed enzymatic hydrolysis at high temperature for 12 h. The yeast proliferation experiment was completed in the fermenting tray (length × width × height: 50 cm × 30 cm × 20 cm, the thickness of the material layer: about 10 cm). After enzymatic hydrolysis, the material was transferred to the fermentation tray. After enzymatic hydrolysis, the material was transferred to the fermentation tray and inoculated with 16% (v/w) seed liquid, using an inoculation ratio of *Bacillus subtilis*: *Candida utilis*: *Saccharomyces cerevisiae* of 1:1:10. Simultaneously, 5% (w/w) ammonium sulfate was added as non-protein nitrogen source. The mixture was thoroughly stirred and fermented at a constant-temperature incubator at 28 ±1°C. The contents were turned over every 12 h until the end of fermentation process.

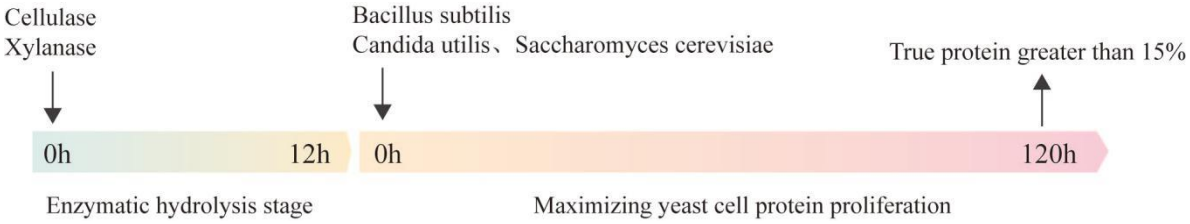

Fig. 1. Microbial-enzyme synergistic fermentation process

Methods for experimental analysis

The determination methods for crude protein and true protein are described in Su *et al*. (2021). The cellulose and hemicellulose contents in the samples were determined by the quantitative saccharogenic method (Pang *et al*. 2017).

For qPCR, samples weighing 3 g were collected from the 0 h, 24 h, 48 h, 72 h, 96 h, and 120 h time points of corn husk protein feed undergoing microbial-enzyme

synergistic fermentation. Each sample was added to 27 mL of 0.9% NaCl solution and subjected to gyratory oscillation of approximately 5 min, followed by a 5-min standing period. The bacterial solution was then diluted 10^3- to 10^9-fold using physiological saline solution. Fresh organisms of standard strains with logarithmic growth (OD_{600} = 0.8) were collected. Bacterial deoxyribonucleic acid (DNA) as well as genomic DNA from *Candida utilis* and *Saccharomyces cerevisiae* were extracted using the Yeast Genome Extraction Kit (TIANGEN). The primer sequences, sizes of the amplified fragments, annealing temperatures, reaction system, and reaction conditions are described in Liu *et al*. (2023).

Data statistics and analysis

Three parallel samples were included in each experimental group. The experimental data were integrated using Microsoft Excel software, and the results were analyzed as "mean ± standard deviation". Differential significance analysis was performed using SAS 9.4 software, with differential significance determined at $P < 0.05$ and $P < 0.01$.

The driving factor analysis method (He *et al*. 2016, 2020; Deng *et al*. 2020) was employed to identify the environmental parameters affecting microorganisms at different fermentation stages in the microbial-enzyme synergistic fermentation process. These factors were derived by VIF, RDA/canonical correspondence analysis (CCA), and variance decomposition analysis (VPA) to analyze their contribution in the process. The correlation coefficients between the environmental factors and selected species were calculated using Spearman's rank correlation coefficients, and the results were visualized with a graph.

RESULTS AND DISCUSSION

Environmental Factors and Dynamic Behaviors During Microbial-enzyme Synergistic Fermentation Process

Dynamic behaviors of temperature during microbial-enzyme synergistic fermentation

As shown in Fig. 2, a significant temperature difference was observed in various fermentation stages.

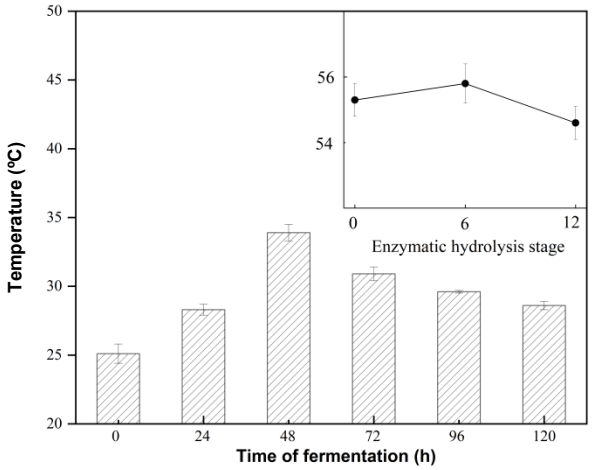

Fig. 2. Changes in temperature ºC during microbial-enzyme synergistic fermentation

During the enzyme digestion stage, the temperature was maintained at 55 ℃ to meet the suitable degradation temperature for cellulase and xylanase. After 24 h of yeast

proliferation, the temperature began to rise due to the rapid proliferation of *Bacillus subtilis*, which inhibited yeast growth, in line with the changes in microbial community. The temperature was up to 35.4 ℃ at 54 h. After 72 h, the temperature of the fermentation substrate stabilized to match that of the constant-temperature incubation, indicating the absence of microbial metabolic heat production (Huang *et al*. 2021).

Dynamic behaviors of moisture content during microbial-enzyme synergistic fermentation

The yeast fermentation process was carried out in an open environment, allowing the yeast cells to have sufficient contact with ambient air, which is conducive to their rapid proliferation. During microbial reproduction, respiration metabolism generates a large amount of biological heat, leading to a sharp increase in the fermentation substrate temperature and resulting in water evaporation loss of the substrate. As shown in Fig. 3, when the fermentation was continued for 72 to 96 h, the moisture content decreased by nearly 20%, which is consistent with the observed temperature trend (Zhou *et al*. 2017; Mageshwaran *et al*. 2024).

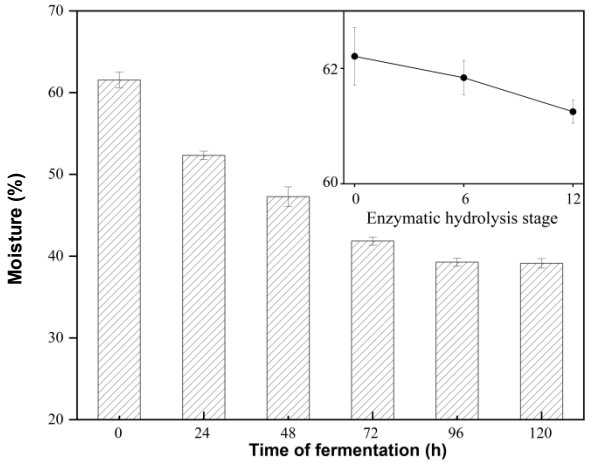

Fig. 3. Changes in moisture content (%) during microbial-enzyme synergistic fermentation

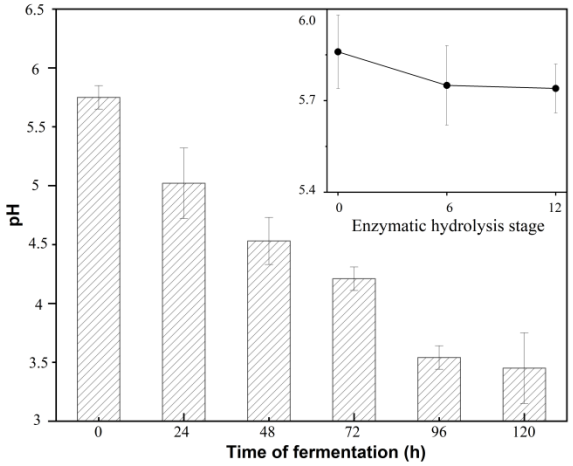

Fig. 4. Changes in pH during microbial-enzyme synergistic fermentation

Dynamic behaviors of pH value during microbial-enzyme synergistic fermentation

Figure 4 demonstrates that the pH value during the microbial-enzyme synergistic fermentation process initially remained stable, then decreased, and finally stabilized, which was lower than 3.5 at the end of fermentation. This drop in pH is caused by the largest proportion of *Saccharomyces cerevisiae* inoculation and organic acids such as malic acid, succinic acid, citric acid, and lactic acid produced by *Saccharomyces cerevisiae* during fermentation. These substances make the fermentation environment acidic, which further inhibits microbial growth (Meng *et al*. 2015; Conde-Ávila *et al*. 2023).

Cellulose in different periods during microbial-enzyme synergistic fermentation and its dynamic behaviors

As demonstrated in Fig. 5, the cellulose content decreased by 49.3% and the hemicellulose content decreased by 41.9% after 12 h of enzymatic hydrolysis. The extents of degradation of cellulose and hemicellulose were 55.5% and 61.5%, respectively, after 120 h of fermentation. The experimental results indicated that the microbial-enzyme synergistic fermentation effectively improved the fiber content of corn husks, which significantly decreased following enzymatic hydrolysis. Additionally, the 12-h enzymatic hydrolysis using cellulase and xylanase enzyme enriched the substrate nutrient content, producing oligosaccharides that alleviated feedback inhibition during subsequent microbial fermentation, with enzyme preparations playing a key role.

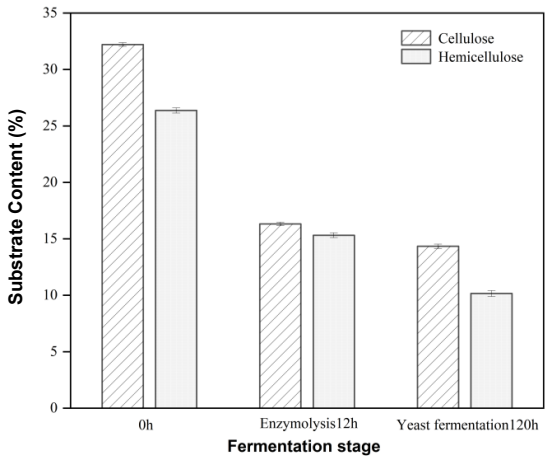

Fig. 5. Changes in cellulose and hemicellulose contents (%) during microbial-enzyme synergistic fermentation

Dynamic behaviors of crude protein and true protein during microbial-enzyme synergistic fermentation

Figure 6 shows that the true protein content increased significantly after fermentation with an increase of 103% due to microbial protein synthesis, which is consistent with the changes in microbial community. The crude protein content showed a gradual decline followed by stabilization. From a material conservation perspective, the crude protein content was highest at 0 h after the addition of 5% (w/w) ammonium sulfate at the beginning of experiment. During fermentation, microorganisms metabolized some nitrogen, producing volatile ammonia, which contributed to the reduction in crude protein content. In the later stage of fermentation, as microbial metabolism activity slowed, the crude protein content tended to be stabilized (Shi *et al*. 2017; Zhang *et al*. 2022).

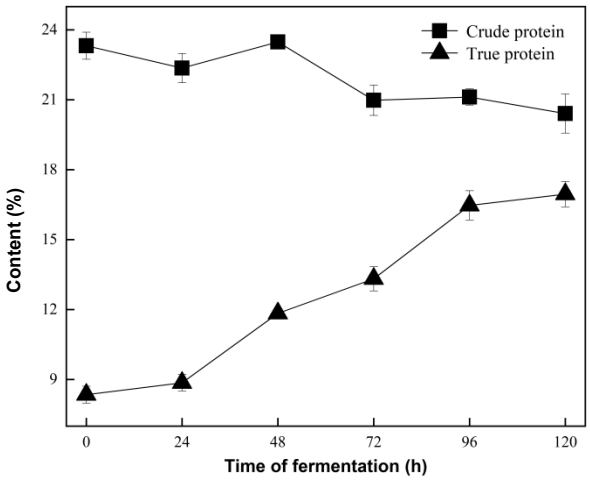

Fig. 6. Changes in crude protein (%) and true protein (%) during microbial-enzyme synergistic fermentation

Dynamic Behaviors of Artificial Microbial Community

Establishment of standard curves of strains

At the yeast proliferation stage, qPCR was applied to monitor the dynamic behaviors in artificial microbial community during fermentation, with the standard curves of the strains used illustrated in Fig. 7 (Kawase *et al*. 2022).

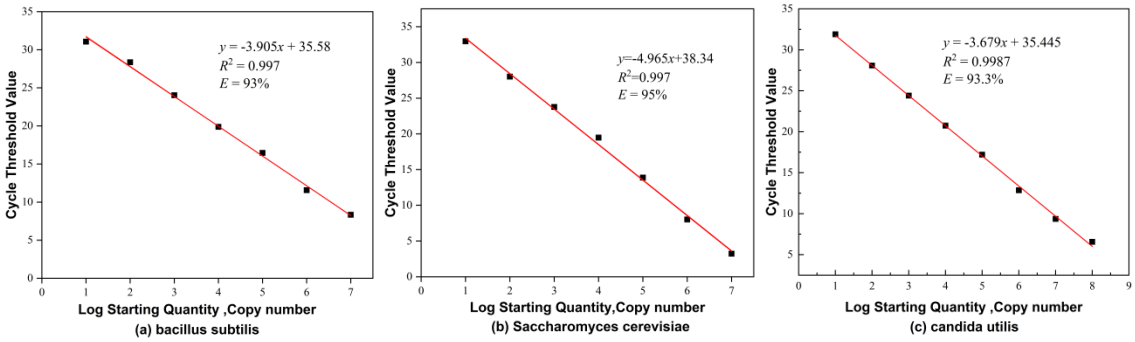

Fig. 7. Standard curves, (a) *Bacillus subtilis* CICC 10090; (b) *Saccharomyces cerevisiae* CICC 32236; (c) *Candida utilis* CGMCC 2.2878

Burdukiewicz *et al*. (2018) reported that the slopes of the standard curves were between -3.1 and -4.0, the amplification efficiencies were between 80% and 110%, and the correlation coefficients $R^2 \geq 0.98$, which were all consistent with the results in this research.

Dynamic behaviors of microbial community

As illustrated in Fig. 8, *Bacillus subtilis* CICC 10090 multiplied rapidly from 0 to 24 h of fermentation, with its copy number increasing by 29.9% and reaching its peak at 24 h, followed by a decreasing trend. In contrast, *Saccharomyces cerevisiae* CICC 32236 and *Candida utilis* CGMCC 2.2878 showed a decreasing trend from 0 to 24 h, followed by an increasing trend at 24 h, and then reaching the peak value at 96 h with 8.5 log copies/g and 9.4 log copies/g, respectively. The substrate temperature was around 25 °C at the beginning of fermentation. *Bacillus subtilis* CICC 10090 had a short initiation period and rapid growth and reproduction, promoting further degradation of cellulose and

hemicellulose. As the fermentation substrate temperature increased, yeast fermentation accelerated growth and multiplication, inhibiting the growth of *Bacillus subtilis.* The inhibited metabolites of yeast mainly manifested as acid production, which lowered the pH, ethanol stress, and macromolecular antimicrobial substances (Jiranek *et al*. 2019). The changes in pH during fermentation indicate that yeast acid production dramatically reduced the pH of the fermentation environment, and the growth of *Bacillus subtilis* stopped after 96 h, aligning with the previous results that *Bacillus licheniformis* growth occurs at pH 4.0 (Peng *et al*. 2014; Tang *et al*. 2022). After 120 h, yeast entered the death phase, microbial activity decreased, and yeast began to accumulate intracellular substances, accompanied by autolysis.

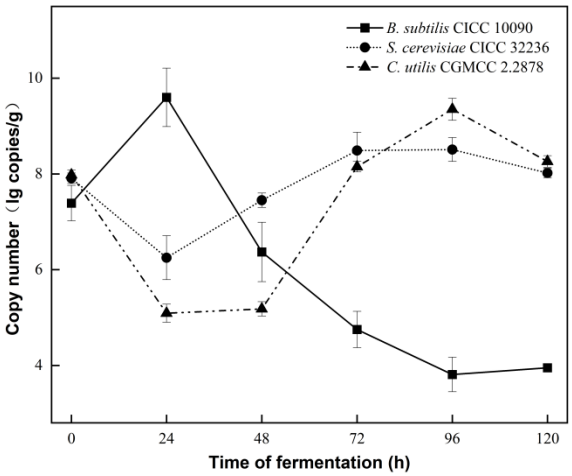

Fig. 8. Changes in microbial communities (%) during fermentation

Analysis of Key Driving Factors Affecting the Yeast Fermentation Process

Microbial growth and metabolic processes are affected by environmental factors such as substrate moisture content, temperature, pH, and the extent of fiber degradation. The VIF analysis of collinearity between environmental factors and fiber degradation revealed that pH and hemicellulose content had VIF values greater than 10, indicating significant collinearity that should be filtered out. In contrast, the VIF value for moisture content, temperature, and cellulose content were below 5, suggesting that these factors should be included in the RDA analysis.

RDA

Figure 9 shows that the total explained variance of RDA was 0.75 (P=0.001), indicating that the three environmental factors significantly influenced the changes in microbial abundance differences at different fermentation times. RDA1 and RDA2 accounted for 72.4% and 2.4% of the explained variance, respectively, with RDA1 being the most important axis. The highest moisture content, with a value of -0.68981 and an explained variance of 0.29 (P value), emerged as the most important environmental factor (Table 2). Therefore, moisture content was judged to be the primary environmental factor affecting the differences in microbial abundance at various fermentation times (Ma *et al*. 2022; Tang *et al*. 2022).

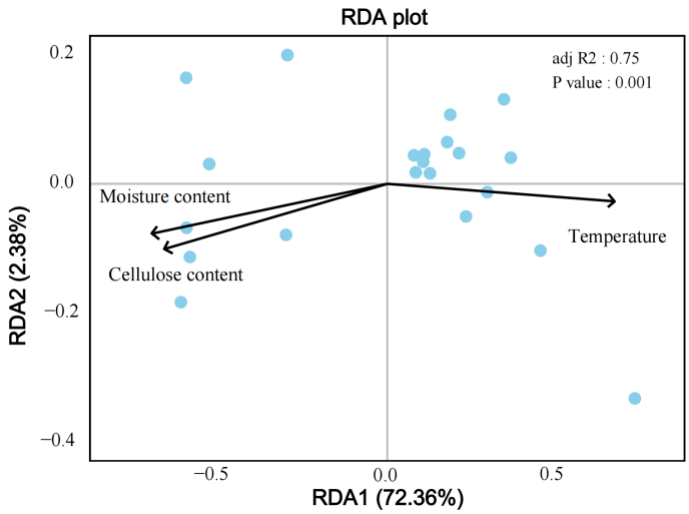

Fig. 9. RDA of environmental factors and microbial abundance

Table 3. Scores of Environmental Factors

Factor	RDA1	RDA2	R^2	P-value
Moisture content	-0.68981	-0.07649	0.287749	0.001
Temperature	0.668289	-0.02698	0.143982	0.001
Cellulose content	-0.65438	-0.1003	0.037397	0.042

VPA

The impact of the three factors identified by RDA on the change in yeast abundance was validated by VPA. According to Fig. 10 and Table 3, cellulose content during yeast fermentation made the largest individual explanatory contribution at 11.2%, followed by moisture content at 2.2%. The combined explanatory contribution of cellulose content and moisture content reached 19.4%, indicating that these two factors had the greatest influence on yeast fermentation.

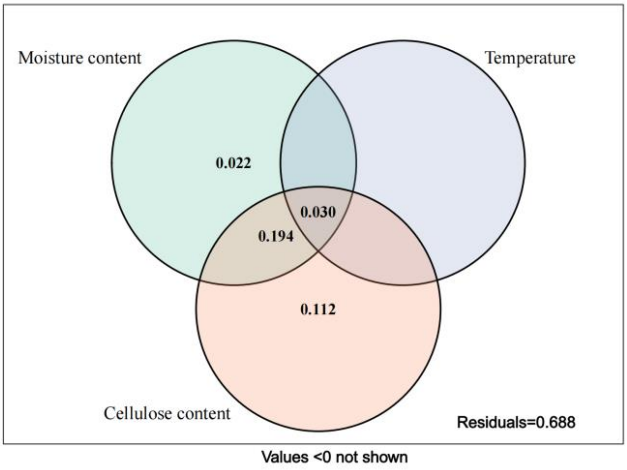

Fig. 10. Explanatory contributor of various environmental factors in VPA

RDA and VPA of environmental factors, cellulose, and hemicellulose during the yeast proliferation stage revealed that moisture content was the most important driving factor of yeast abundance in terms of fermentation time. The most significant factors promoting yeast proliferation were cellulose content and moisture content from a contribution perspective. These findings are consistent with the results of Ma *et al*. (2022) in their study of the driving factors in the fermentation of strongly flavored dacquoise.

CONCLUSIONS

1. A two-step microbial-enzyme synergistic fermentation process was applied to produce corn husk mycoprotein feed, resulting in a 103% increase in true protein content, with the main contribution coming from yeast mycoprotein growth.
2. From the perspectives of statistics and changes in microbial communities, the most important influencing factor during the yeast proliferation process is moisture. Specifically, a moisture content of around 50% is most conducive to yeast growth.
3. This study on the drivers of the fermentation process provides a theoretical foundation for achieving adjustable and controllable microbial mycoprotein feed in industrial production. Furthermore, it offers opportunities for subsequent in-depth research on the key regulatory factors for rapid yeast proliferation.

ACKNOWLEDGMENTS

The authors are grateful for the support of the Inner Mongolia Key Research and Development and Achievement Transformation Plan Project (2023KJHZ0006), Science and Technology Major Projects of Inner Mongolia (2019ZD021).

REFERENCES CITED

Bai, J., Liu, F., Li, S., Li, P., Chang, C., and Fang, S. (2020). "Solid-state fermentation process for gibberellin production using enzymatic hydrolysate corn stalks," *BioResources* 15(1), 429-443. DOI: 10.15376/biores.15.1.429-443

Burdukiewicz, M., Spiess, A. N., Blagodatskikh, K. A., Lehmann, Y., Schierack, P., and Rödiger, S. (2018). "Algorithms for automated detection of hook effect-bearing amplification curves," *Biomolecular Detection and Quantification* 16, 1-4. DOI: 10.1016/j.bdq.2018.08.001

Chaudhary, A., and Kastner, T. (2016). "Land use biodiversity impacts embodied in international food trade," *Global Environmental Change-Human and Policy Dimensions* 38(000), 195-204. DOI: 10.1016/j.gloenvcha.2016.03.013

Conde-Ávila, V., Loera-Corral, O., Díaz, R., and Sánchez, C. (2023). "Cutinolytic esterases are induced by growth of the fungus *Trichoderma harzianum* on glyceryl monostearate in solid-state fermentation," *BioResources* 18(4), 8515-8527. DOI: 10.15376/biores.18.4.8515-8527

Deng, L., Mao, X., Liu, D., Ning, X. Q., Shen, Y., Chen, B., Nie, H. F., Huang, D., and Luo, H. B. (2020). "Comparative analysis of physicochemical properties and microbial composition in high-temperature Daqu with different colors," *Frontiers in Microbiology* 11, article 588117. DOI: 10.3389/fmicb.2020.588117

Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K., and Prasanna, B. M. (2022). "Global maize production, consumption and trade: Trends and R&D implications," *Food Security* 14(5), 1295-1319. DOI: 10.1007/s12571-022-01288-7

Han, M. L., An, Q., He, S .F., Zhang, X. L., Zhang, M. H., Gao, X. H., Wu, Q., and Bian, L. S. (2020). "Solid-state fermentation on poplar sawdust and corncob wastes for lignocellulolytic enzymes by different *Pleurotus ostreatus* strains," *BioResources* 15(3), 4982-4995. DOI: 10.15376/biores.15.3.4982-4995

He, H., Willems, L. A. J., Batushansky, A., Fait, A., Hanson, J., Nijveen, H., Hilhorst, H. W. M., and Bentsink L. (2016). "Effects of parental temperature and nitrate on seed performance are reflected by partly overlapping genetic and metabolic pathways," *Plant & Cell Physiology* 57(3), 473-487. DOI: 10.1093/pcp/pcv207

He, G. Q., Huang, J., Wu, C. D., Yao, J., and Zhou, R. Q. (2020). "Bioturbation effect of fortified Daqu on microbial community and flavor metabolite in Chinese strong-flavor liquor brewing microecosystem," *Food Research International* 129, article 108851. DOI: 10.1016/j.foodres.2019.108851

Huang, X., Duan, C. S., Yu, J. H., Dong, W. Y., and Wang, H. J. (2021). "Response of VFAs and microbial interspecific interaction to primary sludge fermentation temperature," *Journal of Cleaner Production* 322, article 129081. DOI: 10.1016/j.jclepro.2021.129081

Jiranek, V., Bauer, F., and Takagi, H. (2019). "Editorial: Yeast ecology and interaction," *FEMS Yeast Research* 19(8), 73-74. DOI: 10.1093/femsyr/foz073

Kawase, J., Sakai, T., Iwaki, M., Umeda, K., Fukuma, A., Fujisawa, N., Kawakami, Y., Hayashi, H., and Wada M. (2022). "Rapid detection and discrimination of potentially toxigenic *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* by multiplex real-time PCR and amplicon melting curve analysis," *Journal of Microbiological Methods* 195, article 106454. DOI: 10.1016/j.mimet.2022.106454

Liu, J. X., Wang, S. L., Wang, Z., Shen, C. H., Liu, D., Shen, X. J., Weng, L. F., He, Y., Wang, S. M., Wang, J. X., Zhuang, W., Cai, Y. F., Xu, J. L., and Ying, H. J. (2023). "Pretreatment of Luzhou distiller's grains for feed protein production using crude enzymes produced by a synthetic microbial consortium," *Bioresource Technology* 390, article 129852. DOI: 10.1016/j.biortech.2023.129852

Liu, Z., Yan, Y. P., Cui, J. N., Liu, W., Liu, M., Sun, H., and Liu, Z. Y. (2023). "Establishment of a qPCR method for detection of microbial community in corn husk by solid-state fermentation," *Feed Research* 246(7), 110-116. DOI: 10.13557/j.cnki.issn1002-2813.2023.07.022

Ma, S. Y., Luo, H. B., Zhao, D., Qiao, Z. G., Zheng, J., An, M. Z., and Huang, D. (2022). "Environmental factors and interactions among microorganisms drive microbial community succession during fermentation of *Nongxiangxing* Daqu," *Bioresource Technology* 345(0), 126549-126549. DOI: 10.1016/j.biortech.2021.126549

Ma, H., Mei, J., Yuan, X. Y., Gu, X. M., Sun, H., Wang, Z. P., and Kong, L. M. (2024). "Bacteriozyme cooperated with solid-fermented walnut glutenin to prepare bacteriostatic peptides," *Science and Technology of Food Industry* 45(12), 112-120. DOI: 10.13386/jissn1002-0306 2023070037

Mageshwaran, V., Satankar, V., and Paul, S. (2024). "Solid-state fermentation for gossypol detoxification and nutritive enrichment of cottonseed cake: A scale-up of batch fermentation process," *BioResources* 19(1), 1107-1118. DOI: 10.15376/biores.19.1.1107-1118

Meng, X., Wu, Q., and Xu, Y. (2015). "Interactions between *Saccharomyces cerevisiae* and *Bacillus licheniformis* and interaction mechanisms based on proteomic analysis," *Microbiology China* 42(09), 1679-1688. DOI: 10.13344/j.microbiol.china.150032

Pang, J, Liu, Z. Y., Hao, M., Zhang, Y. F., and Qi, Q. S. (2017). "An isolated cellulolytic *Escherichia coli* from bovine rumen produces ethanol and hydrogen from corn straw," *Biotechnology for Biofuels* 10(1), 1-10. DOI: 10.1186/s13068-017-0852-7

Peng, S. Q., Wu, Q., and Xu, Y. (2014). "Tolerance characteristics of *Bacillus licheniformis* CGMCc 3963 and tolerance mechanisms based on transcriptome analysis," *Microbiology China* 41(12), 2395-2403. DOI: 10.13344/j.microbiol.china.140172

Qu, H., Cao, J., Chen, Y., Li, R., Wang, P., and Chen, M. (2018). "Enhancement of biogas production from bundled rice straw solid-state fermentation by adding microbial agents," *BioResources* 13(4), 8723-8737. DOI: 10.15376/biores.13.4.8723-8737

Shi, C. Y., Zhang, Y., Lu, Z. Q., and Wang, Y. Z. (2017). "Solid-state fermentation of corn-soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* for degrading antinutritional factors and enhancing nutritional value," *Journal of Animal Science and Biotechnology.* 8(4), 925-933. DOI: 10.1186/s40104-017-0184-2

Spiller, M., Muys, M., Papini, G., Sakarika, M., Buyle, M., and.Vlaeminck, S. (2020). "Environmental impact of microbial protein from potato wastewater as feed ingredient: Comparative consequential life cycle assessment of three production systems and soybean meal," *Water Research* 171(0), 115406-000. DOI: 10.1016/j.watres.2019.115406

Su, W. F., Jiang, Z. P., Hao, L. H., Li, W. T., Gong, T., Zhang, Y., Du, S., Wang, C., Lu, Z. Q., Jin, M. L., and Wang, Y. Z. (2021). "Variations of soybean meal and corn mixed substrates in physicochemical characteristics and microbiota during two-stage solid-state fermentation," *Frontiers in Microbiology* 12, article 688839. DOI: 10.3389/fmicb.2021.688839

Su, W. F., Jiang, Z. P., Wang, C., Xu, B. C., Lu, Z. Q., Wang, F. Q., Zong, X., Jin, M. L., and Wang, Y. Z. (2022). "Dynamics of defatted rice bran in physicochemical characteristics, microbiota and metabolic functions during two-stage co-fermentation." *International Journal of Food Microbiology* 362, 109489-109489. DOI: 10.1016/j. ijfoodmicro.2021.109489

Tang, J., Chen, J., Chen, D. M., Li, Z. J., Huang, D., and Luo, H. B. (2022). "Structural characteristics and formation mechanism of microbiota related to fermentation ability and alcohol production ability in Nongxiang Daqu," *Foods* 11(17), 2602-2602. DOI: 10.3390/foods11172602

Zhang, T., Jiang, D., Li, Y. M., Zhang, H., Jing, Y. Y., Lu, C. Y., Zhang, Y., Xia, C. X., and Zhang, Q. G. (2022). "Lignin removal, reducing sugar yield and photo-fermentative biohydrogen production capability of corn stover: Effects of different pretreatments," *Bioresource Technology* 346, article 126437. DOI: 10.1016/j.biortech.2021.126437

Zhou, W. P., Shen, W. J., Li, Y. E., and Hui, D. F. (2017). "Interactive effects of temperature and moisture on composition of the soil microbial community," *European Journal of Soil Science* 68(6), 909-918. DOI: 10.1111/ejss.12488

Zhu, X. Y., Chen, Y. L., Hao, S. X., and Li, X. Q. (2023). "Improvement of the nutritional quality of rapeseed meal through solid-state fermentation with *B. subtilis*, *S. cerevisiae*, and *B. amyloliquefaciens*," *Fermentation* 9(5), article 492. DOI: 10.3390/fermentation9050492

Article submitted: February 9, 2025; Peer review completed: May 9, 2025; Revised version received: May 9, 2025; Accepted: June 7, 2025; Published: June 24, 2025.
DOI: 10.15376/biores.20.3.6476-6489