Optimization of fermentation factors to enhance rice straw degradation ability using a microbial consortium LZF-12

Guoxiang Zheng[1,2,3*], Chenyang Zhou[1,3], Ting Yin[1,3], Zhaoxin Lu[1,3], Shuang Ai[1,3]

(1. *College of Engineering, Northeast Agricultural University, Harbin 150030, China*;
2. *Key Laboratory of Pig-breeding Facilities Engineering, Ministry of Agriculture and Rural Affairs, Harbin 150030, China*;
3. *Heilongjiang Key Laboratory of Technology and Equipment for the Utilization of Agricultural Renewable Resources, Harbin 150030, China*)

Abstract: Biological pretreatment has broad application prospects in agricultural waste treatment because of its economic benefits, environmental protection and energy saving characteristics. In this study, a microbial consortium LZF-12 was applied for a biological pretreatment to degrade rice straw. Batch experiments were performed under hydrolysis conditions by using the method of Box-Behnken factorial design (BBD). The results showed that the model multiple correlation coefficient R^2 was 0.9816, and the effects of three factors on the degradation of rice straw of LZF-12 as descending order were the initial rice content, chicken manure content, and initial pH value. The interaction between straw concentration, chicken manure concentration, and initial pH value had significant effects on the degradation of a microbial consortium. Under the optimum conditions of 0.86% rice straw, 0.5% chicken manure and the initial pH value of 7.0, the degradation rate of rice straw reached 72.4%. There is only a small difference of 0.55% between the experimental value and predicted value from BBD model. Therefore, it is feasible for the established model due to the consistent results between the prediction and experimental value. The microbial consortium LZF-12 has high cellulase enzyme activities and degradation ability over a wide range of temperature and pH value, indicating that it has a good development potential and application prospects in waste biodegradation and biomass energy production.

Keywords: microbial consortium, biological pretreatment, biodegradation, rice straw, optimization
DOI: 10.25165/j.ijabe.20191203.4638

Citation: Zheng G X, Zhou C Y, Yin T, Lu Z X, Ai S. Optimization of fermentation factors to enhance rice straw degradation ability using a microbial consortium LZF-12. Int J Agric & Biol Eng, 2019; 12(3): 168–173.

1 Introduction

Fossil fuels are the main energy source to satisfy the worldwide energy demands. However, the energy demands are increasing and the supply of fossil fuels is decreasing, thus many countries are looking for other fuel sources[1]. Straw is one of promising alternatives to fossil-based fuels resulting from its abundant, renewable and can be utilized by the microorganisms[2]. However, due to the complex chemical structure of straw, cellulose is encapsulated by lignin, which makes straw difficult to use efficiently. Therefore, the degradation of straw by microorganisms has become a new research hotspot. Because of the complexity of straw structure and the single structure of pure bacterial enzymes, more and more attention has been paid to the microbial consortium. In this study, used rice straw as carbon source, screened out a highly efficient and stable rice straw degradation microbial consortium LZF-12 from cellulose-rich soil[3]. In nature, the interaction between many species of microorganisms, mainly including some fungal and bacterial genera produces various cellulolytic and hemicellulolytic enzymes under both aerobic and anaerobic conditions[4]. However, it is hard for microorganisms to utilize rice straw without pretreatment due to complicated structure of biomass[5]. During the past decades, some cellulose-degrading microbial consortiums from enriched cultures have been studied extensively. Gu et al.[6] reported that the pretreatment of straw using the combination of bacillus and decomposing lipoperoxidase achieved degradation rate of 45.5% at 30 ℃. Du et al.[7] reported the pretreatment of corn using white-rot fungi achieved 82% degradation rate after 28 d. Han et al.[8] established a stable microbial consortium by multiple screening to degrade more 60% rice straw at 50 ℃ after 4 d. These studies proposed an efficient biomass pretreatment via a mixed native microbial consortium in favor of feedback regulation and metabolite repression compared to a single bacterium culture. Although biological pretreatment offers many advantages such as low-capital cost, low-energy input and high yields without polluting byproducts[9], it is a relatively time-consuming process. To save time and carbohydrate during pretreatment, the optimization of strain and culture condition is necessary[10]. For industrial bioconversion, moreover, both medium and culture condition need to be optimized to obtain higher volumetric productivity and lower manufacturing cost[11]. Optimization studies on medium components and fermentation conditions can provide the important knowledge for effective biomass application in terms of composting, anaerobic digestion, enzymatic biomass saccharification[12,13]. In previous studies, a stable microbial consortium named LZF-12 was screened by a succession of enrichment cultures to degrade various cellulosic materials (e.g., filter paper, cotton, and rice straw) under facultative anaerobic conditions in our own lab[14]. Some environmental factors, such as temperature, pH, chemical environment, osmotic

Received date: 2018-09-04 **Accepted date:** 2019-03-09

Biographies: Chenyang Zhou, Master candidate, research interest: agricultural biological environmental and energy engineering, Email: zhouchenyang24@163.com; **Ting Yin**, Master candidate, research interest: agricultural wastes treatment; **Zhaoxin Lu**, Master candidate, research interests: agricultural biological environmental and energy engineering; **Shuang Ai**, Master candidate, research interests: environmental microbial utilization.

***Corresponding author: Guoxiang Zheng**, PhD, Professor, research interests: agricultural biological environmental and energy engineering, Northeast Agricultural University, Harbin, 150030, China. Tel: +86-451-55191746, Email: zgx720331@126.com.

pressure, et al.[15], will affect the growth and fermentation characteristics of microbial consortium in practical application. Thus, it is necessary to investigate the key factors affecting LZF-12's fermentative characteristics and determine the optimal cultural conditions, which will provide important theoretical and practical reference for follow-up waste biodegradation and biogas application.

2 Materials and methods

2.1 Microorganism and medium

A mesophilic microbial consortium LZF-12 was used in this study for rice straw degradation from Biomass Laboratory of Northeast Agricultural University[3]. Rice straw was obtained from a local farm, pretreated with 1% (W/V) NaOH for 24 h, then dried at 50 ℃, and was cut into pieces with about 1 cm length[16]. The basic medium was prepared with 0.5% peptone, 0.5% NaCl, 0.3% $CaCO_3$, 0.1% yeast powder. The microbial consortium was cultured in 250 mL Erlenmeyer flasks containing 50 mL medium at 35 ℃.

2.2 Analytical methods

The pH was analyzed by the portable pH meter (Accuracy: 0.01). The cell concentration was determined by spectrophotometric method measuring absorbance value of the sample at 600 nm. The straw degradation ratio was determined as described by Liu[17], and the degradation ratio was calculated by following equation:

$$Degradation\ rate = (Mt - Mr)/Mt \times 100\% \quad (1)$$

where, M_t is the total weight of the cellulosic biomass before the degradation and M_r is the weight of the residual biomass after the degradation. The experiments were performed independently in triplicate and the averages of the results were reported[18].

The cellulase (CMCase) activity was determined by incubation 500 μL of 1% CMC in 50 mM sodium phosphate buffer (pH 7.5) with 500 μL cell-free culture for 30 min at 50 ℃. The reaction was terminated by adding the 1 mL 3 mL, 5 mL dinitrosalicylic acid (DNS) reagent and boiled in a water bath for 10 min[19,20]. After cooling at room temperature, the amount of glucose released was determined by measuring absorbance at 540 nm[21].

The volatile fat acid (VFA) was determined by a gas chromatography (GC-6890N, Agilent Inc., USA) equipped with a flame ionization detector and a 30 m×0.25 mm×0.25 μm fused-silica capillary column. Nitrogen was used as the carrier gas with split injection method with the split ratio of 20:1[22,23].

3 Results and discussion

3.1 Key factors affecting the enzyme production of LZF-12 in fermentation

3.1.1 Temperature optimization

20 ℃, 25 ℃, 30 ℃, 35 ℃, 40 ℃ and 45 ℃ were used to investigate the effect of different cultivation temperature on rice straw degradation of LZF-12 microbial consortium in terms of the pH, OD value, liquid products and cellulose activity in 1×10^5 Pa pressure for 20 min. Figures 1 and 2 show that different temperatures had a significant effect on the growth and fermentation of microbial consortium LZF-12. When the temperature ranged within 20 ℃-35 ℃, the physiological and metabolic functions of microbial consortium gradually increased with the temperature. In the same time, such an increase in temperature would be expected to result in increased enzyme activity, rate of substrates consumption, concentration of cell and metabolites. The OD of bacteria solution reached the maximum value of 0.68 at 35 ℃ for 7 d. The concentration of acetic acid and butyric acid as main metabolites in the microbial consortium also reached the maximum values of 2.48 and 0.82 g/L, respectively. However, the OD and liquid product of the microbial consortium showed a decreasing trend with the temperature. These results indicated that the temperature above 35 ℃ limited the biological metabolism of microbial consortium affecting the growth of microorganisms and inhibiting the degradation and conversion of the substrate.

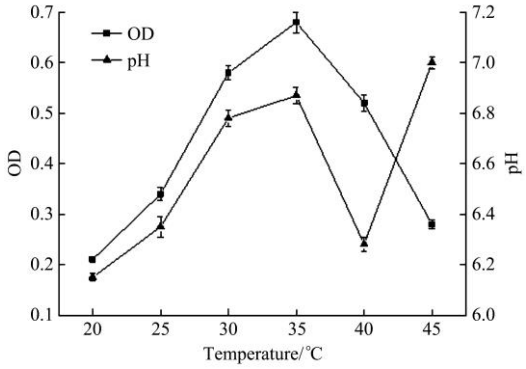

Figure 1 OD and pH of microbial consortium LZF-12 at different cultivation temperatures

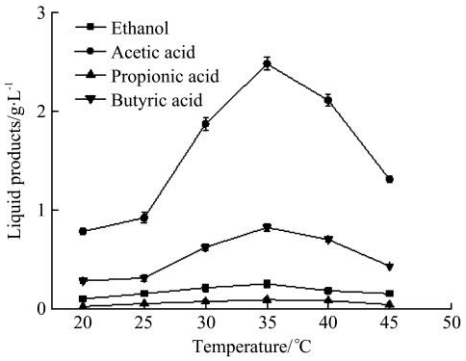

Figure 2 Liquid products of microbial consortium LZF-12 at different cultivation temperatures

Figure 3 is the comparison of CMCase activity in microbial consortium at different cultivation temperatures. When temperature was about 20 ℃-30 ℃, the CMCase activity of the microbial consortium was relative low, even the highest activity in entire cultivation process was only about 6 U. While CMCase activity increased rapidly with the temperature and reached a peak activity of 41.8 U at 35 ℃ for 7 d. When the temperature exceeded 35 ℃, CMCase activity started to decline sharply to only 9.7 U in 45 ℃. The results showed that the excessively high and low temperature could inhibit the fermentation of microbial consortium LZF-12.

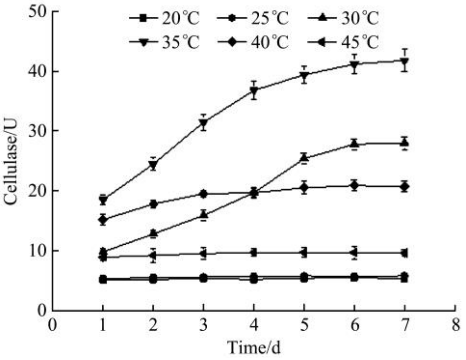

Figure 3 CMCase activity of microbial consortium LZF-12 at different cultivation temperature

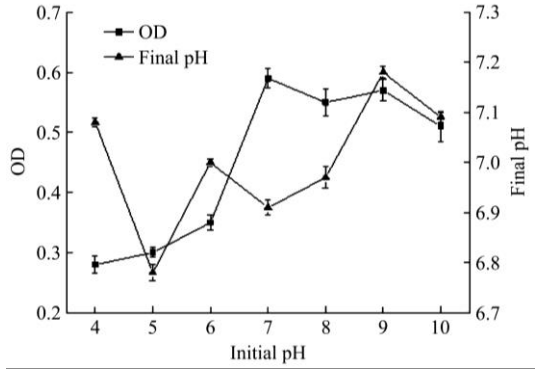

Figure 4 OD and final pH values of microbial consortium LZF-12 at different initial pH values

3.1.2 pH optimization

With 1% concentration rice straw as the sole carbon source, initial pH value of culture medium at 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 was studied to evaluate the OD, pH in the microbial consortium LZF-12 fermentation for 7 d at 35 ℃. As shown in Figure 4, the pH value of fermentation broth increased gradually to 7.0 with the proliferation of bacteria when the initial pH was 4.0-6.0, and the final pH value was between 6.8 and 7.0 after microbial consortium LZF-12 cultivated for 7 d. Nevertheless, the bacterial density grew slowly with the initial pH value was between 4.0 and 6.0, and OD also was at a low level from 0.28 to 0.35. With the initial pH value elevated, the proliferation of the microbial consortium LZF-12 increased reaching the maximum OD of 0.59 at the initial pH value of 7.0. The OD maintained from 0.51 to 0.59 when the initial pH value ranged 7.0-10.0. The final pH value was between 6.91 and 7.18, indicating that the microbial consortium LZF-12 bears a good adaptation on a wide range of pH value in the degradation of lignin-rich straw biomass.

Figure 5 shows that comparison of cellulase activity in the reaction system of the microbial consortium at different initial pH value. In the initial pH value of 4.0-6.0, the cellulase activity remained at a low level of 6.1-10.6 U during the transformation of the substrates. The ability of substrates transformation into metabolites enhanced and the cellulase activity of the microbial consortium was significantly improved with the initial pH increased. The cellulase activity in the reaction system of each group which fermented for 7 d maintained at a high level when the initial pH value was between 7.0 and 10.0, was about 33.2-42.5 U. This indicates that the neutral alkaline environment is beneficial to the biosynthesis of LZF-12, improving catalytic activity of the corresponding enzyme in the biochemical reaction process, thereby increased the rate and ability of microorganisms to convert substrates.

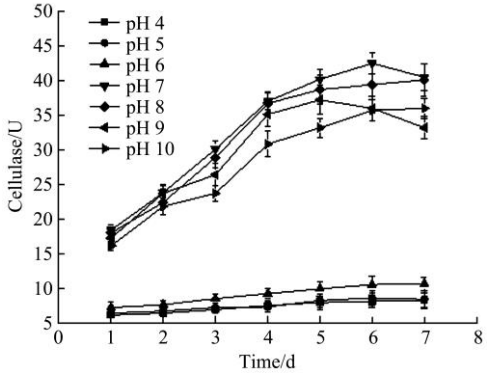

Figure 5 Cellulose activity of microbial consortium LZF-12 at different initial pH values

3.1.3 Nitrogen source optimization

Given 1% rice straw as a sole carbon source, ammonium chloride, sodium nitrate, urea, chicken manure, corn starch and yeast powder as the nitrogen source substituted peptone were investigated in different basal medium. The nitrogen contents were 25.2%, 15.7%, 45.5%, 1.63%, 4.5%, 8.1% and 14.2% in ammonium chloride, sodium nitrate, urea, chicken manure, corn starch, yeast powder and peptone, respectively. Figure 6 showed that the decomposition rate of rice straw exceeded 65% with organic nitrogen source (peptone, yeast powder and corn syrup) as a nitrogen source. In contrast, the degradation rate of rice straw with inorganic nitrogen source (ammonium chloride and sodium nitrate) was relatively weak and less than 20% till the fermentation end. What is noteworthy is that the degradation rate of rice straw reached 49.2% and 60.1% for urea and chicken manure as a nitrogen source.

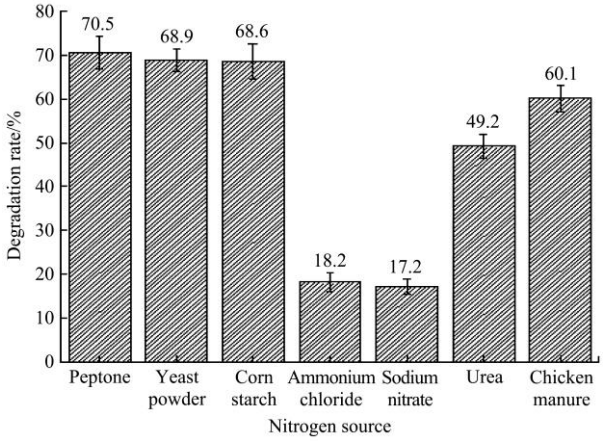

Figure 6 Straw degradation ability of microbial consortium LZF-12 at different nitrogen sources

Given rice straw as a carbon source and yeast powder as a sole nitrogen source, the degradation ability of microbial consortium LZF-12 to straw at different concentration of nitrogen source was studied. As shown in Figure 7, with yeast powder concentrations ranging from 0.4% to 0.7%, the metabolic ability and conversion ability of bacteria was strong, leading to the degradation rate of straw of 70.2% and 64.2% at 7 d respectively. With nitrogen concentration continued to increase, the growth and fermentation of bacteria was inhibited, whereas the low concentration of nitrogen source would affect the synthesis of proteins, lipids and nucleic acids in bacteria, affecting the growth and proliferation of bacteria, and then the conversion of substrates.

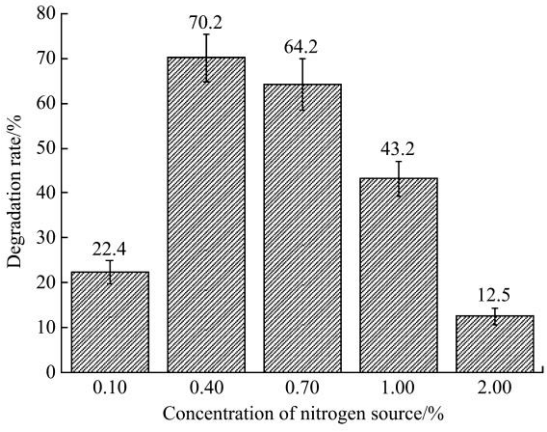

Figure 7 Straw degradation ability of microbial consortium LZF-12 at different yeast concentrations

3.2 Optimization of experimental design

Based on the single factor study, BBD model of response surface was applied to optimize the condition combination in microbial consortium LZF-12 fermentation. Compared with peptone and yeast powder, chicken manure is cut-price, broad and replaceable nitrogen sources (Figure 6), so in this study, chicken manure was used to be only nitrogen source instead of yeast powder and peptone in subsequent optimization experiments. The degradation rate of rice straw was denoted as the experimental index (dependent variable), three external factors of rice straw content, chicken manure content, and initial pH value were investigated as the study factors (independent variable) and were expressed as x_1, x_2, and x_3 with + 1, 0, 1 representing the high, medium and low level.

The independent variables are encoded according to the equation $x_i=(X_i–X_0)/X$. X_i is the encoded value of the independent variables. X_0 is the true value of the independent variable at the experimental center, X is the change step of the independent variable, factor coding and level are shown in Table 1.

Table 1 Levels and codes variable for CCD

Variable	Code		Coding level		
	Coded	Not encoded	–1	0	+1
Rice straw content/%	x_1	X_1	0.4	0.8	1.2
Chicken manure content/%	x_2	X_2	0.2	0.4	0.6
Initial pH value	x_3	X_3	6.5	7.0	7.5

Note: The relationship between the encoded value and the true value is: $x_1=(X_1–0.8)/0.5$, $x_2=(X_2–0.4)/50$, $x_3=(X_3–7.0)/0.5$.

The data in Table 2 was analyzed using multiple regression analysis by Design Expert 7.1.3. Response surface method developed a corresponding quadratic regression model for rice straw degradation rate as shown in the following equation obtained for the coded factors:

$$Y=70.74–3312.5+106.54375x_1–321.4125x_2+970.92x_3+16.5625x_1x_2+3.5x_1x_3+58.5x_2x_3–78.95312x_1^2–110.8125x_2^2–71.23x_3^2$$

where, Y represented rice straw degradation rate; x_1, x_2, and x_3 represented the content of rice, chicken manure and initial pH, respectively.

Table 2 BBD matrix of variables and actual and predicted values of DW

Test group	Straw content/%	Chicken manure content/%	Initial pH	Degradation rate of rice straw (Y)/%	
	x_1	x_2	x_3	Actual value	Predictive value
1	0	0	0	70.2	70.74
2	0	0	0	69.5	70.74
3	1	–1	0	51.2	54.35
4	–1	–1	0	48	47.93
5	1	0	0	71.5	70.74
6	1	0	0	70.9	70.74
7	1	0	0	32.5	35.01
8	1	–1	–1	52.5	51.86
9	–1	0	1	32.5	35.01
10	1	0	1	46.2	45.49
11	0	–1	1	42.5	40.06
12	–1	0	–1	35.8	36.51
13	1	1	0	62	62.07
14	0	1	1	56.2	56.84
15	0	1	–1	42.8	45.24
16	1	0	–1	46.7	44.19
17	0	1	0	53.5	50.35

Based on the variance analysis of the equation (ANOVA, Table 2), F_{model} = 41.49, this model was highly significant at the level of α = 0.01. Linear defects of rice content (x_1), chicken manure content (x_2), as well as quadratic effects of rice content (x_1^2), chicken manure content (x_2^2), initial pH (x_3^2), and interaction between chicken manure content (x_2) and initial pH (x_3) significantly impacted rice straw degradation rate at level of α = 0.05. The model multiple correlation coefficient R^2 was 0.9816, indicated that there was a good fitness between the experimental value and the predicted value of rice straw degradation. The adjusted R square R^2_{Adj} was 0.9579, implying that the adjusted model could explain the variance in the rate of 95% straw degradation. In the Table 3, the effects of three factors on the degradation of rice straw of LZF-12 as descending order were as follows: the initial rice content, chicken manure content, initial pH value.

The optimal conditions for the degradation of rice straw in LZF-12 fermentation based on regression model were as follows: 0.86% rice straw, 0.49% chicken manure and the initial pH value of 7.01. The predicted results showed that the degradation rate of LZF-12 was 71.52% under optimal condition.

Table 3 Regression coefficients and their significance of straw degradation rate model

Source of variance	Sum of square	Degrees of freedom	Mean square	F vvalue	Prob > F
Model	2633.59	9	292.62	41.49	<0.0001*
x_1	164.71	1	164.71	23.36	0.0019
x_2	51.51	1	51.51	7.3	0.0305
x_3	0.02	1	0.02	0.88	0.356
x_1x_2	7.02	1	7.02	1	0.3516
x_1x_3	1.96	1	1.96	0.28	0.6143
x_2x_3	136.89	1	136.89	19.41	0.0031*
x_1^2	671.92	1	671.92	95.28	<0.0001*
x_2^2	82.72	1	82.72	11.73	0.011
x_3^2	1335.19	1	1335.19	189.33	<0.0001*
Residual	49.36	7	7.05		
Mismatch F value	46.19	3	15.4	19.42	0.0076*
Absolute error	3.17	4	0.79		
Total deviation	2682.96	16			
R^2=0.9816			R_{Adj}^2=0.9579		

Note: * is a significant factor.

3.3 Contour map and response surface analysis

All three independent variables directly or indirectly affected the ability of the microbial consortium LZF-12 in the straw degradation. Figure 8 showed that the degradation rate of rice straw increased with the increasing of chicken manure concentration as the content of straw from 0.4% to 0.8%. Once the rice straw content was larger than 0.8%, the rice straw degradation rate showed a decreasing trend even if the chicken manure content increased. Figure 9 showed that the interaction between the initial pH and the rice straw was not significant. The degradation ability of LZF-12 increased with the increasing of initial pH value with the content of rice straw from 0.4% to 0.8%. Upon the content of rice straw was more than 0.8%, the declining trend of rice straw degradation rate was not obvious, indicating that the pH value had a relatively weak impact on the growth and fermentation of the microbial consortium LZF-12. The rice straw degradation rate changed rarely even if the concentration of substrate straw had a big change. Figure 10 contours

demonstrated that the degradation rate of rice straw increased with the increasing of chicken manure content with initial pH value from 6.5 to 7.0. Once the initial pH value above 7.0, the degradation rate of rice straw decreased with the increasing of chicken manure, indicating that the interaction between chicken manure content and initial pH of fermentation was relatively large.

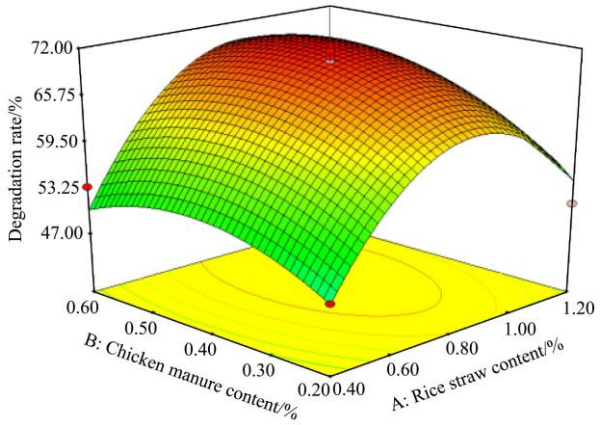

Figure 8 Effects of rice straw content and chicken manure content on rice straw degradation rate

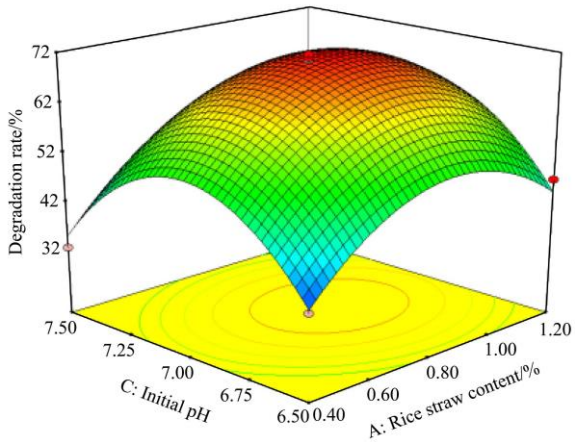

Figure 9 Effects of rice straw content and initial pH value on rice straw degradation rate

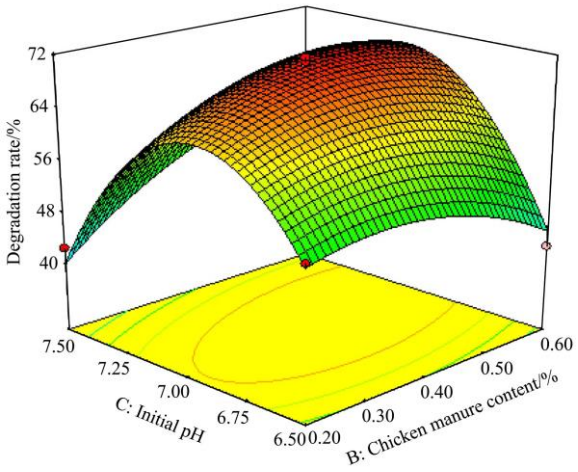

Figure 10 Effects of chicken manure content and initial pH value on rice straw degradation rate

3.4 Model validation

In order to verify the practicability and effectiveness of the model developed by BBD, four sets of experiments were conducted selectively at the experimental level. Table 4 shows that with rice straw of 0.86%, chicken manure of 0.5% and the initial pH value of 7.0, the true value of degradation of rice straw obtained from the experiment was 72.4%. There is only small different of 0.55% between the experimental value and predicted value from the model, suggesting that developed model can provide a reliable guide for the microbial consortium LZF-12 for the degradation of rice straw.

Table 4 Model validation tests

Test group	Rice straw content/%	Chicken manure content/%	Initial pH	Degradation rate /%	
				Measured value	Predictive value
1	0.80	0.40	6.80	71.0	69.8
2	0.83	0.55	7.20	71.8	72.0
3	0.86	0.50	7.00	72.4	72.8
4	0.89	0.45	7.40	70.2	70.4

4 Conclusions

Cellulose is the main substrate in the hydrolysis stage of anaerobic digestion of agricultural wastes[24]. But natural rice straw is difficult to be decomposed by a single microorganism or enzyme due to its complex structure. To combat this problem, co-cultivation of organisms has the ability to produce multi-cellulase complexes in adequate quantity. In this study, medium composition was systematically optimized for rice straw degradation rate through batch tests by a microbial consortium LZF-12. The effects of temperature, pH value, carbon source and nitrogen source on the degradation of microbial consortium LZF-12 were determined by single factor test. Three factors that have remarkable effects on degradation capacity were identified by batch experiments. These factors are rice straw, chicken manure, and initial pH value. Further optimization was carried by Box-Behnken factorial design. A mathematical model with a R^2 value of 95.79% was realized which estimated a rice straw degradation rate of 72.4% with the optimal medium. Model validation showed that the predicted values agreed well with the experiments data. In conclusion, microbial consortium LZF-12 used in this study is a community that was screened from natural environment by the succession of subcultures, which was stable and excellent cellulolytic capabilities in optimal conditions using low-cost wastes, suggesting that has a widely prospect of applying to environmental governance and the potential for the developing of biomass energy production.

Acknowledgements

This work was supported by Heilongjiang Province Natural Science Fund (E2015023), The National Key Research and Development Program of China (2017YFD0700705) and Heilongjiang Province Postdoctoral Launch Fund (LBH-Q13023).

[References]

[1] Kang P, Gary M, Zhang X L, Wang T P, Tan Z F, Agarwal J. Systematic comparison of hydrogen production from fossil fuels and biomass resources. Int J Agric & Biol Eng, 2017; 11: 192–200.

[2] Souichiro K, Shin H, Zong J C, Masaharu I, Yasuo I. Effective cellulose degradation by a mixed-culture system composed of a cellulolytic clostridium and aerobic non-cellulolytic bacteria. FEMS Microbiology Ecology, 2004; 51: 133–142.

[3] Zhong Y T. Research on screening of rice straw degradation microbial system and its fermentation characteristics. Harbin, Northeast Agricultural University, 2011. (in Chinese)

[4] Souichiro K, Shin H, Zong J C, Masaharu I, Yasuo I. Stable coexistence of five bacterial strains as a cellulose-degrading community. Applied and

Environmental Microbiology, 2005; pp: 7099–7106.

[5] Soundar S, Chandra T S. Cellulose degradation by a mixed bacterial culture. Ind Microbial, 1987; 2: 257–265.

[6] Gu F, Wang W, Jing L, Jin Y. Effects of green liquor pretreatment on the chemical composition and enzymatic digestibility of rice straw. Bioresource Technology, 2013; 149: 375–382.

[7] Du W, Yu H, Song L, Zhang J, Weng, C, Ma F, et al. The promoting effect of byproducts from *Irpex lacteus* on subsequent enzymatic hydrolysis of bio-pretreated cornstalks. Biotechnology for Biofuels, 2011; 11(4): 37–48.

[8] Haruta S, Cui Z, Huang Z, Li M, Ishii M, Igarashi Y. Construction of a stable microbial consortium with high cellulose-degradation ability. Applied Microbiology and Biotechnology, 2002; 59(4-5): 529–534.

[9] Han X, Song W, Liu G, L, Z, Yang P, Qu Y. Improving cellulase productivity of *Penicillium oxalicum* RE-10 by repeated fed-batch fermentation strategy. Bioresource Technology, 2017; 227: 155–163.

[10] Jung D U, Yoo H Y, Kim S B, Lee J H, Park C. Optimization of medium composition for enhanced cellulase production by mutant *Penicillium brasilianum* KUEB15 using statistical method. J. Ind. Eng. Chem, 2015; 25: 145–150.

[11] Matkar K, Chapla D, Divecha J, Nighojkar A, Madamwar D. Production of cellulase by a newly isolated strain of *Aspergillus sydowii* and its optimization under submerged fermentation. Int. Biodeterior. Biodegrad, 2013; 78: 24–33.

[12] Ortiz G E, Guitart M E, Cavalitto S F, Alberto E O, Fernandez-Lahore M. Characterization, optimization, and scale-up of cellulases production by *Trichoderma* reesei cbs 836.91 in solid-state fermentation using agroindustrial products. Bioprocess. Biosyst. Eng, 2015; 38: 2117–2128.

[13] Guo P, Zhu W B, Wang H, et al. Functional characteristics and diversity of a novel lignocelluloses degrading composite microbial consortium with high xylanase activity. Journal of Microbiology and Biotechnology, 2010; 20(2): 254-264.

[14] Zhong Y T, Li W Z, Zheng G X, Screening of rice straw degradation microbial consortium and its growth characteristics. Journal of Northeast Agricultural University, 2011; 42(8): 56-61.

[15] Amnuaycheewa P, Hengaroonprasan R, Rattanaporn K, Kirdponpattara S, Cheenkachorn K. Enhancing enzymatic hydrolysis and biogas production from rice straw by pretreatment with organic acids. IndCrops Prod, 2016; 87: 247–254.

[16] Song Z, Sun X, Yang G, Yan Z, Yuan Y, Li D, et al. Effect of NaOH pretreatment on methane yield of corn straw at different temperatures by anaerobic digestion. Journal of Chemical Industry and Engineering, 2014; 65(5): 1876–1882. (in Chinese)

[17] Liu S. Characteristic and flora development of a microbial consortium for degradation of lignocelluloses. Harbin, Northeast Agricultural University, 2009. (in Chinese)

[18] Chen L, Luo J, Dong B, Song C, Wan X, Qi Y, et al. Pretreatment with composite microbe and NaOH to improve anaerobic performance of corn straw. Transactions of the CSAE, 2013; 29(7): 185–190. (in Chinese)

[19] Yin L L, Liu S, Wang M Y, Ju W C, Wei D H, Li W Z. Formation process and properties of hydrogen-producing granular sludge in UASB reactor. Int J Agric & Biol Eng, 2018; 11(5): 224–229.

[20] Mackulak T, Prousek J, Svorc L. Increase of biogas production from pretreated hay and leaves using wood-rotting fungi. Chemical Papers, 2012; 66(7): 649–653.

[21] Kuijk S, Sonnenberg A, Baars J, Hendriks W, Cone J. Fungal treated lignocellulosic biomass as ruminant feed ingredient: A review. Biotechnol Adv, 2015; 33: 191–192.

[22] Zidan A S, Sammour O A, Hammad M A, Megrab N A, Habib M J, Khan M A. Quality by design: understanding the formulation variables of a cyclosporine a self-nanoemulsified drug delivery systems by Box-Behnken design and desirability function. International Journal of Pharmaceutics, 2007; 332: 55.

[23] Chandra R, Takeuchi H, Hasegawa T, Kumar R. Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. Energy, 2012; 43(1): 273–282.

[24] Liu S, Wang C Y, Yin L L, Luo L N. Optimization of hydrogen production from agricultural wastes using mixture design. Int J Agric & Biol Eng, 2017; 10(3): 246–254.