Trichoderma reesei utilized tea residue for the production cellulase

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Abstract—A study on the utilization of tea residues together with cellulase production is a meaningful research topic.Objective: This study aimed to make full use of tea residues. Methods: In this study, tea residue was utilized as a solid culture medium to produce the enzyme of cellulase by T. reesei, we used the methods of orthogonal experiments to optimize the fermentation conditions. Results: The results were shown that under these optimum fermentation conditions, the enzyme of cellulase activity was achieved of 6.27 IU/g dry medium. Conclusion: This study provides theoretical basis for make full use of the tea waste, Sichuan is a big tea resources province this study has great application potential.

Keywords-Trichoderma reesei QM9414, Cellulase, 2．2．1Culture conditions; Utilize.

I. INTRODUCTION

In the earth, we all know that the cellulose is the most abundant renewable resource[1]. The large supply of cellulose should be fully utilized to address social problems, such as production the sugar and fiber products[2]. Thus, a study of this method will produce positive effects. At present, researchers produce cellulase by submerged fermentation. Sichuan province of China is a large tea-producing province, sichuan is not only the major tea producing province, and also the big province which used the tea, in the Sichuan, the tea resources are very rich, Emei Mountain fuzz tip et al was the famous tea varieties.Several tea residues are produced in this area every year, thus, a study on the utilization of these tea residues together with cellulase production is a meaningful research topic. This study aimed to make full use of tea residues, this study has great theoretical value and application value, after the results obtained in this study, it will provide a practical reference for how to make full use of tea waste.

II. EXPERIMENTAL

A. The microorganisms.T. reesei

QM9414 was kindly provided by the lab. of biochemistry, Southwest University for Nationalities.

B. Culture conditions.

The Cant cultivation were cultivation under 192 h at 30 ℃ in an incubator. The fermentation cultivation conditions were under 120 h at 30 ℃ in an incubator.

C. Extraction of crude enzyme

The crude liquid enzyme was extraction according to the method description by Nong et al (2013)[10]. The 0.1 M of d-NaAc buffer (pH 4.8) (20 times amount), was mixed with the solid-state fermentation medium. Extraction 1 h oscillation at 30 ℃.

D. Measurement the cellulase activity (filter paper method)

In this study, cellulase activity was measurement by filter paper activity according to Nong et al (2013)[10]. The enzyme activity unit in IU (μmol/min/ml)[4].

III. RESULTS

A. Effect of different moisture contents on enzyme production in the medium

The medium was prepared by combining 1.5 g of wheat bran and 2 g of tea residue. Different moisture contents were incorporated in the mixture by adding 9, 10, 11, 12, 13, 14, and 15 mL of Mandels nutrient solution. Exactly 1 mL of fungi spore suspension with a concentration of 1 × 109 spores/mL was added in the culture medium, after culture 5d, extraction of crude enzyme liquid and measuring enzyme activity. The result was shown in Fig .1. The amount of moisture that

produced the highest enzymatic activity in the culture was 12 mL of Mandels nutrient solution.

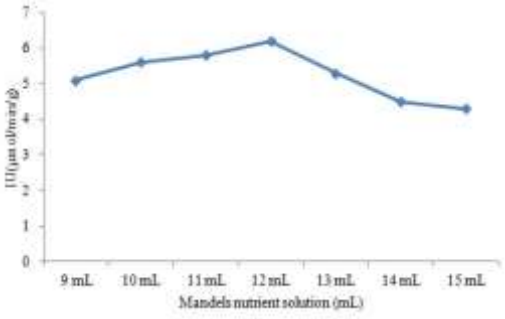

Figure 1. Effect of moisture content on enzyme production

The presence of moisture significantly affected enzyme production in the medium because a certain amount of moisture in soil is required for mold growth. When moisture content is small, the required humidity necessary for spore germination and mycelial growth is not achieved.

B. Effect of different initial pH values on enzyme production in the medium

In this step, the conditions were the same with the above steps except the pH value. The initial pH values of the different media prepared were 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, and 11. The result was shown in Fig .2. The medium with an initial pH of 5.0 produced the highest enzymatic activity.

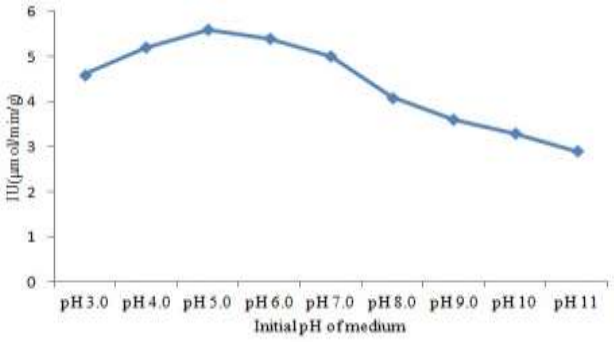

Figure. 2. Effect of initial pH on enzyme production in the medium

The pH of the medium did not significantly affect the production of cellulase. Growth and production of T. reesei QM9414 were optimized when pH of the medium was between 3 and 7, indicating that the strain exhibited excellent acid resistant characteristics; However, the strain should not be cultivated in an alkaline environment.

C. Effect of different proportions of wheat bran and tea residue on enzyme production in the medium

Different amounts of tea residue and wheat bran were combined (i.e., tea residue/ wheat bran were 3:0.5, 2.5:1, 2.0:1.5, 1.5:2, 1.0:2.5, 0.5:3.0). The different media prepared were placed in six triangular bottles (150 mL), and 12 mL of Mandels nutrient solution was added to them. The fungi spore suspension and cultured conditions was the same with above.

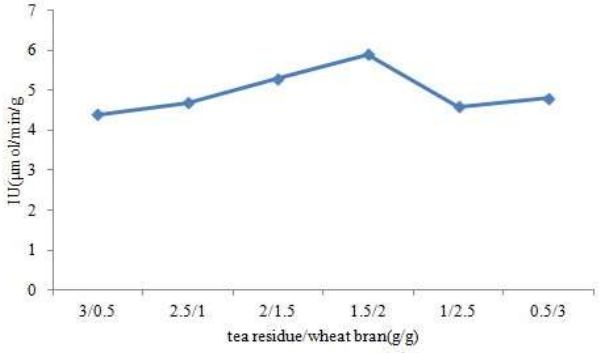

Figure. 3. Effect of tea residue/wheat bran ratio on enzyme production

The results are shown in Fig .3 Highest cellulase enzyme production was achieved when the proportion of tea residue and wheat bran was 1.5 g: 2 g. Wheat bran acted as an inducer of cellulose in the medium. but if the ratio of residue and wheat bran is not suitable of tea residue were higher, 12 mL of Mandels nutrient solution added was substantial absorption by tea residue and the medium was too dry, so it is not conducive to the growth of T. reesei QM9414 producing cellulase enzyme.

D Effect of different nitrogen sources on enzyme production in the medium

The medium component was contain wheat bran (2 g) , tea residue (1.5 g) and Mandels nutrient solution (12 mL). Mandels nutrient solution that contained different amounts of nitrogen from different sources (Table 1) was added to the medium. The fungi spore suspension concentration, cultured conditions and the method of measuring enzyme activity were the same with above.

TABLE 1: EFFECT OF NITROGEN SOURCE ON ENZYME PRODUCTION

Nitrogen source	enzyme activity, IU（μmol/min/g）
0.50% Urea	4.52
0.50% peptone	5.36
0.50% Ammonium sulfate	5.84
0.25% Ammonium sulfate + 0.25% Urea	4.25
0.25% Ammonium sulfate + 0.25% peptone	4.32

Different nitrogen sources affected enzyme production, and the different strains had certain selectivity of nitrogen source by production the cellulase [5]. In this study, the best nitrogen source of T. reesei QM9414 was ammonium sulfate.

E. Effect of different content of ammonium sulfate on enzyme production in the medium

The medium was prepared by the best condition description above. The difference was the different amounts of ammonium sulfate. Exactly 12 mL of Mandels nutrient solution that contained different amounts of ammonium sulfate (0.25%, 0.50%, 0.75%, 1%, 1.25%, 1.5%) was added to the medium. The results are shown in Fig .4 Highest cellulase enzyme production was achieved when the added amounts of ammonium sulfate was 1%.

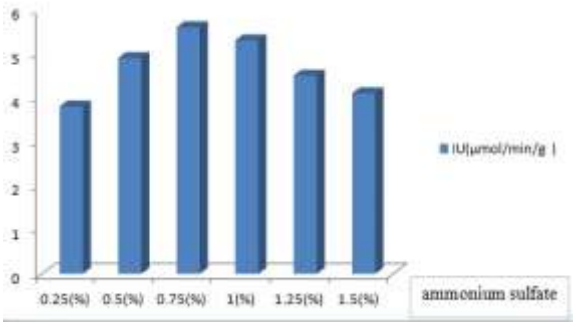

Figure 4. Effect of amounts of ammonium sulfate on enzyme production.

F. The comprehensive effect of enzyme production (orthogonal)

We using the L9(34) orthogonal test to investigate the comprehensive effect of enzyme production by four factors of the initial pH, moisture contents, proportion of tea residue and wheat bran and the amount of ammonium sulfate. The experimental results were shown in table 2.

TABLE II $L_9(3^4)$ ORTHOGONAL TEST AND THE RESULT

Number of tests	Factors				Enzyme activity(IU(μmol/min/g))			
	A	B	C	D	1	2	3	Average of enzymeactivity
	Initial pH	moisture content(mL)	Ammonium sulfate（%）	Tea residue /wheat bran				
1	4	11	0.5	2.5/1	5.88	6.06	5.96	5.97
2	4	12	0.75	2/1.5	5.95	5.86	5.78	5.86
3	4	13	1	1.5/2	5.32	5.54	5.23	5.36
4	5	11	0.75	1.5/2	6.08	6.22	6.26	6.19
5	5	12	1	2.5/1	6.29	6.17	6.22	6.23
6	5	13	0.5	2/1.5	5.46	5.78	5.69	5.64
7	6	11	1	2/1.5	5.85	6.03	5.78	5.89
8	6	12	0.5	1.5/2	4.58	4.36	4.54	4.49
9	6	13	0.75	2.5/1	4.33	4.27	4.20	4.27
∑K1	51.88	54.12	48.31	49.38				
∑K2	54.17	49.75	48.95	52.18				
∑K3	43.94	45.82	52.43	48.13				
R	10.23	8.3	4.12	4.05				

From the size of range, factor A (initial pH) was the maximum factor of impact of the T. reesei QM9414 producing cellulase by using the tea residue.The initial pH great influence on enzyme production, because of T. reesei QM9414 may not fit in the alkaline environment, the orthogonal experiments showed that the initial pH is the first major factors on enzyme production which is verified the results of single factor experiments. According to the range size, order the experimental factors was A > B > C > D, in each factor, the maximum value

were A2, B1, C3 and D2 respectively, so the optimum conditions of fermentation for A2B1C3D2.

IV. DISCUSSION

At present, the method to production the cellulase was still used the solid state fermentation. Solid-state fermentation exhibits several advantages compared with liquid fermentation. Such as the survival of microorganisms is similar to that in their natural state. And the "three wastes" discharge of solid-state fermentation does not occur, and low equipment investment et al. In 2000, Zhang et al[6] screened a high cellulase-producing strain TR-G from seven strains of Trichoderma reesei. And found that the maximum filter paper enzyme fermentation culture of cellulase activity to 308 mg /g.h. Li et al[7] investigated the coordination culture and solid-state fermentation of Trichoderma and Aspergillus niger. Results showed that key factors affecting cellulase activity were moisture and inoculation time. Alkali resistant strains was isolated and used to cellulase producing [8]. Liu et al[9], isolation and identification of strains producing cellulase at low temperature. In this study, moisture contents that were not suitable for the growth of T. reesei QM9414. In this study, the results of the single factor and orthogonal experiments showed that the optimum culture conditions were as follows: 2 g of the tea residue and 1.5 g of the wheat bran were prepared in the medium, and about 11 mL of the Mandels nutritional liquid which containing 1.0% of nitrogen source [(NH4)2S04], the pH was adjustment for 5.0, and the cultivation temperature was 30 ℃, and the production time was 120 h. Under these optimum fermentation conditions, cellulase activity reached 6.27 U/g dry medium. Future experiments will deal with more single factors, in order to obtain the optimal fermentation conditions, we will set the more single factors. In the experiment, the tea leaves which using as the sample were collected after the bubble tea house. The solid state fermentation medium and the composition of the relevant is very important, if the nutrition liquid amount is high, the composition too wet, the water is too large may also influence the effect of fermentation. In addition, we are also exploring the culture volume increased after the fermentation effect. The results show that, the amount of amplification effect on fermentation results after tea, more than any other single factor, more likely to affect. The next step, we will collect more tea samples, studied a lot more factor, at the same time in different varieties of tea, tea varieties to study the effects of enzyme production. Different varieties of tea, the bioactive component contains different, can provide nutrient elements, different for the microorganisms therefore, if the conclusion of the experiments is different tea varieties are significant difference on the fermentation effect. Then, our future experiments can be starting from the analysis of tea components, and can carry out a lot of tea residue experiments, opens up a new research way for making full use of the cellulose waste of tea dregs. It will be an interesting and contents which worthy further study.

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REFERENCES

[1] Tomme, P. Warren A.J. and Gilkes N.R., et al. Cellulose hydrolysis by bacteria and fungi. Microb. Physiol., 1995. 37, pp :1-4.

[2] Wang, C.Y. Hsieh, Y.R. and Ng, C.C. et al. Purification and characterization of a novel halostable cellulase from Salinivibrio sp strain NTU-05. Enzyme Microb. Technol., 2009, 44, pp:373-377.

[3] Gallo,B.Andreotti,R, and Roche,C, et al. Cellulase production by a new mutant strain of Trichoderma reesei MCG77. Biotechnol Bioeng.Symp.,1978. 8: pp: 89-101.

[4] Ye X.Y. and Cheng, K.J. Purification and characterization of a cellulase from the ruminal fungus Orpinomyces joyonii cloned in Escherichia coli. Int. J. Biochem. Cell Biol, 2001.33, pp: 87-92.

[5] Sun, J.S Li, X. Dong. J. X.Q Induced breeding and conditions of producing cellulase by Trichoderma koningi. Beijing Forestry Uni.2002. 2, pp: 83-85.

[6] Zhang, C.J Wang Q.F et al.J. Food and Fermentation Industry. 2000. 26, pp: 21-23.

[7] Li, Y. Huang. W. B J. Food and Feed industry. 2002.9, pp: 29-31.

[8] Lu, Z.L Zhang H, S. et al.J. Guangxi Sci. 2014.1, pp: 22-27.

[9] Liu, X. H. Gao, X. M et al.J. Shangdong Agricultural Sci. 2014.3, pp: 54-57.

[10] Nong, X Wu H and Xu C,H et al. Study on the cellulase components β-endoglucanase purification by chromatography. Asian journal of chemistry. 2013.25(3).pp: 1687-1690.