Advance Journal of Food Science and Technology 10(11): 833-837, 2016
DOI: 10.19026/ajfst.10.2270
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: May 20, 2015 Accepted: June 19, 2015 Published: April 15, 2016

Research Article

Optimization of Fermentation Technology for Producing Single Cell Protein from Yam Starch by Orthogonal Test

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Abstract: Using Yam starch as raw material, the fermentation technology of Single Cell Protein (SCP) was optimized through by active dry yeast fermentation through single factor and orthogonal test in this study. The optimal conditions were showed as followed: 30 mL the medium volume in 250 mL flask, inoculum size17%, fermentation time 69 h, pH 5.0. Under these conditions, the production of SCP reached 241.54±0.15 (g wet weight/100 g Dry starch.

Keywords: Active dry yeast, orthogonal test, SCP, yam starch

INTRODUCTION

Protein is a basic material to sustain life, which is composed of human organs, organizations, enzymes in the body, the hormone and immune globulin of the main components (Kosseva, 2013). Protein deficiency problems of the whole world have been existed for many years, the biotechnology development of single cell protein is an important way to solve this problem (Buschke *et al*., 2013). Single cell protein is a modern important source of protein in feed industry and food industry.

Single Cell Protein (SCP) is defined as a kind of protein from yeast or bacteria and other microorganisms for feeding animals and even humans (Rajoka *et al*., 2004). Single cell protein can be used to feed, also can be used to edible (Chanda and Chakrabarti, 1996). Microbial variety, large number, wide distribution, breeding fast, as a new resource of protein has great development potential (Wijeyaratne and Jayathilake, 2000). According to the determination of protein content of single cell protein is 40-80%. Among them, yeast is 45-55%, mold class is 30-50%, bacteria are 69-80% and algae are 60-70% (Nasseri *et al*., 2011; Anupama and Ravindra, 2000). Single cell protein have rich nutrients, including amino acid composition is complete, lysine and other essential amino acid content is higher, at the same time, rich in vitamins, can be used as a substitute for vitamins (Guo and Xiao, 2006; Ren, 2007). Therefore, the use of non food resources and waste resources (such as residues of agricultural and industrial liquid waste) has become the development and promotion of microorganisms to produce single cell protein is one important way of supplementary feed protein source is insufficient (Ravindra, 2000; Nigam, 1998). So, it is of great significance.

Yam as a very useful medicinal material in traditional Chinese medicine is known as "the medical gold ". Its tuber contains rich dioscin, the hydrolyzing product of which is diosgenin (Chen *et al*., 2008). It can be used as the foundation raw material and the outset intermediate to synthesize the hormone medicine and has high value for medicinal purposes (Trouillas *et al*., 2005; Bertrand *et al*., 2009; Jayachandran *et al*., 2009). Yam not only contains a small amount of dioscin, but also contains 40-50% of starch and 40-50% of cellulose (Wang *et al*., 2008; Huang *et al*., 2008). At present, domestic factories employ the traditional pre-ferment acid hydrolysis in diosgenin production (Wen *et al*., 2008). But the technology was not effectively use yam starch, cause a lot of pollution. At present, people have been looking for clean production technology of diosgenin. Cellulose of the method was not separated before the acid hydrolysis, the acid hydrolysis with acid, BOD, COD value was high and pollution was serious, the low utilization rate of yam (Zhao *et al*., 2004; Cheng *et al*., 2009). Therefore, our research term first isolated cellulose and starch from yam and then hydrolyzed the residue to produce diosgenin by acid hydrolysis. This paper studied the fermentation technology of Single Cell Protein (SCP) using yam starch fermentation through single factor and orthogonal test, so as to achieve low pollution, high recovery and resources comprehensive utilization.

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MATERIALS AND METHODS

Materials: Yam starch was obtained from laboratory. Active dry yeast was obtained from Angel Yeast Co. Ltd. (Hubei, China). Thermostable α-amylase (15000 U/mL) and glucoamylase (10000 U/mL) were provided from Wuxi star of biological engineering co., Ltd (Jiangsu, China).

Activation of yeast: Take a certain amount of active dry yeast put in sugar water with 10 times weight and 5% sterilization, activated 50 min in the 38°C water bath and can be used for inoculation (Liang *et al*., 2003).

Liquefaction and saccharification of starch: $CaCl_2$ was added to 8% starch slurry and reached0.15% as amylase protecting, then added thermostable α- amylase (100 u/g dry starch), under the condition of 90°C water bath to keep 1 h make it liquefaction completely, then cool to 60°C and add glucoamylase (100 u/g dry starch) in 60°C water bath to keep 1 h so that it is completely saccharification, the saccharification liquid was used as medium for SCP prodcution (Ma *et al*., 2006).

Initial fermentation conditions for producing SCP: The Initial fermentation conditions for producing SCP from yam starch were as follows: 25 mL medium in 250 mL flask, the inoculums 15%, pH 4.5, 28.5°C, 200 r/min condition shaker fermentation 60 h.

The determination of SCP production: The broth was centrifuged at 3500 r/min for 10 min, removed the supernatant and weighed wet weight of SCP. The production was calculated by wet weight of SCP from the 100 g dry starch.

RESULTS AND DISCUSSION

The effect of liquid volume on SCP production: The saccharification liquid of 8% yam starch was added to 250 mL flask with different volume (10, 20, 30, 40 and 50 mL, respectively), sterilized, cool to room temperature and inoculated activation of the active dry yeast. The fermentation conditions for producing SCP from yam starch were the same as Initial fermentation conditions except medium volume, then centrifugal and wet weight measurements of SCP under this condition. The results were shown in Fig. 1.

By the results of Fig. 1 showed that the SCP production had no obvious change when the medium volume for 10 and 20 mL, reached a maximum at 30 mL, after decreasing with the increment of liquid volume. Dissolved oxygen was one of the important limiting factors of the aerobic growth of yeast. The liquid volume directly affected of the ventilation effect of medium and dissolved oxygen concentration. In the flask fermentation conditions with a constant rotating speed of the vibrating bed, by changing the liquid volume of medium to change the way of dissolved oxygen. The production of SCP was higher at 30 mL liquid volume in 250 mL flask, so the optimum liquid volume was 30 mL in 250 mL flask at rotation speed of 200 r/min.

The effect of culture time on SCP production: The fermentation conditions for producing SCP from yam starch were the same as Initial fermentation conditions except liquid volume was 30 mL in 250 mL flask, under this condition, the SCP (wet weight) was determined at culture time interval of 12 h. The result was shown in Fig. 2.

The production of SCP had no obvious change at the initial stage of growth, but began to accelerate after 36 h, reached a peak at 60 h. The SCP production increased with the increase of culture time. Yeast cells were autolyzed more than 60 h. SCP production decreased with the decrease of the number of cells, so the best fermentation time was 60 h.

The effect of pH on SCP production: The fermentation conditions for producing SCP from yam starch were the same as Initial fermentation conditions except liquid volume was 30 mL in 250 mL flask and different pH (3.5, 4.0, 4.5, 5.0 and 5.5), the result is shown in Fig. 3.

The initial pH also affected the growth of yeast, the concentration of hydrogen ion change cell plasma membrane colloid charge. So, the plasma membrane of certain substances and ion permeability will change with the change of the concentration of hydrogen ion. This indicates that the speed of nutrients into the cells was related to pH. As shown in Fig. 3, the optimum initial pH was 5.0, but the optimum pH was 4.5 during

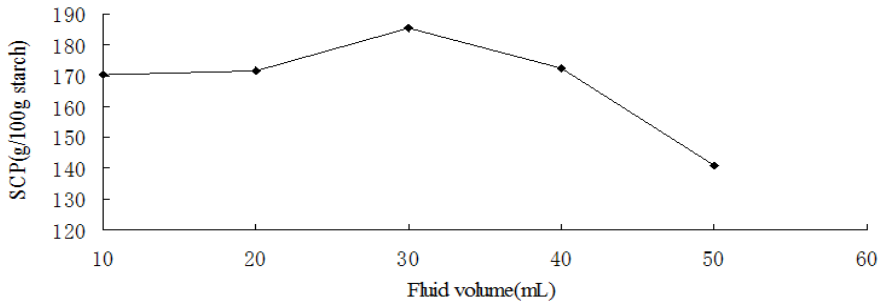

Fig. 1: The effect of quantity of zymatic fluid to SCP

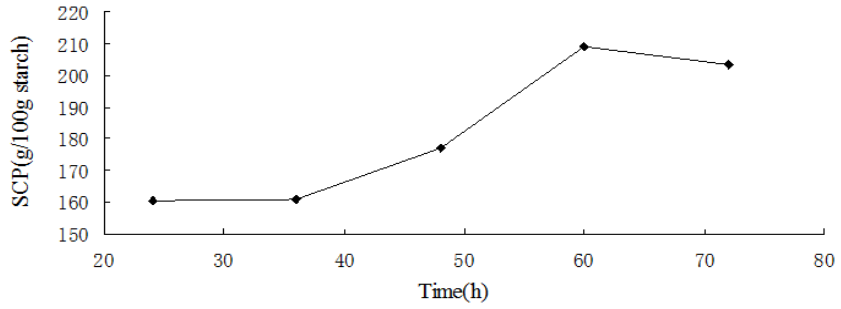

Fig. 2: The effect of cultivating time to SCP

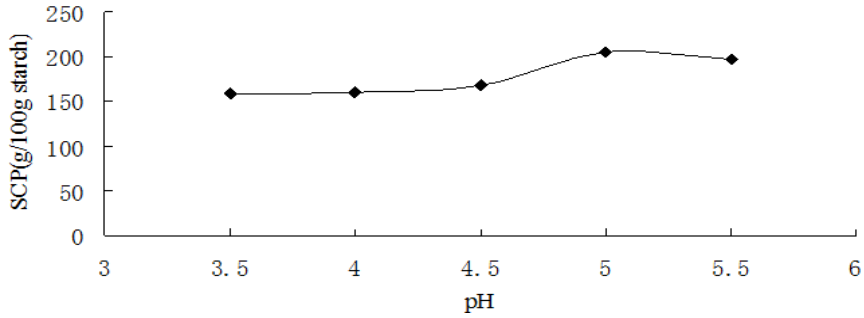

Fig. 3: The effect of pH to SCP

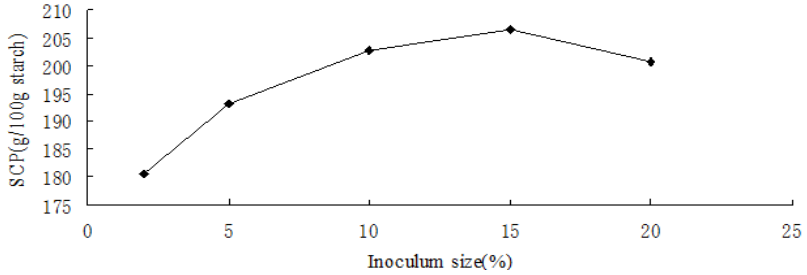

Fig. 4: The effect of inoculum size to SCP

Table 1: Table of factors and levels

Factor	Fluid volume A (mL)	Inoculum size B (%)	Time C (h)	Initial pH D
1	25	13	51	4.7
2	30	15	60	5.0
3	35	17	69	5.3

fermentation to produce ethanol, so optimum initial pH was 5.0 for SCP production.

The effect of inoculum size on SCP production: The fermentation conditions for producing SCP from yam starch were the same as Initial fermentation conditions except liquid volume was 30 mL in 250 mL flask, pH 5.0 and different inoculum size (2, 5, 10, 15 and 20%, respectively). The result was shown in Fig. 4.

The SCP production was higher when the 15% inoculum size. The fermentation incomplete for inoculum size was decreased, making the low yield of SCP. The concentration of bacteria in liquid culture was increase with more inoculum size, making increase the demand for nitrogen source, carbon source. Therefore, nitrogen content and residual sugar content of the culture medium were decrease, nutrient deficiency makes yeast growth slowed, bacteria decline ahead of time, eventually the concentration of bacteria cell decreased, thus affecting the level of fermentation. Production of SCP by starch fermentation optimum inoculum size was 15%.

Optimization of technological conditions for production of SCP: According to the results of single factor experiment, liquid volume, initial pH, culture time, inoculum size were selected to carry out four factors three levels orthogonal experiment, to determine the optimum conditions for producing SCP. The factor levels were shown in Table 1, experimental results and analysis were shown in Table 2 and Fig. 5.

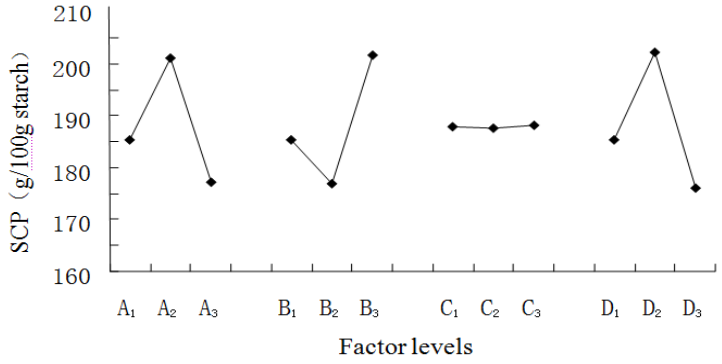

Fig. 5: The yield trends of SCP

Table 2: Experimental design and results analysis

Factor	Fluid volume A (mL)	Inoculum size B (%)	Time C (h)	Initial Ph D	SCP (g/100 g starch)
1	1	1	1	1	180.20
2	1	2	2	2	188.40
3	1	3	3	3	187.67
4	2	1	2	3	186.50
5	2	2	3	1	187.71
6	2	3	1	2	228.92
7	3	1	3	2	189.29
8	3	2	1	3	154.15
9	3	3	2	1	187.96
K_1	556.27	555.99	563.27	555.87	∑ = 1690.80
K_2	603.13	530.26	562.86	606.61	
K_3	531.40	604.55	564.67	528.32	
k_1	185.42	185.33	187.76	185.29	
k_2	201.04	176.75	187.62	202.20	
k_3	177.13	201.52	188.22	176.11	
Optimal levels	A_2	B_3	C_3	D_2	
R_{ij}	23.91	24.77	0.60	26.09	
Primary and secondary order	$R_D>R_B>R_A>R_C$				

Table 3: The validation results

Number	1	2	3	Average value
SCP (g/100 g dry starch)	241.65	241.37	241.60	241.54±0.15

From the above results, the optimum fermentation conditions of production of SCP from yam starch was $A_2B_3C_3D_2$ with 30 mL liquid volume in 250 mL flask, 17% inoculum size, fermentating time 69 h, initial pH5.0. The most important factor for producing SCP from yam starch was the initial pH, followed by inoculum size and liquid volume, the least was time.

The optimum fermentation conditions of the $A_2B_3C_3D_2$ was not in the orthogonal test, so made the validation test under the optimal condition, the results as shown in Table 3.

The yield of SCP was 241.54 (g wet weight/100 g dry starch), which was consistent with the results of the analysis.

CONCLUSION

The optimum condition that fermentating SCP from yam starch was 30 mL the medium volume in 250 mL flask, inoculum size 17%, fermentation time 69 h, pH 5.0. Under the optimal conditions, the production of SCP reached 241.54 (g wet weight/100 g Dry starch).

ACKNOWLEDGMENT

The project was partly supported by the Science and Technology Overall Planning for innovation Engineering project of Shaanxi Province, China (No. 2013KTDZ03-01-03).

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