Study on the Adsorption Technology of Chitosan to the Protein in Tofu Yellow Slurry Water

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Abstract: In this paper, the protein removal rate was used as an indicator to study the effects of the amount of chitosan added, reaction time, reaction temperature, and pH on the process of chitosan's adsorption of protein in tofu yellow slurry water. Orthogonal experiments were used to determine the optimal reaction conditions. The results show that, optimum conditions for chitosan to adsorb protein in tofu yellow slurry water: chitosan added 1.0g/L, pH 7, temperature 20°C, the response time is 3h, the removaling rate is 51.64%. The results show that chitosan can be utilized to the process of the recycling of soybean protein and Tofu yellow slurry water.

1 INTRODUCTION

Soybean is one of the main food and oil crops in the world. Processed products using soybeans as raw materials are favored by consumers because soybeans are rich in high-quality protein, calcium, potassium, iron, copper, zinc and other trace elements, vitamin B1 and B2, organic acids and cellulose and other nutrients (Diao, 2015, Wang, 2019). The most popular food developed with soybeans as a raw material by consumers is traditional tofu. A large amount of yellow slurry water is generated in the production of tofu. It is estimated that 8-10 tons of wastewater will be generated from processing 1 ton of soybeans (Du, 2008). Tofu yellow slurry water is produced in the process of extracting tofu from soy milk and alkali-dissolved supernatant. According to the current research status (Che, 2017), yellow slurry water contains a lot of organic matter, which contains about 1700 mg/L of oil, 3800~4000mg/L of protein, 7000~20000 mg/L of total sugar, and 800~1700 mg of total nitrogen (TN). /L, total phosphorus (TP) 100~200mg/L, BOD5 value is about 5000~10000mg, COD value is 20000~24000mg, it is a kind of wastewater with high concentration and high degree of biodegradability (Xie, 2009). Therefore, if the protein in the fermented tofu yellow slurry water can be effectively recovered, not only the utilization of raw materials will be improved, but the sewage will be purified, and the pressure of subsequent sewage treatment will be reduced, thereby realizing the unification of environmental and economic benefits.

Chitosan is a natural biopolymer of basic amino polysaccharides, which is the product of deacetylation of chitin. Chitosan has excellent biocompatibility, non-toxicity and easy chemical modification (Xie, 2013, Chen, 2001). Studies have found that chitosan can be used as a flocculant for water treatment. It has good effects in the treatment of food wastewater, papermaking wastewater and urban domestic wastewater. This paper studies the flocculation conditions of chitosan to adsorb protein in tofu yellow slurry water, which can provide a certain theoretical basis for the treatment of tofu yellow slurry wate.

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2 MATERIALS AND METHODS

2.1 Materials and Reagents

Soybeans, Northeast soybeans are commercially available. Chemical reagents such as sodium hydroxide, hydrochloric acid, acetic acid, and methyl orange are all analytically pure. Chitosan, Zhejiang Golden Shell Biochemical Co., Ltd.; Bovine Serum Protein, Jilin Boshen Biotechnology Co., Ltd..

2.2 Instruments and Equipment

Model 752 UV-Vis Spectrophotometer, Shanghai Spectrometer Co., Ltd.; HZS-H Water Bath Oscillator, Harbin Donglian Electronic Technology Development Co., Ltd.; PHS-25 Digital Display pH Meter, Shanghai Precision Scientific Instrument Co., Ltd.; ALC-2100 electronic balance, Shanghai Precision Instrument Co., Ltd.; DHG-9123A electric heating constant temperature blast drying oven, Shanghai Yuejin Medical Equipment Factory; DK-98-II electric heating constant temperature water bath, Shanghai Yuejin Medical Equipment Factory; JB-1 timing two-way Magnetic stirrer, Jiangsu JintanRonghua Instrument Manufacturing Co., Ltd.; 80-2 centrifuge, Shanghai Anting Instrument Factory; HZQ-C air bath oscillator Harbin Donglian Electronic Technology Development Co., Ltd., DHP-9162 electric heating constant temperature incubator Shanghai Yiheng Technology Co., Ltd..

2.3 Method

2.3.1 Preparation of Tofu Yellow Slurry Water

Evenly take the tofu whey wastewater (yellow slurry water) discharged when the traditional method is used to produce tofu. 1 kg of raw soybeans produces 2.5 kg of tofu and discharges 8.95 kg of yellow water.

2.3.2 Preparation of Chitosan Solution

The experiment uses glacial acetic acid to prepare 1% chitosan solution. Specific steps: (1) Configure 1% acetic acid solution. (2) Add an appropriate amount of chitosan to 1% acetic acid solution, and prepare 1% chitosan solution. A magnetic stirrer can be used to help chitosan dissolve uniformly in acetic acid.

2.3.3 Determination of the Degree of Deacetylation of Chitosan

In the experiment, the degree of deacetylation of chitosan was measured by acid-base titration. The specific steps are as follows: accurately weigh 0.3~0.5g of chitosan dried to constant weight at 105℃, put it in a 250mL Erlenmeyer flask, add 30mL of 0.1mol/L hydrochloric acid standard solution, and stir at 20℃~25℃ until it is completely dissolved, Add 2~3 drops of methyl orange indicator, titrate free hydrochloric acid with 0.1mol/L sodium hydroxide standard solution to the end point (WU S H, 2009). Deacetylation degree calculation formula:

$$\text{Free amino content}(-NH_2)\% = \frac{(C_1V_1-C_2V_2)\times 0.016\times 100\%}{G}$$

$$\text{Degree of deacetylation} = \frac{(-NH_2)\%\times 100\%}{9.94\%}$$

Where:

C1-Concentration of hydrochloric acid standard solution, mol/L

C2-Concentration of sodium hydroxide standard solution, mol/L

V1-The volume of hydrochloric acid standard solution added, mL

V2-The volume of sodium hydroxide standard solution consumed during titration, mL

G-Sample weight, g

0.016-The amount of amino group equivalent to 1mL 1 mol/L hydrochloric acid solution, g

9.94%-Theoretical amino content.

2.3.4 Single-factor Experiment of Protein Adsorption in Tofu Yellow Slurry Water

The Effect of Chitosan Addition on Protein Removal Rate. Take five 50mL colorimetric tubes, and add 50mL tofu yellow slurry water, labeled 1~5. Maintain the pH of the original wastewater. Add 1% chitosan solution to No. 1~5 respectively, that is, the amount of chitosan added: 0.4g/L, 0.6g/L, 0.8g/L, 1.0g/L, 1.2g/L. The protein removal rate is used as an indicator to determine the optimal amount of chitosan added.

The Effect of pH on Protein Removal Rate. Take eight 50mL colorimetric tubes, add 50mL of tofu yellow slurry water, labeled 1~8, add chitosan to 0.8 g/L, and adjust the pH to 3.08, 3.99, 5.04, 5.97, 6.14, 6.52, 7.07, 7.98, respectively. The protein removal rate is an indicator to determine the optimal pH.

The Effect of Temperature on Protein Removal Rate. Take five 50mL colorimetric tubes, label 1~5 and add 50mL tofu yellow water. The amount of chitosan added was 0.8 g/L, the pH was 7.07, and the reaction temperature was controlled at 5°C, 17°C, 25°C, 35°C, and 45°C. The protein removal rate was used to determine the optimal reaction temperature.

The Effect of Reaction Time on Protein Removal Rate. Take five 50mL colorimetric tubes, and add 50mL tofu yellow slurry water, labeled 1~5. The amount of chitosan added was 0.8 g/L, the pH was 7.07, the temperature was 25°C, the reaction time was controlled to 1h, 2h, 3h, 4h, 5h, and the protein removal rate was used to determine the optimal reaction time.

2.3.5 Orthogonal Experiment on the Absorption of Protein in Tofu Yellow Slurry Water

According to the results of the single factor experiment, the reaction time is controlled to 3h, and the addition amount of chitosan, pH, and reaction temperature are selected as factors for L9 (34) orthogonal test. The protein removal rate is used as an indicator to determine the best process conditions. The experimental design is shown in Table 1.

Table 1: Test factor level table.

Level	A pH	B the addition amount of chitosan/g · L^{-1}	C T/°C
1	6.5	0.6	20
2	7	0.8	25
3	7.5	1.0	30

3 RESULTS AND ANALYSIS

3.1 Determination of the Degree of Deacetylation of Chitosan

The degree of deacetylation of chitosan was determined according to 1.3.3, and the degree of deacetylation of chitosan used in the test was 72.4%.

3.2 Single-factor Experiment of Protein Adsorption in Tofu Yellow Slurry Water

3.2.1 The Effect of Chitosan Addition on Protein Removal Rate

The relationship between the amount of chitosan added and the protein removal rate is shown in Figure 1:

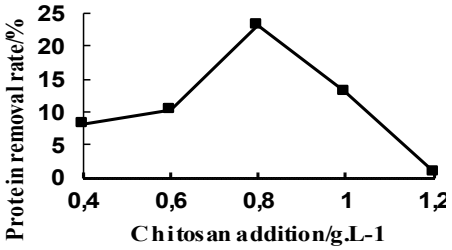

Figure 1: The effect of chitosan addition on protein removal rate.

It can be seen from Figure 1 that as the amount of chitosan added increases, the protein removal rate shows a trend of first increasing and then decreasing. When the added amount reaches 0.8g/L, the protein removal rate reaches the maximum, indicating that the adsorption and separation effect of chitosan on protein is the best at this time. The analysis believes that when the amount of chitosan added is small, the protein cannot fully bind to the chitosan, so it cannot completely react to form a protein-chitosan complex; when the amount of chitosan added is too high, it will inhibit the formation of the protein-chitosan complex, and the reaction consumes too much chitosan, which is a waste of resources, so the optimal addition amount of chitosan is 0.8g/L.

3.2.2 The Effect of pH on Protein Removal Rate

The relationship between the pH of tofu yellow slurry water and the protein removal rate is shown in Figure 2:

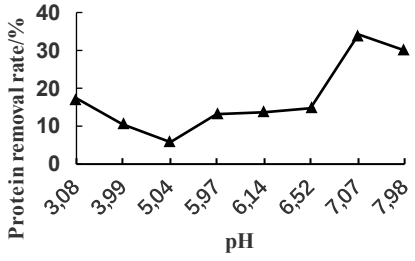

Figure 2: The effect of pH on protein removal rate.

It can be seen from Figure 2 that when the pH is 3~5, as the pH of the protein increases, the protein removal rate decreases. This is because the protein is positively charged at this time, and the chitosan is also positively charged, and the two repel each other, so when the pH is 5, the protein removal rate reaches the lowest. When the pH is between 5 and 7, the protein removal rate increases as the pH increases. This is because when the pH of the protein is greater than the isoelectric point, the protein is negatively charged and attracts the positive charge of chitosan to form a protein-chitosan complex. When the pH is 7.04, the protein removal rate reaches the maximum. Therefore, the optimal pH value is 7.04.

3.2.3 The Effect of Temperature on Protein Removal Rate

The relationship between temperature and protein removal rate of tofu yellow slurry water is shown in Figure 3:

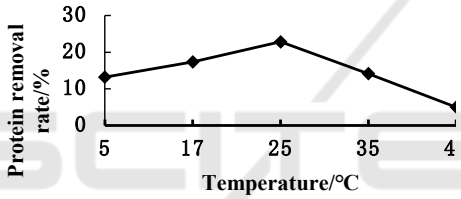

Figure 3: The effect of temperature on protein removal rate.

It can be seen from Figure 3 that too high temperature will inhibit the formation of chitosan-protein complexes. As the reaction temperature increases, the protein removal rate gradually increases, which proves that the increase in temperature is beneficial to the adsorption and separation of proteins by chitosan. When the temperature reaches 25°C, the protein removal rate reaches the maximum, and chitosan has the best effect on protein adsorption and separation. After that, the protein removal rate decreases with the increase of temperature. Excessive temperature affects the adsorption and separation effect. Therefore, the optimal reaction temperature is 25°C.

3.2.4 The Effect of Time on Protein Removal Rate

The relationship between time and the protein removal rate of fermented bean curd syrup is shown in Figure 4:

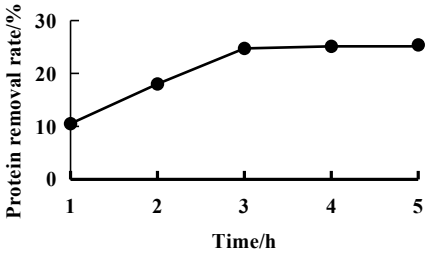

Figure 4: The effect of time on protein removal rate.

It can be seen from Figure 4 that the prolonged reaction time can promote the contact and reaction between chitosan and protein. In 1~3h, the protein removal rate increased obviously. In3~5h, the increasing trend of protein removal rate was not obvious, indicating that the adsorption and separation of protein by chitosan was basically completed at this time. Therefore, the optimal reaction time is 3h.

3.3 Orthogonal Experiment on the Adsorption of Protein in Tofu Yellow Slurry Water

Table 2 shows the orthogonal experiment results of chitosan adsorbing protein in tofu yellow slurry wate.

Table 2. Orthogonal test results.

Number	A pH	B Chitosan addition/g. L^{-1}	C Temperature/ ℃	D Empty column	Protein removal rate/%
1	1	1	1	1	32.74
2	1	2	2	2	20.29
3	1	3	3	3	24.96
4	2	2	3	1	27.51
5	2	3	1	2	49.87
6	2	1	2	3	24.18
7	3	3	2	1	38.97
8	3	1	3	2	24.96
9	3	2	1	3	21.84
K_1	77.99	81.88	104.45	99.22	
K_2	101.56	69.64	83.44	95.12	
K_3	85.77	113.80	77.73	70.98	
k_1	25.997	27.293	34.817	33.073	
k_2	33.853	23.213	27.813	31.707	
k_3	28.590	37.933	25.910	23.660	
R	23.57	44.16	26.72	28.24	
Major factors→Secondary factors			BCA		
Optimal scheme$A_2B_3C_1$					

From the range R value in Table 2, it can be seen that the primary and secondary order of the three factors affecting the protein removal rate is: B>C>A, that is: chitosan added amount>reaction temperature>pH, according to the orthogonal experiment statistical calculations show that the superior level is $A_2B_3C_1$. Therefore, a verification test was carried out, and the protein removal rate of the combination $A_2B_3C_1$ was measured three times. The results are shown in Table 3.

Table 3. Protein removal rate of experimental combination $A_2B_3C_1$

Number	1	2	3	Average value
Protein removal rate/%	50.93	52.33	51.65	51.64

From Table 3, the optimal process conditions for chitosan to adsorb protein in tofu yellow syrup were determined: chitosan was added at 1.0 g/L, pH was 7, temperature was 20°C, reaction time was 3h, and protein removal rate was 51.64%.

4 CONCLUSIONS

In this paper, tofu yellow slurry wate is used as the raw material, The effect of chitosan on the protein removal rate of tofu yellow syrup was investigated through single factor experiment, and the optimal process conditions for chitosan to adsorb protein in tofu yellow slurry water were determined through orthogonal experiment design. Get the following conclusions: (1) The degree of deacetylation of chitosan was determined by chemical analysis. The degree of deacetylation of chitosan used in the test was 72.4%. (2) Using chitosan as a flocculant, we studied in detail the influence of pH, chitosan addition, reaction temperature, and reaction time on the protein removal rate in tofu yellow slurry wate. (3) We adopted an orthogonal experiment design and used the protein removal rate as an indicator to determine the best conditions for chitosan to adsorb protein in tofu yellow slurry water: pH is 7, the addition amount of chitosan is 1.0g/L, reaction temperature is 20℃, The reaction time is 3h. At this time, the removal rate of protein in the tofu yellow slurry water was 51.64%.

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