Residual sludge catalyzed by hydrolysis of thermophilic bacteria

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Abstract. In order to improve the utilization rate of the surplus sludge, this experiment investigated the efficient hydrolysis of the residual sludge, explored the effect of sludge nitrogen source, analyzed the influence on the content of protein, amino acid, polypeptide and particle size of the sludge hydrolysis index, and the extraction conditions were optimized by response surface analysis. The maximum protein extraction rate of the residual sludge at the reaction pH of 60℃ and =7. Here, the particle size of S sludge was gradually reduced, reduced by 1.1% by 500 m-100 um, 7.5% by 30-50 μ m, 5.4% by 10 μ m-30 μ m, and 0-10 μ As m decreases 13% and sludge increased 13% Nt particles become thinner, larger than surface area and more developed pore structure.

1 Introduction

In recent years, with the rapid development of economy, the growth of population and industrialization level, lead to the increase of sewage treatment plant, sewage treatment capacity is inclined to increase rapid and stable development, in addition to harmful substances such as heavy metals in residual sludge, at the same time, the residual sludge has a large number of plant nutrients and organic matter, including organic matter in addition to polysaccharide, fat, 30%~60% of dry sludge is not used protein, This part of the protein can be used as a raw material for foaming agent and animal feed. [1] Current sewage treatment plants mainly treat sewage through activated sludge [2] and biofilm [3] Both methods belong to aerobic biological treatment. The principle of pri activated sludge method is to decompose organic matter through floating activated sludge in the aeration tank. The biofilm method relies on the carrier of the microbial membrane surface to release organic matter. According to the study, the treatment and disposal costs of the residual sludge exceeded 65% of the total operating costs of the WWTP [4].

Biohydrolysis of organic matter by thermophiles can improve the sludge degradation rate and dissolution performance of [5], which is considered as an effective treatment method. This experiment introduced additional elements and then applied the cellular enzymes of the thermophiles, avoiding the effect of antibiotics on the production of the thermophilic enzymes n, achieving the residue Efficient hydrolysis of s explored the influence of sludge nitrogen source on peptide content and particle size of sludge hydrolysis index, and the extraction conditions were optimized by response surface analysis.

2.Materials and Methods

Selection of dominant thermophiles that produce proteases and amylases from the matrix in heat and compost, Amino acids are determined by automatic amino acid analyzer. The principle is to use cation exchange chromatography separation-ninhydrin column derivative method to analyze the type and content of amino acids. The content of peptides in the supernatant was determined using the biuret reagent assay. The sample was first diluted to an appropriate concentration, after which 1 mL of supernatant was added t o the tube, followed by 4 mL of double urea reagent. After sufficient shaking, the colorimetric measurement was performed at room temperature (20~25℃) for 30min and at 540nm. Tube without protein solu tion were used as blank control solution. The average value of the three groups was determined, with the protein content as the abscissa and the light absorption value as the ordinate. The standard cu rve made is shown in Figure Figure 2-5, with the standard equation of y=0.0549x-0.0032, R2=0.9999. The total protein of the sludge was determined by (VELP, UDK159, Italy) in four steps: digestion, distillation, absorption and titration. By adding concentrated sulfuric acid and catalyst to the sludge, fully shaking and heating digestion and decomposition, the organic nitrogen in the sludge is converted into ammonium sulfate, alkalized and distilled to free ammonia, then absorbed with boric acid, and then titrated with hydrochloric acid standard titration solution, the consumption of hydrochloric acid is recorded, and the protein content is calculated. The tic proteins in the supernatant were determined using a modified BCA method. In 25 μ L of the supernatant sample, 25 μ L solution D, 37℃ for 30 min, then 1 mL working solution, 37℃ for 30min, finally left at room temperature for

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10min, and the absorbance at 562nm was measured. The standard curve was drawn with the standard solution of bovine serum protein. Solsoluble protein units were mgCOD / L, and 1g soluble protein was converted to 1.5g COD / g protein.

3.Results and Discussion

3.1. Effect of the hydrolysis reaction time

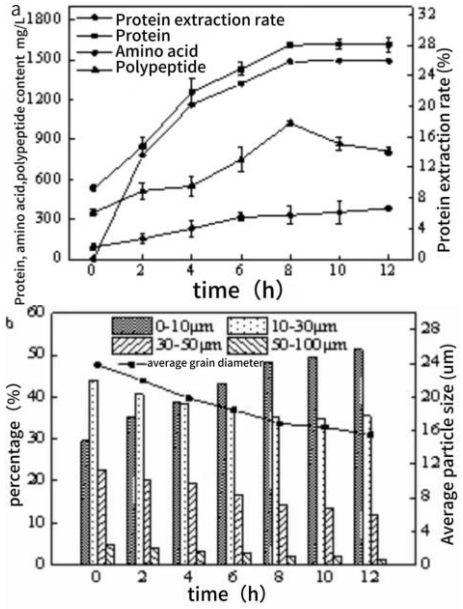

Figure 1 Effect of time on the content of protein, amino acid, peptide and (b) particle size of sludgeamino acid, peptide and (b) particle size of sludge

As can be seen from Figure 1 (a), with the increase of time, in the sludge reaction system supplemented with crude enzyme solution of hyperthermophilus, all indicators are constantly increasing, which has an obvious effect on protein promotion. At the reaction time of 2 h, the protein, amino acid, peptide content and protein extraction rate were 848.3 mg/L, 153.6 mg/L, 513.7 mg / h and 13%, respectively, while at the reaction time of 8 h, the data were 1611.3 mg/L, 230.5 mg/L, 1023.7 mg/L and 25.8%, indicating the three organic species from 2 to 8 h The contents were nearly doubled, which is consistent with the results of the [6] study by Liu et al. With the increase of time, all the substrates have been combined with the crude enzyme solution of hyperthermophilus. However, the growth trend slowed down after 8 h, and the protein extraction rate no longer increased. With the progress of the reaction, the polypeptide was decomposed into amino acid small molecules by the enzyme, and the polypeptide showed a trend of increasing the first step and then decreasing. From a practical working perspective, too long reaction time is accompanied by increased energy consumption and cost, and a long reaction can lead to an increase in short chain protein, so the optimal reaction time is 8 h.

The formation process of residual activated sludge flocs is very complex, and some studies show that micro-growth The flocculation is mainly a self-protection phenomenon produced by bacteria to resist the harsh environment. With the progress of the test reaction, the sludge flocs changed significantly. The color changed from the original dark brown to light brown, and the particles became fine. The color change of the sludge was consistent with the result that Song Yong [7] added the hydrolytic enzyme to the activated sludge system. See Figure 1 (b) for the sludge size change. Compared with the residual sludge not treated with thermophilus, the particle size of the residual sludge treated for 12 h increased significantly from 1~10 μ m, the particle size of 30-100 μ m decreased significantly, and the average particle size decreased by 8.345 μ m . The results showed that the cytololytic action of the crude enzyme solution of hyperthermophilus led to the disintegration of the flocs, the bacteria in the sludge, and then the enzyme acted on the bacteria to break it, and the particle size decreased significantly. Chen et al.[8] compared the effects of several enzymes on improving the hydrolysis and biodegradability of residual sludge, and concluded that the enzyme plays a significant role in crushing the complex structure of residual sludge.

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3.2 Influence of different inoculum sizes of hyperthermophiles

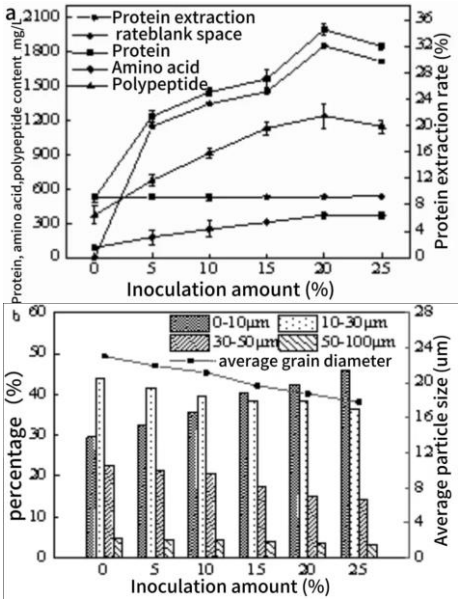

Figure 2 Effect of inoculum volume on (a) protein, amino acid, polypeptide content and (b) particle size in the sludge

The inoculum is the volume ratio of the crude enzyme solution of hyperthermophilus to the remaining activated sludge. As can be seen from Figure 2 (a), the content of protein, amino acids and peptides all increased rapidly when the inoculum amount was 5%~20%, and the trend was slowed down or decreased at 20%~25%. The specific data are as follows. In the stage of 5%~20% of the inoculum, the content of protein, amino acid and polypeptide increased by 760 mg/L, 192.01 mg/L and 564.67 mg / L, respectively, and the protein extraction

rate increased by 12.19%. In 20% to 25% stages, protein and peptide content as well as protein extraction rate were reduced 14 At 6.67 mg/L, 182.15 mg/L, and 2.35%, the amino acid content increased by 3.47 mg / L. Comparative data can be obtained. After 20% of the inoculation volume, the reaction between sludge and enzyme reaches saturation, excessive enzyme decomposition of protein leads to the decrease of its content [9], and the polypeptide decomposed by protein is also decomposed into amino acids by excessive enzyme. Therefore, when the inoculation amount is 20%, the enzyme makes full contact with the substrate to achieve the best effect. As can be seen from Figure 2 (b), the sludge particle size decreased continuously, and the particle size of 1~10 μ m increased by 16.4%, and its average particle size decreased from 23.01 μ m to 17.7 9 μm。Liu et al. [10] used the combination of lysozyme and rhamnolipids to treat the remaining sludge, and the particle size of the treated sludge was greatly reduced. This test is consistent with its results.

3.3 Influence of different solid content of sludge

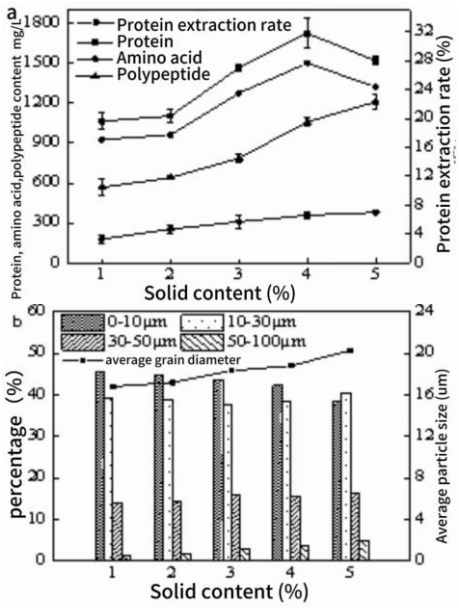

Figure 3 Effect of solid content on (a) protein, amino acid, polypeptide content and (b) particle size in sludge

According to Figure 3 (a), amino acid and peptide content kept increasing at 1% to 4%, protein content decreased at 4% to 5%, and protein extraction rate also decreased. The specific data are as follows, amino acid and peptide increased by 199.53 mg/L and 642.31 mg / L, respectively, protein content increased by 660.1 mg/L from 1% to 4%, extraction rate increased by 10.6%, and 206.67 mg/L at 4% to 5%, and extraction rate decreased by 3.3%. As shown in Figure 3 (b), the sludge particle size increases with the retention rate Moreover, the average particle size increased by 3.49 μ m. Its main reason is that enzymes in the catalytic reaction, the first and sludge form ES (enzyme-substrate complex) network, intermolecular catalysis into molecular catalytic [11], and content of 5%, flocs structure is relatively close, sludge and enzyme mass blocked, lead to enzyme difficult to form ES complex with sludge, which affect the hydrolysis efficiency, protein content drops, which cannot combine with substrate enzymes will dissolve into amino acids and peptides, so the content rise.

3.4 Optimization experiment of protein response surface method for residual sludge extracted from thermophilic bacteria

Table 1 Experimental factors and levels of the response surface

factor	Horizontal value		
A: reaction time	6	8	10
B: inoculum concentration/%	15	20	25
C: Solid rate/%	3	4	5

Table 2 Experimental design and results of response surfaces

Experimental number	A	B	C	Protein extraction rate/%
1	6	3	20	21.9
2	10	3	20	23.7
3	6	5	20	20.4
4	10	5	20	23.4
5	6	4	15	24.0
6	10	4	15	26.1
7	6	4	25	25.7
8	10	4	25	29.7
9	8	3	15	19.9
10	8	5	15	20.3
11	8	3	25	23.8
12	8	5	25	25.9
13	8	4	20	32.0
14	8	4	20	32.3
15	8	4	20	31.4
16	8	4	20	31.8
17	8	4	20	32.7

As show in table 1and 2.

(1) Establishment of the regression model and the statistical analysis

Analythe results of the table with Design Expert software, the regression equation was: R1 = 32.09 + 1.36A + 0.11B + 1.84C + 0.32AB + 0.48AC + 0.43BC-2.88A2-6.78B2-2.77C2.

Table 2-3 shows the F value of the model is 44.54 and (Prod> F) <0.0001, indicating that the model is extremely significant. Correlation coefficient R2 = 0.9828> 0.9, experimental values Good correlation with the predicted value. The coefficient of determination is R2Adj = 0.9608, and the model is able to explain 96.08% of the change in response value and has better regression. The signal to noise ratio Adeq Precision 16.879> 4 indicates that the equation is well fit, so it can be used for extracting protein process optimization of residual sludge from thermophiles. The misfitting term (P> 0.05) is not significant, indicating that the fitting effect of the equation is good. Compared with the F value of the independent variable, it can be concluded that different factors affect the protein extraction rate: C (inoculum amount)> A (time)> B (including solid rate).

Table 3 Analysis of variance of the regression equation model for protein recovery rate

Source of variance	The deviation is square and	free degree	mean square	F	Prod > F
model	328.77	9	36.53	44.54	<0.0001
A	14.87	1	14.87	18.13	0.0038
B	0.091	1	0.091	0.11	0.7482
C	27.22	1	27.22	33.18	0.0007
AB	0.41	1	0.41	4.10	0.0416
AC	0.93	1	0.93	1.13	0.3233
BC	0.73	1	0.73	0.89	0.3764
A2	34.96	1	34.96	42.63	0.0003
B2	193.80	1	193.80	236.28	<0.0001
C2	32.42	1	32.42	39.52	0.0004
residual	5.74	7	0.82		
Unplanned item	4.63	3	1.54	5.56	0.0655
pure error	1.11	4	0.28		
amount to	334.52	16			

(2) Response to curved surface analysis and condition optimization

The shape of the response surface slope and the contour can reflect the strength of the interaction between the factors, and the influence of the interaction of the factors on the protein extraction rate. The surface diagram and the contour diagram are shown in Figure 5~ Figure 6.

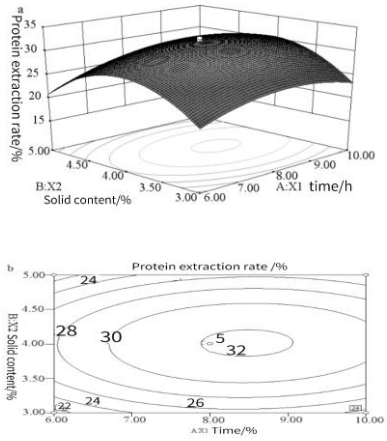

Figure 4 Surface plot of response to protein extraction rate (a) and contour plot (b)

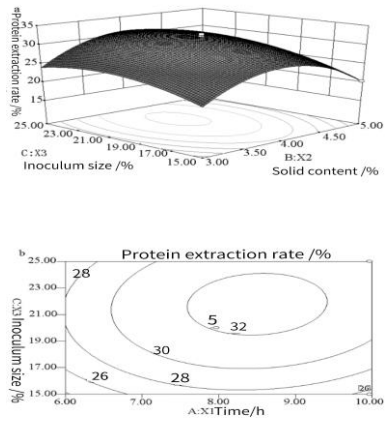

Figure 5 Effect of inoculum and reaction time on protein extraction rate Response surface curve (a) and contour plot (b)

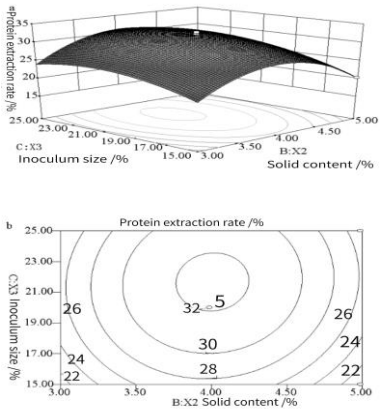

Figure 6 Response surface plot (a) and contour plots of the effect of residual sludge retention content on protein extraction rate (b)

The darker the color of the response surface, the more significant the result, the better the protein extraction effect. The steeper the slope of the response surface, the more sensitive the response is to changing the experimental conditions, and the evolution of the contour from ellipse to circle indicates the significance of the interaction. As can be seen from Figure 3-4 (a), the protein extraction rate (R) increased and then decreased with the increase of reaction time (A) and retention rate (B), and the surface slope was large. In the ANOVA in Table 3, the p value of AB was 0.0416 <significant value 0.05, indicating that there was obvious interaction between factor AB and significant influence. From the figure We can also find that the slope of the surface along the A axis is greater than that of the surface along the B axis B, and the significance of the F value in Table 3 is greater than that of B, indicating that the influence of reaction time on the protein extraction rate is greater than the sludge retention rate. As can be seen from Figure 4 (b), the contour lines are relatively dense and oval, which also indicates a significant AB interaction.

As can be seen from Figure 5 (a), the protein extraction rate (R) increases and then decreases with the increase of inoculation volume and time, and the surface slope is small. In the ANOVA in Table 3, the p value of AC is 0.3288> 0.05, indicating that the influence of factor AC is not obvious outstanding. From the figure, the slope of the surface along the C axis is greater than that of the A axis, and the significance of F value C in Table 3 is greater than A, indicating that the influence of enzyme inoculation on the protein extraction rate is greater than the reaction time. As in Figures 3-5 (b), the contour lines are sparse, which also indicates that the AB interaction is not significant.

As can be seen from Figure 3-6 (a), the protein extraction rate (R) increases with the increase of inoculum (C), and the solid rate (B) increases, the surface is relatively flat, and the p-value of BC in Table 3 is 0.3764> 0. 05, the illustrated factors BC is not significant. From the figure, the slope of the surface along the C axis is greater than that of the B axis, and the significance of F value C is greater than that of B,

indicating that the influence of enzyme inoculum on the protein extraction rate is greater than the sludge retention content. From the Figure 3-6 (b) contour plot, the contour shape is circular, indicating an unsignificant BC interaction.

In conclusion, in the process of thermophilus hydrolysis of residual sludge protein extraction, the time and inoculation amount alone had a significant impact on the protein extraction rate, but from the perspective of pairwise binding, the reaction time and enzyme inoculation amount, enzyme inoculation amount and sludge content were found There was no significant interaction between rates, while there was an interaction between inclusion and reaction time.

(3) Validation of the results of the experiment

Based on the results of the Box-Behnken test design and the secondary multinomial regression equation, the optimal reaction conditions for protein extraction were predicted by Design Expert software as 4% of sludge fixation content, 20% of enzyme inoculum, and reaction time of 8h. Under the above conditions, the protein extraction rate is more than 32%, and the accuracy of the model can be monitored by hydrolysis under the optimal hydrolysis conditions. Three sets of parallel experiments were performed, and the sludge protein extraction rate (32.1 1 ± 0.13)%, so that it is feasible to optimize the optimal enzymatic digestion conditions by using the response surface analysis method. This model can accurately simulate the influence of each factor on the protein extraction rate, which has a high application value.

4. Conclusion

Through single factor test and response surface optimization test of residual sludge solid content, thermophilic crude enzyme solution, and reaction time factors, to determine the optimal extraction conditions for protein extraction of residual sludge by thermophilus hydrolysis method is: 4%, 20%, reaction time of 8h, reaction temperature 60℃, reaction pH=7.The maximum value of the protein extraction rate of the residual sludge under this extraction condition can reach 32.2%.

Through response surface analysis, the influence of residual sludge protein extracted by hydrolysis of thermophiles was significant from strong to weak: inoculation capacity of thermophiles> reaction time> retention content of residual sludge.

Sludge form sludge particle size gradually decreases with the reaction, in which sludge decreases by 50-100 um by 1.1%, 30-50 μ m by 7.5%, 10-30 μ m by 5.4%, 0-10 μ m by 13%, and sludge Fculent particles become thinner, with a larger specific surface area and more developed pore structure.

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