Extraction of protein from excess sludge by enzymatic hydrolysis

L Qin[1], Y X Yan[1,2], J L Gao[1] and C Y Miao[1]

[1]School of Water Conservancy and Environment, Zhengzhou University, Zhengzhou, Henan, China

E-mail: yxyan@zzu.edu.cn

Abstract. The effects of enzymatic hydrolysis on the extraction of sludge protein and the sludge dewatering performance were investigated. Results showed that the enzymatic hydrolysis effectively extracted protein and improved the sludge dewatering. The most effective condition for protein extraction was as following: enzyme dosage of 2%, enzymolysis time of 4h, temperature of 55°C, pH of 8 and sludge moisture content of 94%. The maximum protein extraction rate was 58%, and the specific resistance of sludge (SRS) reduced from 1.7×10^{10} S^2/g to 1.050×10^{9} S^2/g.

1. Introduction

Recently, the excess sludge amount sharply increased with the accelerating process of urbanization in China [1,2]. Thus it is necessary to implement various technologies for resource utilization of excess sludge [3], among which the method of extracting protein from sludge has been rapidly developed [4]. The content of protein in the sludge is about 30% to 60%. The protein extracted can be used as foliar fertilizer, animal feed additive, foam extinguishing agent, etc [5,6].The common extraction methods are chemical [7,8], physical [9-11] and enzymatic hydrolysis [12]. Although chemical hydrolysis method has been widely studied, there are shortcomings of high temperature, second pollution, easily destroying the protein in the sludge, etc. Physical methods such as ultrasonic method also play a certain role in the extraction of sludge protein, but high energy consumption makes it unsuitable for practical production.

According to Su *et al* [12], the alkaline protease showed high efficiency for sludge proteolysis. However, the sludge dewatering performance after protein extraction which directly determines the subsequent processing was not considered. Therefore, the effects of protein extraction effect and the sludge dewatering performance were studied under different conditions of enzyme dosage, enzymolysis time, temperature, pH and moisture content. In this way, the enzymatic hydrolysis condition for protein extraction was optimized accordingly.

2. Experimental materials and methods

2.1. Experimental materials

The alkaline protease (BR) used in this study was from Beijing Ao Bo Xing Biological Co., Ltd.

Experimental sludge was from the concentrated tank of a wastewater treatment plant in Zhengzhou, Henan province of China. The characteristics of the experimental sludge are shown in table 1.

Table 1. Properties of the original sludge.

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Parameters	Full name	Value
MLSS (g/L)	Mixed liquid suspended solids	60.26±0.64
MLVSS(g/L)	Mixed liquor volatile suspended solids	39.13±1.45
TCOD(mg/L)	Total chemical oxygen demand	56002.7±3478.4
SCOD(mg/L)	Soluble chemical oxygen demand	476.9±69
TKN(mg/L)	Total Kjeldahl nitrogen	2508±771
SRS($\times 10^{10} S^2/g$)	Specific resistance of sludge	1.7±0.5

2.2. Experimental process

Enzymatic hydrolysis experiments were performed in a thermostatic oscillator. Hydrolysis experiments were performed using 5 single-factor variables such as pH, enzyme dosage, temperature, time, and water content. Three parallel samples were made for each experiment. After the hydrolysis was complete, the enzyme was inactivated immediately by placing it in boiling water for 15 minutes. The sludge hydrolysate was centrifuged at a speed of 4000 rpm for 30 minutes, and then the centrifuged supernatant was filtered through a 0.45μm filter. The optimum hydrolysis process conditions were determined by measuring the protein and SCOD content in the supernatant and SRS of the hydrolyzed sludge.

2.3. Analytical methods

MLSS, MLVSS, SCOD and SRS were determined using Standard Methods [13,14]. pH was measured with a pH meter. Protein content was calculated by Kjeldahl method.

SCOD dissolution rate (SCOD%) calculation:

$$SCOD\% = \frac{SCOD_2 - SCOD_0}{SCOD_1} \times 100\% \quad (1\text{-}1)$$

In the Formula 1-1, $SCOD_0$ was the SCOD content in raw sludge supernatant; $SCOD_1$ was the SCOD content of sludge extract; $SCOD_2$ was the total SCOD content of sludge.

3. Results and analysis

3.1. Effects of enzyme dosage

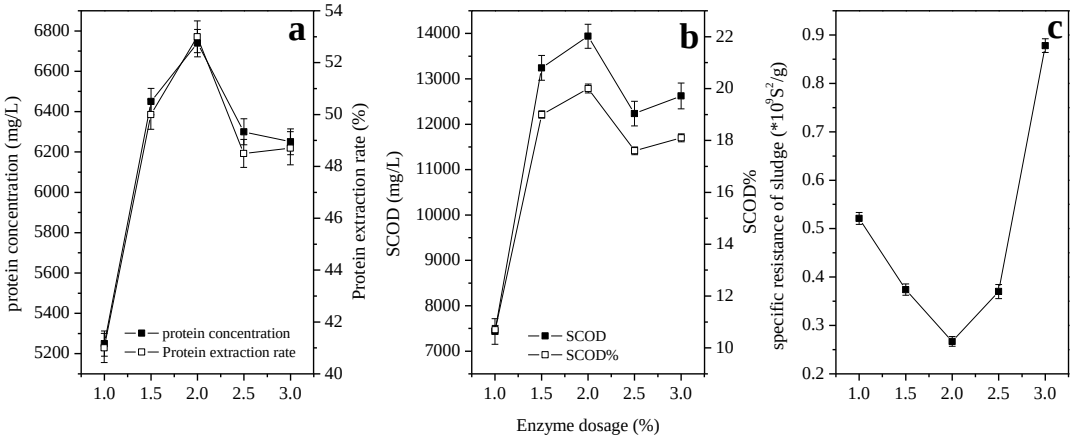


Figure 1. Effects of enzyme dosage on protein (a), SCOD (b) and SRS (c) of sludge (pH 8, 4h, 55°C, moisture content 94%).

During the experiment, the effect of enzyme dosage on each index is shown in figure 1. With the increase of the enzyme dosage, the protein concentration and the protein extraction rate of the supernatant increased firstly and then decreased. The protein concentration and the protein extraction rate reached the maximum when the enzyme dosage was 2%. Likewise, The SCOD and SCOD% in the supernatant

reached the maximum at enzyme dosage of 2%. Correspondingly, the SRS decreased first and then increased with the increase of the enzyme dosage. When the enzyme dosage was 2%, the SRS reduced by several times compared with the original sludge.

The increment of enzyme dosage increases the dissolution of the insoluble protein. However, reduction of protein in supernatant occurred with the increment of enzyme dosage. The exact mechanism was not very clear yet; maybe the extracted protein was further hydrolyzed by the excess enzyme. In addition, enzyme hydrolysis causes the reduction of the sludge viscosity, thus the SRS decreased with the enzyme dosage. Therefore, the optimum dosage of enzyme was 2%.

3.2. Effects of enzymolysis time

During the experiment, the influence of enzymolysis time on each index is shown in figure 2. With the increase of hydrolysis time, the protein concentration in the supernatant and protein extraction rate increased firstly and then decreased, the maximum was obtained when the heating time was 4h. Similarly, the SCOD and SCOD% in the supernatant reached the maximum at the hydrolysis time of 4h. As for SRS, although the minimum value was obtained at 3h, the difference was relatively small athydrolysis time of 4h.

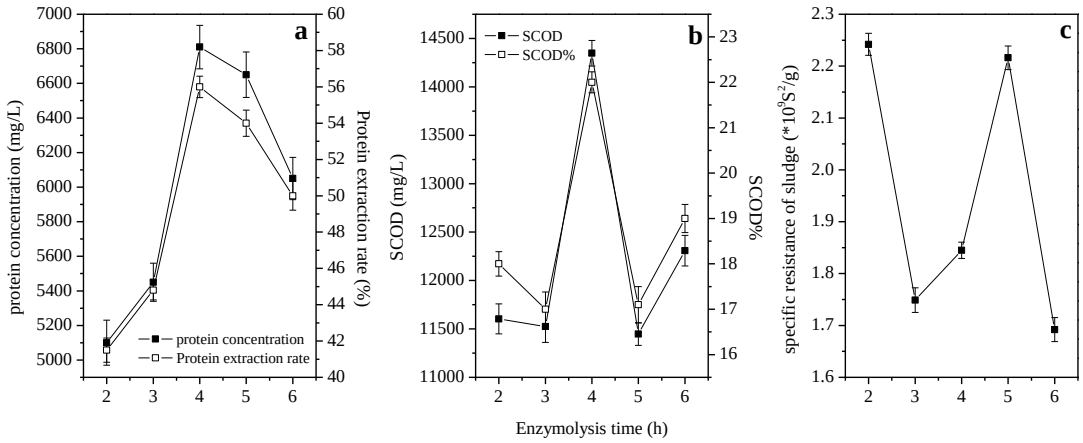


Figure 2. Effects of enzymolysis time on protein (a), SCOD (b) and SRS (c) of sludge (pH 8, enzyme dosage 2%, 55°C, moisture content 94%).

At the beginning of the hydrolysis reaction, high substrate concentration and enzyme activity induced the rapid increment of conversion rate. However, with the increase of the enzymolysis time, the enzyme activity may be inhibited by the gradually accumulated products [15], resulting in the decline of protein extraction rate. Although the decrement of sludge viscosity occurred during the hydrolysis, but overlong hydrolysis time caused crashing of the sludge floc, and the resultant increment of filtration resistance was disadvantageous to sludge dewatering. Comprehensive consideration suggested that 4h was the best hydrolysis time.

3.3. Effects of enzymolysis temperature

During the experiment, the influence of enzymatic temperature on each index is shown in figure 3. The protein concentration and the protein extraction rate of the supernatant increased first and then decreased with the increment of temperature, and the maximum was obtained at the temperature of 55°C. SCOD and SCOD% showed the same trend. The SRS was the lowest at the temperature of 50°C, but there was no significant difference in comparation with the SRS at temperature of 55°C.

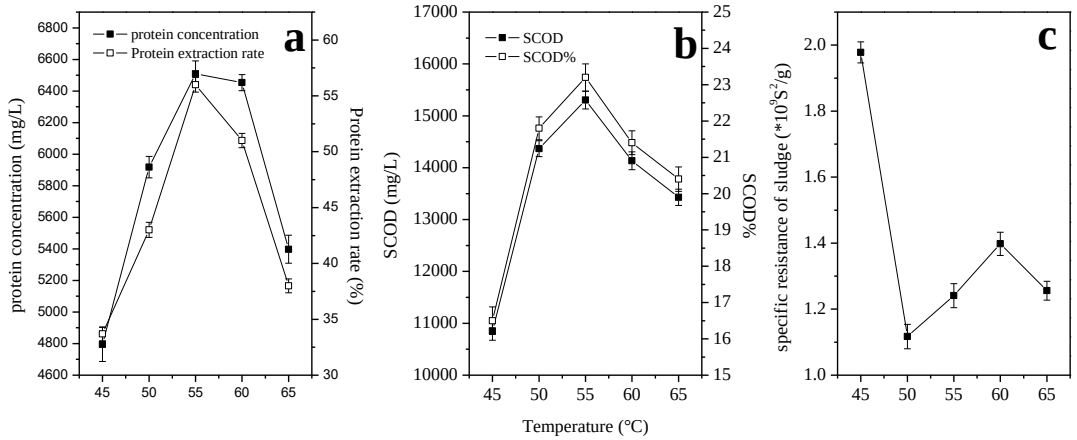


Figure 3. Effects of temperature on protein (a), SCOD (b) and SRS (c) of sludge (pH 8, enzyme dosage 2%, 4h, moisture content 94%).

There are specific temperature ranges for different enzymes, and too high or too low temperature can affect the enzymatic activity and consequently the protein extraction and sludge dewatering performance. Thus, it can be concluded that 55°C was the suitable temperature for alkaline protease extracting sludge protein.

3.4. Effects of enzymolysis pH

During the experiment, the influence of enzymatic pH on each index is shown in figure 4. The protein concentration and protein extraction rate in the supernatant increased first and then decreased with the increase of pH, and the protein concentration and protein extraction rate reached the maximum at pH of 8. The change of SCOD and SCOD% was consistent with the protein. As for SRS, the minimum of SRS occurred when pH was 9.

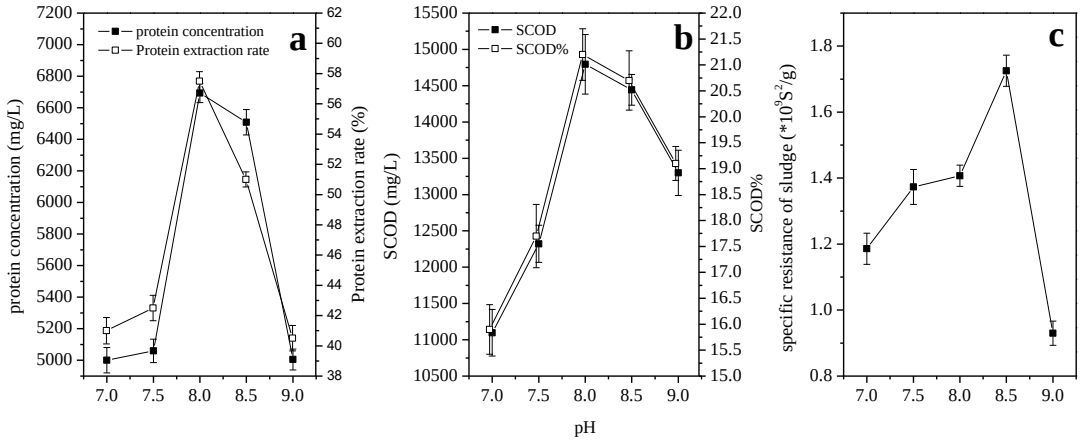


Figure 4. Effects of pH on protein (a), SCOD (b) and SRS (c) of sludge (55°C, enzyme dosage 2%, time 4h, moisture content 94%).

Similar to temperature, specific pH range is necessary for different enzymes, and the enzyme activity exists only when pH falls in this range. Besides, alkaline hydrolysis can improve the sludge dewatering significantly, so the pH at which the minimum of SRS occurred was not consistent with that of protein and SCOD. Comprehensively, the pH of 8 was preferred for protein extraction by alkaline protease

hydrolysis.

3.5. Effects of sludge moisture

During the experiment, the influence of Sludge Moisture on each index is shown in figure 5. The protein concentration decreased with the increase of moisture content, while the extraction rate of protein increased first and then decreased with the minimum extraction rate was obtained when the moisture content was 94%. Correspondingly, a similar change took place for SCOD and SCOD%. Also, the minimum of SRS was obtained when the moisture content was 94%.

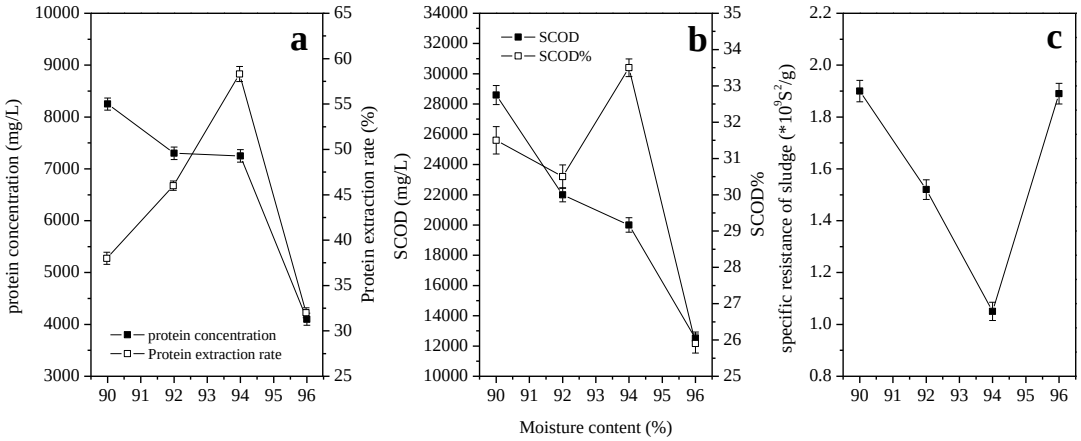


Figure 5. Effects of sludge moisture content on protein (a), SCOD (b) and SRS (c) of sludge (pH 8, 55°C, enzyme dosage 2%, time 4h).

Moisture content is an important limiting factor affecting the protein hydrolysis rate. The probability of interaction between enzyme and substrate is directly affected by the amount of water involved, and the mass transfer rate is also affected at the same time. In a certain range, the higher the substrate concentration, the greater the chance of exposure of the enzyme to the substrate, inducing the better protein extraction. However, too high substrate concentration will affect the mass transfer which is unfavorable for the enzymolysis reaction. It can be seen from figure 5 that the best protein extraction can be obtained under the conditions of sludge moisture content of 94%. In addition, excessive substrate concentration increases the sludge viscosity, which is not conducive to sludge dewatering. Therefore, the sludge moisture content of 94% is suitable for protein extraction.

4. Conclusions

In this paper, the factors affecting protein extraction and sludge dewatering by alkaline protease were studied. The hydrolysis conditions were optimized as enzyme dosage 2%, hydrolysis time 4h, temperature 55°C, pH 8.0 and sludge moisture content 94%. Under this condition, the protein extraction rate of 58% and SRS of 1.050×10^{9} S^2/g were achieved. Therefore, enzymatic hydrolysis of sludge protein extraction is an effective method of sludge stabilization, reduction, resource disposal, has broad prospects for development.

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