Extraction of protein from excess sludge by thermal pretreatment assisted enzymatic hydrolysis

J L Gao¹, Y C Wang¹, Y X Yan¹,² and Q C Yue¹

¹School of Water Conservancy and Environment, Zhengzhou University, Zhengzhou 450001, Henan, China

E-mail: yxyan@zzu.edu.cn

Abstract. In this paper, the hydrolysis conditions to extract protein from excess sludge by thermal pretreatment assisted enzymatic were studied. The optimum technological conditions were the sludge moisture content of 95%, thermal pretreatment temperature of 100°C, heating time of 3h, initial pH of enzymolysis sludge of 11, enzyme dosage of 3000U/g dry sludge, enzymolysis temperature of 55°C and time of 3h. Up to 52.8% of the protein content of the sludge was extracted, and the dehydration performance of the hydrolyzed sludge was 87.46% higher than that of the original sludge. Therefore, thermal pretreatment-assisted enzymolysis can effectively extract protein from excess sludge and improve its dewatering performance.

1. Introduction

A large amount of excess sludge will be produced with increasing sewage treatment capacity. The recovery of biomaterials from residual sludge has aroused people's concern. About 50% to 70% of the protein is present in excess sludge [1,2]. The extracted protein can be used to prepare protein feed [3], foam extinguisher [4], organic fertilizer [5] and so on to realize its resource utilization and reduction. At present, physical [6], chemical [7,8] and biological methods [9,10] are mainly used to extract protein from residual sludge. Among them, thermal hydrolysis can effectively disintegrate sludge, and the technology is relatively mature [11]. According to Barber [12], thermal hydrolysis as a pretreatment can improve the biodegradability and dewatering performance of sludge.

Therefore, in our work, the excess sludge was firstly pretreated by thermal hydrolysis, and then the hydrolysis of alkaline protease was carried out. The effects of this method on protein extraction rate and sludge dewatering performance were analyzed. Due to the lack of research on this combination method, it is expected that this paper will provide a reference for future related research.

2. Materials and methods

2.1. Materials

An excess sludge was taken from the improved oxidation ditch of a sewage treatment plant in Zhengzhou, Henan Province. The basic properties of the excess sludge are shown in table 1. Alkaline protease (Bacillus subtilis serine protease) was purchased from Beijing Ao Bo Xing Biological Co., Ltd.
Table 1. Basic properties of raw sludge.

| pH     | Moisture content (%) | MLSSa (mg/L) | MLVSSb (mg/L) | Total protein (mg/L) | SRSc (×10^9 S^2/g) |
|--------|----------------------|--------------|---------------|----------------------|-------------------|
| 6.8~7.4| 99                   | 9.7~9.9      | 4.85~6.01     | 15000~16000          | 8.8~9.5           |

a Mixed liquid suspended solids.
b Mixed liquor volatile suspended solids.
c Specific resistance of sludge.

2.2. Pyrolysis reactor

Pyrolysis was carried out in a self-made high-pressure reactor. The device parameters are shown in table 2.

Table 2. Parameters of the high-pressure reactor.

| Volume (mL) | Heating power (W) | Maximum temperature (℃) | Working pressure (MPa) | Stirring speed (rpm) |
|-------------|-------------------|-------------------------|------------------------|----------------------|
| 1500        | 300~3000          | 400                     | 0~22                   | 0~100                |

2.3. Experimental methods

The single factor variables of thermal preconditioning temperature and time, initial pH of sludge, enzymolysis temperature and time, enzyme dosage and sludge moisture content were analyzed. Three parallel samples were set up in each experiment.

The thermal pretreatment was carried out in a high-pressure reactor at a fixed temperature, time and stirring speed of 25 r·min⁻¹. After thermal pretreatment, the pH of sludge was adjusted by dilute sodium hydroxide, and then enzymatic experiments were carried out in a thermostatic oscillator at the temperature of 40℃, 45℃, 50℃, 55℃ and 60℃, time of 1 h, 2 h, 3 h, 4 h and 5 h, and enzyme dosage of 2000 U/g, 3000 U/g, 4000 U/g, 5000 U/g and 6000 U/g, respectively.

After enzymolysis, the enzyme was inactivated in boiling water for 15 minutes. The obtained sludge hydrolysis samples were used to determine the SRS. The hydrolyzed sludge of 50 mL was then put into a centrifugal tube and centrifuged at 4000 r·min⁻¹ speed for 30 min, and then filtered by 0.45 μm filtration membrane to obtain the supernatant. The supernatant was used to determine protein concentrations.

2.4. Analytical methods

Protein concentration and specific resistance of sludge(SRS) were determined by Bicinchoninic acid (BCA) [13] and vacuum filtration [14].

Protein extraction rate (P%) calculation:

\[ P = \frac{P_2}{P_1} \times 100\% \]  \hspace{1cm} (1)

In equation (1), P1 represented the total mass of protein in raw sludge; P2 indicated the mass of soluble proteins in the supernatant.

Sludge dehydration performance improvement rate (Dw%) calculation:

\[ Dw = \frac{D_0 - D_1}{D_0} \times 100\% \]  \hspace{1cm} (2)

In equation (2), D0 represented the SRS of the original sludge; D1 indicated the SRS of sludge after hydrolysis.

3. Results and analysis

3.1. Effect of thermal pretreatment temperature
The effects of thermal pretreatment temperature on protein extraction and sludge dehydration are shown in figure 1. As the temperature increased, the extraction rate and concentration of protein in supernatant firstly increased and then declined, reached the maximum value at the temperature of 100°C. The SRS decreased with the prolongation of temperature, and sludge dewatering performance enhanced, which was convenient for the separation of protein solution after enzymolysis.

![Figure 1. Effects of thermal pretreatment temperature on protein extraction (a) and sludge dehydration (b) (sludge moisture content 95%; thermal pretreatment 4 h; enzymolysis pH10, 55°C, 4 h, 6000 U/g).](image)

With the increase of thermal pretreatment temperature, the damage to EPS and cell wall was gradually enhanced. At this stage, not only some proteins were released to the liquid phase, but also a better environment was provided for the subsequent enzymatic hydrolysis reaction. When the temperature was greater than 100°C, some proteins maybe hydrolyzed. Therefore, the optimum temperature for thermal preconditioning was 100°C.

3.2. Effect of thermal pretreatment time
The effects of thermal pretreatment time on protein extraction and sludge dehydration are shown in figure 2. As time increased, the extracted protein concentration and extraction rate firstly enhanced and then decreased, reaching a maximum at 4 h. The SRS declined firstly and then added with time, reaching the minimum value at 4 h.

![Figure 2. Effects of thermal pretreatment time on protein extraction (a) and sludge dehydration (b) (sludge moisture content 95%; thermal pretreatment 100°C; enzymolysis pH10, 55°C, 4 h, 6000 U/g).](image)

At an experimental temperature of 100°C, sludge took a certain amount of time to absorb heat and then destroy the sludge flocculant structure to release protein. Therefore, the increase of protein
concentration was slow from 1 h to 2 h. When the time was more than 4 h, the hydrolysis of protein in the supernatant may lead to a decrease of the concentration, and the effect of time on the specific resistance of sludge was limited. Combined with the economic cost, the suggested time of thermal preconditioning was 3 h.

3.3. Effect of sludge initial pH
The effects of sludge pH on protein extraction and sludge dehydration are shown in figure 3. With the increase of sludge pH, the extracted protein concentration increased at first and then declined, and reached the maximum at a pH of 11. The SRS firstly enhanced and then decreased with increasing pH. And the rate of decline was faster at pH 10 to pH 11.

![Figure 3](image1.png)

**Figure 3.** Effects of initial sludge pH on protein extraction (a) and sludge dehydration (b) (sludge moisture content 95%; thermal pretreatment 100°C, 3 h; enzymolysis 55°C, 4 h, 6000 U/g).

Under alkaline conditions, the catalytic activity of alkaline protease was enhanced, and it was beneficial to release intracellular combined water and reduce the specific resistance of sludge. However, pH greater than 11 may affect the spatial conformation of the enzyme catalytic center, leading to the inactivation of alkaline protease and the reduction of hydrolysis efficiency [15]. Combined with the above, the optimum pH of raw sludge was determined to be 11.

![Figure 4](image2.png)

**Figure 4.** Effects of enzymolysis temperature on protein extraction (a) and sludge dehydration (b) (sludge moisture content 95%; thermal pretreatment 100°C, 3 h; enzymolysis pH11, 4 h, 6000 U/g).

3.4. Effect of enzymolysis temperature
The effect of enzymolysis temperature on the indexes in this experiment is shown in figure 4. As the temperature increased, the extracted protein concentration enhanced at first and then declined, and
reached a maximum at 55°C. The SRS decreased with increasing temperature, and the rate of dehydration performance added.

The reaction rate between enzyme and substrate increased with the increase of temperature from 40°C to 55°C. With the increase of temperature, the solids in the sludge were transformed into a liquid phase, which reduced the SRS. However, when the temperature exceeded 55°C, the enzyme activity may have inhibited or the substrate has denatured, resulting in a decrease of the protein extraction rate. Therefore, the optimum operating temperature of the alkaline protease was 55°C.

3.5. **Effect of enzymolysis time**

The effects of enzymolysis reaction time on protein extraction and sludge dehydration are shown in figure 5. With the prolongation of enzymatic hydrolysis time, the extracted protein concentration and extraction rate enhanced rapidly, and the basic stability was stabilized after 3 h. The SRS dropt at first and then added with the prolongation of time, and reached the minimum at 3 h.

![](image.png)

**Figure 5.** Effects of enzymolysis time on protein extraction (a) and sludge dehydration (b) (sludge moisture content 95%; thermal pretreatment 100°C, 3 h; enzymolysis pH 11, 55°C, 6000 U/g).

The reaction between alkaline protease and substrate tended to be complete at 3 h. When the time was more than 3 h, some proteins in the supernatant were hydrolyzed into amino acids. Figure 5 shows that the dewatering performance of sludge begins to decrease when the time exceeds 3 h. Therefore, the optimum hydrolysis time was determined to be 3 h.

3.6. **Effect of enzyme dosage**

The effect of enzyme dosage on the indexes is shown in figure 6. With the increase of enzyme dosage in the range from 2000 U/g to 3000 U/g, the extracted protein concentration enhanced rapidly. When the enzyme dosage was more than 3000 U/g, the increase slowed down. SRS added at first and then declined with the add of enzyme dosage, and reached the minimum at 5000 U/g.

When the enzyme dosage was less than 3000 U/g, the protein concentration increased rapidly with the enzyme dosage because the concentration of the enzyme was lower than the saturated concentration. However, when the enzyme dosage was higher than 3000 U/g, it may cause the protease hydrolysis itself [16] and hydrolyze the protein in the supernatant. Therefore, it is recommended that the most economical enzyme dosage was 3000 U/g.
3.7. Effect of sludge moisture content

The effect of sludge moisture content on protein extraction and sludge dehydration is shown in figure 7. With the increase of sludge moisture content, the protein concentration showed a decreasing trend. The SRS declined with the increase of sludge moisture content. Under optimized conditions (sludge moisture content 95%; thermal pretreatment 100℃, 3 h; enzymolysis pH 11, 55℃, 3 h, 3000 U/g), up to 52.8% of the protein content of the sludge was extracted, and the sludge dewatering performance was improved by 87%. Compared with Li P et al [17] study on hydrolysis of sludge by single enzyme, thermal pretreatment-assisted enzymolysis not only increased the protein extraction rate, but also reduced the enzymolysis time.

When the moisture content of sludge was low, the phenomenon of uneven heating may occur during thermal pretreatment, which may affect the enzymatic hydrolysis reaction. The proper moisture content of sludge made the protease fully contact with the substrate, and rapidly dispersed the enzymatic hydrolysis products to promote the enzymatic hydrolysis reaction. From figure 7, it can be seen that the SRS of sludge with high moisture content is greatly reduced after hydrolysis. However, if the moisture content is too high, the effective enzyme concentration and protein concentration will be reduced, which is not conducive to industrial production. Therefore, it is recommended that the optimum moisture content of the sludge was 95%.

4. Conclusion
In this paper, the effects of thermal pretreatment assisted alkaline protease hydrolysis on the protein extraction rate in excess sludge and the SRS were reported. The optimum hydrolysis conditions were obtained as the sludge moisture content of 95%, thermal pretreatment temperature of 100℃, heating time of 3 h, enzymolysis initial sludge pH of 11, enzyme dosage of 3000 U/g dry mud, enzymolysis temperature of 55℃ and time of 3 h. Under these conditions, the protein extraction rate was 52.8%, and the sludge dewatering performance was improved by 87%. Therefore, this method can effectively extract protein from excess sludge and improve the dewatering performance of sludge at the same time.

Acknowledgments
This work was supported by the project of the Department of Science and Technology of Henan Province (project number 182102210194).

References
[1] Li P, Yan G, Li D, Su R and Chen Z 2012 Determination of protein content in sludge Chem World 53 151-3
[2] Chen Y, Jiang S, Yuan H, Zhou Q and Gu G 2007 Hydrolysis and acidification of waste activated sludge at different pHs Water Res 41 683-9
[3] Hwang J, Zhang L, Seo S, Lee Y and Jahng D 2008 Protein recovery from excess sludge for its use as animal feed Bioresour Technol 99 8949-54
[4] Wang C, Liang H, Li Y and Hua J 2006 Study on preparation of foam extinguishing agent using excess sludge China Water Wastewater 22 38-42
[5] Ni H, Fan X, Guo H, Liang J, Li Q, Yang L, Li H and Li H 2017 Comprehensive utilization of activated sludge for the preparation of hydrolytic enzymes, polyhydroxyalkanoates, and water-retaining organic fertilizer Prep Biochem Biotech 47 611-8
[6] García M, Urrea J L, Collado S, Oulego P and Díaz M 2017 Protein recovery from solubilized sludge by hydrothermal treatments Waste Manage 67 278-87
[7] Xiao K, Chen Y, Jiang X, Seow W Y, He C, Yin Y and Zhou Y 2017 Comparison of different treatment methods for protein solubilization from waste activated sludge Water Res 122 492-502
[8] Xiang Y, Xiang Y and Wang L 2017 Kinetics of activated sludge protein extraction by thermal alkaline treatment J Environ Chem Eng 5 5352-7
[9] Li P, Lei Y and Li D 2014 Extraction of Protein from excess sludge by combination of ultrasonic and biological treatment China Water Wastewater 30 22-5
[10] Zhang W, Su R and Li D 2012 Extraction of proteins from excess activated sludge by enzymatic hydrolysis Environ Sci Technol 35 7-10
[11] Pilli S, Yan S, Tyagi R D and Surampalli R Y 2015 Thermal pretreatment of sewage sludge to enhance anaerobic digestion: A review Crit Rev Envi Sci Tec 45 669-702
[12] Barber W P F 2016 Thermal hydrolysis for sewage treatment: A critical review Water Res 104 53-71
[13] Ras M, Girbal-Neuhauser E, Paul E, Sperandio M and Lefebvre D 2008 Protein extraction from activated sludge: An analytical approach Water Res 42 1867-78
[14] Xue X, Jin Q, Zhu W and Guo X 2006 Influence of ultrasound on the rheological characterization and the flocculating dewaterability of sludge Acta Sci Circumstantiae 26 897-902
[15] Lü F, Wang J, Shao L and He P 2016 Enzyme disintegration with spatial resolution reveals different distributions of sludge extracellular polymer substances Biotechnol Biofuels 9 29
[16] Gonzalez-Tello P, Camacho F, Jurado E, Paez M P and Guadix E M 1994 Enzymatic hydrolysis of whey proteins: I. Kinetic models Biotechnol Bioeng 44 523-8
[17] Li P, Li D, Su R and Yan G 2011 Study on hydrolysis of by two excess sludge protein treatment methods Chin J Environ Eng 5 2859-63