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Thermal alkali hydrolysis pretreatment of dewatered sludge for protein extraction at low temperature

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ABSTRACT

Protein recovery from wasted sludge has become hot research. This work provided a new approach with protein extraction for dewatered sludge by the thermal alkali hydrolysis (TAH) at low temperatures (<100°C). Effects of alkali addition, temperature, and time on extraction effect were investigated. The protein extraction rate of 33.07% and concentration of 4,702.37 mg L^{-1} were achieved under the optimal conditions (alkali amount at 30 g kg^{-1} dewatered sludge, at 80°C and 5 h). Mechanism for protein extraction was investigated via X-ray photoelectron spectroscopy (XPS), three-dimensional excitation emission (3D-EEM), and Fourier-transform infrared spectroscopy (FTIR). The results of XPS indicated that the protein extraction from flocculent sludge to liquid phase might be related to the significant protein-N conversion that occurred at the optimal treatment conditions which gave the highest dominant protein. 3D-EEM showed the tryptophan-like protein predominance, which has the potential to be feed additives. FTIR showed that the protein structure has undergone first breaking the chain and then oxidizing. Based on Pearson's correlation and principal component analysis (PCA), the protein extraction was correlated with the release of organic compounds, suggesting that proteins in the flocculent sludge entered the liquid phase with treatments. The alkali amount and time were the key factors of protein extraction. The concentration of protein obtained from sludge treated with thermal alkali was four times that of sludge treated by pure alkali.

Keywords: Dewatered sludge; TAH; Low temperature; Protein extraction; N-containing component

1. Introduction

Since the 1910s, the activated sludge process has been widely used to treat a variety of wastewater due to its low cost and excellent adaptability [1]. However, massive waste-activated sludge (WAS) generated during the treatment process of the wastewater has been considered a thorny issue. In terms of building and operating a wastewater plant, the operational cost of WAS treatment/disposal may account for more than 50%, which poses a significant challenge to the operation of wastewater treatment plants [2]. WAS has long been regarded as a potential source of bioenergy. It has a complex flocculent structure formed by cations and microbial extracellular polymeric substances (EPS) which consists of different microorganisms and organic matter [3]. Whereas WAS contains a number of economical and useful organic substances, such as proteins, enzymes, amino acids, and nucleic acids, recovering valuable components from WAS has attracted more and more attention in recent years because of the stricter management regulations on WAS and scarce natural resources [4]. Based on the previous studies, proteins have been considered as the major organic matters of WAS.

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It is estimated that sludge proteins account for about 40% of WAS [5] and 50% of the dry weight of bacterial cells in WAS [6]. The procedure for recovery of proteins or their derivatives such as various amino acids from sludge typically contains treatments, filtration, precipitation, and drying [7]. The recovered protein can be used as animal protein feeds, which was comparable with the nutrient compositions of commercial feed. Also, it has been found that heavy metals could be removed after the protein recovery process [8]. The recovered amino acids can be employed to prepare the trace element-chelated fertilizer [9]. Sludge from a kraft paper mill was used as a source of biomass to recover the protein to prepare wood adhesive, which would help to mitigate global energy shortage [10].

Solubilization of WAS is necessary for protein recovery. The methods of WAS solubilization have been studied for many years, including biological methods [11], physical [12], and chemical [13] such as neuter protease [11], ultrasonic [14], and alkali treatments [15]. There are also many methods of combining physics and chemistry to treat sludge, for example, ultrasonic alkali [16], thermal alkali [17], and ultrasonic enzyme methods [18]. With the advantages of simple operation and high efficiency, alkali and thermal hydrolysis are the most widely used methods to solubilize sludge and destruct microbial cells [19]. Alkali can be employed to solubilize the membrane proteins to damage the microbial cell [20]. During alkali treatment of WAS, soluble protein increased by 200 mg L^{-1} at pH 8.0; 600 mg L^{-1} at pH 9.0; 1,000 mg L^{-1} at pH 10.0; and 1,010 mg L^{-1} at pH 11.0 [21]. Besides, the soluble chemical oxygen demand (SCOD) in alkali conditions is significantly higher than that in neutral or acidic pH conditions [22]. Alkali treatment at pH 12.0 showed the highest protein dominance with a net saving of $ 25.57 per ton wet sludge compared to conventional sludge treatment and disposal method [16]. During thermal treatment of WAS, soluble protein increased by 50 mg L^{-1} at 35°C, 300 mg L^{-1} at 80°C, 600 mg L^{-1} at 100°C, and 1,600 mg L^{-1} at 120°C [23]. Compared with pure thermal method and pure alkali method, the combined thermal alkali method has been widely studied for its superior sludge destruction ability. Shimin et al. [24] found that the optimal conditions of thermal alkali extraction of sludge protein were temperature of 175.6°C, pH of 13.0, and time of 1.18 h.

However, in previous studies, the combined thermal alkali method has been tested at temperature above 100°C. The high temperature (>100°C) and high pressure (>10 M) treatment required high energy input and special equipment [25]. Meantime, the polysaccharides contained in WAS flocs or microbial cells are also released during hydrolysis treatment. During thermal treatment at 220°C, the decomposition of polysaccharides would produce a large amount of monosaccharides and disaccharides [26]. These reducing sugars could easily combine with amides (amino acids and proteins) via the Maillard reaction, which was unfavorable for protein recovery. In order to obtain a more appropriate and economically feasible treatment method, it is necessary to attract more attention to the mild temperature treatment of protein extraction (<100°C).

Previous studies have characterized recovered protein based on protein content [26], protein composition [27], and molecular weight [28]. For example, protein molecular weight would greatly affect the performance of the final protein product (e.g., wood adhesive) by affecting contact surface and interacting groups [29]. However, the protein type and the relationship with soluble substances were worth studying. It is necessary to understand the solubilization of sludge proteins during treatment processing to improve protein recovery and provide an alternative approach for collecting renewable resources from WAS. The nitrogen content in the solid/liquid was related to the degree of protein dissolution or decomposition [30]. Therefore, understanding the distribution of nitrogen species in solid fractions would provide the mechanistic principles of nitrogen transformation during protein extraction. Besides, it is not clear if the treatment methods would affect the change of nitrogenous species. Moreover, the correlation between N-containing compounds and protein extraction has not been investigated in detail.

This work was to study the extraction effects of sludge protein by thermal alkali hydrolysis (TAH) treatment at low temperature (<100°C). The optimal condition including the amount of alkali addition, time, and temperature were determined. The qualitative characteristics of proteins and the relationship between proteins and soluble substances were investigated. The main protein types in the extraction of protein were studied. The change of nitrogenous species and the variation of associated functional groups in solid phases during protein extraction were investigated. The economic practicability of this method was analyzed. The results here would be helpful to understand the mechanism in sludge protein recovery at low temperatures.

2. Materials and methods

2.1. Sludge samples

The sludge samples used in this study were municipal sludge, which was obtained from the dewatered sludge conveyor belt of the Nanjing Jinling Environment Co., Ltd. (Nanjing, China). The sludge samples were stored at 4°C before testing. The characteristics are listed as below: total solids (TS): 61.65% ± 0.12%; volatile solids (VS): 52.25% ± 0.03%; total COD (TCOD): 8,581.63 ± 10.33 mg L^{-1}; soluble COD (SCOD): 236.43 ± 10.12 mg L^{-1}; moisture content: 81.87 ± 0.06 wt.%; pH: 7.11 ± 0.54; crude protein: 35.21% ± 3.22%.

2.2. Methods of extraction

2.2.1. Sludge mixture

All of the sludge samples were mixed with water by a ratio of 1:4. A series of experiments were conducted in a 250 mL glass Erlenmeyer flask with 10 g dewatered sludge and 40 g water mixed by magnetic stirrer for 10 min. The sludge mixture was used for further experiments.

2.2.2. Sludge treatment

After adding 10 g dewatered sludge and 40 g water in a conical flask, all of the sludge samples were mixed equably by a magnetic stirrer for 10 min. Then, a certain ratio (10, 15, 20, 25, 30, and 35 g kg^{-1} dewatered sludge) of NaOH was added. The samples were placed on the thermostatically

controlled magnetic stirrer at a range of different temperatures (50°C, 60°C, 70°C, 80°C, 90°C, and 100°C ± 0.5°C) for hydrolysis and lasted for variable time periods (1, 2, 3, 4, 5, and 6 h). All experiments were repeated three times and the average value was regarded as the final result. Single factor experiments were designed by fixing two of the three above-mentioned parameters while varying one parameter.

2.2.3. Centrifugation of sludge mixture

After the experiments, all of the sludge mixtures were centrifuged at 4,000 rpm for 15 min. The supernatant collected was regarded as the protein hydrolysis solution, which was filtered through a 0.45 μm cellulose nitrate membrane. The residual sludge was firstly freeze-dried for 72 h, and then ground and filtered through a 60 mesh sieve [30]. The filtered residual sludge samples were stored in a –20°C refrigerator before further use. The drying method of dewatered sludge was the same as the residual sludge solids. The above samples were used for subsequent analysis.

2.3. Characteristics of sludge samples

TS, VS, and moisture content of dewatered sludge were based on the steps described in the standard methods [31]. SCOD and total COD (TCOD) were measured according to Chinese standard GB/T 34500.2-2017.

The total Kjeldahl nitrogen (TKN) of sludge was measured with an automatic TKN analyzer (KDT(N)-1000, Tianwei instrument Co., Ltd., China), which was measured to represent total organic nitrogen (crude protein) in solid sludge samples [32].

2.4. Analysis of protein hydrolysis solution

2.4.1. Total organic carbon, SCOD, protein, and ammonium measurements

The protein hydrolysis solution content was characterized by different parameters. Total organic carbon (TOC) was measured using a TOC/TN analyzer (TOC-L_{CSH}, Shimadzu Corporation, Japan). SCOD was measured with Chinese standard GB/T 34500.2-2017. Protein was measured with the modified Lowry method [33].

2.4.2. Three-dimensional excitation emission

3D EEM can qualitatively characterize substances in the protein hydrolysis solution. The protein type in the solution was determined with a luminescence spectroscope (HORIBA, Japan), with 230–520 nm excitation wavelength at intervals of 10 nm and 230–545 nm emission wavelength. The voltage of the photomultiplier tube was 700 V, and the power of the excitation light source Xenon arc lamp was 150 W. The excitation and emission slit bandwidths were 5 nm for spectra, and the scan rate was recorded at 1,200 nm min^{-1}.

The different styles of proteins were identified by the different EEM spectrum which was divided into different regions. They were associated with tyrosine-like proteins (Region I; excitation (Ex) wavelengths less than 250 nm), tryptophan-like protein (Region II, emission (Em) wavelength less than 380 nm), fulvic acid-like materials (Region III, Ex/Em wavelengths: 230–250/380–545 nm), microbial by-product-like materials (Region IV, Ex/Em wavelengths: 250–280/230–380 nm), and humic acid-like organic compounds (Region V, Ex/Em wavelengths: 280–520/380–545 nm) [16].

In order to optimize different operational parameters, the protein extraction rate of all test groups was calculated by Eq. (1):

$$R = \frac{m}{M} \times 100\% \tag{1}$$

where R is protein extraction rate (%); m is the content of the protein in hydrolysis solution (g); M is content of the protein in dewatered sludge (g).

2.5. Analysis of residual sludge solids

X-ray photoelectron spectroscopy (XPS) was used to determine the elemental composition and the evolution of N-containing compounds in sludge solid residues. XPS measurements were characterized on an ESCALAB Xi+ X-ray photoelectron spectroscopy (Thermo Fisher Scientific Ltd., USA) equipped with monochromatized Al-Kα radiation. The XPS spectra of all solid powder samples have been corrected for an adventitious carbon C 1s level of 284.8 eV. All XPS peaks were fitted using Shirley background together with Gaussian-Lorentzian function using CASA XPS software. The N peaks can be assigned to inorganic-N, protein-N, pyridine-N, pyrrole-N, quaternary-N, and nitrile-N at respective binding energy values of 402.5, 400.0, 398.8, 400.3, 401.4, and 399.7 eV, which were based on the methods described in the existing research [34].

Fourier-transform infrared spectroscopy (FTIR) was used to identify the functional group of compound to determine the type and structure of samples. A FTIR spectrometer (Nicolet IS5, Thermo, USA) was used to determine the dewatered sludge and the residual sludge samples prepared under the amount of alkali addition at 10, 30, and 35 g kg^{-1} dewatered sludge, the temperature at 80°C and time at 5 h.

Statistical analyses, including both principal component analysis and correlation analysis, were processed using the SPSS software (version 24.0, IBM Inc., New York, USA).

2.6. Energy consideration and economic analysis

According to the experimental method and data, the energy balance and economic cost in this study were calculated. Two treatment methods of thermal treatment and alkali treatment were used in this study, respectively. The thermal treatment cost was mainly electricity associated with energy input, while the alkali treatment cost was mainly sodium hydroxide consumption.

The energy (input, heat) was calculated based on Eq. (2) [16]:

$$Q = \rho \times V \times C \times \left(T_f - T_i\right) \tag{2}$$

where Q is the heat energy in sludge thermal treatment (kJ), ρ is the density of sludge (kg m^{-3}), V is the volume of sludge treated (m^3), C is the specific heat of sludge (kJ kg^{-1} °C) (4.2 kJ kg^{-1} °C), T_i and T_f are the initial and final temperatures (°C) of the sludge, respectively.

3. Results and discussion

3.1. Effects of various parameters on the extraction of protein

The changes in protein, COD, and TOC concentrations were measured at different conditions. Ammonium concentration was also measured in order to know whether soluble proteins were degraded by treatments. Fig. 1 shows the effects of various parameters on the protein extraction rate and concentration. The experiment results were investigated by analysis of variance. Furthermore, the results of the significant difference analysis have been added to Fig. 1. *$p < 0.05$ stands for significant difference between groups. **$p > 0.05$ stands for no significant difference between groups.

3.1.1. Effects of the amount of alkali addition

As shown in Fig. 1a, the effect of alkali amount at 80°C for 5 h on the protein extraction was studied, which was the most significant parameter in the experiment. As illustrated in Fig. 1a, both protein extraction rate and concentration increased significantly with the increase of alkali amount, but while the alkali amount was increased from 30 to 35 g kg^{-1} dewatered sludge (the following texts are expressed in g kg^{-1}), the protein extraction rate and concentration began to decline. In Fig. S1a, ammonium concentration increased with the addition of alkali but decreased after alkali amount at 20 g kg^{-1}. As presented in Fig. S2a, the trends of COD and TOC concentrations in hydrolysis solution were similar, that is, increased as alkali amount increased. Both the decrease of protein and the increase of ammonium concentration could probably be related to the difference in protein degradation and extraction in treatment [35]. The results suggested that the optimal amount of alkali addition was selected as 30 g kg^{-1} with a protein extraction rate of 31.68% and a concentration of 4,504.66 mg L^{-1}.

3.1.2. Effects of extraction temperature

The effect of extraction temperature was investigated as shown in Fig. 1b. The extraction time was still 5 h and the alkali amount was 30 g kg^{-1} as previously selected. The results depicted that both protein extraction rate and concentration increased significantly with the increase of extraction temperature. However, the protein extraction rate and concentration began to decline after the temperature at 80°C, as the higher temperature is usually beneficial for the reaction between alkali and sludge. As shown in Fig. S1b, ammonium concentration reached the peak at 80°C. Simultaneously, the concentrations of COD and TOC also reached the peak at 80°C in Fig. S2b. These results showed a violent reaction at 80°C. In consideration of energy-saving (<100°C), 80°C was selected as the extraction heating temperature with the protein extraction rate of 32.60% and concentration of 4,635.27 mg L^{-1}.

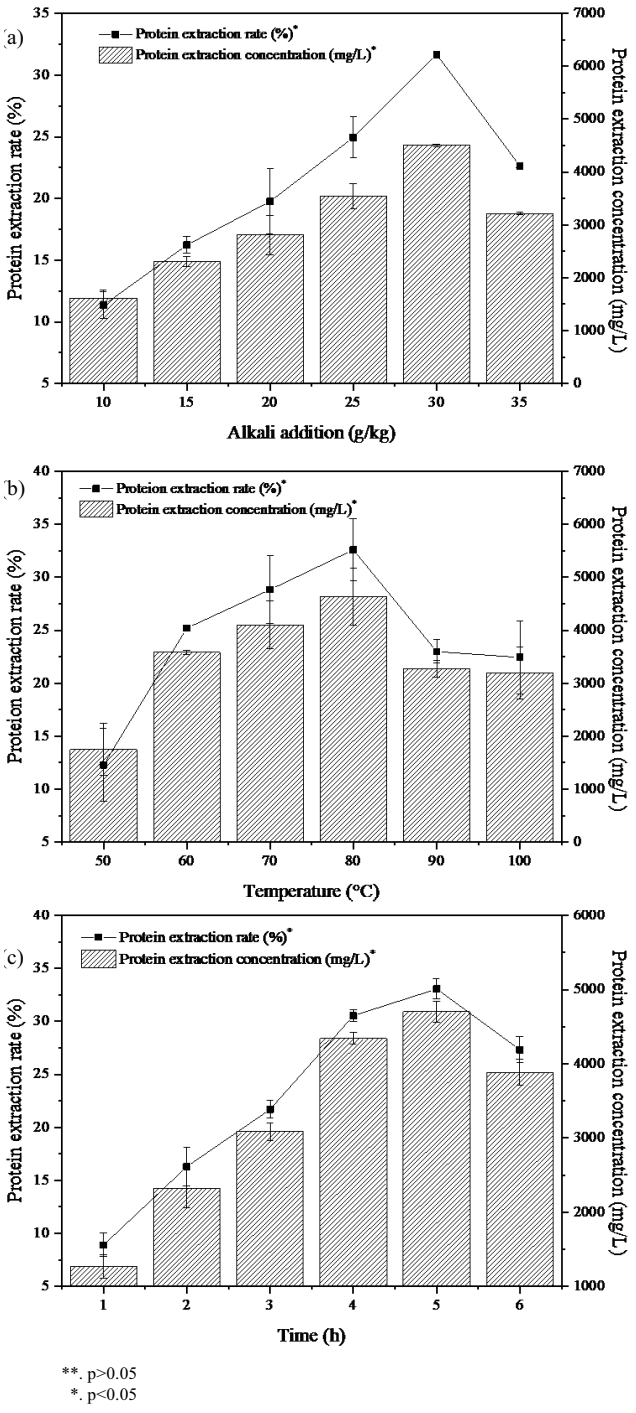

Fig. 1. Effects of different conditions on protein extraction: (a) the amount of alkali addition, (b) the extraction temperature, and (c) the extraction time.

3.1.3. Effects of extraction time

The extraction time was another important parameter affecting the final protein extraction and was optimized based on the condition of alkali amount (30 g kg^{-1}) and temperature (80°C). As shown in Fig. 1c, both protein extraction rate and concentration enhanced rapidly with the

increasing time from 1 to 5 h. After that, the two indicators declined lightly with a further increase of time. Obviously, the change trends of COD and TOC concentrations in Fig. S2c were similar to the above results. Consequently, the optimal extraction time was chosen as 5 h with the protein extraction rate of 33.07% and concentration of 4,702.37 mg L^{-1}. Based on the above conclusions, the optimal conditions for protein extraction were alkali amount at 30 g kg^{-1}, the temperature at 80°C, and time at 5 h.

3.2. Further analysis for the product of protein extraction process

3.2.1. 3D EEM analysis

To determine the effects of parameters (amount of alkali addition, extraction temperature, and time) on the changes of protein types in the protein hydrolysis solution, 3D-EEM fluorescence spectroscopy was employed. 3D-EEM fluorescence spectroscopy is a rapid and sensitive technique to measure the fluorescence compounds in the protein hydrolysis solution.

The results of fluorescence region integration are summarized in Table 1. In all the protein hydrolysis solutions, any tyrosine-like proteins and fulvic acid-like materials were hardly detected. However, the fluorescence intensities of tryptophan-like protein and humic acid-like organic compounds were relatively higher than other organic compounds, which was in line with previous research [16]. The dominance of tryptophan-like protein and humic-acid-like substances may be related to the changes of the aromatic structures and acidic functional groups of macromolecular structure induced by various treatments [36]. During ultrasonic treatment, a similar amount of tryptophan-like protein and humic-acid-like substances were present [37]. From Table 1, the fluorescence intensities of all the organic compounds increased as alkali amount increased but decreased as temperature increased. With the increase of time, the fluorescence intensities of all the organic compounds began to increase and cut back after 5 h.

Moreover, when the alkali amount was 30 g kg^{-1}, the temperature was 80°C and time was 5 h, the highest production of tryptophan-like protein compared to other conditions were reached and shown in Fig. 2. Tryptophan (Region II) was an important amino acid with demonstrated bioactivities and was also a precursor molecule to many important hormones and neurotransmitters [38]. The recovery of this type of protein with tryptophan as its main component from WAS may be used as feed additives.

3.2.2. XPS analysis

In this study, XPS technique was employed to analyze the distribution of N-containing compounds and composition in raw sludge and treated sludge solid samples, which would help to elucidate the nitrogen transformation during different treatment processes. Fig. 3 shows the results of dewatered sludge and residual sludge under the optimal condition (the alkali amount at 30 g kg^{-1}, the temperature at 80°C, and time at 5 h), which were marked as (a) and (b), respectively.

As shown in Fig. 3, organic protein-N (with the peak of 399.7 eV) was predominant N-containing compounds in all sludge solid samples, but their proportions of organic protein-N were different. In the dewatered sludge sample, the proportion of organic protein-N was 63.67%, while the proportion of organic protein-N was 28.78% in the residual sludge sample. Hence, TAH treatment (>100°C) applied in

Table 1
EEM FRI of soluble organics in different regions

Factors	Conditions	Region 1	Region 2	Region 3	Region 4	Region 5
Alkali amount (g kg^{-1})	10	5.0	188.2	3.9	21.8	187.1
	15	5.0	108.6	3.9	27.1	109.9
	20	3.9	115.2	3.9	16.1	124.8
	25	3.9	158.9	3.9	12.9	195.1
	30	3.9	249.6	5.0	11.6	387.1
	35	4.1	287.1	4.1	12.9	417.9
Temperature (°C)	50	4.9	332.7	4.9	25.4	433.6
	60	4.9	275.8	4.9	11.1	377.3
	70	4.9	262.6	5.0	12.2	370.4
	80	4.9	243.9	4.9	9.3	386.0
	90	5.0	224.4	5.0	9.0	386.1
	100	4.1	205.5	4.1	9.8	411.6
Time (h)	1	3.9	202.6	3.9	14.8	247.0
	2	3.9	190.8	4.1	12.5	264.4
	3	4.0	191.2	4.0	8.7	272.7
	4	4.0	215.9	4.0	16.7	307.7
	5	4.1	228.4	4.1	13.0	296.2
	6	3.9	191.4	3.9	10.6	243.5

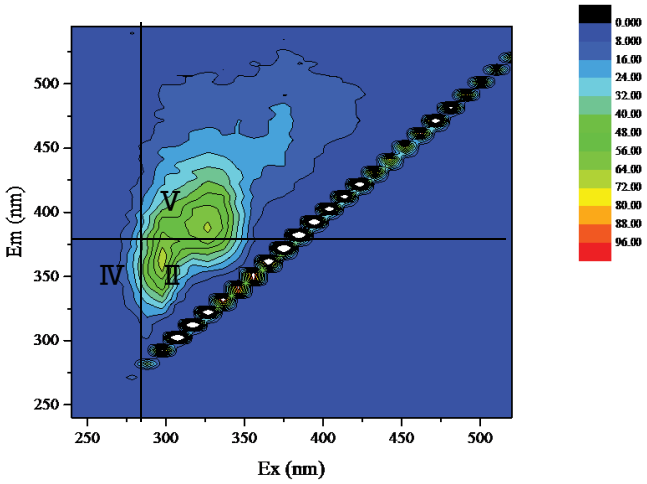

Fig. 2. EEM FRI of soluble organics under the optimal treatment conditions.

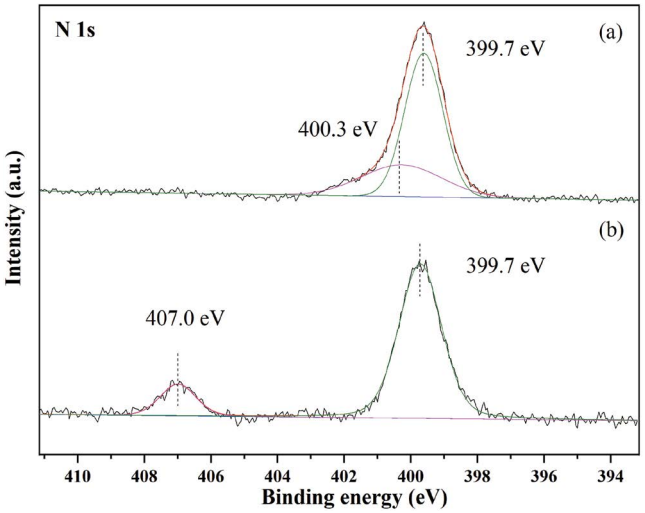

Fig. 3. Evolution of N 1s XPS spectra for sludge samples including: (a) dewatered sludge and (b) residual sludge.

this study can release organic protein-N sufficiently [39]. Strong basic conditions promoted the extraction of protein and its subsequent degradation [40].

The protonated amine in protein can be easily converted to volatile ammonia in the gas phase at alkali conditions [41]. The cyclization of amine-N intermediates obtained from protein decomposition may generate heterocyclic-N compounds [42], which is shown in Fig. 3 with a peak of 407.0 eV. This high depletion of protein-N in the solid phase at optimal condition contributed to its highest protein extraction efficiency as mentioned above. However, the detailed formation mechanism remains unclear and needs to be investigated in the future.

3.2.3. FTIR analysis

FTIR is a convenient method that provides information about the sludge conformation. FTIR spectra of sludge

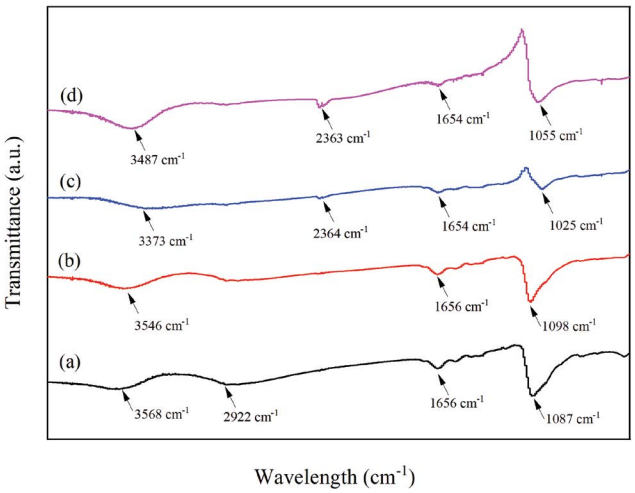

Fig. 4. FTIR spectra of sludge samples including (a) dewatered sludge, (b) at 10 g kg^{-1}, 5 h, and 80°C, (c) at 30 g kg^{-1}, 5 h, and 80°C, and (d) at 35 g kg^{-1}, 5 h, and 80°C.

samples are displayed in Fig. 4, including (a) dewatered sludge (b) at 10 g kg^{-1}, 5 h and 80°C (c) at 30 g kg^{-1}, 5 h and 80°C, and (d) at 35 g kg^{-1}, 5 h, and 80°C.

The results of FTIR spectra show the four changes of absorption peaks. With the changes of alkali amount, the absorption peak at 1,656 cm^{-1} showed redshift which was resulted from Amide I (mainly protein C=O stretching). The redshift indicated the weakening of hydrogen bonding of C=O, which would destroy the spatial structure of protein molecules [43]. The organic material in the samples, which was shown at 3,568 cm^{-1} –OH and 1,087°C–C to 3,487 cm^{-1} N–C and 1,055 cm^{-1} C=C, indicating the breakage of nitrogenous substances [44] and aromatic substances [45]. And the appearance of the absorption peak at 2,363 cm^{-1} C=C=C and 2,364 cm^{-1} C=C–O may show that the structure of the protein has undergone the process of first breaking the chain and then oxidizing [46]. The results showed that as the alkali amount increased, the element component of C=O (chemical composition of the peptide bond in protein-N) increased while the element component of N–C (chemical bond in pyridine-N) decreased, which was in line with the results of XPS analysis.

3.3. Statistical analyses

In addition to the above qualitative results of mechanism investigations, quantitative statistical results, including both correlation analysis and PCA, were given.

The relationships between solubilized protein and concentrations of other dissolved matters were investigated based on Pearson's correlation, which is shown in Table S1. In respective experimental groups (including changes of alkali amount, temperature, and time), the results indicated that protein extraction rate and concentration were positively related to organic compounds in hydrolysis solution (i.e., COD and TOC). Such as the group of alkali amounts, correlation coefficients were 0.946 for TOC ($P < 0.01$) and 0.954 for COD ($P < 0.01$). There was a significant relationship between alkali and time groups on the protein extraction

concentration and rate with a correlation coefficient of 0.967 ($P < 0.01$). Moreover, the results supported this finding that the ammonium concentration was also positively related to protein extraction concentration in alkali and time groups. Two points mentioned above explained that alkali amount and time were the key factors of protein extraction. The results also suggested that organic compounds including protein in the floc sludge were solubilized and entered the liquid phase with treatments [19].

The results of PCA of different parameters on protein extraction are shown in Fig. 5, where PCA was used to reassemble various indicators that have certain relevance into a new set of unrelated comprehensive indicators to replace the original ones. Fig. S3 demonstrated that all the initial variables could be well-explained by three principal components (PCs) with eigenvalue $\lambda > 1$, in which the λ was 3.388, 1.338, and 1.103, respectively. The first (PC1), second (PC2), and third PC (PC3) were responsible for 44.92%, 20.60%, and 17.75% of the total variance in the data sets, respectively. Thus, those PCs accounted for 83.27%. Fig. 5 shows that TOC, COD, and protein were situated with high scores in PC1 which explained that PC1 was on behalf of the soluble organic component. The ammonia nitrogen accounted for the highest weight in PC2, whereas the variable with a high influence in PC3 was time. Considering the above PCA, the primary factors in the liquid phase which affect the protein extraction effect were the degree of disintegration of cells (e.g., COD, TOC, and protein) and the change of ammonia nitrogen. In addition, the primary factor in the experimental conditions was time.

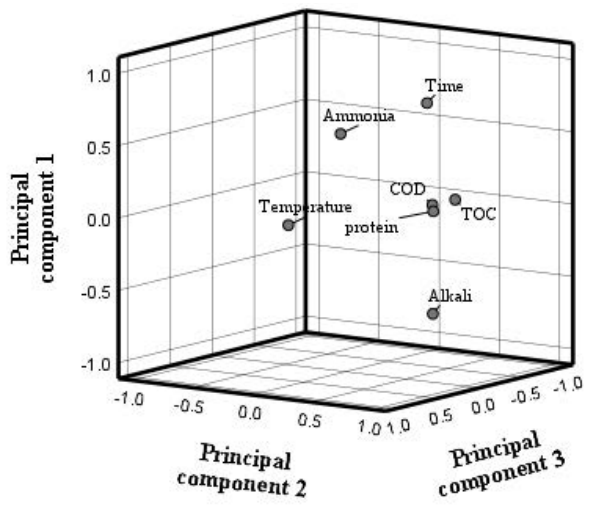

Fig. 5. Component 3D diagram of the first three principal component vectors (seven parameters of protein extraction performance).

3.4. Energy balance and economic analysis

The method of thermal alkali was analyzed economically and compared with the conventional methods (pure thermal and pure alkali) [16] under the optimal conditions. Sludge treatment systems would also increase costs, which was not included in the economic analysis. In Table 2, the energy cost (1 kWh) was about 0.23 $ [47]. The chemical cost (the average price of NaOH) was 333 $ ton^{-1} [48]. Assumed 90% protein can be recovered from the released protein [7]. The cost for protein recovery and isolation was about 28 and 40 $ lb^{-1} was assumed for an isolated protein price [49]. Credit from protein recovery was calculated based on Eq. (3).

$$\text{Credit from protein recovery} = \text{Market credit from isolated protein} - \text{Cost for protein isolation process} \quad (3)$$

Cost of sludge transport and disposal referred to the previous study [50]. The net saving compared to conventional treatment was calculated by Eq. (4).

$$\text{Net saving cost} = \text{increase in protein recovery} - \text{cost of sludge transport and disposal} - \text{energy cost} \quad (4)$$

Table 2 shows that the method in this study can significantly increase the release of protein from sludge, compared to the conventional method, which showed economic feasibility. The sludge pretreated by thermal alkali had a higher protein concentration, which was four times as high as that of pure alkali. This will greatly increase the value of the product to offset the cost. The method of thermal alkali at the optimal conditions (alkali amount at 30 g kg^{-1}, the temperature at 80°C, and time at 5 h) showed the highest

Table 2
Energy balance and cost analysis

Indexes	Raw	Thermal alkali	Thermal (°C)		Alkali	
		Optimal conditions	60	80	pH 10	pH 12
Energy cost ($ ton^{-1} wet sludge)	0	147.60	9.39	14.76	/	/
Chemical cost ($ ton^{-1} wet sludge)	0	1.81	/	/	0.18	0.47
Protein concentration (mg L^{-1})	0	4,702.37	589.63	681.47	343.87	1,109.00
Credit from protein recovery ($ ton^{-1} wet sludge)	0	746.4	93.59	108.17	54.58	176.04
Cost of sludge transport and disposal ($ ton^{-1} wet sludge)	150	0	0	0	0	0
Net saving compared to conventional treatment ($ ton^{-1} wet sludge)	–150	448.80	–65.80	–56.59	–95.60	25.57

net saving of 448.80 $ per ton wet sludge. However, due to the long processing time, the released protein might be greatly damaged during the processing process, which would affect the protein value. The cost of recovering and isolating protein from released protein remains high, and the market price of crude protein is relatively low. More efforts are needed to find cost-effective ways to ensure protein quality, which must be easy to scale up and have huge commercial benefits.

4. Conclusions

According to the above results, the protein extraction rate of 41.47% and concentration of 4,702.37 mg L^{-1} were detected under the optimal treatment conditions (alkali amount at 30 g kg^{-1}, the temperature at 80°C, and time at 5 h). The optimal treatment conditions gave the highest dominant protein (Tryptophan), which could be used as feed additives. The recovery of this type of protein might be related to the significant removal of protein-N in the solid phase as indicated by XPS. The study also showed that the structure of the organic protein has undergone the process of first breaking chain and then oxidizing. The protein extraction rate and concentration were correlated with the release of organic compounds (COD and TOC), which suggested that proteins in the floc sludge entered the liquid phase with treatments. The method of thermal alkali at the optimal conditions showed the highest net saving of 448.80 $ per ton of wet sludge.

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Supplementary information

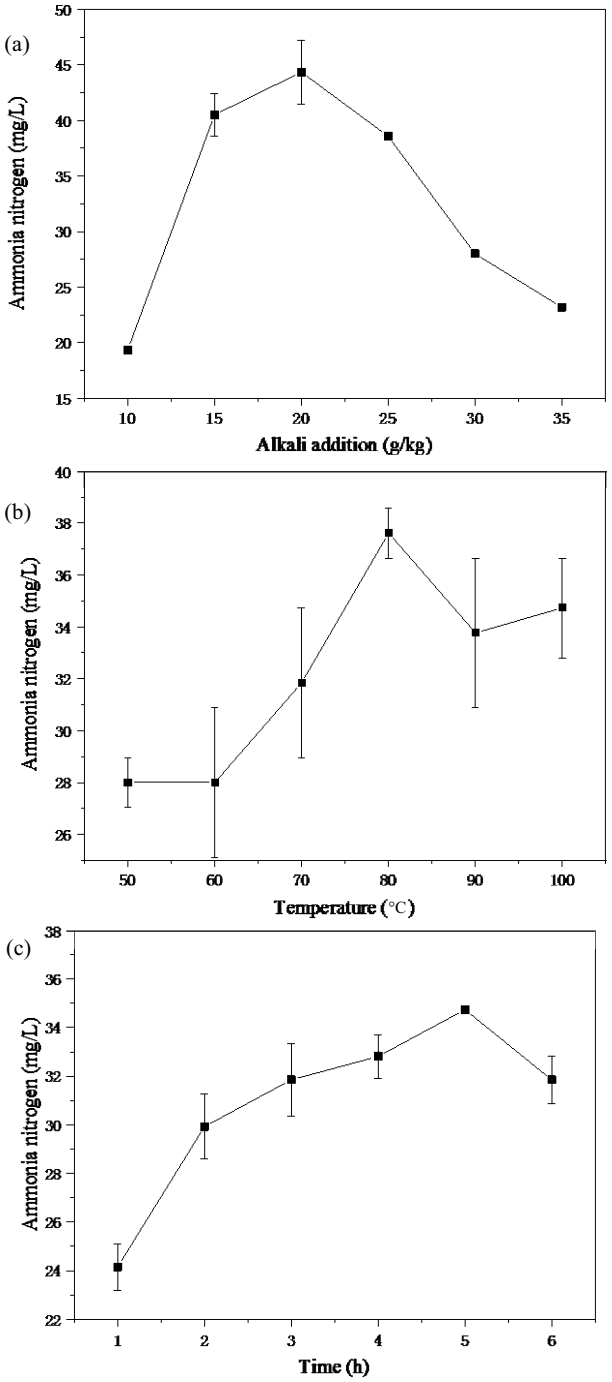

Fig. S1. Effects of different conditions on ammonia nitrogen: (a) the amount of alkali addition, (b) the extraction temperature, and (c) the extraction time.

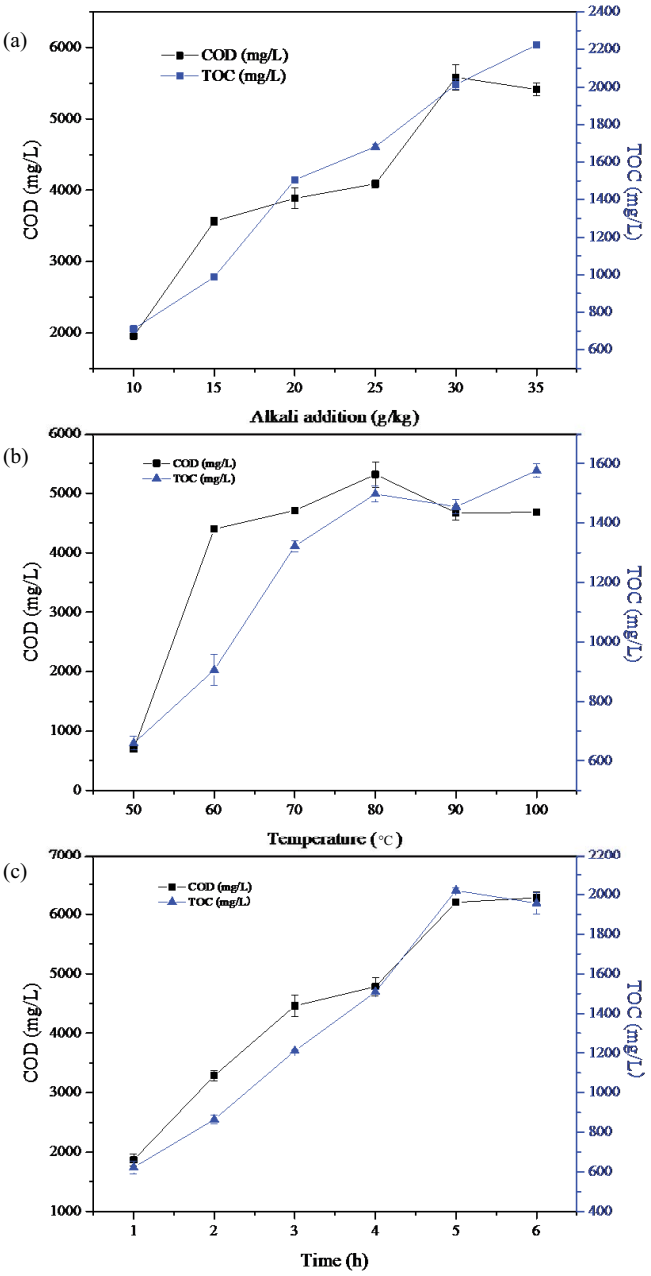

Fig. S2. Effects of different conditions on COD and TOC: (a) the amount of alkali addition, (b) the extraction temperature, and (c) the extraction time.

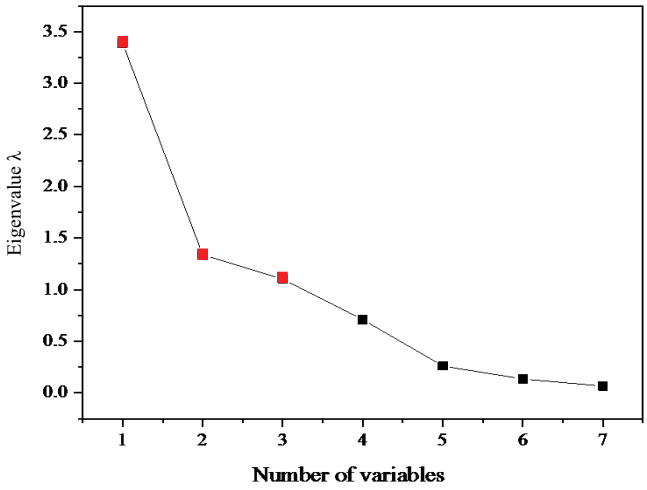

Fig. S3. Eigenvalue screen plot.

Table S1
Correlation between solubilized protein and dissolved organic matters in hydrolysis solution

	Protein extraction rate (alkali)	Protein extraction rate (time)	Protein extraction rate (temperature)	Protein extraction concentration (alkali)	Protein extraction concentration (time)	Protein extraction concentration (temperature)	Ammonia nitrogen (alkali)	Ammonia nitrogen (time)	Ammonia nitrogen (temperature)	TOC (alkali)	TOC (time)	TOC (temperature)	COD (alkali)	COD (time)	COD (temperature)
Protein extraction rate (alkali)	1	0.967**	0.499	1.000**	0.967**	0.499	0.957**	0.920**	0.762	0.946**	0.995**	0.870*	0.954**	0.958**	0.698
Protein extraction rate (time)	0.967**	1	0.625	0.967**	1.000**	0.625	0.980**	0.939**	0.882*	0.910*	0.965**	0.899*	0.923**	0.963**	0.762
Protein extraction rate (temperature)	0.499	0.625	1	0.499	0.625	1.000**	0.477	0.741	0.629	0.619	0.453	0.792	0.523	0.541	0.935**
Protein extraction concentration (alkali)	1.000**	0.967**	0.499	1	0.967**	0.499	0.957**	0.920**	0.762	0.946**	0.995**	0.870*	0.954**	0.958**	0.698
Protein extraction concentration (time)	0.967**	1.000**	0.625	0.967**	1	0.625	0.980**	0.939**	0.882*	0.910*	0.965**	0.899*	0.923**	0.963**	0.762
Protein extraction concentration (temperature)	0.499	0.625	1.000**	0.499	0.625	1	0.477	0.741	0.629	0.619	0.453	0.792	0.523	0.541	0.935**
Ammonia nitrogen (alkali)	0.957**	0.980**	0.477	0.957**	0.980**	0.477	1	0.859*	0.886*	0.859*	0.963**	0.821*	0.882*	0.933**	0.624
Ammonia nitrogen (time)	0.920**	0.939**	0.741	0.920**	0.939**	0.741	0.859*	1	0.727	0.907*	0.904*	0.905*	0.959**	0.953**	0.901*
Ammonia nitrogen (temperature)	0.762	0.882*	0.629	0.762	0.882*	0.629	0.886*	0.727	1	0.732	0.772	0.818*	0.640	0.764	0.608

Table S1 (*continued*)
Correlation between solubilized protein and dissolved organic matters in hydrolysis solution

	Protein extraction rate (alkali)	Protein extraction rate (time)	Protein extraction rate (temperature)	Protein extraction concentration (alkali)	Protein extraction concentration (time)	Protein extraction concentration (temperature)	Ammonia nitrogen (alkali)	Ammonia nitrogen (time)	Ammonia nitrogen (temperature)	TOC (alkali)	TOC (time)	TOC (temperature)	COD (alkali)	COD (time)	COD (temperature)
TOC (alkali)	0.946**	0.910*	0.619	0.946**	0.910*	0.619	0.859*	0.907*	0.732	1	0.933**	0.959**	0.879*	0.890*	0.760
TOC (time)	0.995**	0.965**	0.453	0.995**	0.965**	0.453	0.963**	0.904*	0.772	0.933**	1	0.853*	0.948**	0.968**	0.652
TOC (temperature)	0.870*	0.899*	0.792	0.870*	0.899*	0.792	0.821*	0.905*	0.818*	0.959**	0.853*	1	0.802	0.843*	0.847*
COD (alkali)	0.954**	0.923**	0.523	0.954**	0.923**	0.523	0.882*	0.959**	0.640	0.879*	0.948**	0.802	1	0.974**	0.762
COD (time)	0.958**	0.963**	0.541	0.958**	0.963**	0.541	0.933**	0.953**	0.764	0.890*	0.968**	0.843*	0.974**	1	0.733
COD (temperature)	0.698	0.762	0.935**	0.698	0.762	0.935**	0.624	0.901*	0.608	0.760	0.652	0.847*	0.762	0.733	1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).