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Protein extraction from excess sludge by thermal-acid pretreatment

Jianlei Gao[a], Qikun Wang[a], Yixin Yan[a,*], Zheng Li[b], Wei Weng[a]

[a]School of Water Conservancy Engineering, Zhengzhou University, Zhengzhou 450001, China, emails: yxyan@zzu.edu.cn (Y. Yan), gaojianlei@zzu.edu.cn (J. Gao), wangqikunzzu@163.com (Q. Wang), wengwei0711@163.com (W. Weng)
[b]Zhengzhou University Multi-Functional Design and Research Academy Co., Ltd., Zhengzhou 450002, China, email: 18339900083@163.com (Z. Li)

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ABSTRACT

Thermal-acid pretreatment could represent an efficient sludge hydrolysis technique. The effects of the operation parameters (pH, temperature, and time) and moisture content on protein extraction by thermal pretreatment and the foaming properties of the extracted protein were investigated in this study. The results revealed the following the optimal conditions: pH of 0.5, reaction temperature of 130°C, reaction time of 4 h, and moisture content of 92%. Under these conditions, the protein content reached 522.1 mg/g VSS with an extraction rate of 91.4%. In addition, a polypeptides content of 9,499.6 mg/L was favorable for foaming. The foamability and foam stability of the protein solution were 660% and 88%, respectively, which met the relevant standards for foam extinguishing agents. Moreover, the dewatering performance of hydrolyzed sludge was improved by 91.4%, which was convenient for subsequent protein utilization.

Keywords: Thermal-acid pretreatment; Excess sludge; Protein extraction; Dewatering performance; Foaming properties

1. Introduction

Activated sludge process, as one of the most widely used wastewater treatment processes, produces various amounts of byproducts (excess sludge). Millions of tons (dry weight) of excess sludge are produced in China every year [1]. Excess sludge is characterized by high moisture content and poor dewatering performance. Given that excess sludge contains toxic and harmful substances, such as refractory organic matter, heavy metals, and pathogens, it may cause secondary pollution and increase difficulties in sludge management if not properly handled [2,3]. The cost of conventional handling treatments is expensive, accounting for approximately 50% of the total wastewater disposal cost [4].

Protein is one of the main components of organic matter in excess sludge, accounting for 50%–60% [5,6]. This protein will have high commercial value if it can be separated from the sludge. Most organics are encased within microbial cell membranes. However, the cells are protected from lysis given the semi-rigid structure of the cell wall [7]. Therefore, an appropriate pretreatment technology promoting sludge hydrolysis is a prerequisite for protein extraction from sludge. Several efforts have been made, such as physical pretreatment [8,9], thermal pretreatment [10], chemical pretreatment [11], biological pretreatment [12], and several combinations of different pretreatments [13]. Among these pretreatments, thermal-acid pretreatment has attracted wide attention due to its effective conditioning of sludge. Su et al. [14] recovered amino acids from sludge, and the total volatile solid substance (TVSS) was reduced by 85.74% under optimized conditions (hydrochloric acid content of 3.25 mol/L, 100°C,

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* Corresponding author.

14 h, and moisture content of 99.05%). Neyens et al. [15] reported that the optimum pretreatment at pH 3 and 130°C for 1 h efficiently reduced sludge amounts and improved dewatering performance. Assawamongkholsiri et al. [16] investigated the effects of different pretreatments (acid, heat, and combined acid-heat) on solubilization of organic matter from activated sludge, and the highest percentage increase in soluble chemical oxygen demand (SCOD), carbohydrate, and protein content were achieved by acid-heat pretreatment. Thermal-acid pretreatment has the potential for efficient protein extraction from excess sludge.

The protein extracted from excess sludge exhibits satisfactory foaming properties and can be utilized as a raw material of foam extinguishing agents and foam concrete [17,18]. Compared with chemical foaming agents, it is cheaper and more environment-friendly. During thermal-acid pretreatment, protein is further hydrolyzed to polypeptides and amino acids [19]. The foaming properties of a protein are related not only to the protein content but also to the degree of hydrolysis [20]. A limited degree of hydrolysis is advantageous to foaming properties [21–23]. Polypeptides formed by sufficient hydrolysis of macromolecular protein are flexible linear molecules with low molecular weights and simple structures. Compared with protein, polypeptides migrate faster to the air-water interface [24]. Therefore, the foamability after proteolysis may be improved. Polypeptides with low molecular weight exhibit low surface tension, allowing them to form stable bubbles [24]. Excessive hydrolysis will reduce the viscosity of the solution, which has negative impacts on foam stability [25]. Therefore, in the pretreatment of excess sludge, controlling the incomplete hydrolysis of protein and producing more polypeptides are critical to its use as a foaming agent.

The aim of this work was to investigate the effects of thermal-acid pretreatment of excess sludge on protein extraction, polypeptides content, and sludge dewatering performance. Previous studies have mainly focused on the extraction effects of protein, whereas few studies have reported the compositions and the superiority of the foaming properties of the extracted protein. In this work, the contents of protein, polypeptides, and amino acids were used to help determine the effects of operation parameters and moisture content on the degree of hydrolysis. The foaming properties (foamability and foam stability) of the extracted protein were determined to evaluate its feasibility as a foaming agent. In addition, since the extracted protein need to be separated before it was further utilized, the dewatering performance of hydrolyzed sludge was analyzed (indicated by specific resistance to filtration, SRF), which helped to provide a reference for the practical application of this technology.

2. Materials and methods

2.1. Sludge characterization

Sludge was obtained from the sludge concentration machine room of a local municipal wastewater treatment plant with pre-anoxic A^2/O process. The moisture content of sludge was approximately 94.5%, and the characteristics of sludge are shown in Table 1. The samples were stored at 4°C before use.

2.2. Apparatus

The thermal-acid reactions in the study were performed in a self-made hydrolytic reactor. Its main body was made of 316 stainless steel, and the lining was made of polytetrafluoroethylene (PTFE). The structure diagram is shown in Fig. 1, and the main parameters of the reactor are shown in Table 2.

2.3. Thermal-acid pretreatment

First, acid pretreatment was performed by adding 98% sulfuric acid (H_2SO_4) to 1,000 mL of excess sludge stepwise until the desired pH values (0, 0.5, 1.0, 1.5, and 2.0) were achieved as measured using a pH meter. Then, the sample was added to the preheated hydrolysis reactor. The hydrolysis reaction was performed at certain experimental temperatures and times, and the stirring speed was maintained at 50 rpm. Given that high temperature (greater than 90°C) efficiently destroy sludge floc and the cell wall [8,26], the thermal pretreatment temperature range used in this study was 100°C–140°C. After the hydrolysis reaction was completed, the vent valve could not be opened until the reactor was cooled to 60°C. The hydrolyzed sludge and

Table 1
Characteristics of excess sludge

Parameter	Value
TSS[1] (g/L)	60.255 ± 0.64
VSS[2] (g/L)	39.125 ± 1.45
TCOD[3] (mg/L)	56,002.7 ± 3,478.4
TKN[4] (mg/L)	2,508 ± 771
SRF (×10^{12} m/kg)	16.66 ± 4.9

[1] TSS: Total suspended solids.
[2] VSS: Volatile suspended solids.
[3] TCOD: Total chemical oxygen demand.
[4] TKN: Total kjeldahl nitrogen.

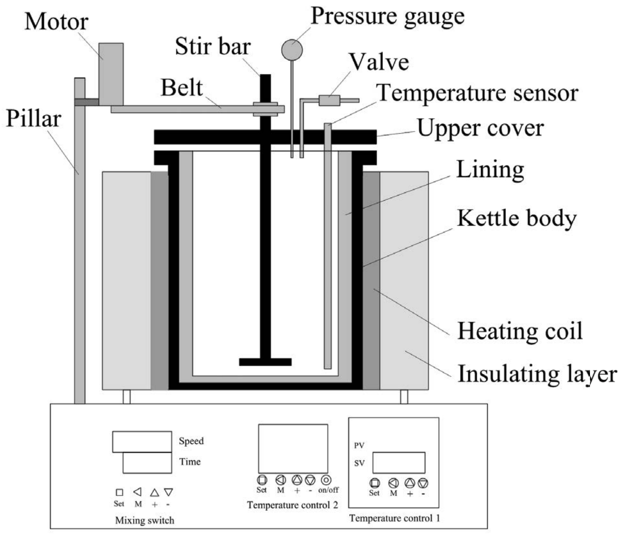

Fig. 1. Schematic diagram of the reactor structure.

Table 2
Parameters of the reactor

Parameter	Value
Active volume (mL)	1,500
Heating power (W)	300–3,000
Maximum heating temperature (°C)	400
Working pressure (MPa)	0–22
Stirring rate (rpm)	0–100

its supernatant were obtained for measurement after the reactor was further cooled to room temperature.

2.4. Analytical procedures

The pH value of sample was monitored with a pH meter (PHSJ-4A, INESA, China). TKN of the raw sludge was determined by the resolution method [27]. Total chemical oxygen demand (TCOD) and SCOD were determined by the potassium dichromate method [27]. The polypeptides were determined by the biuret method [28]. Amino acids were determined by using the ninhydrin calorimetric method [29].

The protein content was determined using the bicinchoninic acid (BCA) kit (P1511, APPLYGEN, China) [30]. Briefly, 0.05 mL assay sample was added to 1 mL of working reagent that was prepared by mixing BCA reagent and Cu reagent at a 50:1 volume ratio. Samples were incubated (25°C) for 120 min. The absorbance of standard and sample were measured at 562 nm with a spectrophotometer (Cury60, CSOIF, China). Bovine serum albumin (BSA) was used as the standard for protein quantification.

The dewatering performance of sludge was expressed as SRF, which represented the resistance of sludge of the unit mass to the unit filtration area under a certain pressure. SRF was measured by the Buchner funnel test [31]. Briefly, 100 mL of sludge was poured into the Buchner funnel fitted with a filter paper and filtered under vacuum pressure of 35.46 kPa for 20 min or until the vacuum could not be maintained. The volumes of filtrate collected at different times were recorded.

Supernatant was obtained by centrifuging the sludge at 4,000 rpm and used for the analyses of SCOD, protein, polypeptides, amino acids, foamability, and foam stability. Sludge samples were used to analyze pH, TCOD, and SRF. The measurements were performed in triplicates for all samples.

2.5. Definition of some indexes

- The protein extraction rate (RP) was calculated as shown in Eq. (1):

$$\text{RP}(\%) = \frac{M_1}{M_0} \times 100\% \tag{1}$$

where M_1 and M_0 denote the protein quality of the hydrolyzed sludge supernatant and the original sludge, respectively.

- The dissolution rate of SCOD (RS) was calculated as shown in Eq. (2):

$$\text{RS}(\%) = \frac{\text{SCOD}_1 - \text{SCOD}_0}{\text{TCOD}} \times 100\% \tag{2}$$

where SCOD_1 and SCOD_0 denote the SCOD in the supernatant of hydrolyzed sludge and the original sludge, respectively, and TCOD denotes the total COD of the original sludge.

- SRF was calculated as shown in Eq. (3) [31]:

$$\text{SRF} = \frac{2pA^2b}{\mu C} \tag{3}$$

where p is the filtration pressure; A is the filtration area; μ is filtrate viscosity; b is the slope of filtrate discharge curve (filtrate time/filtrate volume vs. filtrate volume); and C is the dry weight of the filter residue per unit Volume of filtrate, $C = \frac{1}{\left[\left(100 - C_i\right)/C_f - \left(100 - C_f\right)/C_f\right]}$, where C_i is the initial moisture content and C_f is the final moisture constant.

- The increasing rate of sludge dehydration performance (DW) was calculated as shown in Eq. (4):

$$\text{DW}\left(\%\right) = \frac{\text{SRF}_0 \text{ - } \text{SRF}_1}{\text{SRF}_0} \times 100\% \tag{4}$$

where SRF_0 and SRF_1 denote the SRF of the original sludge and the hydrolyzed sludge, respectively.

- The foaming properties (foamability and foam stability) of protein solution were determined by the Ross–Miles method after the pH value was adjusted to neutral [32]. Foamability and foam stability were calculated as shown in Eqs. (5) and (6), respectively:

$$\text{Formability}\left(\%\right) = \frac{V_i}{100} \times 100\% \tag{5}$$

$$\text{Foam stability}\left(\%\right) = \frac{V_{30}}{V_i} \times 100\% \tag{6}$$

where 100 denotes the sample volume is 100 mL before agitating; V_i denotes the foam volume at initial time; and V_{30} denotes the foam volume that remained at 30 min after agitating.

3. Results and discussion

3.1. Effects of operation parameters on sludge hydrolysis

3.1.1. Initial pH value

First, the effects of initial pH value on sludge hydrolysis were investigated at a temperature of 120°C for 3 h, and the moisture content of sludge sample was maintained at 94%. Fig. 2a shows the protein content increased rapidly as the initial pH decreased from 2 to 0.5, and the maximum protein content (477.6 mg/g VSS) was reached at pH

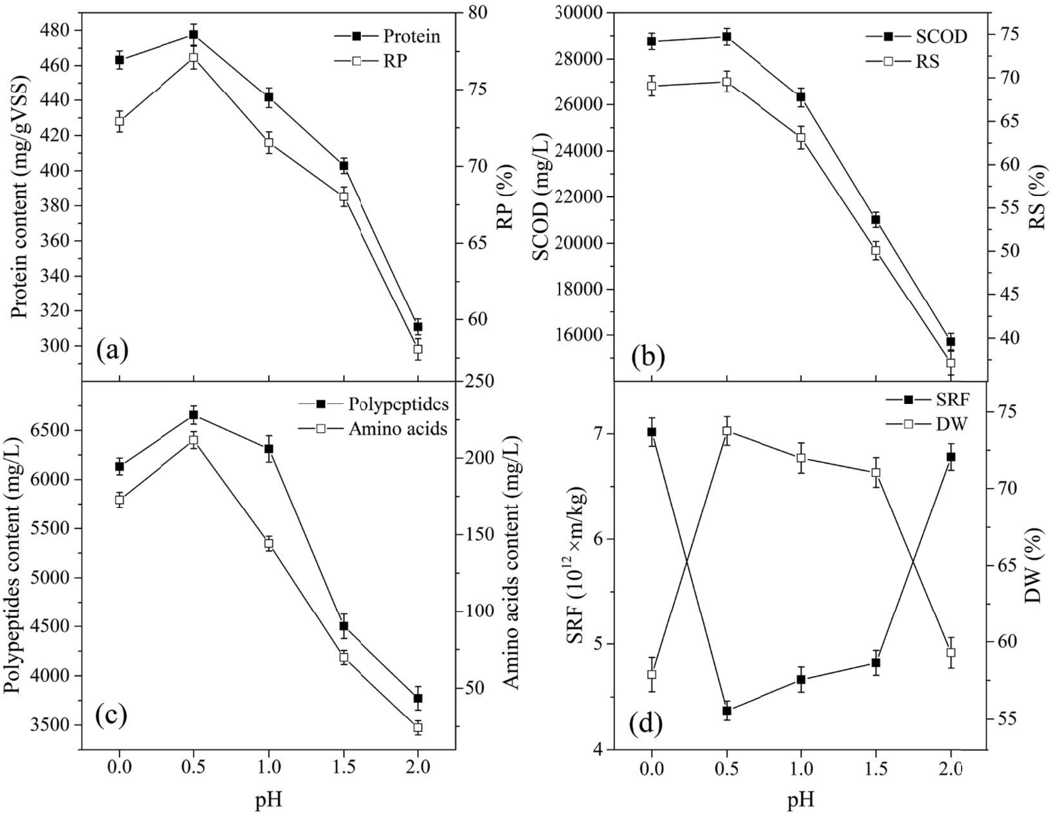

Fig. 2. Effects of pH on sludge hydrolysis: (a) protein, (b) SCOD, (c) polypeptides and amino acids, and (d) dewatering performance.

0.5. Microorganisms in sludge are unable to perform normal metabolism in extremely acidic environments. As a result, numerous microbial cells were lysed, and intracellular substances were released into the liquid phase, which facilitated the further hydrolysis of macromolecular protein. Wang et al. [18] reported similar results, indicating that the RP of thermal-acid pretreatment was the highest at pH 0.5. The solubilization of organic matters in sludge after pretreatment can be characterized by SCOD. Xue et al. [33] reported that protein and SCOD were positively correlated after thermal pretreatment (R^2 = 0.9). Therefore, as the pH value decreased, SCOD increased obviously (Fig. 2b). Devlin et al. [7] reported that the protein content increased to approximately 450 mg/L under pretreatment at pH 1 for 24 h. Compared with this study, protein content was 441.5 mg/g VSS (equivalent to 17,275 mg/L) at the same pH value after thermal hydrolysis for 3 h. Obviously, the efficiency of protein extraction by combining acid with thermal hydrolysis was better. In addition, the minimum SRF was noted at pH 0.5, and the dewatering performance was improved by 73.8% (Fig. 2d). Therefore, an initial pH of 0.5 was used in the subsequent optimization of operation parameters.

3.1.2. Reaction temperature

Temperature plays an important role in thermal-acid pretreatment of excess sludge. To determine the optimal pretreatment temperature, the effects of reaction temperature on sludge hydrolysis were assessed under the conditions of an optimized pH of 0.5 for 3 h, and the moisture content of the sludge sample was maintained at 94%. Fig. 3a shows that the increase in temperature improved the protein content. The protein content and RP achieved maximum values (464.9 mg/g VSS and 68.4%, respectively) at 130°C. High temperatures helped to destroy the floc structure and sludge cells, which is similar to that noted for acid pretreatment [34]. Especially for acid-treated sludge, its resistance to high temperature sharply decreased. With an increase in temperature, the changes of SCOD, polypeptides, and amino acids were similar to protein (Figs. 3b and c). In tests, the protein solution was tan, and the color was darkens as the amount of protein and polypeptides increased. As noted in Fig. 3d, the DW increased rapidly from 67% to 90.5% as the temperature increased from 100°C to 130°C and then decreased slowly. Therefore, the optimal temperature of 130°C was selected.

Moreover, compared with previous studies, the effects of temperature on the thermal hydrolysis of sludge were similar, that is, the solubilization of organic matters was not strongly affected by temperature until the temperature increased to a certain level. This temperature was 100°C as reported by Yan et al. [26], 115°C as reported by Val Del Río et al. [35], and 150°C as reported by Wilson and Novak [36]. Val Del Río et al. [35] attributed this finding to the fact that extracellular polymeric substances (EPS) lost their gel-forming properties at high temperature, and their solubilization subsequently increased. The difference in temperature may be attributed to differences in

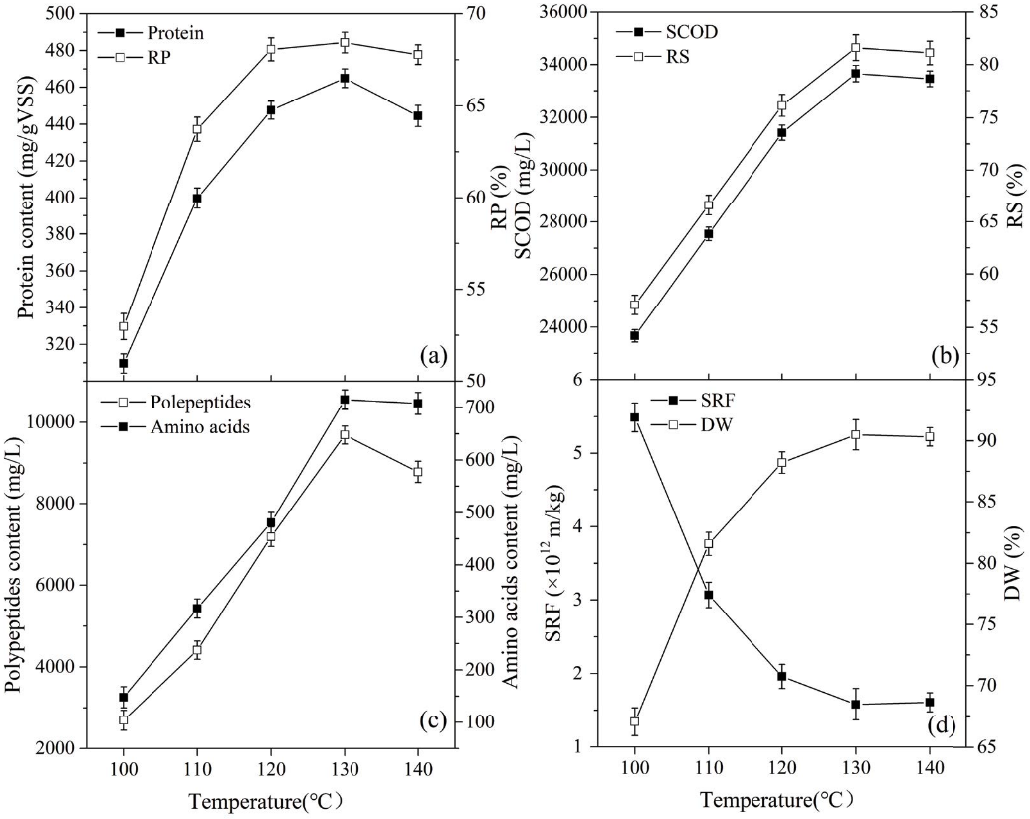

Fig. 3. Effects of reaction temperature on sludge hydrolysis: (a) protein, (b) SCOD, (c) polypeptides and amino acids, and (d) dewatering performance.

the physicochemical properties of raw sludge used in studies. From this study, it was also observed that RS increased quickly as temperature increased from 100°C to 130°C.

3.1.3. Reaction time

The effects of reaction time of thermal-acid pretreatment on sludge hydrolysis were assessed under the conditions optimized pH (0.5) and time (3 h), and the moisture content of the sludge sample was maintained at 94%. Fig. 4 reveals that a longer reaction time was more beneficial to sludge hydrolysis. The protein content increased quickly as the reaction time increased and reached a maximum of 480.3 mg/g VSS at 4 h (Fig. 4a). The trends for SCOD, polypeptides, and amino acids contents were similar to protein (Figs. 4b and c). The SRF also reached the minimum value (1.01×10^{12} m/kg) at reaction time of 4 h, which was suitable for mechanical dewatering (Fig. 4d). Therefore, the reaction time in the next pretreatment was set as 4 h.

Moreover, compared with previous studies of sludge pretreatment with only pH regulation, the addition of acid reagent promoted the sludge solubilization to some extent, but prolonged pretreatment time did not improve the solubilization efficiency [6,16]. Chen et al. [6] explained this finding based on the competitive balance between release and degradation of protein. Although thermal hydrolysis disrupted this balance and significantly improved the hydrolysis efficiency in a short time, higher temperatures were required. Xue et al. [33] reported that thermal pretreatment for 180 min increased the COD solubilization to 34.7%, 42.5%, and 53.4% at 140°C, 160°C, and 180°C, respectively. Compared with results in Fig. 4b, it indicated that the combination of acid and heat greatly improved the efficiency of hydrolysis at a short period of time without excessive temperature requirements.

3.1.4. Dewatering performance after pretreatment

Thermal-acid pretreatment also improved the dewatering performance of sludge. The increased acidity (Fig. 2d) and continuous input of energy (Figs. 3d and 4d) reduced SRF. The sludge flocs were destroyed after thermal-acid pretreatment. Liu et al. [37] reported that the dewatering performance of sludge after thermal hydrolysis was positively correlated with zeta potential. High temperature destabilized some anionic groups, which increased the zeta potential of sludge and reduced the electrostatic repulsion between the sludge particles. The repulsive force between the particles were reduced or even disappeared, which helped the floc particles to re-aggregate into a tighter entity [38]. Acid pretreatment had similar effects. The addition of acid caused the protons (H^+) in the liquid to neutralize the negative charge of the floc particles, especially the negative functional groups on the surface of EPS [38,39]. In addition, it was well-known that the bound water seriously affected the dewatering performance of sludge: the lower the

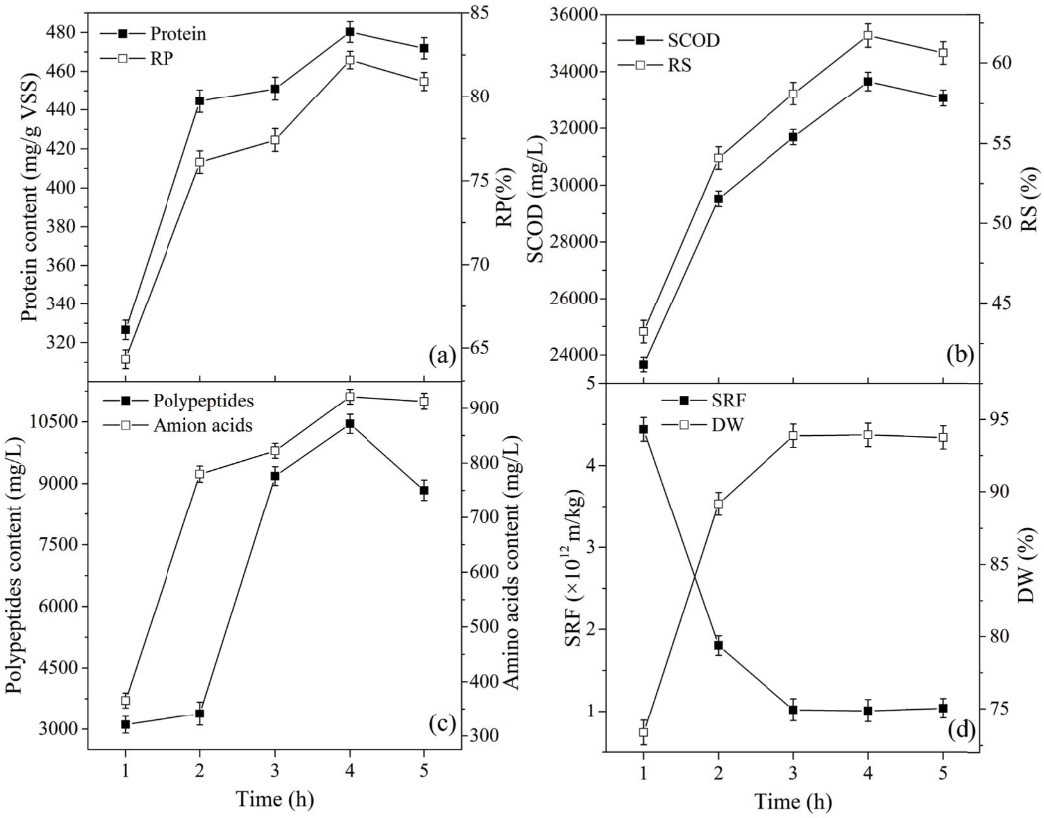

Fig. 4. Effects of reaction time on sludge hydrolysis: (a) protein, (b) SCOD, (c) polypeptides and amino acids, and (d) dewatering performance.

combined water, the more free water, and the better dewatering performance. Because EPS with high viscosity and high hydration was released after thermal pretreatment, the content of bound water decreased, and the clogging of filter holes by these biological macromolecules reduced [40]. Moreover, both acid and high temperature pretreatment could greatly reduce the viscosity of the sludge, which reflected the reduction of EPS to a certain extent [33,41]. In this study, only a thin layer of sludge cake remained at the bottom of the centrifuge tube after the hydrolysate was centrifuged. This finding suggested that the sludge floc was largely disintegrated, and excess sludge was completely dissolved.

3.1.5. Negative effects of excessive pretreatment

However, excessive acidity and excessive energy input also had negative effects on protein extraction. When the pH was less than 0.5 (Fig. 2), the temperature was greater than 130°C (Fig. 3) or time was greater than 4 h (Fig. 4), protein content, and SCOD decreased. This finding was attributed to the fact that the protein was decomposed into amino acids, carbon dioxide, and water [19]. Although increasing the pretreatment temperature or time improved sludge hydrolysis, the effects of temperature were far greater than time [34]. However, negative effects were noted if the temperature was excessive. Lu et al. [42] reported the presence of slowly biodegradable or nonbiodegradable steroid-like compounds and aromatics (e.g., benzenoids, flavonoids, and pyridines) in the residue after thermal hydrolysis at 172°C. Dwyer et al. [43] reported that melanoidin (a product of the Maillard reaction) was found during thermal hydrolysis at 140°C–165°C. Melanoidin was problematic in protein extraction because protein was incorporated into the melanoidin structure in the Maillard reaction. However, the Maillard reaction was inhibited at acidic pH values [44]. In this study, the addition of acid made it possible to achieve high hydrolysis efficiency without excessive temperatures; thus, similar negative effects were avoided to some extent.

On the other hand, with excessive pretreatment, the sludge floc and EPS were broken into numerous undissolved biological macromolecules [37,45]. These macromolecules clogged the filter holes, increasing the SRF, and reducing dewatering performance [40]. For example, the SRF of hydrolyzed sludge was 7.02 × 10^{12} m/kg at pH 0, which was 62.8% increase compared with that at pH 0.5 (Fig. 2d).

3.2. Effects of moisture content of sample on sludge hydrolysis

As a basic physical characteristic of sludge, a high moisture content has serious effects on the management and transportation of excess sludge. Differences in moisture content result in differences in organic matters content. The effects of the moisture content of raw sludge on hydrolysis were assessed under the optimized conditions reported above (pH 0.5, temperature 130°C, time 4 h). For sludge with high moisture content, the heat transfer coefficient was increased compared with that with lower moisture content [46]. In addition, sludge with high solid content tended to resist the stirring process, which might cause the sludge to be heated unevenly and affect the degree of sludge hydrolysis. Therefore, RP increased rapidly when the moisture content increased from 90% to 92% (Fig. 5a). The SCOD, polypeptides, and amino acids trends were similar to that noted for RP (Figs. 5b and c). However, excessively high moisture content may have negative effects. Higher moisture content also causes the organic matter to be more diluted after hydrolysis. When the moisture content was greater than 92%, it was possible that the effects of dilution were greater than that of hydrolysis; thus, the contents of SCOD, polypeptides, and amino acids decreased. As shown in Fig. 5d, SRF decreased as moisture content increased. When the moisture content of the raw sludge was 92%, SRF was approximately 1.25 × 10^{12} m/kg, indicating that the sludge exhibited good dewatering performance (Fig. 5d). The higher moisture content was favorable for operation but increased the subsequent treatment costs. Therefore, a moisture content of 92% was appropriate.

3.3. Foaming properties of protein

To analyze the change rules of foaming properties of protein and further optimize protein extraction conditions, a four-factor three-level orthogonal experiment was designed. The orthogonal experiment with the value of RP as the target index was employed to assign the four considered factors (initial pH value, reaction temperature, reaction time, and moisture content). The influence of each factor on RP was estimated by range value (*R*) as shown in Table 3, and the order was as follows: initial pH > temperature > moisture content > time. According to the mean values (k_i) for different levels of each factor in Table 3, the optimal levels for the four factors were as follows: pH of 0.5, reaction temperature of 130°C, reaction time of 4 h, and moisture content of 92%. This result was consistent with the single factor test results reported in the previous sections.

Fig. 6 presents the foaming properties results. The properties of the protein extracted under the optimum process parameters were also tested (named by Exp. 10). The result was as follows: RP was 91.4%; polypeptides was 9,635 mg/L; and the foamability and foam stability were 660% and 88%, respectively, which met Chinese relevant standards for foam extinguishing agents and foam concrete. In the process of hydrolysis, protein was first hydrolyzed into polypeptides, and then polypeptides were further hydrolyzed into amino acids [19]. Wilson and Novak [36] reported that the increase in temperature reduced the size fractionation of the protein in thermal hydrolysis of sludge, which was converted into smaller molecular weight peptides and VFA. Since the small molecule peptide could quickly enter the gas–liquid interface, unfold and recombine the interface, the foaming properties increased [47]. The optimal conditions controlled the hydrolysis of protein into polypeptides and inhibit their further hydrolysis into amino

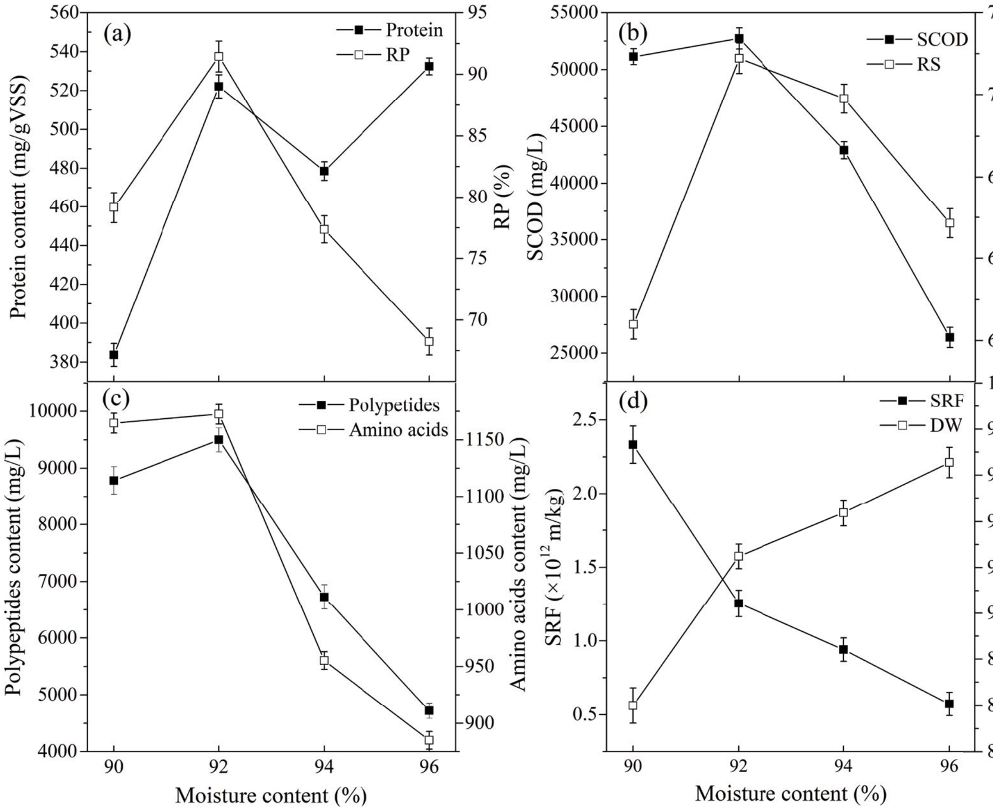

Fig. 5. Effects of moisture content on sludge hydrolysis: (a) protein, (b) SCOD, (c) polypeptides and amino acids, and (d) dewatering performance.

Table 3
Intuitionistic analysis of orthogonal experiment

No.	pH	Time (h)	Temperature (°C)	Moisture content (%)	RP (%)
Exp. 1	0.5	3	120	92	89.4
Exp. 2	0.5	4	130	94	92.4
Exp. 3	0.5	5	140	96	82.5
Exp. 4	1	3	130	96	72.8
Exp. 5	1	4	140	92	83.7
Exp. 6	1	5	120	94	50.1
Exp. 7	1.5	3	140	94	67.4
Exp. 8	1.5	4	120	96	59.9
Exp. 9	1.5	5	130	92	79.7
*k*1	88.1	76.5	66.5	84.3	
*k*2	68.9	78.7	81.6	70.0	
*k*3	69.0	70.8	77.9	71.7	
Range	19.2	7.9	15.2	14.3	

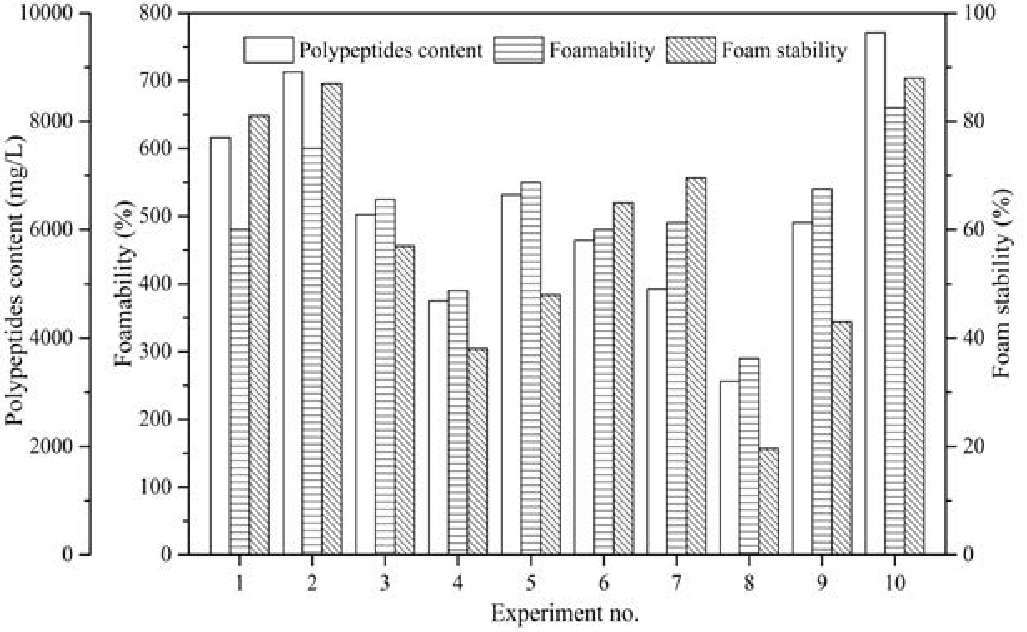

Fig. 6. Orthogonal experiment results.

acids, which facilitated foamability and foam stability. The Pearson correlation coefficient between polypeptides and foamability was 0.89, whereas the correlation coefficient between polypeptides and foam stability was 0.84. Comparing Exp. 1 with Exp. 10 in this study, the protein and polypeptides contents of the latter were 2.0% and 25.2%, respectively, increased compared to the former. Meanwhile, the foamability and foam stability of the latter were 37.5% and 8.6% increased, respectively, compared with the former. This result suggested a close correlation between polypeptides and foaming properties. In addition, the surface performance of foam was improved with the optimization of hydrolysis conditions. The foam produced in optimized conditions was milky white, fine and uniform, whereas that in Exp. 8 was uneven and brown.

4. Conclusions

This study showed the effects of operation parameters (pH, temperature, and time) and moisture content on protein extraction from excess sludge by thermal-acid pretreatment. The optimized conditions were pH of 0.5, reaction temperature of 130°C, reaction time of 4 h, and moisture content of 92%. Under the optimized conditions, the protein extraction rate reached to 91.4%, and the polypeptides content was 9,499.6 mg/L. The foamability and foam stability of the protein solution were 650% and 88%, respectively, indicating that the protein solution could be potentially used as a raw material for foam agents. The dewatering performance of pretreated sludge was improved, and the SRF decreased to 1.25×10^{12} m/kg, which facilitated subsequent protein separation.

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