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Protein extraction from different sludge types by alkaline-thermal hydrolysis

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ABSTRACT

Dewatered sludge (DS) from wastewater treatment plants has been widely studied for protein extraction using alkaline thermal treatment to prepare foam extinguishers or foamed concrete. To explore the protein extraction effects of other types of sludge generated in different treatment stages, this study compared the protein recovery from sludge from the primary settling tank, sludge from the secondary settling tank (SS), thickened mixed sludge (TS), anaerobically digested sludge and DS under the same optimized alkaline thermal hydrolysis conditions (pH = 12, reaction time 4 h, temperature 120°C, moisture content 92%–93%). The results showed that the protein contents in the hydrolysates of TS and SS were approximately 30% higher than that of the commonly used DS, and the protein extraction rate exceeded 90%. The polypeptide content, which is associated with foaming performance, produced by TS and SS was also 30% higher than that of DS. The specific resistance of sludge after hydrolysis of TS was the lowest, which was more beneficial to the subsequent separation of sludge protein. In addition, the moisture content of 95%–96% of TS was closer to the required moisture content of 92%, which was beneficial to the pre-regulation of moisture content. Therefore, TS could be used to replace the commonly used DS for sludge protein recovery.

Keywords: Protein extraction; Alkaline-thermal hydrolysis; Thickened mixed sludge; Dewatered sludge; Polypeptide

1. Introduction

During urban sewage treatment, biological treatment based on the activated sludge process often produces a large amount of excess activated sludge, and its treatment and disposal have become major problems in sewage treatment plants. Excess sludge is mainly composed of microorganisms, microbial oxidation residues, and organic and inorganic substances on the surface of activated sludge [1]. Excess activated sludge contains 20%–60% protein, and it can be extracted to prepare foam extinguishing agents, foamed concrete and other products with high added value [2,3]. Therefore, the hydrolytic extraction of proteins from excess activated sludge has aroused widespread interest.

At present, protein can be extracted from excess activated sludge by acid-thermal [4], alkaline-thermal [5], and biological enzyme methods [6]. Alkaline-thermal treatment has been widely studied and applied because of its high protein extraction rate (R_p), simple equipment and convenient operation [7]. Dai et al. [8] found that alkali-thermal treatment could effectively hydrolyse proteins of different molecular weights. Many studies have revealed that the suitable moisture content of sludge for protein extraction is 92%–94% [9,10]. However, the commonly used

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sludge for protein extraction is dewatered sludge (DS), whose moisture content is usually approximately 80% [11,12]. Therefore, the moisture content must be adjusted in advance to meet the requirements of alkaline-thermal hydrolysis.

In municipal sewage treatment plants, in addition to DS, excess sludge can be obtained from the primary settling tank (PS, moisture content 95%–97% [13]), secondary settling tank (SS, moisture content 99%–99.5% [14]), thickening tank/equipment (TS, moisture content 94%–96% [15]) and anaerobic digestion tank (AS, moisture content 94%–98% [16]). The moisture contents of these sludge samples are higher than 92%. If the samples could be directly dehydrated to a 92% moisture content, this would greatly simplify the commonly used protein extraction process, in which moisture must be added in advance to adjust the DS moisture content from 80% to 92%, and the energy and chemical consumption of the sludge dewatering process could be effectively reduced.

Sludge protein extracted by the alkaline thermal method is suitable for preparing foaming agents. The foaming performance of the sludge hydrolysate mainly depends on the protein content and degree of hydrolysis. Generating more polypeptides by controlling incomplete protein hydrolysis is a key factor in foam formation and the stability of protein solutions [17]. In addition, the dewatering performance of hydrolysed sludge is a critical index with respect to the subsequent separation of extracted proteins. The specific resistance of sludge (SRS) is a comprehensive index used to represent the filtration performance of sludge. Its physical meaning is the resistance of sludge per unit mass per unit filtration area when filtering is conducted under a certain pressure. According to the SRS value, the dewatering performance of different sludge samples can be compared. The higher the SRS value is, the worse the sludge filtration performance [18].

At present, researchers always use DS to extract sludge protein, but PS, SS, TS, and AS, which are also rich in protein, are seldom considered. After adjusting the moisture contents of PS, SS, TS and AS to 92%, the alkaline-thermal method was used to extract protein, and the protein extraction effect was compared with that of DS (moisture content adjusted to 92%). The optimal sludge source was determined by comparing the protein extraction rate, polypeptide content and dehydration performance of hydrolysed sludge obtained by alkaline thermal hydrolysis of each kind of sludge. This study may provide a reference for the practical application of sludge protein recovery processes.

2. Materials and methods

2.1. Experimental sludge samples

The sludge samples used in the experiment were PS, SS, TS, AS and DS from a municipal sewage treatment plant in Zhengzhou that uses an anaerobic-anoxic-oxic (A^2/O) process (as shown in Fig. 1), and the moisture contents of all sludge samples were adjusted to 92% before hydrolysis. TS was a mixture of PS and SS that was centrifugally concentrated after the addition of 0.1% polyacrylamide (PAM). The properties of the raw sludge samples are shown in Table 1.

2.2. Hydrolysis reactor

The alkali-thermal reaction was carried out in a custom-made fast-open miniature reactor (TSZ-GF series). The equipment is shown in Fig. 2, and the equipment parameters are shown in Table 2.

2.3. Experimental process

The experiment was carried out under optimized conditions (pH = 12, reaction temperature: 120°C, reaction time: 4 h, sludge moisture content: 92%) based on a previous experiment [5] using TS. First, the moisture contents of PS, SS, TS, AS and DS were adjusted to 92%, and then, the pH of each sample was adjusted to 12 with 10 M NaOH. Then, 1 L of each adjusted sludge sample was added to the preheated hydrolysis reactor at 120°C for 4 h. During this reaction, the stirring speed of the preheated hydrolysis reactor was maintained between 50 and 100 rpm. Then, the reactor stopped heating, and the valve was opened to reduce the pressure to 0 MPa when the reactor had cooled to approximately 60°C. After cooling to room temperature, the hydrolysed sludge and its supernatant were analysed.

2.4. Analytical methods

The pH was measured by a pH meter (model: PHSJ- 4A, Shanghai Precision Scientific Instrument Co., Ltd., China). Mixed liquor suspended solids (MLSS) and total Kjeldahl nitrogen (TKN) were determined by the determination

Table 1
Properties of sludge samples

Sludge type	Before adjustment		After adjustment		
	Moisture content (%)	TCOD (mg/L)	Moisture content (%)	MLSS (g/L)	TKN (g/L)
PS	93.7	24,000	92.4	65.86	21,428.9
SS	98.8	9,000	92.5	48.93	22,232.03
TS	95.5	30,000	92.8	51.34	23,215.62
AS	96.0	15,000	92.3	43.65	16,946.09
DS	81.6	98,000	92.1	56.9	21,160.94

TCOD – Total chemical oxygen demand; MLSS – Mixed liquor suspended solids; TKN – Total Kjeldahl nitrogen

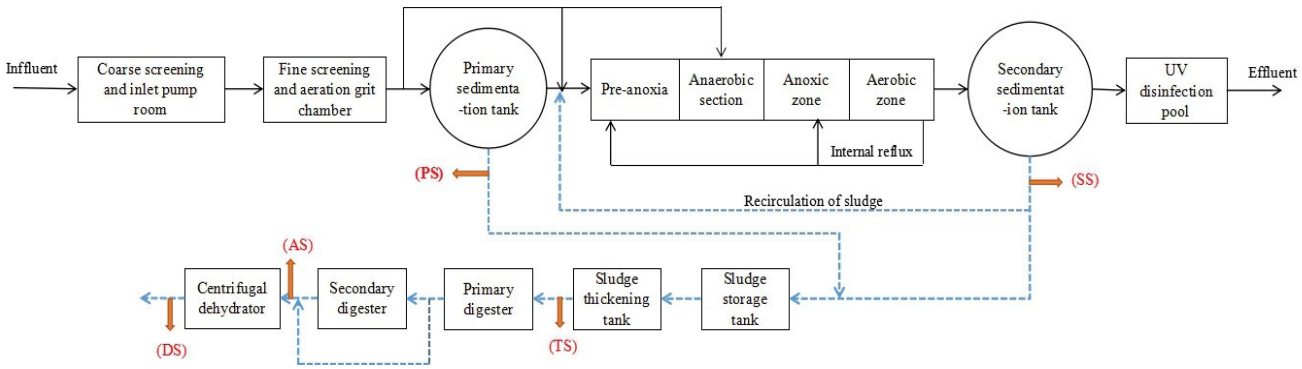

Fig. 1. Process flow diagram of the A²/O wastewater treatment plant.

Table 2
Reactor parameters

Parameters	Values
Volume (mL)	1,500
Heating power (W)	300–3,000
Temperature (°C)	400
Working pressure (MPa)	0–22
Stirring speed (rpm)	0–100

method for municipal sludge in wastewater treatment plants [19].

The protein concentration in the supernatant was measured using a BCA kit (Beijing Pulilai Gene Technology Co., Ltd., China) [20]. Bovine serum albumin (BSA) was taken as the standard sample. Protein was subjected to a complex reaction with Cu^{2+} under alkaline conditions to reduce Cu^{2+} to Cu^{+}, after which the combination of Cu^{+} with the BCA reagent formed a relatively stable purple complex. The absorbance at 562 nm was measured using a UV-visible spectrophotometer, and the protein concentration was calculated according to the standard curve. After this BCA reaction proceeded for 30 min at 60°C, the sensitivity increased to a high level within the range from 5 to 250 μg/mL.

Polypeptides were determined by the biuret method [21], amino acids were stained with ninhydrin [22], total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were determined by the dichromate method [23], and SRS was determined by the Baermann funnel method [24]. The device diagram and process flow of the experimental method are shown in Fig. 3. The viscosity of the filtrate was measured by a viscometer (Changji Geological Instrument Co., Ltd., NDJ-5S, China)

The specific experimental process was as follows: after the filter paper was placed in the Baermann funnel (diameter 65–80 mm), the vacuum pump was turned on, and the vacuum pump valve was closed when the vacuum pressure reached 0.03 MPa. Then, 100 mL sludge was added to the Baermann funnel, and the pressure of the vacuum pump was adjusted. When the pressure reached 0.05 MPa, timing started, and the volume of filtrate in the graduated cylinder was recorded. The volume and viscosity of the filtered liquid were also recorded at

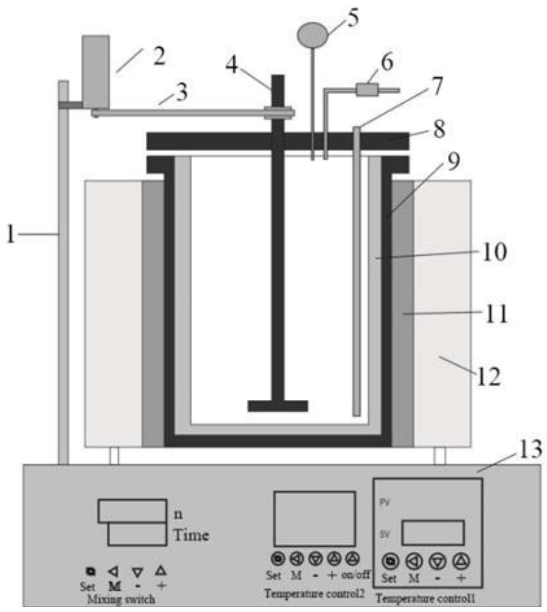

Fig. 2. Fast-open miniature reactor (1 – motor strut; 2 – motor; 3 – conveyor belt; 4 – stirring rod; 5 – pressure gauge; 6 – valve; 7 – temperature sensor; 8 – cover; 9 – reactor body; 10 – polytetrafluoroethylene; 11 – heating ring; 12 – insulating layer; 13 – temperature control system).

intervals until the vacuum environment was destroyed. Then, the vacuum pump valve was closed, and the wet weight of the filtered sludge was determined. The sludge was then placed in an oven at 105°C, and the dry weight was measured after drying. The SRS (s^2/g) was calculated as shown in Eqs. (1) and (2) [25].

$$\mathrm{SRS}\left(\frac{s^2}{g}\right)=\frac{2\mathrm{PF}^2}{\mu}\times\frac{b}{c} \tag{1}$$

$$b\frac{t/V}{V} \tag{2}$$

where P and F denote the vacuum degree (g/cm²) and filtration area (cm²), respectively; μ and b denote the

viscosity of the filtrate (g/cm s) and the slope of the curve of t/V vs. V, respectively; C denotes the dry weight of sludge obtained per unit volume of filtrate (g/mL); V and T denote the volume of filtrate (mL) and the time (s), respectively.

2.5. Calculation methods

The protein extraction rate (R_P) was calculated as shown in Eq. (3) [20].

$$R_P = \frac{M_1}{M_2} \times 100\% \tag{3}$$

where M_1 is the protein content in the supernatant of the hydrolysed sludge, mg/g VSS and M_2 is the protein content in the raw sludge (RS), mg/g VSS.

2.6. Statistical analysis

All analyses were conducted in three groups of parallel tests, the results of which were expressed as the means. The calculations were performed with the statistical program SPSS 20.0 (SPSS Inc., Chicago, USA) and Excel 2013 (Microsoft Office Standard). Analysis of variance was used to determine whether there were any significant differences among the different sludge samples in terms of the protein extraction rate, and differences were considered significant if $p < 0.05$.

3. Results

3.1. Protein contents extracted from different sludge samples

The protein extraction effects of different sludge samples under the same conditions are shown in Fig. 4.

Fig. 4 shows that the protein concentrations of the hydrolysates of TS and SS were relatively high, at 22,019.37 and 19,128.14 mg/L, respectively, and the R_P of TS exceeded 90%. However, the hydrolysed protein contents of PS, AS and DS were relatively low, all of which were between 12,000 and 15,000 mg/L. The R_P of DS, which is commonly used in the protein recovery process, was approximately 30% lower than that of TS. In addition, Fig. 4 shows that although the hydrolysed protein content of DS was higher than that of AS, the R_P of DS was lower than that of AS ($p < 0.05$). Similarly, the difference in protein extraction content between PS and AS was not as significant as that of R_P ($p < 0.05$). This is because PS mainly comprises settleable substances that contain between 40% and 80% organic matter. The quality of the protein extracted from PS was relatively low. The protein content of AS decreased greatly after the stabilization process, but the hydrolysis effect was better.

3.2. Changes in the SCOD of different sludge samples after hydrolysis

SCOD represents the dissolved chemical oxygen demand. The amount of dissolved organic matter in sludge hydrolysate mainly depends on SCOD. SCOD has become the main index to characterize the hydrolysis of sludge and the degree of cell wall breakage [5,7,10].

Fig. 5 shows that the SCOD results for the five kinds of sludge hydrolysate after alkali-thermal hydrolysis were roughly the same as the protein results shown in Fig. 4. The SCOD concentrations of TS and SS were relatively high, 36,372.8 and 32,447.2 mg/L, respectively. However, the SCOD concentration of PS was higher than that of AS and DS, which was different from the concentration distribution of protein. The SCOD concentration of AS was close to that of DS, approximately 19,000 mg/L, which was approximately 50% lower than that of TS.

3.3. Contents of polypeptides and amino acids extracted from different sludge samples

In sludge protein extraction, the obtained protein solution can be used as a foaming agent. Therefore, controlling incomplete protein hydrolysis to facilitate conversion to more peptides is a key factor for foam formation and stability. Fig. 6 shows the amount of peptides and amino acids produced by the hydrolysis of different sludge samples.

It can be seen from Fig. 6 that regardless of what kind of sludge was used, the content of polypeptides obtained

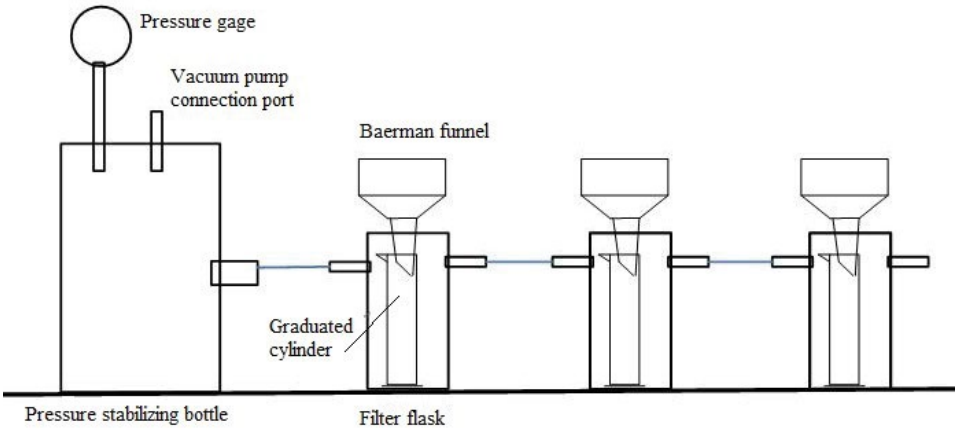

Fig. 3. Device diagram for the determination of SRS.

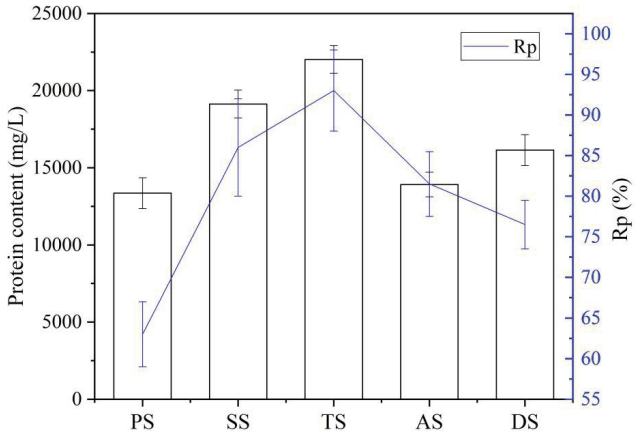

Fig. 4. Protein extraction effects of different sludge samples.

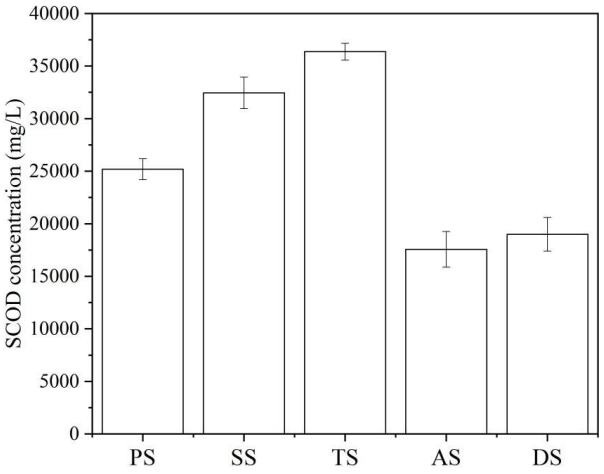

Fig. 5. Changes in SCOD after the hydrolysis of different sludge samples.

by hydrolysis was much higher than that of amino acids, indicating that the alkali-thermal conditions used could effectively control the degree of protein hydrolysis and produce hydrolysis products dominated by polypeptides, which are beneficial to the foaming properties of extracted protein. The contents of polypeptides produced by TS and SS were approximately 6,500 mg/L, and they were 30% higher than those produced by AS and DS.

3.4. Dewatering performance of different sludge samples after hydrolysis

The dewatering performance of sludge after hydrolysis has a direct impact on the subsequent protein separation process. The dewatering performance of the sludge was characterized by SRS. A low SRS value indicates that the sludge has a high dewatering performance.

Fig. 7 shows that the SRS values of different sludge samples after hydrolysis varied greatly. Overall, the SRSs of PS and AS were higher than those of SS and DS. The minimum SRS (1.40×10^9 s^2/g) of TS was 50% lower than that of PS, indicating that the dewatering performance of TS was 50% higher than that of PS. It is generally considered that sludge with an SRS value of 10^9 to 10^{10} s^2/g is difficult to filter, sludge with an SRS value of 0.5×10^9 to 0.9×10^9 s^2/g has a moderate filtration difficulty, and sludge with an SRS value lower than 0.4×10^9 s^2/g is easy to filter [26]. In this test, the SRS of hydrolysed TS was close to the moderate filtration difficulty level and was more than 10 times lower than that of raw TS (SRS = 1.7×10^{10} s^2/g). Thus, it could be concluded that the dewatering performance of TS increased 10 times after hydrolysis, which was beneficial to subsequent protein separation.

4. Discussion

This experiment focused on the suitability of different kinds of sludge for protein extraction. Under the action of NaOH and hydrothermal treatment, the floc structure and microbial cell structure of sludge are destroyed, and intracellular substances can be released [27]. As shown in Fig. 4, the protein contents obtained by hydrolysis of SS and TS were the highest. SS and TS contained a large number of microorganisms and were rich in protein content, so the protein extraction effect was the best. PS mainly comprised settleable substances that contained between 40% and 80% organic matter. The quality of the protein extracted from PS was relatively low, so its R_p was the lowest among the five kinds of sludge. The protein contents of AS and DS decreased greatly after the stabilization process [28], so the extracted protein concentrations were lower than those of SS and TS. Further comparison between SS and TS showed that the protein concentration and R_p of TS were slightly higher than those of SS. SS consists of flocculent sludge particles with a high moisture content produced in the process of biological sewage treatment and is mainly composed of suspended matter in sewage, organic matter adsorbed by microorganisms and microbial metabolic products. Therefore, under the condition of the same moisture content, the content of microbial protein in SS should be higher than that in TS. However, the experimental results showed that the protein content of TS after hydrolysis was higher than that of SS. Therefore, it could be inferred that the addition of PAM to TS interferes with the determination of protein [29]. Similar results were also obtained for AS and DS. Both AS and DS were stabilized sludge, and their protein contents should be similar. However, the addition of 0.3% PAM containing amidogen to DS induced an increase in the measured protein concentration and a decrease in the calculated R_p.

The difference in the SCOD of each sludge sample (Fig. 5) was similar to that of protein concentration in Fig. 4, indicating that the alkaline-thermal hydrolysis process proceeded via cell wall breakage that dissolved protein and released organic matter. Therefore, the SCOD concentrations of TS and SS were similarly high. PS included a large number of settling organic compounds. Alkaline-thermal hydrolysis can promote the hydrolysis of complex polymers and dissolve them into small-molecular organic compounds, so the SCOD concentration of PS was also greatly increased. However, the organic matter contents of AS and DS were low, so the SCOD was relatively low after hydrolysis.

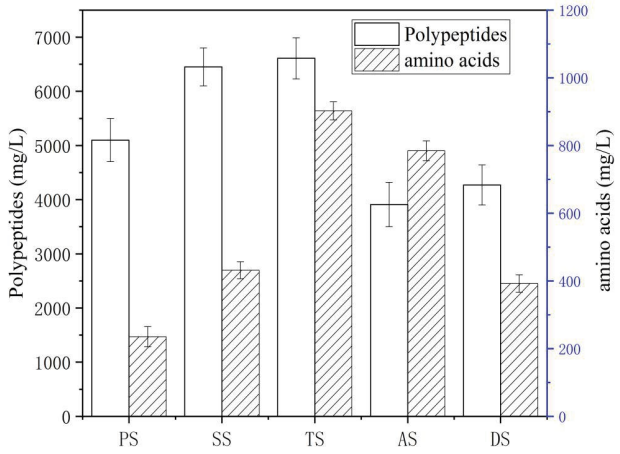

Fig. 6. Polypeptides and amino acids produced by the hydrolysis of different sludge samples.

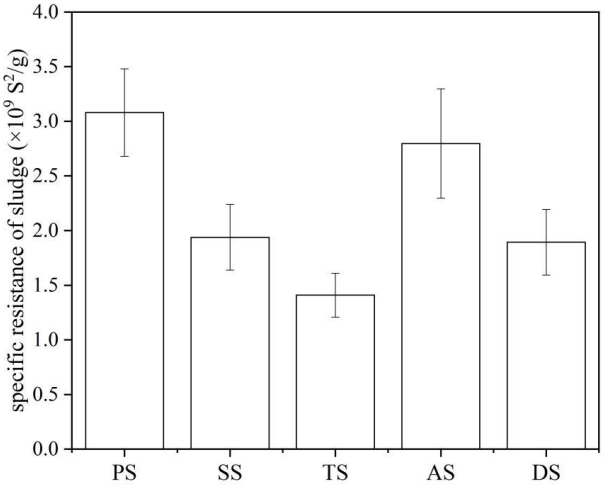

Fig. 7. SRS values of different sludge samples after hydrolysis.

During the protein hydrolysis process, various secondary bonds and cross-linking bonds in protein molecules are gradually destroyed, inducing the dissolution of sludge proteins and breakage of peptide chains; thus, sludge proteins are gradually hydrolysed into polypeptides, and some proteins can be further hydrolysed into amino acids [30]. The recovery of sludge protein is mainly performed to obtain a substrate for the production of foaming agents. Therefore, alkaline thermal conditions should be controlled to hydrolyse proteins into polypeptides and inhibit their further hydrolysis into amino acids. As shown in Fig. 6, the polypeptide contents of these five kinds of hydrolysed sludge were much higher than the contents of amino acids. Further analysis showed that TS and SS had higher protein contents, as shown in Fig. 4, and the highest polypeptide contents, as shown in Fig. 6, so these sludge types would be more suitable than the other types for the preparation of foaming agents by hydrolysis.

For microbial-rich SS and TS, alkaline-thermal hydrolysis could promote the destruction of sludge flocs and microbial cell structure. This kind of damage could not only release interstitial water but also part of the intracellular water so that the dewatering performance of the sludge could be greatly improved. AS and DS were stable, and their hydrolysis effects were limited, so their SRSs were higher than those of TS and SS. However, the addition of PAM [29] to TS and DS increased the size of the sludge particles due to anion interactions, thus reducing SRS and improving the dewatering performance. This improvement mainly occurred because cationic groups on the branched chains of PAM can achieve highly efficient electrical neutralization, adsorption bridging and intermolecular trapping. TS and DS exhibited obvious reductions in SRS, as shown in Fig. 7. For PS, alkaline-thermal hydrolysis hydrolysed and dissolved complex organic and inorganic matter, so the size of PS decreased, and the resultant fragments had a greater specific surface area, leading to the absorption of more free water. Therefore, the dewatering performance of the hydrolysed PS was the worst among the five kinds of sludge.

Overall, the commonly used DS was not a good choice for protein extraction regardless of the R_p or polypeptide content, while TS and SS were obviously better choices. Further analysis showed that the moisture content of SS was usually 99.2%–99.6%, while that of TS was usually 94%–96%, which is closer to the optimal moisture content of 92%. Therefore, considering the complexity of moisture content adjustment prior to hydrolysis, TS would be a more suitable raw material for the recovery of protein as a foaming agent.

5. Conclusions

The extraction of proteins from PS, SS, TS, AS and DS under certain hydrolysis conditions was compared. The protein extraction rate reached more than 90% when SS and TS were used, which was 30% higher than that of the commonly used DS. The polypeptide content of approximately 6,500 mg/L produced by TS and SS was considerably higher than those produced by other kinds of sludge, indicating that the protein solutions derived from TS and SS have better foaming ability. In addition, the dewatering performance of TS was improved 10 times after protein extraction, providing great convenience for subsequent protein separation. The moisture content of TS (95%–96%) was closer than that of DS to the moisture content required (92%) in alkaline-thermal hydrolysis. Therefore, TS is a more suitable raw material for protein recovery than the commonly used DS.

Residual sludge may also contain a variety of toxic and harmful substances [31]. Future research will explore the harmful substances in different types of sludge and the impact of these harmful substances on protein recovery.

Acknowledgements

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References

[1] A. Walczyńska, M. Sobczyk, E. Fiałkowska, A. Pajdak-Stós, J. Fyda, K. Wiąckowski, Interaction between a Bacterivorous Ciliate *Aspidisca cicada* and a Rotifer Lecane inermis: Doozers and Fraggles in aquatic flocs, Microb. Ecol., 75 (2018) 569–581.

[2] C. Wang, H. Liang, Y. Li, J. Hua, Study on preparation of foam extinguishing agent using excess sludge, China Water Wastewater, 22 (2006) 38–42.

[3] L. Qin, Y.X. Yan, J.L. Gao, C.Y. Miao, Extraction of protein from excess sludge by enzymatic hydrolysis, IOP Conf. Ser.: Earth Environ. Sci., 191 (2018) 012069, doi: 10.1088/1755-1315/191/1/012069.

[4] J. Gao, Q. Wang, Y. Yan, Z. Li, W. Weng, Protein extraction from excess sludge by thermal-acid pretreatment, Desal. Water Treat., 209 (2021) 48–57.

[5] J. Gao, Y. Wang, Y. Yan, Z. Li, M. Chen, Protein extraction from excess sludge by alkali-thermal hydrolysis, Environ. Sci. Pollut. Res., 27 (2020) 8628–8637.

[6] J. Gao, W. Weng, Y. Yan, Y. Wang, Q. Wang, Comparison of protein extraction methods from excess activated sludge, Chemosphere, 249 (2020) 126107, doi: 10.1016/j.chemosphere.2020.126107.

[7] Y. Xue, R. Zhuan, G. Zhang, H. Chang, Y. Li, Research progress on sewage denitrification by disintegrated sludge as endogenous carbon source, Mod. Chem. Ind., 38 (2018) 36–39.

[8] Q. Dai, W. Zhang, P.-F. Yu, H. Yi, J.-X. Liu, B.-Y. Xiao, Effects of heat and heat-alkaline treatments on disintegration and dissolved organic matter in sludge, Chin. J. Environ. Sci., 39 (2018) 2283–2288.

[9] J. Cui, A. Dong, W Zhang, F. Miao, Experimental investigation of extracting protein by alkaline thermal sludge hydrolysis from sludge, Chin. J. Environ. Eng., 3 (2009) 1889–1892.

[10] Y. Yan, L. Qin, J. Gao, Research progress of protein extraction from excess sludge, Environ. Eng., 37 (2019) 146–149.

[11] E. Neyens, J. Baeyens, R. Dewil, B. De Heyder, Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering, J. Hazard. Mater., 106 (2004) 83–92.

[12] T.-S. Anton, M.L. Juan, C. Marta, Energetic and economic assessment of sludge thermal hydrolysis in novel wastewater treatment plant configurations, Waste Manage., 92 (2019) 30–38.

[13] M. Li, W. Ma, Q. Ying, Y. Li, B. Xiao, J. Liu, Analysis of anaerobic digestion efficiency and main influencing factors of sludge from primary settling tank, Water Wastewater, 51 (2015) 40–43.

[14] Y. Lei, Y. Liu, Y. Xi, W. Li, X. Zhang, Study of released COD from sludge in secondary settling pond digested by NaOH, J. China Three Gorges Univ. (Nat. Sci.), 37 (2015) 101–104.

[15] H. Zhu, L. Zhang, X. Wen, X. Huang, Feasibility of applying forward osmosis to the simultaneous thickening, digestion, and direct dewatering of waste activated sludge, Bioresour. Technol., 113 (2012) 207–213.

[16] J. Cao, S. Pan, Experiment and analysis on the change of dewatering performance of anaerobic digested sludge, J. China Municipal Eng., 15 (2012), (in Chinese). Available at: https://kns.cnki.net/kcms/detail/detail.aspx?FileName=ZGSZ201203017&DbName=CJFQ2012

[17] J. Mao, Z. Wu, Sludge dewatering properties study on sludge thickening by self-forming dynamic membrane, J. Anhui Agric. Sci., 40 (2012) 5435–5437.

[18] Z. Guo, L. Ma, Q. Dai, J. Yang, R. Ao, L. Wang, Dewatering performance of sewage sludge under pretreatment with modified corn-core powder, Sci. Total Environ., 684 (2019) 402–412.

[19] Ministry of Housing and Urban-Rural Development of the People's Republic of China: Determination Method for Municipal Sludge in Wastewater Treatment Plant: CJ/T 221–2005, 2005, (in Chinese).

[20] M. Ras, E. Girbal-Neuhauser, E. Paul, M. Spérandio, D. Lefebvre, Protein extraction from activated sludge: an analytical approach, Water Res., 42 (2008) 1867–1878.

[21] Chemometrics; Data on Chemometrics Described by Researchers at Jiangsu University (Monitoring of Polypeptide Content in the Solid-State Fermentation Process of Rapeseed Meal using NIRS and Chemometrics), Food Weekly News, 2018.

[22] R.-H. Maria, C. Esther, L. Jordi, L. Joan, Ultrasound, thermal and alkali treatments affect extracellular polymeric substances (EPSs) and improve waste activated sludge dewatering, Process Biochem., 50 (2015) 438–446.

[23] Environmental Pollution; Studies From University of Patras Yield New Information about Environmental Pollution (Coupling Digestion in a Pilot-Scale UASB Reactor and Electrochemical Oxidation over BDD Anode to Treat Diluted Cheese Whey), Ecology Environment & Conservation, 2014.

[24] W. Eckenfelder, D. Ford, Water Pollution Control Experimental Procedures for Process Design, Shanghai Scientific & Technical Publishers, China, 1981.

[25] L. Shan, R. Wang, Improvement of laboratory apparatus for sludge specific resistance determination, Exp. Technol. Manage., 25 (2006) 62–64.

[26] A. Tiehm, K. Nickel, U. Neis, The use of ultrasound to accelerate the anaerobic digestion of sewage sludge. Water Sci. Technol., 36 (1997) 121–128.

[27] H. Carrère, C. Dumas, A. Battimelli, D.J. Batstone, J.P. Delgenès, J.P. Steyer, I. Ferrer, Pretreatment methods to improve sludge anaerobic degradability: a review, J. Hazard. Mater., 183 (2010) 1–15.

[28] Y. Huang, P. Xian, T. Li, Q. Liu, S. He, Two-phase anaerobic digestion performance of food waste and hot alkali treated sludge, J. Environ. Eng., 36 (2018) 119–124.

[29] H. Sheng, G. Xiaoyang, D. Wenhui, C. Xiaowei, Z. Xingguo, L. Zaoyuan, Degradation of high molecular weight polyacrylamide by alkali-activated persulfate: reactivity and potential application in filter cake removal before cementing, J. Pet. Sci. Eng., 174 (2019) 70–79.

[30] R. Parhat, Y. Gao, A. Mourboul, W. Ahmidin, I.J. Uchkun, A. Ayituxun, M. Palida, Y. Abulimiti, Comparative study of different extract methods for protein and peptides from Chinese medicine bovine bone marrow, Chin. Trad. Herbal Drugs, 49 (2018) 3600–3608.

[31] A. Neisi, M.J. Mohammadi, A. Takdastan, A.A. Babaei, A.R. Yari, M. Farhadi, Assessment of tetracycline antibiotic removal from hospital wastewater by extended aeration activated sludge, Desal. Water Treat., 80 (2017) 380–386.