



Study on Extracellular Polymeric Substances (EPS) Extraction Method of Lhasa Municipal Sludge

Yuhang Fei^{1*}, Gaihua Liu², Lincong Wang¹, Jian Lei¹, Lei Zhang¹

¹The First Engineering Bureau Limited Company of China Water Resources and Hydropower, Chengdu 610000, China

²School of Economics and Management, Tibet University, Lasa 850000, China

Abstract: Sludge contains large number of EPS and the main components are protein, polysaccharide, hemic acid and so on, which is a great kind of carbon source. Therefore, using a method to extract the EPS and reflux to the biochemical tank can not only provide sufficient carbon source for microorganisms, but also solve the problem of sludge treatment and disposal in sewage plants. This passage compared five different extraction methods to study the extraction effect of EPS of Municipal Sludge. The results showed that the content of EPS components obtained by UN method was higher than that of the other four methods, which indicated that the UN method was better than the other four methods in extracting EPS; Through the comparison and analysis of the SCOD content and microscopic morphology of sludge EPS, it was found that the SCOD content obtained by UN method was the highest, and the corresponding sludge flocculant morphology was destroyed more thoroughly, so the best method was UN method.

Key words: Municipal Sludge; Extraction method; Municipal Sludge; Carbon source

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***Corresponding author:** Yuhang Fei, 1278796375@qq.com

1 Introduction

With the improvement of living standard, the water consumption of residents is increasing, which forces the discharge of sewage to increase significantly. As a result, the amount of sludge is also increasing

significantly. At present, there is a lack of carbon source in the biochemical tank caused by the single composition of sewage in Tibet municipal sewage treatment plant, which affect the effect of denitrification and phosphorus removal. Besides, the sludge production of sewage plant is large, and the traditional way of mechanical dewatering and landfill is adopted, which not only causes secondary pollution to the environment, but endangers human health. For solving the above problems, it is urgent to find a suitable EPS extraction method.

Common methods in EPS extraction included physical extraction and chemical extraction. Physical extraction methods mainly include thermal extraction, ultrasonic extraction and so on. Among them, ultrasonic extraction method is widely used because of its good extracting effect and green environmental protection. Cui ultrasonic method was used to study EPS extracting^[1]. It was found that ultrasonication could decompose sludge particles to a large extent and convert protein substances into small molecules. Tian adopted ultrasonic method to extract EPS and analyzed the effect of cracking^[2]. The result showed that the concentration of organic matter released from sludge increases significantly by ultrasonic method.

Although ultrasonic method could promote the dissolution of organic matter in sludge, it was difficult to extract only by ultrasonic method. Therefore, many researchers used chemical extraction method to study the cracking effect of sludge. Chemical extraction methods included NaOH method, cation exchange resin method and so on. Among them, NaOH was widely used because of its easy access and low cost. Pelleria studied

the effects of alkali pretreatment on anaerobic digestion of brown sludge^[3]. It was found that NaOH pretreatment could promote the solubility and biodegradability of brown sludge and also affect the amount of methane produced in subsequent anaerobic process. Zou through using treatment with alkali to study the anaerobic digestion of sludge^[4]. The result showed that alkali treatment could significantly increase the content of soluble organic matter and polysaccharides in sludge. Although the content of organic matter extracted from sludge by NaOH method was high, there were some problems, such as corrosion-prone equipment.

To sum up, this paper mainly aimed at the shortage of carbon source and the difficulty of sludge treatment in the treatment of plateau municipal sewage, by adopting U1,Na,UN, FN and UFN methods to study the extraction effect of EPS sludge. Through determining the content of SCOD, polysaccharides, proteins and DNA in the extract and observing the broken morphology of sludge by electron microscope (EM) and scanning electron microscope (SEM) to evaluate the effect of EPS extraction and provide basic theory and value reference for future research.

2 Materials and methods

2.1 Sample sources

The activated sludge sample came from Lhasa municipal sewage treatment plant. The flocculent structure of sludge was spherical or ellipsoid and dense. Collected sludge was immediately transferred to the laboratory and stored in a 4°C refrigerator before use.

2.2 EPS Extraction methods

This experiment used the untreated sludge samples as a control group and adopted the U1,Na,UN, FN and UFN methods to study the EPS extraction experiment. The specific experiments were as follows:

(1)U1:40 kHz ultrasonic (100w), ultrasonic time was 1-30min;

(2)Na: add NaOH 1mol/L solution to adjust pH to 12, stir 1-30 min with agitator;

(3)FN:add 36.5% formaldehyde solution to the reactor and store 1 h in 4°C refrigerator; then add NaOH 1mol/L to the reactor and store 3 h in 4°C refrigerator;

(4)UFN:follow the steps in steps 3) and 1);

(5)UN:follow the steps in steps 1) and 2).

After extraction, some samples should be placed in centrifuge (10000r/min) for 10min, to filter the

supernatant, and the filtrate would be collected for determination and analysis of related indexes.

2.3 Analysis methods

2.3.1 EPS analysis

EPS was mainly composed of proteins, polysaccharides and DNA. Protein was determined by Lowry method and bovine serum albumin was used as a standard substance; Polysaccharide was determined by anthrone-sulfuric acid colorimetric method and glucose was used as a standard substance; DNA was tested by diphenylamine and sodium salt of calf thymus was used as a standard substance.

2.3.2 sludge degradation characteristics

SCOD was determined by a potassium dichromate method. Calculation formula of SCOD change value:

$$\text{SCOD} = \text{SCOD}_t - \text{SCOD}_1$$

SCOD_t: COD concentration in sludge supernatant after t min was solved by different methods, and the COD concentration in sludge supernatant was SCOD₁.

2.3.3 sludge image observation

To study the destruction degree of sludge flocculant during extraction, the cracking morphology of sludge under the same extraction time was observed by EM and SEM.

3. Results and discussion

3.1 Comparative analysis of EPS components obtained by different extraction methods

3.1.1 Comparative analysis of protein extraction from different extraction methods

Different extraction methods would affect the extraction of each component in the EPS. Fig.1, Fig.2 and Fig.3 reflected the effects of five extraction methods on the amount of protein, polysaccharide and DNA extracted from EPS, respectively.

It could be seen from Fig.1 that the amount of protein extracted increased with the prolongation of extraction time. By comparing the contents of protein extracted by five methods, we found that the UN method of protein extraction was the best, and they were 1.78, 2.07, 3.09 and 4.07 times of Na, UFN, U1 and FN, respectively. With the increase of pH, the protein in the EPS increased obviously. Liss found that EPS surface had a large number of negative charges^[5]. It could affect the electrostatic interaction with other substances and

the formation of hydrogen bonds, and then affect the amount of extraction. Therefore, alkaline conditions could destroy the cell wall of microorganisms and change the water solubility of EPS, so that a large number of proteins could be released, which led to an increase in the amount of extraction^[6]. This could explain that the UN method played a role in promoting protein extraction.

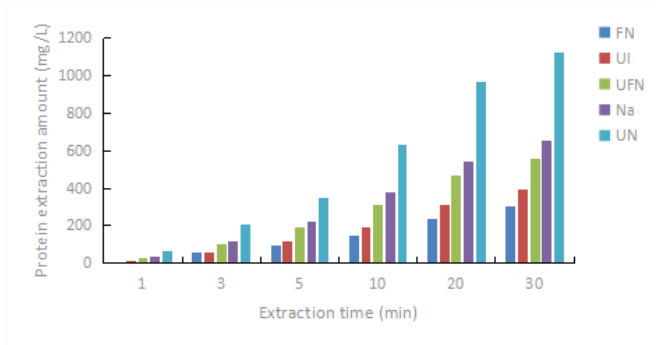


Figure 1. Effect of different extraction methods on protein extraction

3.1.2 Comparative analysis of polysaccharides extraction from different extraction methods

In the process of experiment, the ability of extracting polysaccharides by different extraction methods was different. The change of polysaccharide content extracted by different methods was shown in fig.2.

Fig.2 compared the total amount of polysaccharides extracted by different methods. The order of polysaccharide content from more to less was: UN > UFN > UFN > UI. Among them, the total amount of polysaccharides extracted by the UN method was 1.9 and 2.1 times of the UFN method and Na method, respectively. The total amount of polysaccharides extracted by the UFN and Na methods was very close and it was 163.43 and 180.71 mg/L, respectively. The content of polysaccharides extracted by the FN and UI methods was 83.20 and 109.23 mg/L, which was relatively small.

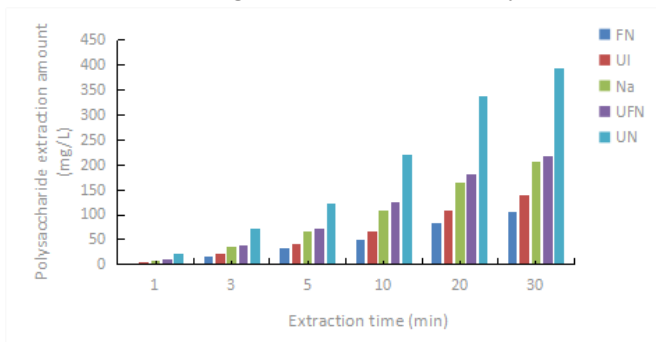


Figure 2. Effect of different extraction methods on extraction of

3.1.3 Comparative analysis of extraction DNA by different extraction methods

It could be seen from fig.3 that the content of DNA components extracted by different extraction methods in the same time was different, and the content of DNA components extracted by the same method in different extraction times was also different. The DNA content extracted by the UN method was the most, which may be due to the fact that the UN method could not only make the EPS dissolve, but also cause the sludge microbial cell wall to destroy and cause the related substances to flow out. Through comparing the FN method and the UFN method with the other three methods, it was found that the DNA effect of the two methods was poor and it may be due to the protection of microbial cells by adding formaldehyde in the experimental operation, which in turn showed that the UN method and the Na method may destroy biological cells in the extraction process. The figure also showed that the DNA content extracted by the UN method was higher than that extracted by the Na method. It could be concluded that the combined method was better than the single method. The conclusion was consistent with Cédric^[7].

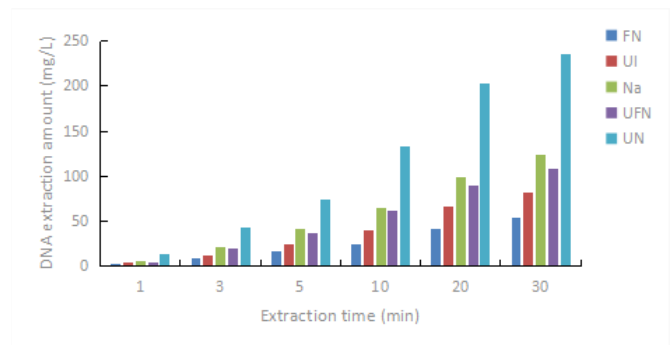


Figure 3. Effect of different extraction methods on DNA extraction

3.2 Effect extraction of sludge EPS

3.2.1 Effects on SCOD values

It could be seen from fig.4 that with the increase of cracking time, the change trend of SCOD value in sludge was very obvious. By comparing the five methods, the release SCOD of the UN method was superior to other methods in sludge cracking effect. 1, 10 and 20 min later, SCOD value added was 285.94, 2826.68, 4329.89 mg/L, respectively and 30 min later, cracking the sludge SCOD increased to 5036.57 mg/L. We could see that when the sludge cracking time changed from 1 to 20 min, the SCOD dissolution value increased fast-

er, and when the time changed from 20 to 30min, the increasing trend of the sludge dissolution value slowed down.

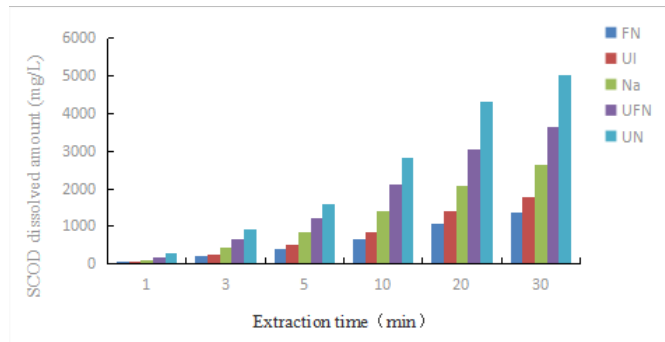


Figure 4. Changes of SCOD values with extraction time under different extraction methods

3.2.2 Sludge Structure Observation and Analysis

(1) EM observation and analysis

Using electron microscope to observe sludge flocculants could directly explain the effect of different methods to crack sludge 20min. Fig.5 showed the changes of sludge flocculent structure in different treatment methods during the same treatment time. It could be seen from fig.5 that the degree of sludge damage would be very different under the same treatment conditions and the same action time. Fig.A showed untreated sludge: flocculent structure was intact, surface was rough, and adhesion between micelles was very close. Through the observation of fig.B to fig.F, it was found that the sludge flocculent became dispersed and the color became lighter and the structure of sludge flocculent was incomplete. As a result, the degree of cracking of fig.F was more thorough and the corresponding method was UN method.

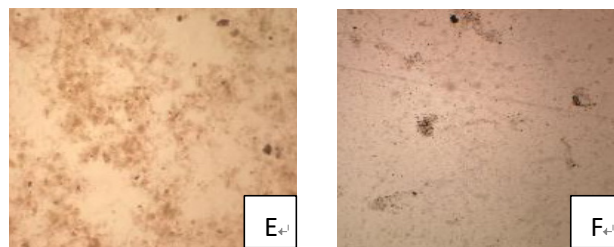
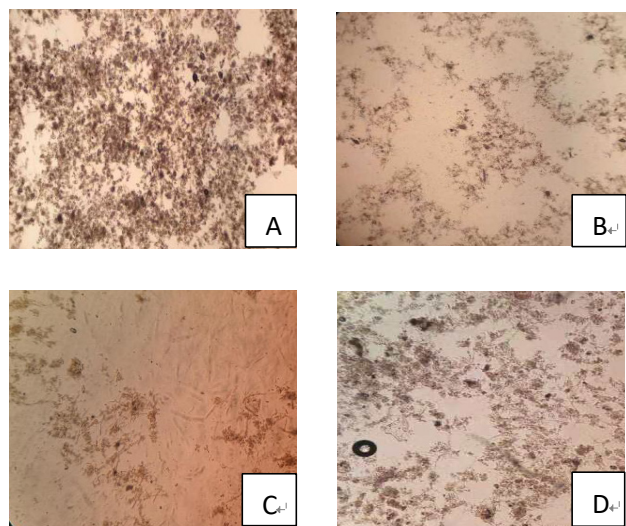


Figure 5. EM Image of Cracking Sludge by Different Methods at the same Time

(2) SEM observation and analysis

SEM can be used to further explore the microscopic morphology of sludge treated by various methods. The composition of EPS matrix in sludge flocculants was complex and uneven, and the spatial distribution of EPS played an important role in floc structure. Fig.6(A) was an original sludge sample, which was characterized by loose accumulation of sludge flocculants and arrangement of sludge flocculants into single pieces. By using U1, FN, UFN, Na and UN methods to crack sludge. Figs.6(B) to (F) were the images observed under scanning electron microscope. By contrast, EPS and bacteria in sludge flocculants were damaged in varying degrees. The most thorough destruction was the UN method, where the sludge flocculent morphology changed significantly, the sludge residue was relatively loose, the volume was small, the filament structure and the bacterial cell wall were completely dissolved.

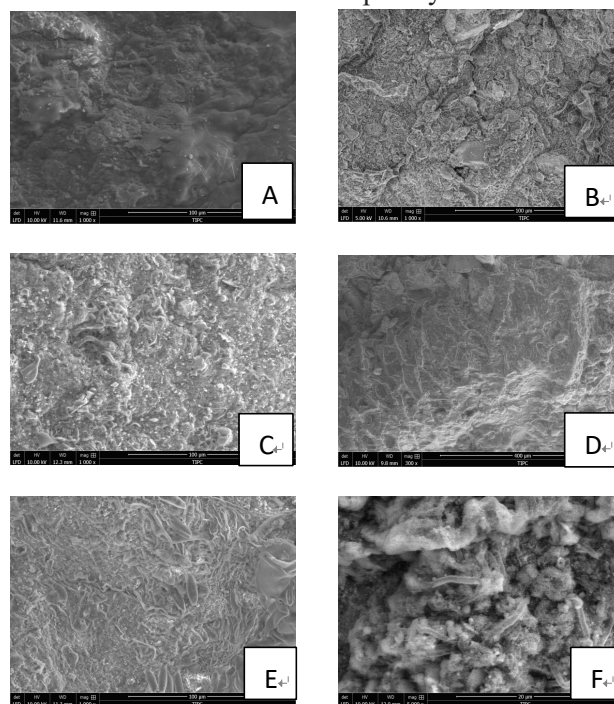


Figure 6. SEM images of sludge cracking using different extraction methods at different extraction times

4. Conclusions

This paper used five methods to extract EPS from sewage treatment plant. The conclusions were as follows:

FN, UI, Na and UFN methods could extract EPS in varying degrees, but because the content of each component extracted was extremely limited, it was necessary to improve the experimental conditions; Through the comparison and analysis of EPS components, SCOD content and sludge cracking morphology obtained by different extraction methods, it was found that the extraction EPS effect of UN method was better than other methods. The contents of the relevant indexes were 962.86, 337.52, 202.50 and 4329.89mg/L, respectively.

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