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The Influences of Sodium Hexametaphosphate (SHMP) and Methyl Orange (MO) on Enzyme Hydrolysis of Excess Sludge

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Abstract: The effect of Sodium Hexametaphosphate (SHMP) and Methyl Orange (MO) on the hydrolysis of sludge were investigated under the optimum condition of alkaline protease hydrolysis. The results showed that as the concentration of SHMP was increased, the content of Soluble Chemical Oxygen Demand (SCOD), protein, and polysaccharide in the sludge hydrolysate gradually raised. When the concentration of SHMP was 0.1g/g·tss, the contents of SCOD, protein, and polysaccharide increased by 17.16%, 50%, and 9.72% respectively. With the increase of MO concentration, SCOD decreased gradually. When the concentration of SHMP was 0.02g/g·tss, the SCOD decreased by 10.3%. An increase of 13% in the protein content was noted, when the concentration was 0.02g/g·tss. On the contrary, there was a slight reduction of the polysaccharide content i.e. 2.4%. The concentration of the polysaccharide was found to be the lowest at the concentration of 0.002g/g·tss. From the aforementioned results combined with three-dimensional fluorescence map of sludge EPS, it was found that SHMP promoted the hydrolysis of sludge by alkaline protease and accelerated the dissolution of intracellular organic matter. Herein, the MO inhibited alkaline protease hydrolysis of sludge, which hindered the dissolution of intracellular organics.

Keywords: Alkaline Protease, Sodium Hexametaphosphate, Methyl Orange, Three-Dimensional Fluorescence Spectroscopy

1. Introduction

With the rapid development of Chinese industry, the effluent of industrial wastewater and industrial sludge is an emerging problem. The discharge of printing and dyeing wastewater accounts for 10% of the country's industrial wastewater. The annual average discharge of textile printing and dyeing wastewater is about 2.1 billion tons and the annual average sludge discharge is about 21 million tons [1]. Printing and dyeing sludge, mainly including biochemical sludge and materialized sludge, comes from the various processes of printing and dyeing wastewater treatment channels. Biochemical sludge remained after biological treatment of printing and dyeing wastewater that mainly comes from the activated sludge processing unit. Its organic content is high (generally less than 50%) [2, 3] and sludge particles are small floccules with high moisture content, relatively low density and poor stability. Materialized sludge is obtained by chemical treatment (such as coagulation, oxidation, and electrolysis) [4]. It mainly comes from the coagulation and sedimentation of wastewater or coagulation flotation unit. Its sludge particles are large and easy to settle. The moisture content is low while relative density is high and stability is good [5].

The chemical composition of printing and dyeing sludge is complex. It contains a large number of toxic organic pollutants (such as dyes, additives, polycyclic aromatic hydrocarbons - PAH, aromatic amines) and heavy metal elements (such as zinc, copper, lead and chromium) [7]. Dyeing sludge is a kind of strong pollutants and was identified as hazardous industrial waste [6]. The nature of printing and dyeing sludge is directly

influenced by the production methods, types, contents of dyes, additives. The composition and properties of printing and dyeing sludge in different factories are different, which increases the difficulty of the treatment of printing and dyeing sludge.

The disposal methods of dyeing sludge in the worldwide are mostly based on the treatment and disposal methods of urban sludge, mainly including landfill, incineration and land use. In recent years, scholars have studied some new sludge treatment technologies. Zhang Hedong et al. used microwave pyrolysis technology to treat dyeing sludge. It was found that adding CaO at 550°C can maximize the surface area of peat. It enriched with large number of heavy metal elements in the dyeing mud on the peat surface [8]; Feng Yinfang used ultrasonic-coupled potassium ferrate to enhance the dewatering of the dyeing sludge and found that low acoustic energy density and short ultrasonic time can promote sludge dewatering performance. The addition of 60mg/g potassium ferrate also contributes to its dehydration [9]; Chen Hao's research shows that the use of acidophilus acid bioleaching combined with Fenton oxidation method can increase the organic carbon content in the sludge supernatant from 20.8 mg/L to 356.6 mg/L and the water content from 88.75% to 82.82% [10].

Luo Kun studied extra-enzymes to strengthen the hydrolysis of excess sludge and found that added enzymes could promote the dissolution of suspended solids in sludge and the degradation of macromolecule. The addition of enzymatic hydrolysis technology can destroy the EPS structure of the sludge and allow the dissolution of intracellular organic substances, thereby reducing the solid sludge and achieving the purpose of reduction. This method has the advantage of short digestion time, good digestion performance [11], and economical efficiency [12], with no environmental side-effect [13]. However, little research has been done to apply this technique to the treatment of printing and dyeing sludge. In this paper, the dyestuff methyl orange and sodium hexametaphosphate (a printing and dyeing assistant) were selected as the research object to simulate the dyeing sludge and to study its effect on the enzymatic hydrolysis of sludge.

2. Material and Methods

2.1. Reagents and Instruments

The sludge was taken from the Songjiang Sewage Treatment Plant in Shanghai. It is a resultant sludge from the secondary sedimentation tank. Samples were stored at 4°C and refrigerated. Sludge properties are shown in Table 1.

Table 1. *Sludge properties.*

Sludge properties	Value
pH	6.88
SCOD ($mg \cdot L^{-1}$)	104
TSS ($g \cdot L^{-1}$)	4.131
VSS ($g \cdot L^{-1}$)	2.77
Polysaccharide ($mg \cdot g^{-1}$)	3.68
protein ($mg \cdot g^{-1}$)	0.48
SOUR ($mgO_2/gMLSS \cdot h$)	11.3

SOUR is the rate of deoxygenation, characterizing sludge activity. (SOUR between 5 and10 means sludge activity is poor, and 10-40 activity is better [14-16])

Alkaline protease was selected for the experiment. The basic parameters of the enzyme are shown in Table 2.

Table 2. *Enzyme parameters.*

Alkaline protease	Value
Enzyme Activity (U/mg)	≥2000
Suitable pH	7-12
Suitable temperature (°C)	40-60

Reagents: Methyl Orange (MO), Sodium Hexametaphosphate (SHMP), Phosphate Buffer Solution pH=8.0, Potassium Dichromate Standard Solution (Analytical Pure), Tricholine Titril (Analytical Pure), Concentrated Sulfuric Acid (Analytical Pure), Sulfuric Acid Silver (Analytical Pure), Mercury Sulfate (Analytic Pure), etc.

Main instruments: Analytical balance (JA31002, Shanghai), water bath thermostatic oscillator (DKY-11, Shanghai), centrifuge (SL-16R, Theermo Fisher, USA), microwave oven (M1-211A 21L, Midea), thermostatic drying oven (BPH -6063, Shanghai), Fluorescence Spectrometer (QuantMaster 40, USA), UV Spectrophotometer (TV-1810, Shanghai), Dissolved Oxygen Meter.

2.2. Analytical Methods

Analytical methods: TSS and VSS were determined by gravimetric method; CODcr was determined by microwave digestion-the potassium dichromate method [17, 18]. The chemical oxygen demand of the supernatant was measured after SCOD was centrifuged at 11000 r·min-1 for 20 minutes. Polysaccharides were determined using the sulfuric acid-anthrone method [19]; Proteins were determined using the Coomassie brilliant blue method [20]. SOUR test method is sensitive electrode-dissolved oxygen analyzer method [14, 16].

2.3. Experimental Procedure

Take 12 250ml conical flasks, numbered 1-12. Add 50 ml of sludge to the 1-12 conical flask and add 0.1g/g·tss of alkaline protease. The 12 Erlenmeyer flasks were divided into two groups, from group 1-6 were group A, and 0g/g·tss, 0.01g/g·tss, 0.03g/g·tss, 0.05g/g·tss, 0.1g/g·tss, and 0.15g/g·tss were respectively added SHMP, Nos. 7-12 are in Group B, and 0g/g·tss, 0.002g/g·tss, 0.005g/g·tss, 0.01g/g·tss, 0.015g/g·tss, and 0.02g/g·tss MO were added respectively.

Adjust the pH to 11. Place the 12 Erlenmeyer flasks in a thermostatic oscillator, reacted at 130r/min and 55°C for 4h and then take them out. Put them into 100 ml centrifuge tubes and centrifuged at 11000r/min for 20 minutes. The sample was filtered.

Take 6 Erlenmeyer flasks, numbered 1-6, add 50ml sludge to the 1-6 Erlenmeyer flask respectively, No. 1 is blank control group, No. 2 sample is added with 0.1g/g·tss alkaline protease. No. 3 and No. 4 were sequentially added with 0.1g/g·tss SHMP and 0.02g/g·tss MO. Sample Nos. 5 and 6 were dosed with 0.1g/g·tss SHMP and 0.02g/g·tss MO, respectively. Adjust the pH to 11. Place the 6 Erlenmeyer flasks in a thermostatic oscillator, reacted at 130r/min and 55°C for 4h and then take them out. Put them into 100ml centrifuge tubes and centrifuged at 11000r/min for 20 minutes. The samples were filtered.

Three-dimensional fluorescence spectrometry conditions: excitation wavelength λex is 220 nm to 550 nm, emission wavelength λem is 240 nm to 700 nm, wavelength interval is 5 nm, and slit width is 0.8 nm.

3. Results and Discussion

3.1. Effect of SHMP on Enzymatic Hydrolysis of Sludge

The effect of SHMP on the hydrolysis of organic matter, proteins, and polysaccharides from alkaline protease hydrolysis sludge is shown in Figure 1 and Figure 2.

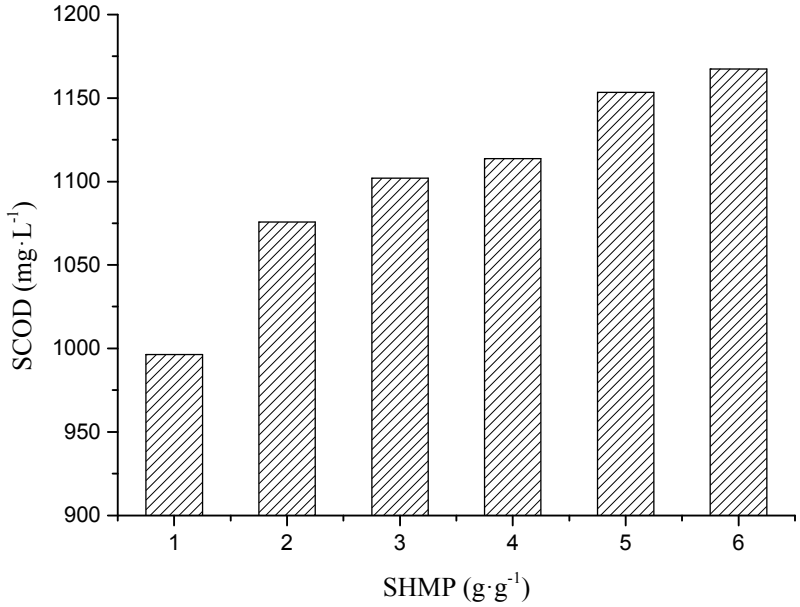
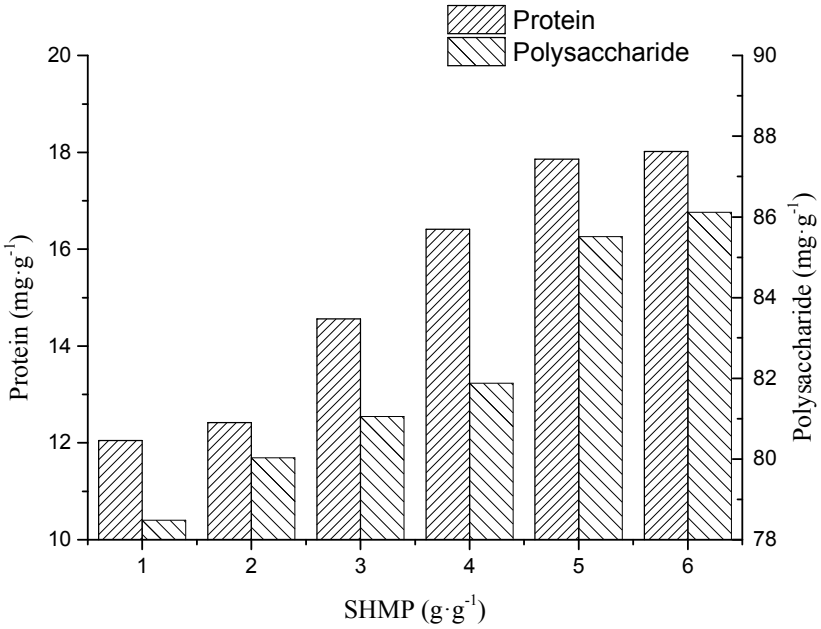


Figure 1. *SCOD changes with SHMP concentration.*



1-6 are SHMP concentrations of 0, 0.01, 0.03, 0.05, 0.1, 0.15g/g·tss, respectively.

Figure 2. *Protein and polysaccharide content changes with SHMP concentration.*

As can be seen in Figure 1, SCOD gradually increases with the increase of SHMP concentration. When the SHMP was added at 0.01g/g·tss, the SCOD value was 1075 mg/L. Compared to the case where SHMP was not added (SCOD was 996.44 mg/L), SCOD increased by 7.9% When the dosage reached 0.1g/g·tss, the SCOD value reached 1153.38 mg/L, an increase of 17.16%.

From Figure 2, it can be seen that with the increase of SHMP concentration, the content of protein and polysaccharide gradually increases. The protein content was 12 mg/g when no contaminants were added. When the dosage was 0.01g/g·tss, the protein concentration reached 18 mg/g, an increase of 50%. The polysaccharide content increased from 78.48mg/g to 86.11mg/g, which was an increase of 9.72%.

The content of SCOD, protein and polysaccharide in the hydrolysate increased after the addition of SHMP. The reason may be that sodium hexametaphosphate acts as a dispersant, which can more evenly disperse the sludge and enzyme particles, thereby increasing the contact between the enzyme and the sludge. At the same time as a surfactant, it can promote the dissolution of carbohydrates and proteins [21]. The EPS structure was broken which increased the contact between the enzyme and the substrate and promoted the hydrolysis of the enzyme.

3.2. Effect of MO on Enzymatic Hydrolysis of Sludge

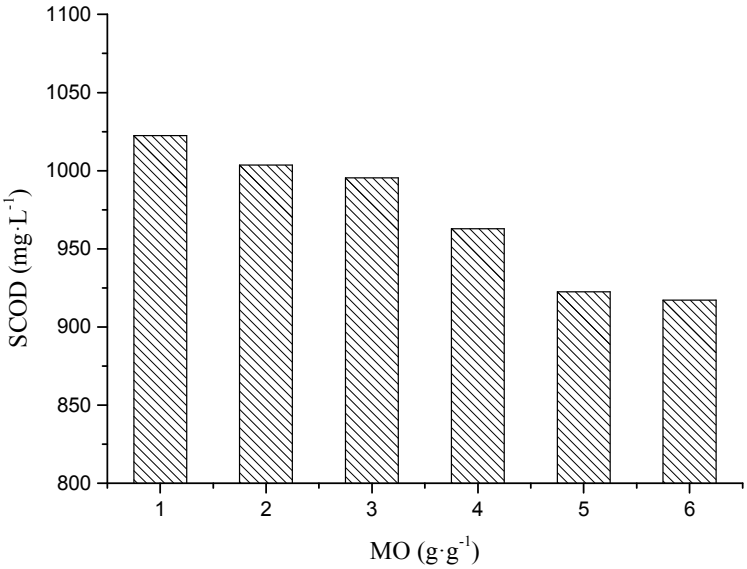
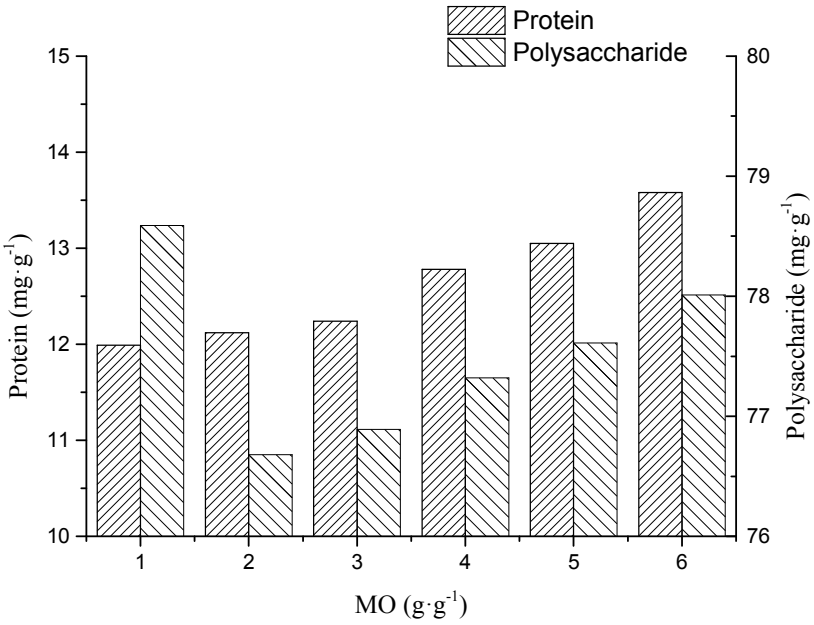


Figure 3. *SCOD changes with MO concentration.*



1-6 are MO concentrations of 0, 0.01, 0.03, 0.05, 0.1, 0.15g/g·tss, respectively.

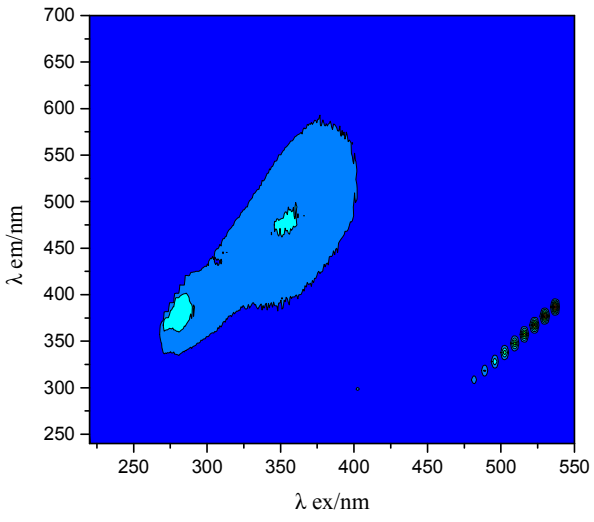
Figure 4. *Protein and polysaccharide content changes with MO concentration.*

From Figure 3, it can be seen that as the MO content increases, the SCOD value gradually decreases. When the MO dosage was 0.02g/g·tss, the SCOD value was 917.27 mg/L. Compared with the non-dosed MO (SCOD value 1022.4 mg/L), the SCOD was reduced by 10.3%.

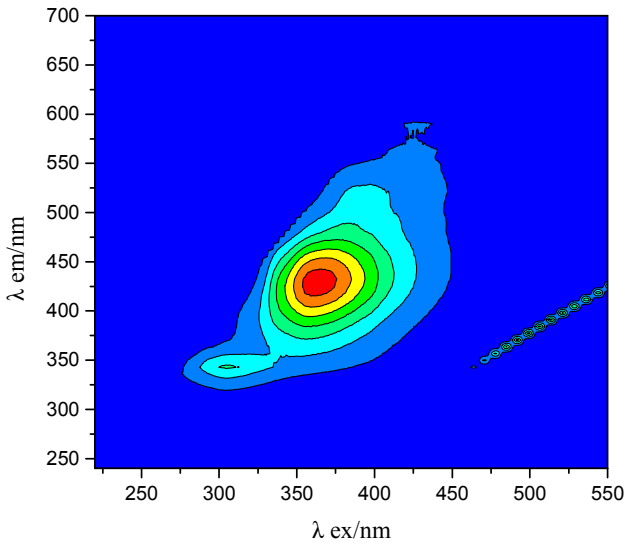
As can be seen from Figure 4, the protein content increased with increasing MO concentration. When 0.02g/g·tss MO was added, the protein content reached 13.58 mg/g, an increase of 13%. With the increase of MO concentration, the polysaccharide content decreased firstly. After the concentration of MO was more than 0.002g/g·tss, the polysaccharide content gradually increased but it was always lower than that when it was not added. When 0.002g/g·tss MO was added the polysaccharide concentration was reduced from 78.59 mg/g when undosed to 76.68 mg/g, a decrease of 2.4%. When the MO dosage was 0.02g/g·tss, the polysaccharide content was 78.01 mg/g and the polysaccharide was reduced by 0.7%.

After the addition of MO, SCOD gradually decreased that indicating the dissolution of organic matter in the sludge was reduced. The reason may be that the added MO will adsorb on the surface of the sludge which hindering the contact of the enzyme with the sludge and at the same time hindering the dissolution of the organic matter [22]. The gradual increase in protein content may be due to the addition of MO to stimulate organisms such as microorganisms and bacteria in the sludge. As the concentration of MO increases the microorganisms in the sludge will secrete more EPS to resist extracellular toxic substances through metabolism in the new city by adsorption in the environment or autolysis of the cells, thereby protecting themselves [23]. The polysaccharide content decreased first, probably due to the adsorption of MO on the sludge surface. It reduces the content of dissolved polysaccharides. At the same time, the amount of polysaccharides secreted by microorganisms is less and with the increase of the concentration of MO the polysaccharides secreted by microorganisms gradually increases.

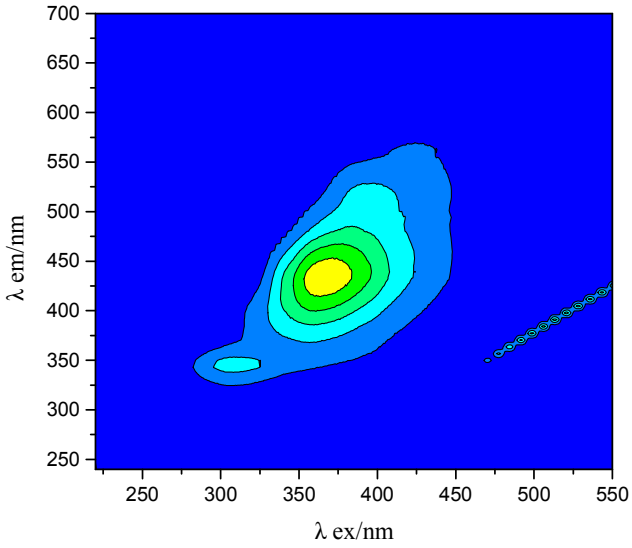
3.3. Three-Dimensional Fluorescence Changes of EPS After Sludge Hydrolysis



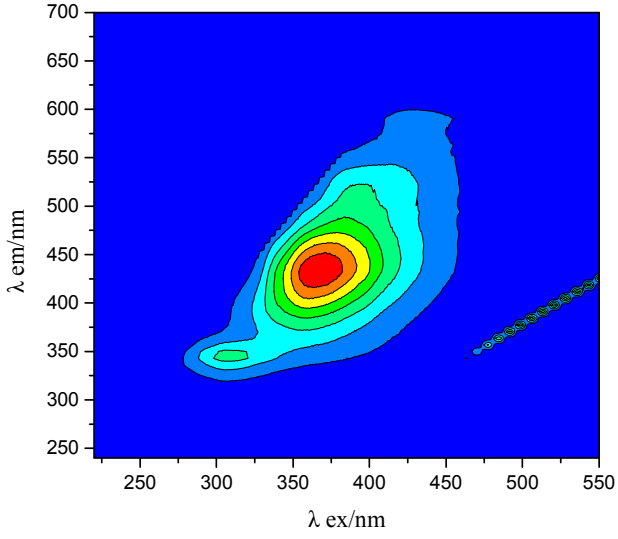
(a)



(b)



(c)



(d)

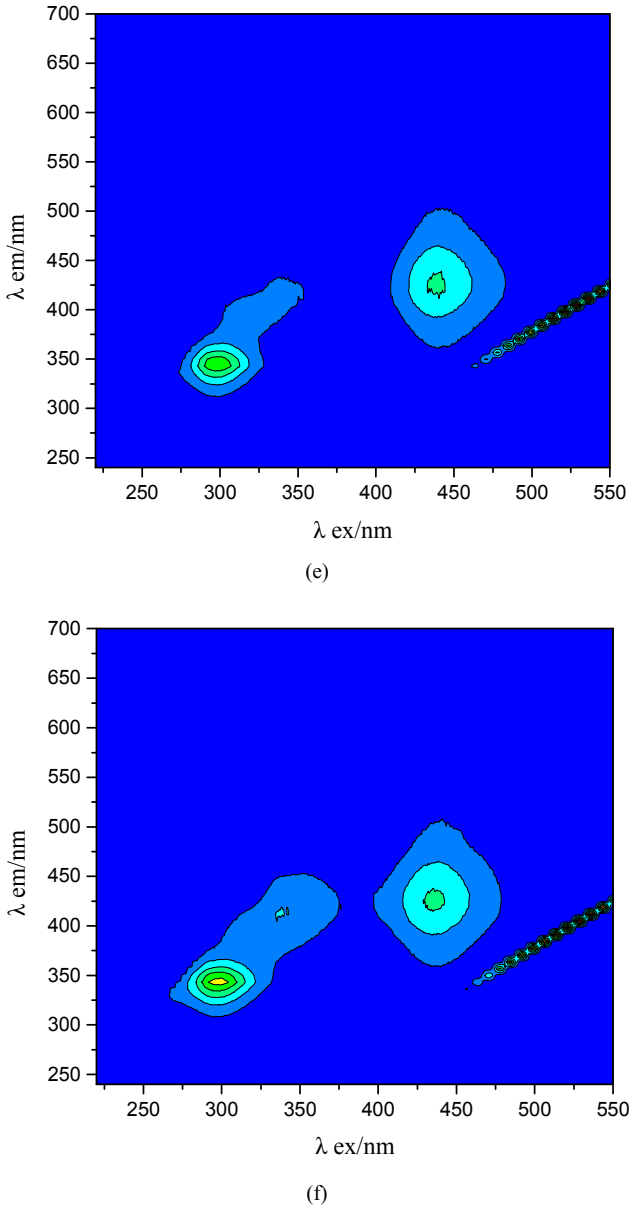


Figure 5. *EPS three-dimensional fluorescence image of sludge hydrolysis.*

(a) raw sludge, (b) sludge + 0.1g/g·tss alkaline protease, (c) sludge + 0.1g/g·tss SHMP, (d) sludge + 0.1g/g·tss alkaline protease + 0.1g/g·tss SHMP, (e) sludge + 0.1g/g·tss Alkaline protease + 0.02g/g·tss MO, (f) sludge + 0.02g/g·tss MO

The fluorescence intensity of c is greater than a, indicating that the addition of SHMP reacts for a large amount of organic matter after being reacted for 4 h at 55°C and pH=11. The fluorescence intensity of d is greater than b, which indicates that under the same reaction conditions, the content of protein and humus dissolved in 0.1g/g·tss was increased compared with the case where it was not added, which is consistent with the results of 3.1 studies. It is better illustrated that SHMP promotes the hydrolysis of sludge by alkaline protease.

In general, the fluorescence intensity of f was less than b, and the fluorescence intensity of humus was greatly reduced after MO was added, but the protein fluorescence intensity of f was higher than that of b. It shows that the total amount of organics dissolved after the addition of MO was reduced, but the protein content was increased. This is because MO is adsorbed on the surface of sludge particles, which hinders the contact of the enzyme with the sludge substrate and limits the dissolution of intracellular organic substances. The toxicity of MO stimulated the living organisms in the sludge to secrete EPS, which is consistent with the results of the study of 3.2, indicating that MO has an inhibitory effect on alkaline protease hydrolysis of sludge.

4. Conclusions

SHMP that acts as a surfactant, enables more uniform dispersion of sludge and enzymes, and accelerates the dissolution of proteins and carbohydrates, thereby destroying the EPS structure and increasing the contact between the enzyme and the substrate. The addition of SHMP can increase the content of SCOD value, protein and polysaccharide, and it can be considered that it promotes the hydrolysis of sludge by alkaline protease.

MO can be adsorbed on the surface of sludge particles, which hinders the dissolution of organic matter. However, due to its toxicity, the microorganisms in the sludge stimulate the secretion of proteins and then form a protective layer on the surface. The content of SCOD and polysaccharide decreases when MO was added, but the protein content increases. According to the three-dimensional fluorescence map, it can be confirmed that it inhibites alkaline protease hydrolysis of sludge.

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