

## Application Progress of Biofloculant on Treatment of Heavy Metal Wastewater

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**Abstract.** Biofloculant is a kind of natural polymer flocculant having a broad application prospect. Because it is non-toxic, biodegradable, no secondary pollution, etc, more and more attention is paid to its unique properties. The sorts of microbial flocculants are introduced briefly, and it also introduces the research advance of microbial flocculants on treatment of heavy metal wastewater, including the sorts and structural characterisation of microbial flocculants, the development and characteristics of biofloculant in heavy metal wastewater treatment, etc.

### Introduction

In recent years, with the high-speed development of Chinese economy and the rapidly expanding population, the discharge amount of Chinese domestic sewage and industrial wastewater increases yearly. The drinking water quality of headwaters has been deteriorating increasingly, which is due to spilling untreated wastewater, misuse of chemical fertilizers and pesticides caused. Water pollution has currently been one of the most important Chinese environmental issues. Wastewater containing heavy metal ions holds a large proportion of industrial wastewater, which mainly comes from mechanical processing, nonferrous metal smelting, electroplating, leather processing and some chemical enterprises, etc. Heavy metal pollutants contain mercury, cadmium, chromium, lead, zinc, copper, nickel, etc, whose sorts, content and existent appearance change with the production conditions and environmental conditions. Heavy metal pollutants usually have acute or chronic toxicity that cannot disappear through they self-purification, which can harm the human health mostly by the means of the enriched food chains. Water heavy metal pollution has become one of the most serious global environmental problems. There are a lot of treatment methods of heavy metal wastewater. The traditional treatment methods of heavy metal wastewater are also various, including chemical precipitation, adsorption, oxidation reduction, solvent extraction, ionic exchange, membrane separation and silica gel adsorption method, etc. But the defects of this methods are of high energy consumption, low removal rate, investment and operation high costs, etc. With the development and the application of the environmental science and technology, application of flocculate-precipitate treatment of heavy metal wastewater is becoming increasingly wider.

### 1 Biofloculant

Biofloculant is a high flocculating activity of microorganism metabolites or chemical modification of natural organic polymer flocculant, by cultivating microbial method, a new type of flocculant. The main chemical composition of it contain glycoprotein, polysaccharide, protein, nucleic acid and cellulose material, etc, which quantity of molecular is about several hundred thousand above. The research of biofloculant begins in the 1950 s, Japanese scholars first found

microbial cultures have flocculating effect. In 1976, J. Nakamura and others had done a special study on microbial producing flocculating effects, creating quite a stir of research of microbial flocculants [1]. In water treatment, the characteristics of traditional chemical flocculant are not only the large amount of additive amount, sludge production, but also the chemical sludge is not easily biodegradable, and its emissions to water which has potential harmful effects on human health and ecological water environment. Therefore, the chemical flocculant in application scope and conditions of use has been curbed, the outlook is not optimistic. The biological flocculating researched and developed since the 1970s, making use of biotechnology in microbial fermentation, extraction, refining, is a new type water treatment agent. It not only has the function of flocculation of ordinary flocculants, but also has the advantage that common flocculants do not have, is less dosage, good flocculation effect, easy separation of floc, easily biodegradable, no secondary pollution, wide applicable scope, etc. Therefore, development and application of biological flocculating agent has become the hot spot and focus in the development of new type of high efficient flocculant. As a new type of water treatment technology, microbial flocculant has been widely used in the treatment of high concentration organic wastewater, the decolorizing of dye wastewater, activated sludge processing and other waste treatment, with showing powerful life. Friedman etc. [2] in 1968 reported a flocculant made by *Zoogloea* 115 have the effect of removing  $\text{CO}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Ni}^{2+}$ , then some literatures subsequently reported a variety of microbial treatment effect of metal ions.

We classify biological flocculants according to their source, which can be divided into the following three categories:

(1) The flocculants made use of microbial cell wall extract: flocculation mechanism of flocculating yeast is combining the cell outer wall mannan with cell surface protein causing the cell agglutination [3]. In addition, the yeast cell wall glucan,  $\text{N}_2$ -acetylglucosamine, filamentous fungi cell wall polysaccharides etc. have a strong flocculation for many microbial cells and other negatively charged particle.

(2) The flocculants made use of microbial cell wall waste: the PHB, intracellular polymer of *Zoogloea* rain igera, extracellular fibers attached on the cell surface, produced by *Saariana ventriculi* EC – 1, are directly making these cells have the flocculating activity.

(3) The flocculants directly made use of microbial cell: In 1976, Nakamura, etc. [5] selected 19 microbes with ability of flocculation from the mold, bacteria, actinomycetes, yeast, etc. Flocculant directly made use of microbial cell which is produced by *Aspergillus sojae* AJ7002. In 1986, Kurane [6] found microorganism flocculant Noc-1 that was made from *Rhodococcus erythropolis*, which had apparent flocculation and decolorization effect in *Escherichia coli*, yeast, muddy water, river water, ash water, activated carbon water, bulking sludge and pulp sewage, etc. Since then, the typical flocculant found by Suh etc. in 1997 [7] was flocculant DP – 152. This was the first time found rod-shaped bacteria can also produce flocculant.

## **2 Progress of bioflocculant on treatment of heavy metal wastewater**

According to the different sources and compositions of flocculants, microbial flocculants can be divided into three categories: extracellular metabolites flocculant, intracellular extract flocculant and bacteria flocculant.

### **2.1 Extracellular metabolites flocculant**

The main components secreted out of microbe cells are polymer molecules such as polysaccharide and a small amount of peptide, protein, lipid and its compounds, etc., immobilized metal ions by adsorption, complexation and oxidation reduction methods. Polymer molecules mesh structure contribute to the flocculation process of heavy metals, which is called Extracellular Polymeric Substance flocculant, is traditionally called bioflocculant. Liu, etc. [8] conducted the experiment on adsorbing of metal ions from wastewater using EPS extracted from activated sludge, finding that the absorption capacity of EPS on  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Ni}^{2+}$  is 60% ~ 90% higher than resin's, showing bright prospect of EPS on treatment of heavy metal wastewater.

Wang Jing, etc. conducted research on flocculation-adsorption method using WJ-I flocculant to remove Cr (VI) from wastewater, and found WJ-I flocculant were produced from fermentation solution of bacteria came from extracellular polymer (*Pseudomonas* sp) GX4-1. When pH=1.5, the largest amount of Cr (VI) adsorption was 80 mg/g on the condition that the dosage of adsorbent was 1.5mg, supplying 40~50mg flocculant, which occur in the Cr (VI) concentration of 200 mg/L and the removal rate was 51%. When pH = 0.5 ~ 2.0, supplying about 1.5 mg biological adsorbent, the adsorption rate of Cr (VI) on flocculant produced by (*Pseudomonas* sp) GX4-1 was very high. It achieved the fast adsorption phase of 75% in 5 min after; when it got to 10 ~ 40 minutes, it achieved one stage reaction kinetics adsorption phase. The reaction tended to become steady in 50 minutes.

Su Chunyan, etc. obtained extracellular polymeric flocculant of biofilm of dominant bacteria in natural water and they had explored the flocculating characteristics and adsorption mechanism of lead by extracellular polysaccharide and extracellular protein obtained from extracellular polymer and its separation. When its extracellular polymers and the extracellular polysaccharide and extracellular protein at 20°C, pH = 6. 0±0.1, the adsorption equilibrium time at 6 h, the largest adsorption capacity of lead was 23.64, 15.8, and 3.48 mg/g, coming to the conclusion that the contribution of extracellular polysaccharide in biological membrane extracellular polymers on the adsorption of lead was greater than the extracellular protein. They concluded, through infrared optical spectrum (4 500~400 cm<sup>-1</sup>) analysis, that amino group of extracellular protein, amide group, hydroxyl of extracellular polysaccharide, etc. were chemical groups which played a key role in the adsorption process of lead by extracellular polymer.

Zheng Lei, etc. used three kinds of activated sludge extracellular polymer (EPS1, EPS2, EPS3) as flocculant which protein content to sugar was 2.5:1, 7:1 and 9:1. They studied the adsorption effect on Cd<sup>2+</sup> and Zn<sup>2+</sup> in water which showed that the adsorption of Cd<sup>2+</sup> and Zn<sup>2+</sup> by EPS related to its composition. When the initial concentration was 20 mg/l, the optimum PH was 6 and the optimum dosage of EPS was 375 mg/L and 250 mg/L, the adsorption of Cd<sup>2+</sup> and Zn<sup>2+</sup> by EPS1, EPS2, EPS3 were 19.5, 27, 17 mg/g and 40.5, 47.5, 37 mg/g. And the adsorption process of Cd<sup>2+</sup> and Zn<sup>2+</sup> by three kinds of extracellular polymers can rapidly reach an equilibrium within 3 h. The adsorption rate of Cd<sup>2+</sup> and Zn<sup>2+</sup> by EPS was the fast, according to reaction kinetics fitting, while the equilibrium adsorption capacity of Cd<sup>2+</sup> and Zn<sup>2+</sup> by EPS was the highest. Maybe because the adsorption process of heavy metal by polysaccharide belongs to the reversible reaction, the sugar and protein of EPS in a certain range can achieve maximum adsorption capacity of heavy metals. And adsorption mechanism of heavy metals by EPS still remains to be further research.

## 2.2 Intracellular extract flocculant

Intracellular material refers to the cell wall, cell membrane, cell cytoplasm and cell organelles and nuclear material. The current mainstream trend of the flocculant discovery research is that macromolecular substance with extensively applied is extracted from cell wall as the flocculating agent. The typical examples are chitin and chitosan. Chitosan is a kind of macromolecular polysaccharide, as the derivative of chitin, with safe non-toxic. Because of chitosan molecules containing amino and hydroxyl, it can generate stable chelate with many metal ions and it can effectively remove metal ions. Studies have shown that adsorption rate of Cu<sup>2+</sup>, Cr<sup>2+</sup>, Zn<sup>2+</sup>, Mg<sup>2+</sup> and other metal ions by chitosan is above 94% and adsorption rate of Pb<sup>2+</sup>, Ni<sup>2+</sup>, Hg<sup>2+</sup> is also above 75%.

Traditional method, chitosan made from shell and other animal scale, has the disadvantages of geographical limitations and causing secondary pollution during production, etc, which makes it becomes necessary to open up new production way. Using microbiological fermentation method to extract chitin and its derivatives is an effective way. In the 1980 s, it found abroad abundant chitin and chitosan in mucor mycelium, easily separated. In the late 80 s, Japan and the United States had managed to successfully substitute bio-fermentation for shrimp shell to product chitosan. Since 1994, Beijing University of Chemical Technology made use of industrial waste, penicillin mycelium, to remove protein, nucleic acid and fat to extract the chitosan, and to direct crosslinking,

biological chitosan flocculant was successfully achieved. Then domestic chitosan dusty with fermentation method has rapidly developed.

Zhou Zhilan, etc., using chitosan as the flocculating agent, conducted experiment on flocculation cadmium removal under the action of sodium sulfate in the electrolyte. When cadmium in water samples mass concentration was not more than 40mg/l, PH=8~9 and the content of chitosan was 1 %, cadmium removal rate was over 99.95%. Under the same condition of treatment of wastewater containing cadmium (contain other metal ions impurities), removal rate of cadmium was more than 99.7%. And the remains of other metal ions were below the national discharge standard. For more than 40 mg/L water samples, it can be adopted the method of secondary treatment, and the result is satisfied. However, it is worth mentioning it is suitable to choose the content of chitosan was 1 %, excessive content easily caused water quality change, reducing the effect of cadmium removal rate.

### **2.3 Bacteria flocculant**

According to different strains of bacteria, it can be divided into 3 classes: bacterial flocculant, fungi flocculant and algae flocculant. Bacterial flocculant consist mainly of making use of bacteria, fungi, actinomycete, yeast, algae and other bacteria to remove heavy metal ions. These bacteria are widely existing in nature. Using directly living cells as a flocculant has the following advantages: it removes extraction costs. It is available that a few strains are inoculated to the fermented liquid to make they breed with no secondary pollution. But the disadvantage is that there must be no factors impairing growth of bacteria in the wastewater with processed.

Bioflocculant concentration process of heavy metal removal include three stages: adsorption, coagulation and flocculation, which adsorption ability tend to determine the performance flocculant. Bacteria flocculant has apparent flocculation effect in metal ions which can be achieved through absorption. The adsorption of heavy metals by microbes depends on two aspects: one is its nature of microorganism, the other is metal with the endophilicity to organisms. A large number of studies have shown that microorganisms such as bacteria, fungi and algae, etc. have strong ability of adsorption on metal ions. Wang Yaxiong had pointed out that nuclear material existing in the Marine environment could be enriched by adsorbing directly marine microorganisms from water and the nature has nothing to do with the function of cells. Many microbial cells, regardless of the life all have the same good adsorption properties. The advantage of using death microbial cell is the cultivation and application of microorganisms can be separated, in order to be better commanding adsorption of heavy metal ions by biological adsorbent.

## **3 Prospect**

The unique advantage of bioflocculant on treatment of heavy metal wastewater over traditional flocculant is high efficiency, no toxicity, easier biodegradation, extensive flocculation objectoriented, non-secondary pollution after being used. It can replace small or all of the existing high polymer flocculant and there will be a far-ranging market prospect. The future research will mainly focus on the following aspects.

1) It can be pointedly bred bacteria produced by efficient flocculant which resist the toxic of heavy metal. Improving the flocculating activity, reducing the dosage of flocculant and reducing the production cost is the key whether bioflocculant can spread in industries. It can try to use genetic engineering and ferment engineering for effectively improving the productivity of bioflocculant.

2) It can be pointedly conducted gene control and expression, cloning technology research which is the efficient flocculation substance gene and resistance gene of microbial flocculant.

3) Enriching rare metal ion in heavy metal wastewater, through the method of microbial flocculation deflocculation, can be re-used and while making the waste profitable on treatment of wastewater, it achieves reuse of waste. We can receive the harvest of the ecological benefit, social benefit and economic benefit.

4) We should conduct an-depth research on the flocculating function mechanism, molecular structure, flocculation kinetic, several interactions between flocculants, through combining the

advantages of traditional flocculant and new type of flocculant, to guide the development of new type of flocculant and super flocculant having the benefits of both.

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