# Screen culture of B-megaterium flocculant and the removal of Cu(II) from wastewater

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ABSTRACT: Isolation and screening of a strain with high efficiency of flocculation effect from the activated sludge of a wastewater treatment plant in Kunming, after identification of strains, flocculating activity experiments, and eventually extracted Bacillus megaterium flocculant and study on its treatment of Cu (II) wastewater removal. Results showed that in the optimal medium: flocculating activity of the bacteria from 78.76% to 90.78%. In the optimal culture conditions, flocculation reaction 20min, flocculant dosage 100mg/liter, Cu (II) initial concentration of 10mg/liter, Cu (II) effluent concentration of 0.404mg/liter, Cu (II) removal rate of 95.96%. By flocculating agent before and after the SEM, XPS, comparative analysis of XPS before and after reaction, wastewater in the Cu (II) replaced by flocculating agent on the -OH or -COOH of a molecule of hydrogen, forming $CH_2CHNH_2COOCu$ colloidal substances, ultimately bridging role by adsorption flocculation and sedimentation Cu (II).
KEYWORD: B-megaterium; screen culture; Heavy metals; flocculant; Heavy metals

## 1 INTRODUCTION

Copper containing wastewater has high economic value, but it is easy to cause harm to the environment and human (Li B. Liu SP, 2008). A lot of researches have been carried out on the production of microbial flocculant in foreign countries, and it has been obtained that many strains of microbial flocculant producing bacteria have different characteristics. A lot of researches have been carried out on the production of microbial flocculant in foreign countries, and it has been obtained that many strains of microbial flocculant producing bacteria have different characteristics (Levy N.Y, 1990). However, the study on the microbial flocculant is the main stay in the laboratory stage and most of the research is still concentrated in the flocculant producing bacteria screening, coagulant composition, flocculating characteristics, flocculation mechanism and application etc (Li ZL, 1999).

In this paper, the treatment of Cu (II) were studied, which is a kind of flocculant, and it is easy to be extracted and stored. At the same time, through the SEM and XPS analysis of the flocculant, the process mechanism of Cu (II) removal was studied, so as to provide theoretical support for the treatment of low concentration wastewater containing Cu (II).

## 2 MATERIALS AND METHODS

### 2.1 *Materials*

The test strains are derived from the sludge of a sewage treatment plant in Kunming. Glucose, sucrose, urea, soluble starch, D-fructose, $(NH_4)_2SO_4$, $KNO_3$, NaCl, $MgSO_4·7H_2O$, $CuSO_4·5H_2O$, $FeSO_4·7H_2O$, $KH_2PO_4$, $K_2HPO_4·3H_20$, NaOH, HCl, $CaCl_2$, $Al_2O_3·2SiO_2·H_2O$, $Pb(NO_3)_2$, $ZnSO_4·7H_2O$, $H_2SO_4$ are domestic analytical reagent; beef extract, peptone, yeast extract agar, domestic biological reagent; yeast extract for domestic biochemical reagents.

Conditions of separation medium are beef 3g extract, 10g peptone, 20g agar, 5g NaCl, 1liter distilled water and pH is 7.0 to 7.2; Conditions of screening medium are 20g glucose, 1.0g yeast extract, 0.5g $KH_2PO_4$, 0.2g $K_2HPO_4$, 0.05g $MgSO_4·7H_2O$, 0.05g NaCl, 1liter distilled water and pH is 7.0. The instruments used in the experiment are vertical pressure steam sterilizer, incubator, vacuum freeze dryer, super net work station, thermostatic oscillator, UV visible spectrophotometer, centrifuge, microscope, DNA sequencing, DNA electrophoresis tank, electric thermostatic water bath, gel imager, atomic absorption spectrometer and PCR Instrument.

 

## 2.2 *Methods*

Isolation and screening of bacteria from sewage sludge under sterile conditions. Using Gram staining method to observe the morphology of Bacillus under oil microscope. The strain shaking culture to the logarithmic growth phase and collected by centrifugation cell, genomic DNA. According to the method of 16S rDNA PCR amplification and sequence analysis, DNA purpose of the strip for purification and recovery, PCR products with PCR primers directly sequenced, will determine the sequence submitted to the GenBank database, after Blastm homology search for identification.

Medium single factor test content including carbon source; carbon source concentration; nitrogen sources; nitrogen source concentration degree; inorganic salts. In addition to the single factor as a variable, the rest of the screening culture medium formula, inoculation amount of 2%, pH is 7, temperature is 30 centigrade, 100rpm shake flask culture 24 hours, take the fermentation broth to test its flocculation activity.

After activation of the huge Bacillus inoculation to 2liter optimal screening culture medium and under the optimal culture conditions for 24 hours, 4000rpm centrifugation 20 minutes, discard supernatant and distilled water washing of thalli were added three times. After centrifugation, remove supernatant. After the wet biomass is dried, it can be used as a bio flocculant for the subsequent experiment.

The fully dried Cu $(NO_3)_2$ and 10mg/liter Cu(II) were prepared into the simulated wastewater containing copper containing wastewater, and the test samples were diluted as needed. The fully dried Cu $(NO_3)_2$ and 10mg/L Cu(II) were prepared into the simulated wastewater containing copper containing wastewater, and the test samples were diluted as needed.

Flocculation conditions of single factor test: samples of initial pH, flocculant dosing amount, flocculation reaction time. Adjusting the initial pH of water samples, we weigh 100mg/liter flocculant dry products by adding water; in addition to the single factor variable and the rest in the temperature of 25 centigrade and 100 rpm response of 20 minutes, water solution after centrifugation, the supernatant, by atomic absorption spectrometry and measuring the content of heavy metal ions.

# 3 RESULTS AND ANALYSIS

## 3.1 *Strain identification*

A strain with high flocculation activity was isolated and screened from the sludge of a sewage plant in Kunming, No. L7. As shown in Figure 1.

Sequence analysis and homology comparison of the rDNA 16S length (1425bp) sequences of the screened L7 strains were performed by Blast program and GenBank data. The results showed that the sequence of the strain had 100% similarity with *Bacillus megaterium strain BS6* and *Bacillus megaterium strain HNS88*. The strain L7 was identified as Bacillus strain (*Bacillus megaterium*).

## 3.2 *Single factor test*

Through the single factor experiment, Bacillus megaterium flocculant producing the optimal medium composition: sucrose 20.00g/liter; yeast extract 2.00g/liter; 0.50 g/liter $KH_2PO_4$; $K_2HPO_4$ 0.20g/liter; $MgSO_4 \cdot 7H_2O$ 0.25g/liter; NaCl 0.05g/liter;metal $Na^{2+}$, $Cu^{2+}$ has little effect on flocculating activity of the bacteria, appropriate concentration of $Mg^{2+}$ have help to enhance the flocculating activity of the bacteria and $Fe^{2+}$ on flocculating activity of the bacteria have inhibitory effect. Optimum culture conditions for Bacillus megaterium flocculant producing: initial pH 7.0; inoculation amount was 2.0% (V/V); culture temperature of 30 centigrade; shaker speed to 100 rpm. Under the optimum medium composition and the optimum culture conditions, the flocculation activity of Bacillus strain was raised from 78.76% to 90.78%.

## 3.3 *Single factor test of flocculation condition*

(1) Effect of initial pH value on adsorption flocculation Cu (II)

Different flocculant had different sensitivity to pH, and the same kind of flocculant had different initial pH requirements. Due to the lowering of the surface charge of the colloidal particles, so as to reduce the mutual repulsion between the particles, and to bridge between particles and flocculant even role in promoting bridge formation and particle precipitation. Is shown in Fig 2, with the increase of the initial pH value, Cu (II) removal rate from pH 1.0 of 28.18% increase to pH value 7.0 of 95.5%. The test of flocculation system optimum initial pH value is 7.0.

(2) Effect of coagulant dosage on the adsorption and flocculation of Cu (II)

When the flocculant dosage is insufficient, pollutant destabilization is not sufficient and the poor treatment effect; when the adding amount of excess, of colloidal particles in wastewater because of over loading and reach to a stable, leading to a decrease in the flocculation ability. As shown in Figure 3, when the coagulant dosage is 100mg/liter, the removal rate of Cu (II) can reach the maximum value of 93.78%. This experiment chooses 100mg/liter as the best dosage.

(3)Effect of flocculation reaction time on adsorption and flocculation Cu (II)

: Because of the high temperature can make the biopolymer degeneration, spatial structure change,

some active groups no longer and suspended particles combined, which showed flocculating activity decreased. The removal rate of Cu (II) was 95.96%. From the figure 4, when the reaction time is 20min with the increase of reaction time, and the removal rate was stable and slightly decreased.

Fig.1 The picture of L7 Bacteria morphology

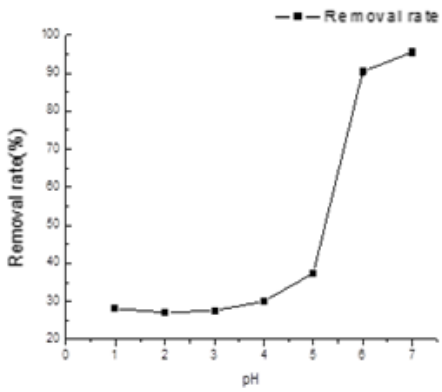


Fig.2 The effect of the initial pH on flocculation of Cu(II)

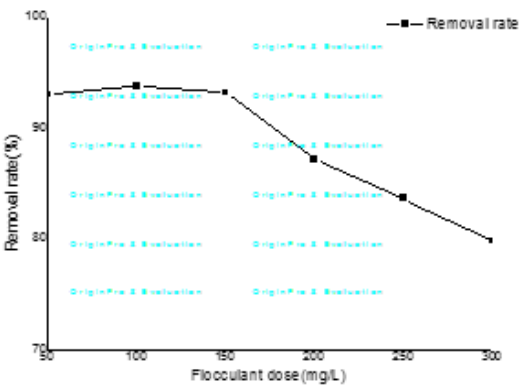


Fig.3 The effect of adding the quantity on flocculation of Cu(II)

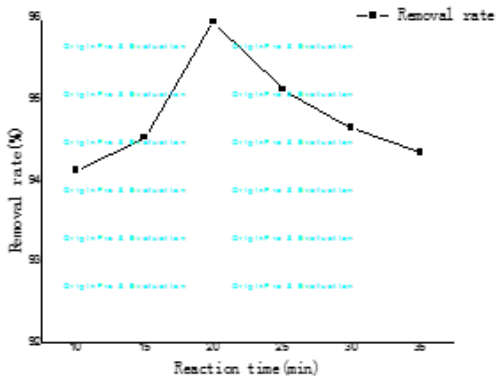


Fig.4 The effect of contact time on flocculation of Cu(II)

### 3.4 *SEM analysis*

It can be seen from the figure 5 that a large number of the reaction before the flocculation of the rod like chain or overlapping arrangement; after the reaction of the flocculation of the rods have obvious aggregation, interwoven into a group of flocculation. Figure 5 (3) shows that the reaction front flocculant exists on the surface of the pore, and after reaction, pore surface of flocculant has a significantly reduced, rod-shaped objects as a whole in the full state, indicating that the heavy metal ion flocculant and wastewater produced adsorption, as shown in Figure 5 (6) is shown. By the appearance of SEM, it was showed that flocculation reaction occurred mainly in the treatment of heavy metal wastewater.

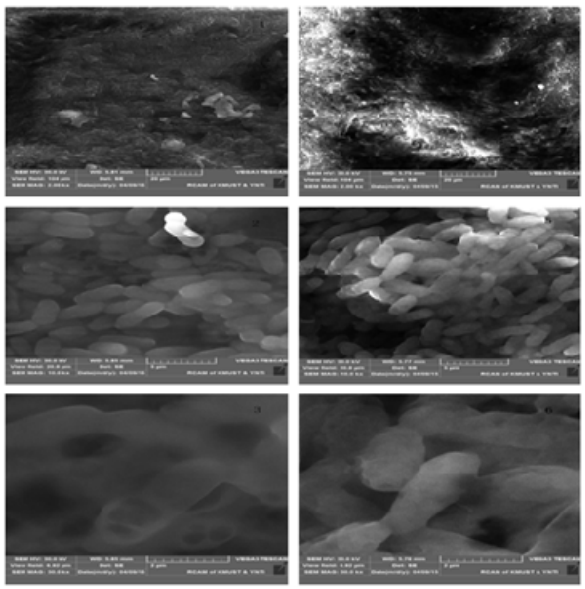

Fig.5 The SEM picture of B-megaterium flocculant before and after removal of wastewater

### 3.5 *XPS analysis*

The results showed that the main forms of C, O and N were the main forms of, and before the treatment

of heavy metal waste water were $[-CH_2CH(C_6H_5)-]_n$, SiC, $NaC_2H_3O_2$, $C_3N_6H_6$, $C_6H_2(NO_2)_3(NH_2)$.

According to the reaction before and after the flocculant XPS contrastive analysis, after the reaction of Cu (II) replaced the –OH or -COOH hydrogen, hydrogen bonding, electrostatic interaction and chemical adsorption, and the formation of such as (-COO)-Cu,-O-Cu,$C_4H_8N$-CuOH,$(C_2H_3O_2)_2$-Cu-$(C_2H_3O_2)_2$ colloidal substances, eventually high molecular flocculant active groups (such as amino, carboxyl) with hydrogen bonding, electrostatic interaction and chemical adsorption to adsorb water in the copper ion and colloid particles, thus the wastewater flocculation copper.

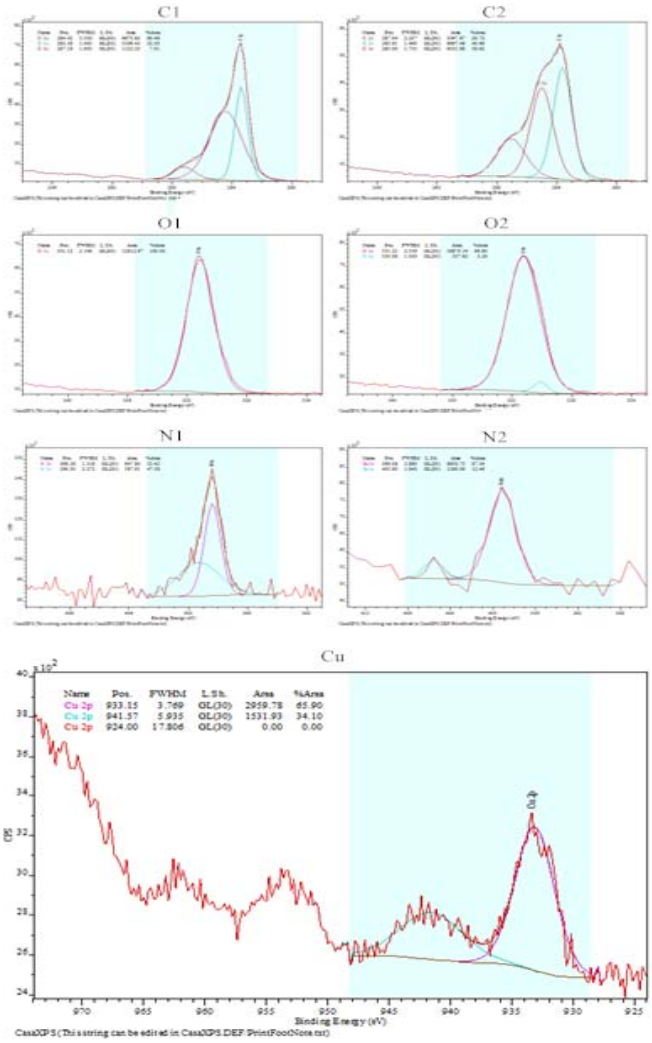


Fig.6 The decomposed XPS spectrum of C,O,N,Cu before and after removal of wastewater
(Remark: The XPS decomposition spectrum of C1,O1 and N1 in the wastewater before treatment with the B-megaterium. The XPS decomposition spectrum of C1,O1 and N1 in the wastewater after treatment with the B-megaterium.)

## 4 CONCLUSIONS

(1) In the optimal medium composition, according to the initial pH value of 7.0, inoculum, 2.0% (V / V) and culture temperature of 30 DEG C, the shaking speed of 100 rpm, culture conditions, Bacillus megaterium flocculating activity by the initial 78.76% up to 90.78%, 1L optimal medium cultured for 24 hours, from extracted flocculant dry products.

(2) The optimal flocculation conditions for the treatment of copper containing simulated wastewater by Bacillus sp. is: the initial pH is 7, the coagulant dosage is 100mg/L, the flocculation reaction time is 20min. Under the optimum flocculation conditions, the Cu (II) removal rate of the simulated wastewater was 95.96% after the flocculation treatment of the wastewater.

(3) Can be drawn through the analysis of the characterization of the flocculant SEM appearance, flocculent surface reaction is a rod-shaped object is chain or overlapping arrangement, and on the surface of the rod-shaped object exist certain pore; after the reaction of flocculant on the surface of the rod-shaped object has a significant aggregation, flocculation interwoven into the group and the reduction of pore surface, rod-shaped objects as a whole was full. By flocculanting XPS photoelectron energy spectrum analysis can be drawn through and Bacillus megaterium flocculant is through microbial flocculant active groups (such as amino, carboxyl) by hydrogen bonding, electrostatic interaction and chemical adsorption to adsorb water Cu ion and colloid particles.

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