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Research Article

Introduction of Compound Bioflocculant and Its Application in Water Treatment

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Abstract: The research background and characteristics of Compound Bioflocculant (CBF) were introduced. The paper focused on research and application of CBF, including screening of strains, optimization of cultivation conditions of flocculant producing bacterium, components analysis of CBF, application of CBF. Finally existent problems in current research and the research trend of CBF in the future were discussed.

Keywords: Application, compound bioflocculant, introduction, research trend

INTRODUCTION

Flocculants are useful agents in the aggregation of colloids, cells and suspended solids and are commonly used for drinking water production, waste water treatment, fermentation processes and food production (Shih *et al*., 2001). In general, flocculants are classified into three groups: inorganic flocculants, such as aluminum sulfate and polyaluminum chloride; organic synthetic flocculants, such as polyacrylamide derivatives and polyethylene imine; and naturally occurring flocculants, such as chitosan, sodium alginate and microbial flocculants. Despite the effective flocculating performance and low cost of the synthetic chemical flocculants, their use has resulted in some health and environmental problems (Xia *et al*., 2008). In contrast, bioflocculants, extracellular biopoly-meric substances secreted by bacteria, fungi, algae and yeast are biodegradable and nontoxic flocculants (Salehizadeh and Shojaosadati, 2001; He *et al*., 2004).

Microbial flocculants is a kind of metabolites produced by the microorganism, the main ingredients are glycoproteins, polysaccharides, protein and DNA *et al*. It is obtained by the fermentation of bacteria and fungi, extraction, purification in use of microbial technology, is a new type of water treatment agent (Li *et al*., 2000; Gan and Gan, 1999; Nakamura, 1976a). Microbial flocculant is biodegradable, non-toxic and safe of high efficient for wastewater treatment. The study on the bioflocculant began in the 1950s, Japanese scholars first discovered microbial culture liquid with flocculation. In Nakamura *et al*. (1976b) studied microbes producing flocculation effect and raised the research hotspot for microbial flocculant (Nakamura *et al*., 1976b). Kurane *et al*. (1986) used *Rhodococcus erythropolis* S1 strain isolated from nature to produce flocculant NOC-1with good flocculation effect. The research of microbial flocculant, has been mainly concentrated on producing strain screening and flocculant fermentation liquid for wastewater treatment in laboratory scale tests, there were many problems in the process of research, for example, high cost for producing bioflocculant, screening the strain was difficult, the flocculation process and mechanism is not clear and so on. In recent years, in order to improve the effect of flocculation, reduce the producing bio-flocculant cost, compound bioflocculant began to receive the much attention of researchers (Fang, 2008; Zhu *et al*., 2010).

Bioflocculants are mainly composed of macromolecular substances, such as polysac-charide and protein (Lu *et al*., 2005; Zheng *et al*., 2008). The composition and properties of bioflocculants depend on type of Bioflocculant- Producing Microorganisms (BPMs), composition of media and environmental conditions (Ahmad *et al*., 2013). Although so many studies about bioflocculants have been done, low flocculating capability and high cost are still the major problems in limiting bioflocculants development for practical application (Li *et al*., 2003). Consequently, screening for microorganisms producing bioflocculants with high flocculating activities, improving the flocculating performance of the biofloccualnts (Ma *et al*., 2003) and seeking for low-cost substrates for producing bioflocculants have become recent hot topics in this field (Fujita *et al*., 2000).

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The concept of Compound Bioflocculant (CBF) was first put proposed by Professor Ma of Harbin Institute of Technology (Ma *et al*., 2003). The raw fermentation material of compound bioflocculant was agricultural waste straw. The high-efficiency combined production of bioflocculant utilized the composition flora of cellulose degrading bacteria and flocculant producing bacteria, through two stage fermentation, realized the coupling of cellulose saccharification section and producing flocculating section. Therefore, compound bioflocculant on the basis of compound bacteria has advantages of bacterial activity, the flocculation effect, the cost of production, compared with single microorganism producing flocculant, becomes the hot spot in the current research.

Ma *et al*. (2003) put forward the conception of CBF, which was produced by mixed culture of bioflocculant-producing microorganisms and could exhibit excellent flocculating ability. The compound bioflocculant in the present research was produced by mixed culture of two bioflocculant-producing microorganisms, *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6. CBF possesses higher flocculating activity than bioflocculants by single culture of F2 and F6 (Wang *et al*., 2011). So far, the domestic scholars mainly concentrated in the screening and optimization of compound bacteria culture (Zhu *et al*., 2004), application of compound bioflocculant for treatment raw water and wastewater. The domestic and international research status of compound bioflocculant in recent years and its future research development were introduced in the study.

SCREENING OF FLOCCULANT PRODUCING BACTERIUM

So far, microorganisms with the flocculating ability have been found more than 20 different kinds, most of which were bacteria, mould and yeast. Two kinds of micro-organisms was co-cultured for producing CBF. It was found that co-cultureing flocculant producing bacteria F2 and F6, flocculating activity was the optimization and are better than the single strain, strain F2 and strain F6 were identified as *Rhizobium radiobacter* and *Bacillus sphaericus*. CBF was identified as mainly polysaccharides of high molecular weight which containing abundant chemical groups, such as hydroxyl, carboxyl and amino groups. In our previous work, it can be concluded that the main coagulation mechanism for CBF was adsorption bridging (Wang *et al*., 2011). Five kinds of flocculant-produced strains which had high flocculating capacity had been obtained from activated sludge and soil. The compound microbial flocculant which was formed by compositing and cultivating Strain 1 and Strain 5. Strain 1 and Strain 5 were both identified as *Bacillus sp.* (Zhao *et al*., 2010). Fang (2008) screened and isolated thirty-seven strains from soil and active sludge, including twelve bacteria stains, eleven mildew strains, seven epiphyte stains and seven actinomyces stains. There were 20 strains with flocculation activity whose flocculating rate was above zero in these thirty-seven strains. The fifteen strains with flocculating rate >20% were mixed one by one with the same proportion and then were cultured in certain condition. The flocculation activity of the complex bioflocculant was much higher than microbial flocculant produced by a single strain. The complex bioflocculant with high flocculating rate above 80% were produced by mixed strains XJ 12 and ZJ6, ZJ6 and MJ9 and ZJ7 and MJI0, whose focculating rate were 86.6, 84.9 and 85.7%, respectively. Lin (2006) respectively cultured the mixed strains one by one preserved in the lab, which included 13 strains bacteria, 6 strains moulds, got flocculant producing bacteria F14 and F15 with high flocculating activity, flocculant producing moulds H8, H9 and H10 with high flocculating activity. Wang (2009a) separated screened four flocculant-producing moulds with high flocculating activity from activated sludge of tannery wastewater. Two kinds of compound bioflocculant produced by one by one mixed culturing were named as MBF I and MBF II, their flocculating rate were respectively 84.4 and 86.9%. In order to increase the output of bioflocculan, improve its efficiency and stability and enlarge its application scope, four yeast-like flocculant producing strains were cultured in distillery wastewater and the optimum culture conditions were investigated. The results indicate that the mixed strain HJ4 has the highest flocculating rate. A compound flocculant MBF4 which is produced by mixed strain HJ4 can remove turbidity and color by more than 80% and COD by 20 to 90% in the actual wastewater, which shows that it is feasible to take advantage of distillery wastewater to culture the mixed yeast-like flocculant producing bacteria (Wang *et al*., 2007b).

Optimization of cultivation conditions of flocculant producing bacterium: The culture conditions of flocculating bacteria directly affects the yield and quality of compound bioflocculant, has an important influence on the application of the flocculant. The study of optimization culture of compound bioflocculant producing bacteria had acquired research achievements of plenty, such as Wang *et al*. (2007b) found in the study, the best culture conditions of mixed strains HJ4: inoculation quantity was 15%, initial pH was 4.5, carbon and nitrogen ratio was 20: 1. The flocculation rate could got 94.7% under the condition. Fang (2008) showed that the optimum culture conditions of flocculating bacterium XZ: the culture time was 48-56 h, culture temperature was 30C, initial pH was 7.5, shaker speed was 160 rpm. Zhang *et al*. (2008a) dealt with the optimization for culture condition on compound bioflocculant producing bacterium. The results indicated that the optimal components of the culture were glucose 2.0 g, $K_2HPO_4 \cdot 3H_2O$ 5.0 g, KH_2PO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.20 g, $(NH_4)_2SO_4 \cdot 7H_2O$ 0.20 g, distilled water 1 L and the initial pH of the culture was 7. The external conditions of the culture medium were as follows: the sterilization pressure is 0.06 MPa, the temperature was 20-30°C and the rotation speed of surge was 100-140 r/min. It reduced transform cost of compound bioflocculant and enhanced of the flocculating capability. Wang's research showed the optimum fermentation conditions of bioflocculant production by F2+F6 (F+) were as follows: 32°C of fermentation temperature, 7.5 of initial pH, 18h of the first phase fermentation time, 24 h of fermentation time, 150 r/min of shaker speed, bottle of 500 mL with 250 mL cultural medium. Under the mixed fermentation conditions, the optimal flocculant producing activity could reach 97.6% (Wang, 2009b).

Components analysis of CBF: Many studies found that the main composition of compound bioflocculant was polysaccharide substances with some protein and other macromolecules. Ma *et al*. (2008b, 2005) put forward CBF was mainly composed of polysaccharide, which is determined by anthrone reaction, coomassie blue reaction and ultraviolet spectroscope method. CBF contains carboxyl, which is determined by Infrared spectra. Distributing of molecular weight of purified CBF is from 10^5 to 10^6, which is determined by gelatin chromatogram. The flocculating activated substances distributed mainly in supernatant, which contains protein and amylase. The characteristics of the complex biofloccultant XZ were studied in the study, it indicated that the complex biofloccultant XZ has the characteristics of high thermo stability and pH stability (Fang, 2008). Wang *et al*. (2010) analyzed components of the compound bioflocculant MFHJ4. The effects showed the compound bioflocculant MFHJ contained hydroxyl and carboxyl was mainly composed of polysaccharide.

APPLICATION OF CBF IN WATER TREATMENT

Treatment of drinking water: The compound bioflocculant achieved good results in surface water treatment. Such as, Ma *et al*. (2004) study showed that when CBF dosage was about 14 mL/L, the pH value was 7.5, the addition of $CaCl_2$ was 1.5 mL/L. The optimal flocculation effect was obtained in the treatment of Songhua River water. The temperature has little influence on the flocculation efficiency.

Treatment of domestic sewage: Domestic sewage with the characteristics of high organic content, complex composition of the water quality, but compound bioflocculant in treatment domestic sewage showed good treatment properties. When CBF was used for treatment of domestic sewage, all kinds of pollutant removal rate was above 60% (Wang *et al*., 2007a); compound bioflocculant XZ has the high removal efficiency for SS and Chroma of domestic sewage, XZ also could improve the related indexes of activated sludge (Fang, 2008).

Treatment of industrial wastewater: Zhang *et al*. (2008b) research showed that when CBF treated starch wastewater, flocculation rate reached 80.0-90.3%. By the compound bioflocculant XJBF-1 for treatment of starch wastewater, printing and dyeing wastewater and landfill leachate, COD removal rate reached 88, 66 and 58%, respectively and the treatment effect of starch wastewater and dyeing wastewater is better than that of the action polyacrylamide (Ji *et al*., 2010). The compound bioflocculant Produced by *Aspergillus sojae* and *Pichia membranifaciens* cultured in soy sauce waster had good effect on the water treatment, the removal ratio of kaolin clay suspension was up to 98.3%, other wastewater such as soy sauce waste, liquor brewing waste, the removal ratio of turbidity and COD was 78.20-92.3% and 64.2-85.2%, respectively (Ren *et al*., 2010).

Combination technology of compound bioflocculant and chemical flocculant: The water pollution is severe now and water quality of domestic sewage, industrial wastewater and water source becomes more and more complex. Combination effect of different kinds of flocculants is one of the effective means of enhanced coagulation for water treatment. CBF could combined with the inorganic flocculants, such as $FeCl_3$, $AlCl_3$, $Al_2(SO_4)_3$, Polymerization Aluminum Chloride (PAC) and poly Aluminum Ferric Chloride (PAFC) and also combined with the organic Flocuulants, such as polyacrylamide (PAM). CBF combined with chemical flocculants could improve the flocculation effect, reduce residual amounts of iron or aluminum and improve the safety of the effluent. Combination of CBF and chemical flocculants could reduce the use amount of CBF, use Cost would reduce further (Zhu *et al*., 2010). Combination technology of Compound bioflocculant has many advantages, so many researchers in the laboratory carried out experiments of Combination technology for treating high concentration organic wastewater, source water and domestic sewage, the results of them were showed in Table 1.

THE RESEARCH TREND OF CBF IN THE FUTURE

There were still many problems in the process of development and application of CBF, for example, high cost for producing bioflocculant, screening the strain was difficult, the flocculation process and mechanism is not clear and so on.

Table 1: Application of complexation of compound bioflocculant and chemical flocculant in water treatment

Combined chemical flocculants	Treatment object	Treatment effect	References
$AlCl_3$, PAC	Songhua River water, Papermaking wastewater, Coal gasification wastewater	The optimum doses of CBF with $AlCl_3$, PAC used in the mixed solution were determined when some kinds of wastewater were treated and the flocculation rate was 98.6%. The results showed that flocculation efficiency of the mixed solution of CBF and chemical flocculant was higher than that of using CBF solely. It made the dose of CBF decrease about 60%-75% and the dose of chemical flocculant used was decreased remarkably.	Wang *et al*. (2007a) and Jin *et al*. (2006)
$FeCl_3$,PAC, PAM	Domestic sewage, Mud wastewater	The uses of CBF in combination with $FeCl_3$ for domestic wastewater could achieve pollutant removal with the efficiency above 65% in terms of the considerable removal of corse particles such as suspended particeles. The quality of the effluent could meet the national standard of the discharge. By investigating the treatment effectiveness of mud wastewater using compound bioflocculants, the optimum dosage of CBF and $AlCl_3$, PAC and PAM was determined in the test for mud wastewater treatment. The flocculating efficiency was maintained as high as 98% on the basis of decrease of CBF and chemical flocculants. The combined use of CBF and chemical flocculants could accomplish much better performance than individual use of CBF and the amounts of both types of flocculants used was decreased, which could, in part dismish or reduce the risks of the inorganic flocculants brought to the human health. The substantial decrease of chemical coagulants could also reduce the residual toxic metals in the effluent.	Zheng (2007)
$FeCl_3$	Printing and dyeing wastewater	The new composite bioflocculant MBF-737 has been prepared by the inexpensive swine wastewater medium. It can be combined with chemical flocculant $FeCl_3$ for treating actual printing and dyeing wastewater. The optimum technical conditions are ascertained.	Yao *et al*. (2014)
PAFC	Reservoir water	The combination of PAFC and CBF were applied into the drinking water system. The quality of the outflow for composited operation of CBF and PAFC is much better than the separateness.	Jin (2009)
PAFC	Algae-laden water;	Coagulation jar tests reveal that the combination of CBF and PAFC has a better effect on turbidity remova.l When the dosages of CBF and PAFC are 12.5 mg/L and 25 mg/L respectively, the turbidity removal rate reaches 93. 5%.	Ma *et al*. (2008a)
PAFC	Surface source water	In CBF and PAFC compound use can effectively improve the removal effect of low temperature and low turbidity water turbidity and aluminum CBF and eliminates the PAFC causes the aluminum concentration increased phenomenon.	Meng *et al*. (2009a and b)
Al2(SO4)3	Kaolin–humic acid solution	The coagulation performance and floc properties of Compound Bioflocculant (CBF)-Aluminum Sulfate (AS) dual-coagulant were compared with that of AS in the treatment of synthetic kaolin-humic (HA) water. Results showed that AS-CBF dual coagulant was more efficient for HA removal. The floc formation, breakage and reformation were investigated under different dosages and shear rates. Precipitate charge neutralization was a dominant mechanism of AS and CBF had good adsorption bridging effect. Due to the combination of two advantages, coagulation efficiency could be enhanced significantly.	Bo *et al*. (2011, 2012)

Further the research would be made in the following six aspects:

- Construct of the bioflocculant producing strain resources database
- Looking for cheap substrate, developing waste pretreatment technology and resource utilization technology
- Development of mixture technology of compound flocculant producing bacteria and new compound bioflocculant
- Further study on flocculation mechanism
- Improvement of purifying method of bioflocculant
- Construct bioflocculant producing engineering bacteriabacteria with high flocculating activity.

CONCLUSION

The compound bioflocculant research at home and abroad had been developed for more than 10 years, a lot of achievements were obtained. But large-scale applications of CBF meet a certain difficulty. Therefore, a single chemical or bioflocculant are not suited to development needs of the current water pollution control engineering technology, the urgent needs of the market should be efficient, environmental friendly composite flocculants products. Research and development of new key technology of composite biological flocculants industry, provided new flocculants products and technology support for the safety of drinking water supply, water pollution control and water deep purification treatment. It would be an important direction for future development of bioflocculant.

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