Optimization of Flocculating Conditions of a Microbial Flocculant by Uniform Test

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Abstract: A microbial flocculant-producing strain was screened from the soil of zuojia nature reserve in jilin province, whose flocculation rate was 52.6%. The flocculating conditions of this microbial flocculant was optimized by uniform test, coagulant aid (1% cacl2) was added in an amount of 5ml/100ml, the amount of flocculant added 3ml/100ml, ph of kaolin suspension was 9.0, the reaction temperature was 20℃. Consequently the flocculation activity was up to 73.58% in the case of the concentration of kaolin suspension was 5g/l.

1. Introduction

Recently water pollution and water crisis are getting worse and serious, and sewage treatment is becoming urgent. The flocculant precipitation method is an efficient, economical and environmentally friendly which commonly used at home and abroad [1]. Common flocculants mainly include inorganic flocculants, organic flocculants and microbial flocculants[2]. Among them, organic polymer flocculants have the advantages of high flocculation ability and low cost, which are most widely used, but the most common organic flocculants such as polyacrylamide have strong neurotoxicity and carcinogenic[3]. At present, a wide range of inorganic flocculants such as aluminum sulfate and polyaluminum chloride are applied in our country, which could cause secondary pollution of the environment[4], and also endanger human health, causing aluminum poisoning and dementia[5]. Microbial flocculants are efficient, non-toxic and non-secondary pollution, which can overcome the shortcomings of organic and inorganic flocculants[6, 7]. It's the secondary metabolites produced by microorganisms through separation and purification[8, 9], and contains various functional groups, which could facilitate colloidal suspensions in water condensing and precipitating each other[10,11].

The research on microbial flocculants is still mainly in the laboratory scale stage, and the research direction mainly includes the screening and separation of high-efficiency microbial flocculant-producing bacteria, optimization of flocculation conditions, improvement of flocculation efficiency and research of flocculation mechanism[12-17]. Low flocculation activity an high production cost are still the main problems in the application of microbial flocculants. Therefore, it is of great significance to develop new microbial flocculants with high flocculation efficiency and low cost.

Microbial resources are abundant in soil. In this study, a microbial strain with high-efficiency flocculation activity was isolated and screened from the soil of zuojia nature reserve in jilin province, and its flocculation conditions were optimized by using a uniform test with the objective of providing foundation for the follow-up research and development application of the highly active microbial flocculant.

2. Materials and Methods

2.1 Test Materials

The Bacterial Flocculant-Producing Strain in This Study Was Isolated and Screened from the Soil of Zuojia Nature Reserve.

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Separation Medium: Beef Extract 0.5%, Peptone 1.0%, Nacl 0.5%, Ph 7.0.

Liquid Medium: Glucose 2.0%, Kh_2po_4 0.2%, $Nano_3$ 0.5%, Yeast Extract 0.2%, $Mgso_4 \cdot 7h_2o$

2.2 Soil Sample Collection

The Sampling Points Are 50m~240m in Altitude and 5~20cm in Depth. Soil Samples Are Collected by the Five-Point Sampling Method. Followed by Standing At Room Temperature (25℃) for 1 to 2 Days.

2.3 Isolation and Purification of Flocculant-Producing Bacteria

10 g of Soil Sample Was Placed in a Sterilized Mortar with Sterile Water and Ground into Small Particles. Pour into a Sterilized 250 Ml Erlenmeyer Flask. Subsequently 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} Dilutions of Soil Were Prepared and Coated on the Separation Medium, Finally Incubated in a Biochemical Incubator At 30 ° C for 1-2 Days.

2.4 Screening of Flocculant-Producing Strain

2.4.1 Preliminary Screening of Flocculant-Producing Strain

A single colony was purified picked up on the separation medium plate,then transferred to a slanted medium for purification, following by being inoculating into a liquid medium and shaking at 160 r / min, 30 ° C, for 72 h.

Flocculation activity measurement was performed: 2 mL of culture solution, 5 mL of 1% $CaCl_2$ solution and kaolin suspension (5 g/L) were mixed and the volume was adjusted to 100 mL. The mixture was stired with magnetic stirrer for 2min, and observed by gent shaking. If the kaolin suspension could be flocculated into large particles, the strain could be identified to posess capablility of flocculating.

2.4.2 Determination of Flocculation Activity

5 mL of the fermentation supernatant was mixed with 5 mL of a 1% $CaCl_2$ solution, and then a kaolin suspension (5 g / L) was added to make up to 100 mL. The above mixture was stirred quickly for 2 min and left to stand for 10 min, followed by measuring the absorbance of the supernatant with an ultraviolet-visible spectrophotometer at 550 nm. The flocculation activity is expressed by the flocculation rate, and its calculation formula[18] is as follows:

$F/\% = (1 - B/A) \times 100$

F: flocculation activity, A: control absorbance value, B: sample absorbance value

2.5 Uniformity Test to Optimize Flocculation Conditions

The uniform test table U6 (64) was used to optimize the flocculation conditions. The coagulant (1% $CaCl_2$) addition amount, the flocculant addition amount (fermentation supernatant), the initial pH value of kaolin suspension and the reaction temperature were selected as four factor. The factors and levels of uniformity test were shown in table 1, The uniformity test was performed according to the uniform test design table (table 2).

Table 1 Table of Factors and Levels

Levels	A/ The amount of coagulant added (mL)	B/ The amount of flocculant added (mL)	C/pH	D/ Temperature(℃)
1	5	3	4	20
2	6	6	5	25
3	7	9	6	30
4	8	12	7	35
5	9	15	8	40
6	10	18	9	45

Table 2 Table of Uniform Test $U_6(6^4)$

Factors / No.	A/ The amount of coagulant added (mL)	B/ The amount of flocculant added (mL)	C/pH	D/ Temperature (℃)
1	7	18	8	40
2	6	6	7	20
3	10	9	9	30
4	5	12	4	35
5	8	3	6	45
6	9	15	5	25

2.6 Strain Isolation and Screening Results

The strain with the highest flocculation activity was rescreened from three strains with strong flocculation activity, whose flocculation activity was 52.6%. The edges of this strain colonies were smooth, moist, milky white, and Gram-positive.

2.7 Optimization Results and Analysis of Flocculant Flocculation Conditions

The six levels of coagulant, the amount of flocculant (fermentation supernatant), the initial pH of kaolin suspension, and the reaction temperature were selected to perform the uniform test. The results of the uniform test design are shown in Table 3.

Table 3 Results of Uniform Test

Factors / No.	A/ The amount of coagulant added (mL)	B/ The amount of flocculant added (mL)	C/pH	D/ Temperature (℃)	Flocculation activity
1	7	18	8	40	0.663
2	6	6	7	20	0.6814
3	10	9	9	30	0.7251
4	5	12	4	35	0.5221
5	8	3	6	45	0.6257
6	9	15	5	25	0.5373

Regression analysis was performed with dps software, the regression equation is as follows: $Y=0.442188095-0.00617142857\,A-0.00393571429\,B+0.0439619048\,C-0.000448095238\,D$

$R=0.998930, F=584.1440, P=0.0310$.

Table 4 t Test of Partial Regression Coefficient

Factors	Partial regression coefficient	t	P
A/ The amount of coagulant added	-0.99012	8.50179	0.01355
B/ The amount of flocculant added	-0.99791	18.59498	0.00288
C/pH	0.99980	60.56212	0.00027
D/ Temperature(℃)	-0.94640	3.52851	0.07178

The partial regression coefficient t test was performed. According to the t test result, it could be known that pH> the amount of flocculant added> the amount of coagulant added> temperature. The P values corresponding to the pH value and the flocculation time were <0.01, which indicates that they have a extremely significant effect on the flocculation rate; the P value corresponding to the amount of the coagulant addition was between 0.01 and 0.05, which indicates that it has a significant effect on the flocculation rate; the P value corresponding to temperature> 0.05, indicating that its effect on flocculation rate was not significant.

All the factors in the above regression equation was derivated, the results illustrated that when A = 5.00, B = 3.00, C = 9.00, D = 20.00004, the extreme value of the regression equation was 0.78622, which meant the maximum value of theoretical flocculation was 78.622%. The optimal values of the corresponding factors are: the amount of coagulant was 5mL / L, the amount of flocculant was 3mL / L, the pH of the soil suspension was 9.0, and the reaction temperature was 20℃.

Based on the optimal values of the above factors, a verification test was performed. The measured flocculation rate was 73.58%, which was less than the theoretical calculation result of the homogeneous test. This indicated that the actual flocculation effect was due to the interaction of various factors in the actual test. It was slightly smaller than the theoretical settlement result.

2.8 Factors Affecting Flocculating Activity of Flocculants

In this study,we found that the most significant factor affecting the flocculating activity of flocculant was pH, and the flocculation rate was the highest when pH was 9.0. This may showed that the active center of the flocculant molecule could be exposed under alkaline conditions, which promoted its binding with colloidal particles. In addition, the amount of flocculant added also had a significant effect on the flocculation rate. When 3mL of flocculant was added, the highest flocculation rate could be reached, indicating that higher concentrations of flocculant will not increase the flocculation rate. The reason might be that when the excess flocculant was added, which adsorbed on the surface of the colloidal particles were difficult to combine with each other due to the like charge, preventing the formation of bridges between the colloidal particles. The addition of coagulant $CaCl_2$ also had a significant effect on the flocculation rate. A certain concentration of Ca^{2+} could neutralize the charge on the surface of colloidal particles and promoted the bridging effect between flocculant molecules and colloidal particles[2]. However, high concentration of Ca^{2+} would occupy the active center of the flocculant molecule and affect the bridging effect between the flocculant molecule and the colloidal particles. Our study also found that the flocculant activity of the microbial flocculant was not sensitive to temperature changes, which illustrated that its flocculant molecules had a certain thermal stability, could exhibit higher flocculating activity at room temperature.

3. Conclusion

In this study, a high-efficiency microbial flocculant-producing strain was screened from the soil of Zuojia Nature Reserve in Jilin Province, with a flocculation activity of 52.6%. The optimal flocculation conditions of this strain optimized by uniform experiments were that the amount of coagulant (1% $CaCl_2$) was 5mL, flocculant was 3mL, the initial pH of the kaolin suspension was 9.0, the reaction temperature was ℃. In this conditions, the flocculation activity could reach 73.58%.

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