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Screening of flocculant-producing microorganisms and flocculating activity

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Abstract: A strain saccharomycete STSM-1 with high flocculanting activity was isolated from activated sludge with conventional methods. The high production rate and the low cost STSM-1 medium was obtained by selecting different kinds of media, carbon source, nitrogen source and inorganic salt ion. The best flocculant-producing conditions were found by changing medium initial pH, culture temperature and ventilation flow. The best flocculating effect was obtained by changing positive ion types, density and concentration of flocculant.

Keywords: microbial flocculants; flocculant-producing microorganisms; flocculating activity

Introduction

Flocculating agents are widely used in industrial processes such as wastewater treatment, downstream processing, and food fermentation processes (Cumming, 1996; Yokoi, 1996; Suh, 1997; Zhang, 1999; Salehizadeh, 1998; 2000; Owenl, 2002; Patience, 2003). They are generally categorized into three classifications (Salehizadeh, 2001): (1) inorganic flocculants: aluminum sulfate and polyaluminum chloride; (2) organic synthetic high polymers: polyacrylamide derivatives; (3) naturally occurring flocculants: microbial flocculants. Inorganic materials such as polyaluminum chloride (PAC) and organic synthetic high polymers such as polyacrylamide derivatives have been used frequently as economical and powerful flocculating agents in wastewater treatment for many years. Among these flocculants, the organic synthetic high polymer-flocculants have most widely applications. However, studies indicated that the monomer of acrylamide was neuro-toxic and was a strong carcinogen in human body (Vanhorick, 1983; Dearfield, 1988). The use of these flocculating agents is therefore harmful to the environment and a dangerous source of pollution that can adversely affect our generations. Thus biodegradable, safe flocculants, which do harmless to the environment, are attacking widely research interest and are urgently required. Recently, it has been reported that some microorganisms could produce flocculating substances (Yokoi, 1996; Suh, 1997; Salehizadeh, 1998; 2000; Mercz, 1997; Shih, 2001), e.g. *corynebacterium* sp., *aspergillus* sp., *dematium* sp., *paecilomyces* sp., *alcaligenes latas*, and *rhodococcus erythropolis*. But these study focused on the microbial flocculant of alcaligenes latas. Microbial flocculants are a kind of natural macro-molecular flocculants and will be broadly used in the future (Lao, 2001; Zhang, 1999; Dermlim, 1999). This paper would discuss the screening process aimed to find a new flocculant-producing microorganism, and study the flocculation activity of microbial flocculants, and the factors that effect on flocculant-producing.

1 Materials and methods

1.1 Screening for flocculant-producing microorganisms

Bioflocculation resulting from synthesis and secretion by microorganisms has been well known in activated sludge. Generally, soil and activated sludge samples are the best sources for isolating flocculant-producing microorganisms. In our study, 32 bacterial strains were isolated from activated sludge of some wastewater works. Each strain was cultured in three different kinds of flocculant-producing liquid media (YE-1, ST-1, GL-1) in 100-ml flasks using a rotary shaker at 30℃ for 70 h. Kaolin suspension was flocculated by the supernatant of centrifuged culture, which was regarded as the index to determine the flocculating activity and ability to produce flocculant (Shih, 2001; Lao, 2001; Zhang, 1999; Dermlim, 1999; He, 2002) (Table 1).

Table 1 Screening medium

Components	Weight percent, %		
	YE-1	ST-1	GL-1
Yeast extract	2	-	0.5
Starch	-	0.05	-
Glucose	-	-	2
KH_2PO_4	0.1	0.05	0.2
$MgSO_4 \cdot 7H_2O$	0.1	0.05	-
$(NH_4)_2SO_4$	-	0.2	0.02
KCl	-	0.05	-
K_2HPO_4	-	-	0.5
NaCl	-	-	0.01
Urea	-	-	0.05
pH	7.0	7.0	7.0

1.2 Flocculation activity

Kaolin clay suspension was used to test flocculating activity. In a 100 ml graduated cylinder, 80 ml of Kaolin clay suspension (5000 ppm), 10 ml of 10% $CaCl_2$ solution, and 0.5 ml of the cultures were mixed, and filled up to 100 ml with distilled water. The pH was adjusted to 7.0. The test cylinder was mixed gently at room temperature and left aside for 5 min, then the formation of visible aggregates was

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observed. By measuring the decrease in turbidity of the upper phase, the degree of flocculation could be measured. The optical density of the upper phase was measured at 550 nm with a spectrophotometer (UV-2102). The flocculating activity was calculated by the following equation(Shih, 2001; He, 2002; Kurane, 1994a; Jorand, 1994; Wilen, 2003).

Flocculating activity (%) = $(A - B)/A \times 100\%$,

where, A is the reference optical density at 550 nm; B is the sample optical density at 550 nm.

1.3 Distribution of flocculating activity in the cultures

Liquid cultures of STSM-1 grew on a rotary shaker at 30℃ for 70 h, usually in a 500 ml flask containing 100 ml of the medium. ST-1, which was chose because of its higher flocculating activity and lower expense. The initial pH of medium was adjusted to 7.0. The supernatant was used as the culture broth. The precipitate including cells was separated from liquid cultures of STSM-1 for use by centrifuging. Flocculating activity in each fraction was assayed as above.

2 Results and discussion

There are many factors that influence the production of microbial flocculant and the bioflocculation process including genotype, physiological and environmental aspects (Salehizadeh, 2001). The environmental aspects involve physical, chemical and biological factors. The carbon and nitrogen concentration (C/N ratio), the culture pH, temperature and the agitation speed used in the fermentor should be optimized for efficiency of production. This is essential because the productivity and distribution of microbial flocculant depend heavily on the culture conditions.

2.1 Screening for flocculant-producing bacteria

Cultures of 32 strains, which were isolated from activated sludge, were tested for the ability to flocculate Kaolin clay. The results are summarized in Table 2.

Table 2 Flocculant-producing strains

Strain	Flocculating activity, %		
	YE-1	ST-1	GL-1
Ⅰ1	9.20	1.21	-
Ⅰ2	35.86	38.63	39.65
Ⅰ3	5.56	6.06	5.30
Ⅱ1	0.49	1.30	1.19
Ⅱ2	2.19	5.54	0.48
Ⅲ1	0.95	2.15	1.91
Ⅳ1	8.07	10.31	14.35
Ⅳ2	30.49	59.19	61.21

Eight flocculant producing strains were obtained. The microorganism that had the strongest flocculating activity among them was STSM-1. STSM-1 strain was studied. By appraisal, STSM-1 was succharmycete. The microbial flocculant of STSM-1 produce was named SUST-1.

2.2 Relationship between cell growth and flocculants production

The STSM-1 growth characteristics were studied and shown in Fig.1. It showed that the organism had a high yield under the conditions, therefore it was suitable to be used as a flocculant. The pH was also monitored and was found to remain constant at around pH 6.5—7.0. The biological flocculation did not occur until the microorganisms had entered into an endogenous phase (Fig. 1, Fig. 2). The flocculating activity increased rapidly with increasing time and reached a maximum value after 2 d (Fig. 2). The result indicated that the production of flocculant occurred in the final phases of a batch culture.

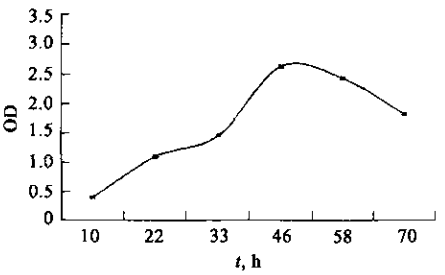

Fig.1 Growth curve of STSM-1

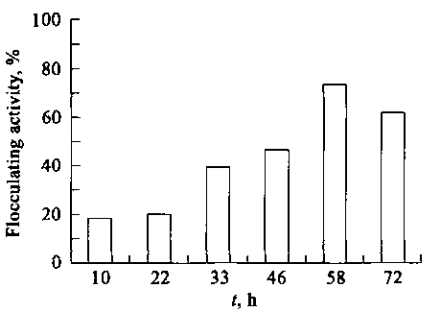

Fig.2 Time course of flocculants production

2.3 Distribution of the flocculating activity

Fig.3 shows the distributions of the flocculating activity in the cultures. Both the culture broth and cells appeared to have flocculating activity and marked them as 1. The flocculating activity of cells was lower than that of the culture broth. It has concluded that more than 95% of flocculating activity was in the culture broth and less than 5% in the cells. The negative number indicated the medium was colorful.

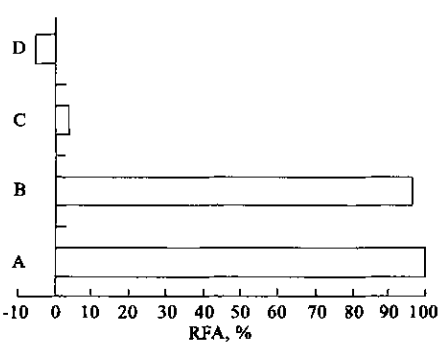

Fig.3 Distribution of flocculating activity

RFA: relatively flocculating activity; A: culture (culture and broth); B: culture broth; C: cell; D: medium

2.4 Effect of carbon sources and nitrogen sources

The importance of carbon and nitrogen sources has been emphasized for flocculant production (Kurane, 1994b). The additional sugars added to the medium would reduce the pH of the culture broth and inhibit the accumulation of the flocculant. Glucose, fructose, sucrose, starch and milk sugar that effect on flocculant formation among the various water-soluble carbon sources were tested (Table 3).

Table 3 Effect of carbon sources

Carbon sources	Flocculating activity, %
Glucose	61.2
Fructose	38.2
Sucrose	54.5
Starch	66.7
Milk sugar	37.1

Starch was found to be the most effective flocculant formation material among the various carbon sources. These five sugar components appeared favorably for cell growth as well as flocculant production. Both peptone, as an inorganic nitrogen sources, and yeast extract, as an organic nitrogen source appeared to be favorable for flocculant production and cell growth among the various inorganic and organic nitrogen sources (Table 4).

Table 4 Effect of nitrogen sources

Nitrogen sources	Flocculating activity, %
Yeast extract	59.7
$Co(NH_2)_2$	23.1
Peptone	60.1
$(NH_4)_2SO_4$	59.3
Beer	47.6
Glutamic acid	57.3
Bean sprout extract	51.3

2.5 Effect of culture temperature

The culture temperature was optimized at 30℃ for both the flocculant production and cell growth compared to 25℃ and 35℃ (Fig.4). At 30℃ the flocculating activity could reach its maximum value in the shortest time.

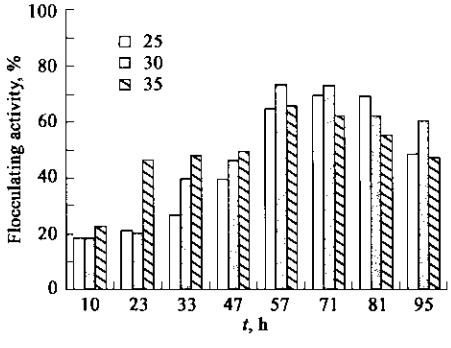

Fig.4 Effect of culture temperature

2.6 Effect on medium initial pH

The initial pH would also affect the growth rate and flocculant production, as shown in Fig. 5. The meta-acid pH, especially pH 6.5 was the best, because the meta-acid pH was beneficial to saccharomycete growth, and stimulated the flocculant production greatly.

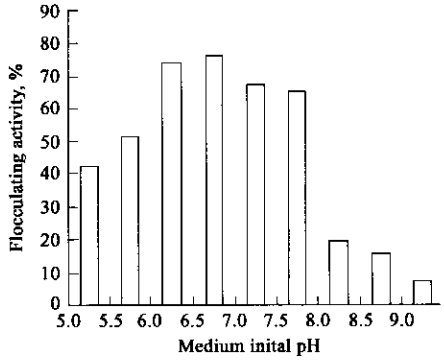

Fig.5 Effect of initial pH

2.7 Cation effects on flocculation

Table 5 shows the effect on the flocculation of a Kaolin suspended solution after adding cations into the reaction mixture. Flocculating activity was stimulated by the additional cations such as Ca^{2+} and Al^{3+}. On the other hand, the activity was not affected by Na^+, while slightly stimulated by NH_4^+ and K^+.

Table 5 Cation effects on flocculant activity

Cation	Flocculating activity, %
Bank	43.3
NaCl	42.8
NH_4Cl	44.7
KCl	43.9
$CaCl_2$	69.4
$Al_2(SO_4)_3$	75.2
$FeSO_4$	61.3
$MgSO_4$	59.6

From the above result, it can be concluded obviously that trivalent metal cations are more suitable for stimulation flocculating activity than divalent cations. It could be assumed that cations stimulate flocculation by neutralization and stabilization of residual negative charges of the carboxyl group of ironic acid, pyruvic acid and acetic acid in an acidic poly-saccharide forming bridge, which bind Kaolin particles to each other.

2.8 Flocculant concentration effects on flocculating activity

The standard Kaolin suspensions containing 1.0 mm of Ca^{2+} were tested with different flocculant preparation concentrations, as shown in Fig.6.

Microbial flocculation is generally attributed to exopolymeric bridging of bacterial cells. Bridging of floc components involves ionic interactions between charged functional groups of biopolymers and divalent cations, and may also be mediated through hydrophobic moieties of exopolymers, specific protein-polysaccharide interactions, or simple physical enmeshing. In addition, electrical double layer effects have been proposed to play an important role in

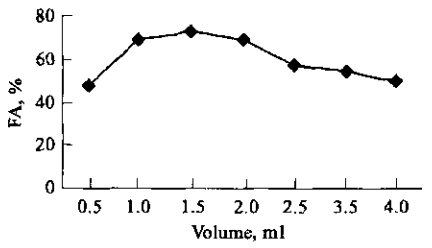

Fig.6 Flocculant concentration effect on flocculating activity
FA: flocculating activity

floc stability(Chaignon, 2002).

3 Conclusions

Flocculant producing microorganisms are of wide distributions in natural circumstances. Humans have been in contact with these microorganisms for a long period in the open environment. In this paper, we screened for bacteria producing flocculants, and obtained 8 flocculant that producing strains. Although STSM-1 could produce the flocculant with a simple medium, some culture conditions were required for efficient production of the flocculant. The high production rate and the low costly medium was 2% starch, 0.05% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.2% $(NH_4)_2SO_4$, 0.05% KCl. The best flocculant producing conditions were initial pH 6.5—7.0, culture temperature 30℃, and culture time 58—65 h. Since the maximum flocculating activity was distributed in the culture broth, we could conclude that the flocculating was not produced by cell autolysis but by biosynthesis. In the process of flocculant isolation, a proper centrifugal condition was recommended because too strong centrifugation may cause obvious decrease in the flocculating efficiencies. Coagulation can be apparently improved when using cation as the flocculating-acid.

The SUST-1 was used for flocculating tests on several kinds of pollution water. The results indicated that they could be purified efficiently and that solid-liquid separation was good. Research and development on microbial flocculants have perspective future.

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