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Microbial flocculant and its application in environmental protection

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Abstract—The microbial flocculant is a kind of natural bio-polymer and has promising future to be used in the fermentation industry and wastewater treatment. It has been studied in details in many countries, such as U. S. A, Japan. This paper reviewed the development on microbial flocculant basic studies and applications, including flocculant-production microorganisms, chemical and components of microbial flocculants, flocculation mechanism of microbial flocculants, capability of microbial flocculants and influence factors, and culture conditions for flocculant production. The application cases of microbial flocculants were also discussed.
Keywords: microorganism, flocculant, flocculation.

1 Introduction

A lot of varied flocculants have been used in wastewater treatment, dredging down-stream processing and some industrial field including fermentation process. These flocculants might be generally classified into three groups, i. e. (1) inorganic flocculants, such as aluminum sulphate and poly-aluminum chloride (PAC); (2) organic synthetic high polymers: polyacrylic acid and others; and (3) naturally occurring flocculants: chitosan and microbial flocculants. Among these flocculants the organic synthetic high polymer-flocculants have been most frequently used since they are both very cost-effective and strong agents. And their use recently is increasing. But it should be pointed that monomers of these synthetic flocculants are not only neural toxicant but also strong human carcinogens (Vanhorick, 1983; Dearlfield, 1988). There is a potential need for strong and biodegradable safe flocculants, which have little or no toxic effect on organisms in water and wastewater treatment in addition to fermentation and food industries.

For thirty years, microbial flocculants (MFs) have been attracting wide interest of researchers from many countries, such as U. S. A (Shilo, 1982; Chen, 1992; Misra, 1991; 1993), Japan (Kurane, 1976a; Kakii, 1985; Tamura, 1986), United Kingdom (Morgan, 1990), Finland (Hantula, 1991a; 1991b), France (Guirand, 1992), Germany (Reinhard, 1989; Dube, 1992), Portland (Sousa, 1992), Korea (Seo, 1993; Kim, 1993; Nam, 1996), Israel (Bar-Or, 1987; Levy, 1990; 1992), Brazil (Fumio, 1991), China (Huang, 1990; Wang, 1994), and so on. Their results indicated that microorganism flocculants, such as the flocculant produced by *Paecilomyces* sp. (Takagi, 1985a), has several advantages over other naturally occurring flocculants. It is also expected that MFs can be produced at a low cost in a large-scale culture growth rate for the following reasons: (1) high growth rates in an inexpensive medium, (2) little fear of bacterial contamination because it is difficult for microorganisms to grow using MFs as substrate, and (3) simple purification processes for the products.

This paper reviewed the development on microbial flocculants studies and applications

including flocculant-producing microorganisms, chemical bases of MFs, mechanisms of MFs flocculation, ability of MFs and its influence factors, the culture conditions for MFs production and the application cases of MFs in environmental pollutants treatment.

2 Flocculant-production microorganisms

Many kinds of microorganisms including bacteria, fungi, antinomyces, yeast and cyanobacteria were found to produce MFs. Some of them are listed in Table 1 and these microorganisms were widely distributed in soils and waters. The MFs produced by these microorganisms might used not only in wastewater treatment and improving the settling of activated sludge but also in the separation process of microorganism fermentation industries.

Table 1 Microorganisms producing microbial flocculants

Microorganisms	Components	References
Bacteria		
Rhodococcus erythropolis	Protein	(Kurane, 1986b; 1992; 1991b; 1988a; 1989a; 1986a)
Alcaligenes latus		(Kurane, 1991a)
Alcaligenes cupidus	Acid polysaccharide	(Toeda, 1991; Kurane, 1989b)
Alcaligenes faecalis		(Shimizu, 1985)
Corynebacterium	Polysaccaride	(Zajic, 1971)
hydrocarbonacalastus	Protein	
Corynebacterium brevicale		(Nakamura, 1976a)
Dematinum sp.		(Kurane, 1986b; Takeda, 1991)
Mycobacterium phlei		(Misra, 1991; Chen, 1992; Dube, 1992; Misra, 1993)
Pseudomonas sp.	Mucopolysaccharide	(Tago, 1977)
Pseudomonas aeruginosa		(Nakamura, 1976a)
Pseudomonas fluorescent		(Nakamura, 1976a)
Bacillus sp.	Poly(-glutamic acid)	(Seo, 1993; Kim, 1993; Yokoi, 1995)
Methylobacterium rhodesianum		(Reinhard, 1989)
Lactobacillus fermentum	Protein	(Fumio, 1991)
Flavobacterium sp.	Protein	(Endo, 1976; Hantula, 1991a)
Brevibacterium insectiphilum		(Nakamura, 1976a)
Staphylococcus aureus		(Nakamura, 1976a)
Zoogloea ramigera		(Krul, 1977)
Kluyveromyces marxianus	Poly peptide	(Saito, 1990)
Kluyvera cryocrescens		(Kakii, 1990)
Antinomyces		
Nocardia amarae	Protein	(Nakamura, 1976a)
Nocardia restricta		(Kurane, 1986b)
Nocardia calcarea		(Kurane, 1986b)
Nocardia rhodnii		(Kurane, 1986b)
Streptomyces vinaceus		(Nakamura, 1976a)
Streptomyces griseus		(Nakamura, 1976a)
Fungi		
Eupenicillium crustaceus		(Nakamura, 1976a)

Table 1 (continued)

Microorganisms	Components	References
Circinella sydowi		(Nakamura, 1976a)
Monascus anka		(Nakamura, 1976a)
Geotrichum candidum		(Nakamura, 1976a)
Sordaria fimicola		(Nakamura, 1976a)
Paecilomyces sp.	Galactosamine polysaccharide	(Takagi, 1985a; 1985b; 1985c)
Aspergillus sojae	Poly-hexosamine	(Nakamura, 1976b; 1976d; Kurane, 1986b)
	Protein, 2-ketogluconic acid	
Aspergillus ochraceus		(Nakamura, 1976a)
Aspergillus parasiticus		(Nakamura, 1976a; Hayashi, 1976)
Aspergillus sp.		(Nam, 1996)
Hansenula anomala	Protein	(Saito, 1990)
Saccharomyces cerevisiae	Poly peptide	(Saito, 1990)
Saccharomyces diataticus		(Guirand, 1992)
Algae		
Phorimidium sp.	Protein	(Shilo, 1982; Fattom, 1990;
	Sulphated heteropolysaccharide	Bar-Or, 1987)
Anabaenopsis circularis	Heteropolysaccharide	(Bar-Or, 1987)
Chlamydomonas mexicana		(Takagi, 1985)
Calothrix desertica		(Bar-Or, 1987)

3 Chemical components of MFs

The flocculant of *Phorimidium* sp. is a sulphated heteropolysaccharide to which fatty acids and protein are bound (Bar-Or, 1987). The polysaccharide backbone is composed of uranic acids, rhamnose, mannose, and galactose. *A. Circularis* flocculant is also an acidic polysaccharide containing keto acid residues and neutral sugars, but to which no fatty acids, proteins or sulphate are linked (Bar-Or, 1987). The flocculant produced by *Asp. sojae* consists of 20.9% galactosamine, 0.3% glucosamine, 35.3% 2-Ketogluconic acid and 27.5% protein (Kurane, 1976b). The constituent sugars of MFs produced by *Alcaligenes cupidus* were glucose, galactose and glucuronic acid (molecule ratio 6.34 : 5.55 : 1.0) (Toeda, 1991). However, not all of the polysaccharides synthesized by microorganisms have flocculating activity. For example only 10 percent of polysaccharide produced by *Pseudomonas* sp. showed flocculating activity (Tago, 1977).

The bioflocculant FIX produced by *N. amarae* YK1 was a mixture of more than three substances, but individual fraction did not exhibit the ability of flocculation (Koizumi, 1991). The chief component of fractions was peptide and one of the ingredients of FIX carried high contents of glycine (25.6%), alanine (13.8%), and serine (12.3%), of which R groups were relatively small (Koizumi, 1991). The research results about *Flavobacterium* also showed that its flocculant was susceptible to pronase (Endo, 1976) and it was proved later that the flocculating factor of one *Flavobacterium* strain was a 140-kDa protein (Endo, 1980; 1981). Other researcher (Kurane, 1988a) found that the flocculating activity of *R. erythropolis* was decreased after 15 min boiling treatment, and both the Xanthoprotein and anthrone reaction were positive with the flocculant of

R. erythropolis. These results suggested that *R. erythropolis* flocculant required the protein portion for the flocculating activity.

Sometimes there were more than one portion responsible for the flocculating activity. The flocculant of *Aspergillus sojae* AJ7002 had three components (Kurane, 1986b) and consisted of 20.9% galactosamine, 35.3% 2-ketogluconic acid and 27.5% protein (Kurane, 1992). It was suggested that the hexosamine moiety in the polymer played a major role in the bio-flocculation, assisted by protein portion in enlargement of the molecule weight of the flocculant, and by 2-ketogluconic acid in endowing it with ball shape (Kurane, 1986b).

Based on the results of these researches, it might be drawn that the most kinds of MFs were polysaccharides produced during metabolism process of microorganisms (Unz, 1976; Tago, 1977; Takagi, 1985c; 1985b; Bar-Or, 1987; Toeda, 1991) and small part of MFs were proteins or peptide (Saito, 1990; Hantula, 1991; Fumio, 1991). Sometimes MFs contained both polysaccharides and proteins (or peptide) (Zajic, 1971; Nakamura, 1976b; 1976c; Bar-Or, 1987). The components of the MFs did not vary largely even when the medium composition or culture conditions changed (Seo, 1993).

Although there is a general agreement on the involvement of cell wall polysaccharides and proteins on flocculation, it is not clear what kind of functional groups are directly implicated. Most of the authors pointed that carboxyl groups play an important role in flocculation (Sousa, 1992), while others refereed to the importance of phosphate groups (Lyons, 1970; 1971). For *Kluyveromyces marxianus*, the sedimentation capacity was reduced after incubation with acid phosphates, suggesting that phosphate groups play a role in flocculation (Sousa, 1992). This is also confirmed by the larger content of phosphate groups on the cell wall of the flocculant *K. marxianus* strain, when compared with the non flocculent one (Teixeira, 1989).

4 Flocculation mechanism of MFs

From adsorption isotherms and zeta-potential measurements, it was concluded that the flocculation process of MFs is based on the bridging mechanism and electrostatic mechanism (Levy, 1992). Other research results also indicated that bridging mechanism was very important for MFs (Toeda, 1991). For the flocculation of pure culture of brewer's yeast, it was generally recognized that flocculation was a specific cell adhesion, with proteinaceous, lectin-like molecules bound to carbohydrates of adjoining cells. The flocculation of yeast cells with the flocculant from *Asp. sojae* was also explained in terms of a bridging phenomenon between discrete cells and linearly extended polymer chains forming a three dimensional matrix that was capable of settling under static condition (Nakamura, 1976c).

The polygalactosamine chain of the flocculant PF-1 produced by *Paecilomyces*, which is adsorbing anionic particles and neutralizing their charges, may form intermolecular bridges that result in a stable floc and precipitation (Takagi, 1985a).

Several negatively charged polysaccharide isolated from bacteria, yeast, and soil fractions are known to be capable of flocculating suspended clay particles by absorbing onto the surfaces of neighboring negatively charged clay particles via cation bridge (Bar-Or, 1987). The effectiveness of such polymers depends on the length of the molecule and the number of charged groups per unit length, i.e., the charge density. These factors determine the extent of inter-particle bridging by the flocculants. It was also said that the Kaolin flock formation occurred by repeated absorption of flocculant produced by *A. cupidus* KT201 on the Kaolin particles and the flocculant macromolecule

was very advantage for this bridging mechanism (Toeda, 1991).

5 Ability of MFs and its influence factors

5.1 Flocculating ability

It was said that low specificity of some MFs, such as the flocculant produced by *Asp. sojae* AJ7002, was because that these flocculants act as polyelectrolytes which aggregate the microbial cells or Kaolin into a floc network by the formation of chemical bridge (Nakamura, 1976a). Other reports showed that the flocculant produced by *Rhodococcus erythropolis* had wide flocculating activity against both organic and inorganic materials. It could effectively flocculate all suspended solids in aqueous solution tested, including microorganisms such as *E. coli* and alcohol yeast, activated sludge, Microcystis aeruginosa, Kaolin clay, muddy water, river dredging muddy water, river bottom sediment, ash from a steam-power station and charcoal (Kurane, 1986b). Another strong MFs was flocculant formed by *Paecilomyces* sp. It also could effectively flocculate all suspended solids in aqueous solution tested and the flocculating activity was partially not affected by ionic strength, pH or temperature (Takagi, 1985a).

However there are some MFs which only flocculate special material (Takagi, 1985a, Saito, 1990). MFs produced by *Aspergillus sojae* might effectively flocculate some microorganisms such as *Brevibacterium lactofermentum* with 100% flocculating efficiency while the flocculating efficiency of it was only 33% for other microorganisms (Takagi, 1985a). An special example was that MFs of *Hansenula anomala* could not flocculate its mutation strain without flocculating activity (Saito, 1990). These results indicated that the flocculating ability of MFs might be remarkably affected by the characteristics of suspended materials. The flocculant produced by *Nocardia amarae* required some activities of *E. coli*. because the rest cells of *E. coli*. were never influenced by the flocculant while active cell was effectively flocculated by the flocculant (Koizumi, 1991).

5.2 Influence factors of flocculating ability

There are other influence factors of flocculating ability beside the characteristics of suspended materials, including temperature, pH, metal cations, the concentrations and molecule weights of MFs.

5.2.1 Temperature

Flocculating ability of some MFs might significantly change with temperature changing. This is partly resulted from that the protein or peptide contained in these MFs denatured under high temperature (Kurane, 1986c). The flocculating ability of MFs, which are consisted of only polysaccharides is not affected by temperature (Takagi, 1985b). The MFs of *Aspergillus sojae* had it maximum activity between 30℃ and 80℃, its flocculating ability would decrease under other temperature outside the above range (Nakamura, 1976c). The activity of MFs produced by *Rhodococcus erythropolis* was reduced by 50 percent after heated in boiling water for 15 mins (Kurane, 1988a). The flocculant from *Bacillus* sp. PY-90 lost its flocculation activity partly at 100℃ (Yokoi, 1995). On the other hand, the *Paecilomyces* sp. MFs which was galactosamine polysaccaride might maintain the same activity from 0℃ to 100℃ (Takagi, 1985b).

5.2.2 pH

Tenny's group (Pavoni, 1972; Tenny, 1973) observed that the flocculation capacity of the extracted polymer for activated sludge for Kaolin solution was greatly enhanced with increasing pH values. The change of pH might vary the charge status of MFs and surface characteristics of

suspended materials, and result in the variation of flocculating ability (Takagi, 1985b; Nakamura, 1976c; Kurane, 1991a). The flocculants produced by the microorganisms might show the strongest flocculation effect in the range of pH where the surface charge of yeast is reduced (Nakamura, 1976c). The MFs of *Paecilomyces* sp. had highest flocculating activity at pH 4—7.5 and the activity decreased quickly to 0 at pH 3 or pH 8 (Takagi, 1985b; 1985a). *Aspergillus sojae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Corynbacterium brevicale* and *Streptomyces vinaceus* show flocculating activity at pH 3—5 while no flocculating ability was observed at pH 7—9 (Nakamura, 1976a; 1976c). This is also true for the flocculant from *Bacillus* sp. PY-90 (Yokoi, 1995). However, flocculation of *Asp. sojae* was observed at pH higher than 7.0 by increasing the concentration of flocculant in the reaction mixture (Nakamura, 1976a; 1976c).

5.2.3 Metal ion

The presence of divalent cations is essential for some flocculants (Nakamura, 1976a; Fattom, 1990; Saito, 1990; Levy, 1992) while some MFs was found to exhibit flocculation activity without metal cations (Toeda, 1991). For instance, *Kluyvera cryocrescens* showed a good flocculant growth in a kind of medium containing a low concentration of Ca^{2+}, but did not from any flocs in the absence of Ca^{2+} (Kakii, 1990). The cell flocs were completely deflocculate not only by Actinase E, but also by EDTA. Therefore, it was thought that Ca^{2+} and cell surface proteins are involved in the flocculation (Endo, 1976). It was also proved that the flocculating strain of *H. anomala* had the same quantity and kinds of fatty acid and amino acid as the non-flocculating strain of *H. anomala* but the concentrations of metal cations including Ca^{2+}, Mg^{2-} and Na^{+} in the former were far higher than those in the latter (Saito, 1990). On the basis of Tezuka's and Endo's studies (Tezuka, 1969; Endo 1980), in addition to the protein factor, the flocculation of *Flavobacterium* was induced by the addition of divalent cations, and floc was dispersed in the presence of EDTA.

The flocculating ability of MFs might be significantly enhanced by the addition of some cations such as Na^{+}, Ca^{2+}, Fe^{3+} and Al^{3+} (Toeda, 1991; Hantula, 1991; Takeda, 1992). Most of the MFs which would be affected by metal cations had protein or peptide composition (Takeda, 1992). The metal ion effects come from neutralization of the zeta potential and enforcement of the bridge between flocculants and suspended materials (Toeda, 1991, Levy, 1992). The flocculant produced by *Alcaligenes cupidus* was found to aggregate a suspended Kaolin solution without cations, but its flocculating ability was significantly enhanced by the addition of bivalent/trivalent cations such as Ca^{2+}, and Al^{3+} (Toeda, 1991). On the other hand, the same effect was not observed for activated carbon powder flocculation. These results indicated that the metal ion effects result from neutralization of the zeta potential. The presence of a $CaCl_2$ significantly increased the flocculation efficiency of the MFs produced by *Chlaymodomonas mexicana* and *Rhodococcus erythropolis* (Kurane, 1986; Levy, 1992). The role of $CaCl_2$ is to increase the initial adsorption of the bio-polymer on the bentonite particles. Ca^{2+} also decreases the negative electrical charge of the bentonite particle and of the bio-polymer molecules (Levy, 1992). In addition, the floc size became larger with increasing concentration of Ca^{2+} (Kakii, 1990). Most authors agreed on the importance of calcium (Mill, 1964; Lyons, 1970; Taylor, 1971; Amri, 1979; Stanford, 1989), due to its specificity to promote flocculation. Other author (Stewart, 1976; Nishihara, 1982; Wang, 1994) claimed that Mg^{2+}, Mn^{2+} as well as other ions were as effective as calcium.

The effect of metal ion might be complex and specific (Endo, 1976; Kurane, 1986b; Levy, 1992). Data from pure culture studies suggested that in some cases inorganic divalent metal ions enhanced flocculation and in others promoted dispersion of cells (Hantula, 1991a). Fe^{3+} and Al^{3+}

might increase the flocculating ability of MFs at low concentration while high concentrations of Fe^{3+} and Al^{3+} would inhibit the activity of MFs (Takeda, 1992). Also, the MFs activity of *Paecilomyces* sp. might be remarkably inhibited by Fe^{3+} and CO_3^{2-} (Takagi, 1985a; 1985b). A decrease in the flocculation rate of *Asp. sojae* was observed at concentrations higher than 0.04 mol/L of Na^+ and flocculation was completely inhibited at concentration of 0.4 mol/L of Na^+ (Nakamura, 1976c).

The same metal ions might have different effects on different flocculants produced by different microorganisms (Sousa, 1992). For *K. marxianus* cells Ca^{2+}, Co^{2+}, Mn^{2+}, Sr^{2+} or Mg^{2+} had all similar effects to promote flocculation. Nevertheless, they were not as efficient as Fe^{2+} or Sn^{2+}, which were the most effective ions tested. The trivalent ions, Ce^{3-} and Al^{3+}, were both capable of promoting flocculation, and Ce^{3+} a slighter lower effect than Al^{3+}. However, *S. cerevisiae* cells had a different behavior in the presence of these ions. Indeed, Ce^{3+} was unable to promote flocculation, even after a 10 mins settling period, while cell flocculated quite well in the presence of Al^{3+}. The divalent ions came into two groups on the basis of their effects on *S. cerevisiae*. One, for Ca^{2+}, Co^{2+}, Mn^{2+}, Mg^{2-} or Fe^{2+}, where very fast and extensive sedimentation occurred, and another where the sedimentation is slower, which occurred in the presence of Sn^{2+} or Sr^{2+} (Sousa, 1992).

According to Endo *et al*. (1976), apart from the direct participation of calcium in flocculation of *Flavobacterium* pure culture, the presence of calcium on the growth medium appeared to be required for the synthesis of some components of cell surface which was essential for flocculation.

5.2.4 Molecule weight of MFs

Molecule weight of MFs is a critical factor determining their flocculating ability. Higher molecule weight means more absorption points and more electric charge and results in stronger bridging and neutralizing capacity. Most of the MFs which had been purified and analyzed are biopolymers such as polysaccharides and proteins with high molecule weight ranging from 10^5 to 10^6 except that *Hansenula anomala* formed lower molecule weight flocculant (37000) (Nakamura, 1976b; Saito, 1990; Toeda, 1991). The flocculants, which were produced by *Flavobacterium* sp. and *Asp. sojae*, had molecule weight of 140000 and 200000 respectively (Nakamura, 1976b). The flocculant from *Arthrobacter* sp. has the molecule weight more than 10^6 Da (Wang, 1994). The molecule weight of *Alcaligenes cupidus* flocculant had reached 2000000 (Toeda, 1991). Decreasing of molecule weight might reduce flocculating activity. For instance, after protein part was removed, flocculating ability went down remarkably due to decreasing of molecule weight (Nakamura, 1976b).

6 Culture condition for MFs production

6.1 Time course of flocculant production

Mickinney (Mickinney, 1952) reported that the floc formation was correlated with the metabolic activities of bacteria, and that the floc could be determined microscopically only after the bacteria had ceased their metabolic activities. *Flavobacterium* showed flocculating activity only in the end of logarithmic growth phase and the beginning of stationary phase (Hantula, 1991).

Other microorganisms, however, had a different behavior with respect to the producing stage of flocculants. The flocculating activity of *Alcaligenes cupidus* culture increased during the first three days and maintain in the rest culture period (Toeda, 1991). For *Paecilomyces* sp. I-1, flocculating activity and accumulation of the flocculant were found in the culture fluid with the

growth of the mycelium, reached the maximum after 5 days culture and maintain that level during the rest period of culture (Takagi, 1985b). In the case of *Alcaligenes latus*, maximum flocculant production was achieved at the middle and latter stage of logarithmic growth phase (Kurane, 1991a), and flocculating activity began to decrease at the latter of stationary phase because of deflocculation enzymes (Tago, 1977; Kurane, 1991a).

These results indicated that it was better to collect flocculants in the latter of logarithmic growth phase and the early stage of stationary phase because the flocculants production would not increase after those stages and at the worst case it would decrease.

6.2 Distribution of MFs in microorganism culture

It was indicated that the flocculants produced by microorganisms were distributed in culture solution and the surface of microbial cells (Nakamura, 1976a; 1976d; Hantula, 1991b; Kurane, 1986c). The flocculating activity was observed both in the culture filtrates and in the washed cells of *Pseudomonas fluorescent* AJ2084, *Pseudomonas aeruginosa* AJ2116 and *Streptomyces griseus* AJ9022 culture (Nakamura, 1976b). However, in the cases of *Aspergillus genus*, *Corynebacterium brevicale*, *Staphylococcus aureus* and *Streptomyces vinaceus*, flocculating activity was mainly shown in the culture filtrates (Nakamura, 1976b). Sometimes the flocculant absorbed on the surface of *Aspergillus sojae* cells was only 5 percent of that in culture filtrates (Nakamura, 1976d). For *R. erythropolis* more than 90% of the flocculating activity was in the culture broth and less than 10% in the cells (Kurane, 1986c).

The distribution of flocculants in the culture filtrates and cells would be affected by culture medium and environmental factors such as pH. The flocculant on the surface of cell was more at pH 6 than that at pH 8 (Nakamura, 1976d). For *R. erythropolis* the flocculant was mainly located on the cell surface in the *n*-pentadecane medium (Kurane, 1994).

6.3 Culture condition for flocculants production

The microorganism flocculants has problems such as expensive compared with synthetic flocculants. Thus, the effects of carbon sources, nitrogen sources, ions, temperature, pH and other factors on the production of MFs were investigated to cut down the medium cost and raise productivity of the flocculant.

6.3.1 Effect of carbon sources

The flocculant of *Paecilomyces* sp. I-1 was accumulated in the medium containing polypeptone and casamino acid (Takagi, 1985b). Addition of yeast extract and meat extract stimulated only the mycelial growth, there being little or no flocculant production. For *Asp. sojae*, the flocculant was well produced when casein or yeast extract was added to the culture medium as carbon and nitrogen sources (Nakamura, 1976d). Polypepton, casamino acids and amino acids, such as alanine and glutamic acid, were also effective as substrate for flocculant production (Nakamura, 1976d).

In the case of *Rhodococcus erythropolis*, glucose, fructose and sorbitol appeared favorable for cell growth as well as flocculant production (Kurane, 1986c). On the other hand, mannose, galactose, arabinose, xylose, lactose, maltose, cellobiose and sucrose were not favorable for either flocculant production or cell growth (Kurane, 1986c). Flocculant production of *Rhodococcus erythropolis* was more in the medium using 0.5% glucose and 0.5% sucrose as carbon sources than in the medium using 1% sucrose or 8% waste sugar syrup (containing glucose, fructose and sucrose)(Kurane, 1991b).

Paecilomyces sp. I-1 efficiently produced the flocculant from all kinds of carbohydrate examined, including glucose, fructose, mannose, galactose, sucrose, maltose, raffinose and starch, except lactose (Takagi, 1985b). Starch appeared especially favorable for both the growth

and the flocculant production (Takagi, 1985b). For *Alcaligenes cupidus* KT201 using glucose, galactose and sucrose as the carbon sources resulted in better flocculation activity than either starch or maltose (Toeda, 1991).

Addition of some organic acids such as 2-Ketogluconic acid, 5-Ketogluconic acid, gluconic acid, succinic acid and so on, would enhance the flocculant production of *Asp. sojae* (Nakamura, 1976d). Among them 2-Ketogluconic acid had the best effect. But some organic acids, such as acetic acid and citric acid, and phenol were not good for the flocculant production (Kurane, 1994).

In searching cost-effective medium for flocculant production, it found that sorbitol, mannitol and ethanol were effective on both cell growth and flocculant production of *R. erythropolis*, as well as glucose and fructose (Kurane, 1994). Other alcohols including *n*-propanol and glycerol were also effective for flocculant production beside ethanol. Flocculating activity per cell growing on the ethanol medium, was highest among all carbon sources consumed (Kurane, 1994). Ethanol is easy to get and handle, and cheap for manufacturing process. In addition, sterilization of culture equipment might be omitted by using 70% ethanol. It appeared that ethanol was a favorable candidate for carbon sources to produce the MFs industrially (Kurane, 1994). The research results also indicated that wastewater containing alcohol was useful for the flocculant production. To date, fish-blood containing wastes from canning factories were found to be suitable carbon sources for flocculant production although which component in fish blood was effective is not known (Kurane, 1988b).

Sometimes the best carbon sources for cell growth were not the best ones for flocculant production. *Rhodococcus erythropolis* grew well in the medium containing 8% waste sugar syrup as carbon sources while flocculant production was not high (Kurane, 1991b). Another example is that olive oil was more effective for cell growth of *R. erythropolis* than glucose and fructose while it greatly reduced flocculating activity (Kurane, 1986c).

6.3.2 Effect of nitrogen sources

Urea and ammonium sulphate as inorganic nitrogen sources appeared favorable for both flocculant production and cell growth of *R. erythropolis* (Kurane, 1986c). On the other hand, ammonium chloride and ammonium nitrate stimulated cell growth but only about 60%—70% flocculant activity was produced compared with urea (Kurane, 1986c). Other results indicated that ammonium sulphate, ammonium chloride and ammonium nitrate was not good nitrogen sources for either cell growth or flocculant production (Nakamura, 1976d). Both the flocculant formation and cell growth were greatly affected by the addition of yeast extract and casamino acids as the organic nitrogen sources (Kurane, 1986c).

The ratio of C/N would also affected the production of MFs. The flocculating activity of *Zoogloea* sp. was highest at C/N of the range from 0.6—11.4 (Unz, 1976).

6.3.3 Effect of other materials in the medium

In the case of *Paecilomyces* sp. I-1, Ca^{2+} drastically stimulated both the growth of cells and the production of flocculant (Takagi, 1985b). Na^{+}, Mg^{2+} and K^{+} ions affected neither of them, while Fe^{2+} and Cu^{2+} ions inhibited the growth. As to anions, PO_4^{3-}, SO_4^{2-}, CO_3^{2-} and CH_3COO^{-} ions inhibited both (Takagi, 1986b). In the case of *Flavobacterium*, high concentration of Mg^{2+} would inhibit the production of flocculant (Hantula, 1991a). Citrate, malate, poly-L-lysine, bovine serum albumin, EDTA and NaN_3 had different effects on flocculant formation of *Flavobacterium* at different concentrations. The change of flocculant production caused by these chemical agents could in principle take place either via regulating gene expression or by physically or chemical modifying floc formation (Hantula, 1991a).

6.3.4 Effect of microbial cells and volume of medium

The flocculant production is induced and stimulated by the presence of some bacteria cells. The cells of *Corynebacterium glutamicum*, *Micrococcus* sp. and *E. coli* I-1 were stimulative for the flocculant production of *Paecilomyces* sp. I-1, but those of *E. coli* B, *Bacillus subtilus* 168, *Saccharomyces cerevisiae* and *Candida utilis* were inhibitory (Takagi, 1985b).

Although the volume of culture medium did not affect the quantity of cells in the culture, it would have effect on the production of flocculants. For *Asp. sojae*, the optical culture volume was 30—50 ml for flocculant production (Nakamura, 1976d), the volume less or more than this would decrease flocculant production.

6.3.5 Effect of temperature and pH

Beside the factors presented above, temperature and pH would also affected flocculant production. It was indicated that the culture temperature of 30℃ was optimal for both the flocculant formation (twice as much) and cell growth of *R. erythropolis* in comparison with 25℃ and 37℃ (Kurane, 1986c).

The alkaline pH, especially pH 9.5, greatly stimulated the flocculant production of *R. erythropolis* (Kurane, 1986c). In the case of *Asp. sojae* (Nakamura, 1976d), the addition of glucose or other saccharide to the medium markedly lowered flocculating activity because decomposition of glucose decreased the pH value of the medium. At pH 8.0 the flocculant was possibly produced in large amounts by *Asp. sojae* and liberated to the culture broth (Nakamura, 1976d).

7 Application of MFs

Recovery of microorganism cells: the flocculant produced by *Asp. sojae* AJ7002 was examined in the capacity of removing the cellular materials from fermentation broth. The results indicated the possibility of using the flocculant for the industrial separation or recovery of microbial cells from fermentation broths (Nakamura, 1976a).

Improvement of effluent quality: the flocculant of *Asp. sojae* AJ7002 was applied to the removal of microorganisms from activity sludge and was compared with other typical flocculants such as ferric chloride. The MFs showed better flocculation activity than the three non-biological flocculants (Nakamura, 1976a).

Livestock wastewater treatment: the flocculant formed by *Rhodococcus erythropolis* S-1 was used for the primary treatment of the pig urine and excrement waste water. Addition of 5 ml *R. erythropolis* culture broth into 100 ml wastewater might decrease TOC, TN and OD_{660} from 1420 mg/L, 420 mg/L and 8.60, to 425 mg/L, 215 mg/L and < 0.02 respectively after 10 min sedimentation (Kurane, 1986b).

Removal of suspended solids: an iron work coke wastewater contains a significant amount of very fine carbine suspended solids called pinfloc. For the primary treatment of this coke wastewater, poly inorganic flocculant has been used, but its treatment efficiency is not very good. The culture broth of *A. latus* was tested as a flocculant. Carbide suspended fine solids were visually flocculated and efficiently settled after addition of 2% culture broth while in the presence of calcium ions. The supernatant suspended solid value in the coke wastewater decreased from 370 mg/L to 80 mg/L (removal rate 78%) but the value after treatment with a poly iron inorganic flocculant only decreased to 195 mg/L (removal rate 47%) (Kurane, 1991a).

Colour removal: it is hard to remove coloured material from a coloured wastewater by

flocculation with current used flocculants such as synthetic highpolymer flocculant. When a blue-coloured wastewater from one paper manufacturing company was treated with *A. latus* culture broth and chitosan, the decolorization ratio was 94.6%, 41% more than the decolorization ratio while only using chitosan (Kurane, 1986a).

Oil water separation: when the crude bioflocculant of *A. latus* was added into a palm acid oil emulsion, some oil drop formed and rose to the surface. A visible oil layer was formed and was easily separated. The COD in the lower water layer decreased from the original value of 450 mg/L to a final value of 235 mg/L (Kurane, 1986a).

Settling characteristic improvement: the settling characteristic of activated sludge was improved by addition of flocculant *Alcaligenes faecalis* ATCC 8750 into the activated sludge. After 30 mine settling, the sludge settling volume of sludge with *Alcaligenes faecalis* ATCC 8750 was 35% while the settling volume of control sludge without that microorganism was 53%. COD removal rates for the two sludge were similar, thus addition of that microorganism improved the settling characteristic while did not decrease the efficiency of organic material removal (Shimiziu, 1985).

References

Amri M A, 1979. European J Appl Microbiol Biotechnol, 7:235—240

Bar-Or Y, 1987. Appl Environ Microbiol, 53(9):2226—2230

Chen S, 1992. Flocculation of mineral fines using by hydrophobic *Mycobacterium phlei*. Waste Process Recycle Min Metal Ind Pro Int Symp, 483—491

Dearlfield K L, Abermathy C O, 1988. Mutant Res, 195:45

Dube J, 1992. Miner Eng, 5(3-5):547—556

Endo T, Nakamura K, Takahashi H, 1976. Agri Biol Chem, 40:2289—2295

Endo T, Takahashi H, 1980. Agri Biol Chem, 44:2413—2418

Endo T, Takahashi H, 1981. Agri Biol Chem, 45:513—516

Fattom A, Shilo M, 1990. Arch Microbiol, 139:421—426

Fumio Y, 1991. Rew Microbiol, 22(1): 12—16

Guirand J P, 1992. Res Microbial, 1433(1):81 91

Hantula J, BaMFsord D H, 1991a. Appl Microbiol Biotechnol, 36:100—104

Hantula J, BaMFsord D H, 1991b. Appl Microbiol Biotechnol, 36:105—108

Hayashi O, Yamade H, Miyazaki T, 1976. Agri Biol Chem, 40(8):1643—1645

Huang S L, 1990. Taiwan Tangye Yanjiuso Yanjiu Huibao, 129:11—19

Kakii K, Kitamura S, Shrakashi T, 1985. J Ferment Technol, 63:263—270

Kakii K, Hasumi M, 1990. Journal of Fermentation and Bioengineering, 69(4):224—227

Kim Y H, 1993. Sanop Misaengmul Hakhoechi, 21(5):478—485

Koizumi J, 1991. J Gen Appl Microbiol, 37:447—454

Krul J M, 1977. Water Res, 11:31—36

Kurane R, 1976a. Agri Biol Chem, 40:907

Kurane R, 1976b. Agri Biol Chem, 40:377

Kurane R, 1986a. Method for increasing production of flocculant from *Rhodococcus erythropolis*. Jpn Kokai Tokyo Koho JP 62, 195, 288(87, 195, 288) (C1.C12P1/04), 28 Aug 1987. Appl 86/36,022,20 Feb

Kurane R, 1986b. Agri Biol Chem, 50(9):2301—2307

Kurane R, 1986c. Agri Biol Chem, 50(9):2309—2313

Kurane R, 1988a. Bio Ind, 5(10):741—749

Kurane R, 1988b. Abstracts of Papers, the Annual meeting of the society for fermentation technology, Osaka, Novermber, 152

Kurane R, 1989a. *Rhodococcus erythropolis* culture medium as flocculant for recovery of products in fermentation. Jpn Kokai Tokyo Koho JP. 02, 215, 387 (90, 215, 387) (C1.C12P1/06), 28 Aug 1990. Appl 89/35, 343, 15 Feb

Kurane R, 1989b. Polysaccharide flocculant manufacture with Alcaligenes. Jpn KoKai Tokyo Koho JP. 03 56, 102 (91, 56, 102) (C1, B01D21/01), 11 Mar 1991 Appl JP 89/53, 717, 08 Mar

Kurane R, 1991a. Agri Biol Chem, 55(4):1127—1129
Kurane R, 1991b. J Ferment Bioeng, 72(6):498—500
Kurane R, Nohora T, 1992. Nippon Kagaku Kaishi, (5):445—463
Kurane R, 1994. Biosci Biotech Biochem, 58(2) 428—429
Levy N, Bar-Or Y, 1990. Colloics Surf, 48(4):337—349
Levy N, Bar-Or Y, 1992. Water Research, 26(2):249—254
Lyons T P, Hough J S, 1970. J Inst Brew, 76:564—571
Lyons T P, Hough J S, 1971. J Inst Brew, 77:300—305
Mickinney R E, Weichlein R G, 1952. Sewager Ind Wastes, 24:117
Mill P J, 1964. J Gen Microbicl, 35:61—68
Misra M, 1993. Miner Metal Process, 10(1):20—23
Misra M, 1991. Bioflocculation of finely divided minerals (Ed. by W Ross). Miner Bioprocess Prec Conf Warrendalp Pa. Met Miner Soc. 91—103
Morgan J W, Forster C F, Evison L, 1990. Water Research, 24(6):743—750
Nakamura J, 1976a. Agri Biol Chem, 40(2):377 381
Nakamura J, 1976b. Agri Biol Chem, 40(3):619—624
Nakamura J, 1976c. Agri Biol Chem, 40(8):1565 1571
Nakamura J, Miyashiro S, Hirose Y, 1976d. Agri Biol Chem, 40(7):1341—1347
Nam J S, 1996. Arch Microbiology, 131:112—115
Pavoni J L, Tenney M W, 1972. J Water Poll Control Fed, 44:414—431
Reinhard P, Walter Z, Andreas S, 1989. Manufacture of flocculant with *Methylbactrium*. Ger. (East) DD 290917 (C1.C12P21/00). 13 Jun 1991, Appl 336, 387, 27 Dec
Saito K, Sato S, Shimoi H, 1990. Agri Biol Chem, 54(6):1425—1432
Sato T, Ose Y, 1980. Water Research, 14:333—338
Seo H H, 1993. Sanop Misaengmul Hakhoechi, 21(5):486—493
Shilo, Moshe, Fattom, 1982. Polymeric substance, and method of separating and culturing bacteria to provide a polymeric substance useful in liquid clarification and soil conditioning. U.S. US 4.649, 110 (C1, 435-68; C112P21/00), 10 Mar 1987, US Appl 403, 047, 29 Jul
Saimiziu N, 1985. J Ferment Technol, 63(1):67—71
Sousa M J, Teixeira J A, Mota M, 1992. Biotechnology Letters, 14(3):213—218
Stanford M, 1989. Yeast, 5:487—496
Stephan S, 1995. Adv Filtr Sep Technol, 9:611—615
Stewart G G, Goring T E, 1976. J Inst Brew, 82:341—342
Tago Y, Aida K O, 1977. Appl Environ Microbiol, 34(3):308—314
Takagi H, Kadowaki K, 1985a. Polygalactosamine produced by a microorganism. Chitin Nat Technol (Proc Int Conf Chitin Chitosan) 3rd. 121—128
Takagi H, 1985b. Agri Biol Chem, 49(11):3151—3157
Takagi H, 1985c. Agri Biol Chem, 49(11): 3159—3164
Takeda M, kurane R, Koizumi J, Nakamura I, 1991. Agri Biol Chem, 55:2309
Takeda M, Koizumi J, Matsuka H, 1992. Journal of Fermentation and Bioengineering, 74(6):408—409
Tamura J, Takagi H, 1986. Manufacture of flocculant PF-102 with the fungus *Paecilomyces*. Ger Offen DE 3704419 (Cl. C08B15/06) 17 Dec 1987, JP Appl 86/134, 799, 12 Jun
Taylor N W, Orton W L, 1975. J Inst Brew, 81:53—57
Tenny M W, Verhoff F H, 1973. Biotechnol Bioeng, 15:1045—1073
Teixeira J A, 1989. Biotechnology Letters, 11(8):579—582
Tezuka Y, 1969. Apppl Microbiol, 17:222—226
Toeda K, 1991. Agri Biol Chem, 55(11):2793—2799
Unz R F, Farrah S R, 1976. Appl Environm Microbial, 31:623—628
Vanhoriek M, Moens W, 1983. Carcinogenesis, 4:1459
Wang Z, 1994. Biotechnol Tech, 8(11):831—836
Yokoi H, 1995. J Ferment Bioeng, 79(4):378—380
Zajic J E, 1971. Developments in industrial microbiology, Washington D.C: American Institute of Biological Science, 1971

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