ENVIRONMENTAL AND WASTE MANAGEMENT: ADVANCEMENTS THROUGH THE ENVIRONMENTAL MANAGEMENT SCIENCE PROGRAM

Organized by

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Symposia Papers Presented Before the Division of Environmental Chemistry
American Chemical Society
Anaheim, CA March 28 – April 1, 2004

MICROBIAL FLOCCULANT FOR NATURE SODA

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INTRODUCTION

Bioflocculant is a kind of biodegradable macromolecular flocculant secreted by microorganisms with strong flocculating activity and harmlessness to humans and environment. Bioflocculant[1-7] is attracting wide research interest and is being applied to microorganism, sludge, waste water, etc. However, Bioflocculant applicated to strong basic system, such as nature soda, has not been reported. *Lactobacillus TRJ21* was obtained in our lab, which showed strong flocculating activity for nature soda.

Methods

Materials

Reagents for cultivation such as nutrient agar was purchased from Tianjin Zhujiang sanitation material factory. Nature soda was gained from Chaganzhuer nature soda mine in Inter Mongolia. Components of Medium, glucose, fructose, peptone, yeast extract, urea, $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$ and NaCl were of the analytical reagent.

Strain and culture condition

Lactobacillus TRJ21 was obtained from our lab. The bacterium was cultured on 30ml medium composed of glucose(10g/L), fructose(10g/L), yeast extract(0.5g/L), urea(0.5g/L), $(NH_4)_2SO_4$(0.5g/L), KH_2PO_4(5g/L), $MgSO_4$(0.2g/L) and NaCl (0.1g/L)in a 250mL flask. The culture was incubated at 37°C, pH8.0 with shaking 180rpm. At the end of the cultivation, the culture broth was centrifuged (20,000xg, 15min) to separate the cells and tested for flocculation activities.

Flocculation activity of culture medium

Assay of flocculation activity used nature soda suspension; 2mL of culture broth and 25mL of nature soda suspension 20g/L were mixed in a test tube. The mixture was stirred with a vortex for 3min. Two milliliters of the supernatant were carefully removed from the upper layer and its absorbance at 550nm(OD_{550}) was measured. Similarly, a blank control was applied without using the culture broth and the absorbance at 550nm (OD_{550}) was measured. The fractional flocculation was calculated by the following equation.

$$\text{Fractional flocculation} = 1/[OD_{550}-(OD_{550})_c]$$

Bioflocculant purification

The culture broth was diluted with two volumes of distilled water and then centrifuged at 20,000xg for 15min. The supernatant was poured into three volumes acetone to precipitate the biopolymer flocculant. The resultant precipitate was collected by centrifugation at 25,000xg for 20min. and re–dissolved in distilled water. After three such acetone precipitation steps, the precipitation was washed for three times using ether. Then, the crude biopolymer obtained was dialyzed at 4°C overnight in de-ionized water and vacuum-dried overnight in a desiccator to obtain pure bioflocculant.

Characterization of flocculant

The total carbohydrate content of the flocculant was determined by the phenol-sulfuric acid method[8] and expressed as the glucose equivalent. The flocculant was hydrolyzed using 6N HCl for 12h. The protein and amino acid moiety in the flocculant molecule was determined by amino acid autoanalyzer (Hitachi L-8800). Hexosamine was investigated using Elson-Morgan method[1].

RESULTS AND DISCUSSION

Production of bioflocculant

The flocculant productivity of *Lactobacillus TRJ21* was investigated using various nitrogen and carbon sources. The effects of carbon sources and nitrogen sources in bioflocculant production are shown in Table 1. Glycerol, as carbon sources, was not favorable for growth and flocculant production. Lactose was favorable for bacterial growth, but not for flocculant production. In contrast, fructose and glucose were more favorable for growth and flocculant production. *Lactobacillus TRJ21* was able to use $(NH4)_2SO_4$ and Urea as nitrogen, but was not use peptone effectively. High C/N ratio was more favorable to *Lactobacillus TRJ21* growth and flocculant production than low C/N ratio. When *Lactobacillus TRJ21* was cultured in medium described in Section 2, it was found that dissolved oxygen changed with bacterial growth. The course of bacterial growth as well as changes of dissolved oxygen, pH and flocculating activity are shown in Figure 1. The bacterial growth reached a maximum after 30h. However, flocculating activity became marked on this occasion and reached a maximum after 60h. The pH and dissolved oxygen profiles showed that the pH and dissolved oxygen first fell, then increased after about 30h and changed a little after 60h. These showed that during the logarithmic growth phase, TRJ21 grew very fast and produced lots of acid metabolite, however, produced little of flocculant. The flocculant was mainly produced during stationary growth phase.

Table 1. Effects of various carbon and nitrogen sources on flocculant production of *Lactobacillus TRJ21*.

		Yield of flocculant (g/l)
Carbon source	Fructose	1.5
	Glucose	1.3
	Glycerol	ND*
	Lactose	ND
Nitrogen source	Urea	1.2
	$(NH4)_2SO_4$	1.5
	Peptone	0.6
C/N ratio	10:1	1.5
	3:1	0.9

*Non-detectable

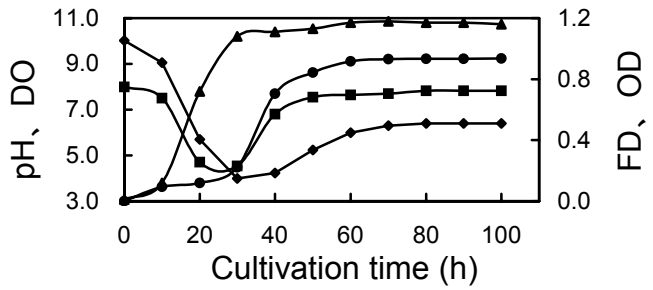

Figure 1. Course of bacterial growth, changes of dissolved oxygen, pH and flocculating activity. (▲)OD; (●)FD: Flocculating activity; (■)pH; (□)DO: dissolved oxygen(mg/l)

Characterization of flocculant

The molecular weight of the flocculant was determined by gel permeation chromatography and found to be 1.38×10^6. The phenol-sulfuric acid method showed that the polysaccharide contained more than 52.63%(w/w) of total sugar. Hexosamine was found in the flocculant using Elson-Morgan method. Amino acid composition of protein moiety in the flocculant are shown in Table 2. The results showed the flocculant contained 27.4%(w/w) of total protein.

Table 2. Amino acid composition of protein moiety in the flocculant

Amino acid	Content/ %	Amino acid	Content /%	Amino acid	Content/ %	Amino acid	Content/ %
ASP	2.336	ALA	2.149	LEU	2.313	ARG	1.354
THR	1.113	CYS	2.067	TYR	1.318	PRO	1.559
SER	0.976	VAL	2.028	PHE	1.082		
GLU	3.658	MET	0.978	LYS	1.368		
GLY	1.414	ILE	1.202	HIS	0.469		

Determination of activity ingredient in flocculant

The results that Flocculating activity changed with processing temperature are shown in Figure 2. The Flocculating activity increased with temperature, however, decreased over 50ºC. The reason was denaturalization of protein in flocculant over 50ºC. These indicated that the activity ingredient was protein.

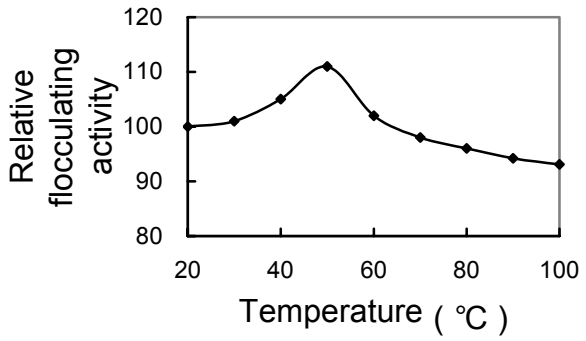

Figure 2. Effect of temperature on flocculating activity

CONCLUSION

Lactobacillus TRJ21 produced an excellent biopolymer flocculant for nature soda when it was grown in a high C/N ratio culture medium. The flocculant was composed of polysaccharide and protein and its activity ingredient was protein.

ACKNOWLEDGEMENTS

This work was supported by National Natural Science Fund 29766003.

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