QUANTITATIVE ANALYSIS OF FLOCCULATION OF *ACETOBACTERIUM WOODII* BY CHEMICAL FLOCCULANTS

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Introduction

Acetobacterium woodii produces acetic acid from carbon dioxide and hydrogen in almost 100% yield.[1] In our previous work,[8] the kinetics of acid production of *A. woodii* was examined in a bubble-column bioreactor using suspended and immobilized resting anaerobes. Higher acetate productivity was found in the culture of suspended cells. From a practical viewpoint the establishment of a continuous cultivation method will be required. As the first step toward this development, a proper method of separating the cells from their media must be found. Flocculation of bacteria will assist in the continuous cultivation using suspended cells.

Bacterial flocculation by the addition of chemical flocculants has been studied.[2-7,9] The mechanism of cell flocculation by chemical additives is highly complex, and such interacting factors as temperature, ionic environment, physiological age, flocculating agent, bacterial genus and surface shear were observed.[5] Various strains of *Acetobacter* species were immobilized on hydrous titanium (IV) oxide or hydrous titanium (IV) chelated cellulose and used in the continuous conversion of a dilute aqueous alcoholic solution into acetic acid.[4]

Although these studies have had limited success, the quantitative relationship between the properties of flocculants and bacterial flocculation is still poorly understood. The objective of this research is to investigate the flocculation of *A. woodii* by various flocculants.

1. Experimental

1.1 Organism

High-density culture was obtained by centrifugation of the late logarithmic culture of *A. woodii* ATCC 29683 that grew heterotrophically in a medium containing fructose as described earlier.[8]

1.2 Flocculation tests

Bacterial cells are charged at neutral pH.[3] However, a survey[2] of a large number of commercial synthetic polymers regarding their effectiveness in flocculating pure cultures of *E. coli* and *Acetobacter aerogenes* revealed that a wide range of polymers, whether anionic, non-ionic or cationic, could flocculate bacteria efficiently. We intended to use a variety of flocculants to test their ability to flocculate *A. woodii.* **Table 1** shows the list of flocculants and their dosage.

In performing flocculation experiments, aliquots of cell suspensions were added to medium containing various flocculants (without fructose) at pH 7.3 in test tubes, to give a total volume of 14 ml. The tubes were then stoppered under an atmosphere of H_2/CO_2 (4:1, v/v) and thoroughly mixed by inverting the tubes approximately 20 times. They were shaken for 64 h at 30°C, then settled for 2 h. The optical density at 600 nm (OD_{600}) and acetate concentration of the culture supernatant were measured. As a measure of bacterial flocculation, the ratio of OD_{600} value of test sample to that of the control, OD_{600}/OD_{600}

Table 1. List of flocculants used in the flocculation of *A. woodii*

No.	Flocculant	Dosage [g/L]
1.	$Al_2(SO_4)_3 \cdot 14$—$18H_2O$	0.5
2.	Sodium alginate	2.0
3.	Sodium polyacrylate	2.0
4.	$FeSO_4 \cdot 7H_2O$	0.5
5.	$FeCl_3 \cdot 6H_2O$	0.5
6.	$ZnCl_2$	0.5
7.	$ZnSO_4$	0.5
8.	MgO	0.5
9.	$MgCO_3$	0.5
10.	Starch	2.0
11.	Gelatin	2.0
12.	Activated charcoal	35.7
13.	SiO_2	35.7
14.	Bentonite	35.7
15.	PAC	0.1
16.	ORUFLOC OA-4(Polyacrylamide)	2.0
17.	ACCOFLOC C485(Polymethacrylic acid ester)	2.0
18.	ACCOFLOC C498T(Polyacrylic acid ester)	2.0
19.	ACCOFLOC C470H(Polymethacrylic acid ester)	2.0

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(cntl), was adopted here.

2. Results and Discussion

Figure 1 shows the results of the flocculation test. The control run, which was performed without any flocculants, is indicated by the number 20. Quantitative results on the effects of flocculants on both the flocculation and acetate production rate of *A. woodii* are shown in **Fig. 2**. From these results, assuming that the partitioning behavior of acetic acid between the culture supernatant phase and the rest phase is constant in all cases, cationic polymeric flocculants having a strong positive charge, such as ACCOFLOC C498T and C470H, could flocculate *A. woodii* effectively without inhibiting the acetate production of the bacterium.

To investigate the effect of the flocculation of cationic polymeric flocculants on *A. woodii* in more detail, an additional test was undertaken using nine commercial homologous series flocculants (C485 and C470H were used in the previous tests). These flocculants, having diverse degrees of cationic strength and polymerization, are made by the copolymerization of acrylamide and dimethylaminoethyl methacrylate-methychloride. The difference in the ability of flocculants to flocculate *A. woodii* is clearly shown in **Figure 3**. **Table 2** shows the OD_{600}/OD_{600} (cntl) values and the properties of the flocculants. Flocculation of dispersed organisms by polymeric flocculants has been interpreted as a process whereby polymers adsorb to and bridge between cell surfaces.[2] From this intuition, the ionic polarity and molecular weight of flocculants would be the most important factors affecting the flocculation. Then we listed the following six properties by considering the possibility of other factors in the flocculation: (1) colloid equivalent (C_{eq}), (2) molecular weight (MW), (3) molar volume (MV), (4) parachor (PA), (5) molar refraction (MR), and (6) cohesive energy density (ε_{coh}). As a measure of ionic polarity, colloid equivalent was determined here by titration by polyvinyl potassium sulfide (PVSK), using toluidine blue as indicator. The values of molecular weight were determined on the basis of viscosity-molecular weight relationships in 1N–$NaNO_3$ at 30°C. Other properties were calculated on the basis of the nonionic molecular structure of flocculants. The methods used for their calculation are shown in the footnote to **Table 2**.

Stepwise linear regression analysis was applied to examine the correlation between OD_{600}/OD_{600} (cntl) and the properties of flocculants. All possible combinations of descriptor paris were examined to identify those pairs that were highly correlated. Significant interrelations ($r>0.970$) were found in the following pairs: MW and MV, MW and PA, MW and MR, MW and ε_{coh}, MV and PA, MV and MR,

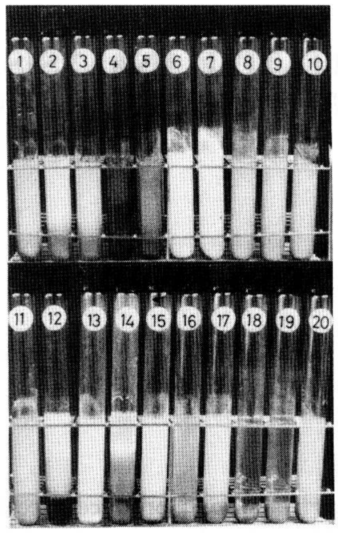

Fig. 1. Flocculation test of *A. woodii* by various flocculants shown in Table 1 (The numbers 1 to 19 indicate the flocculants cited in Table 1. The number 20 indicates the control.)

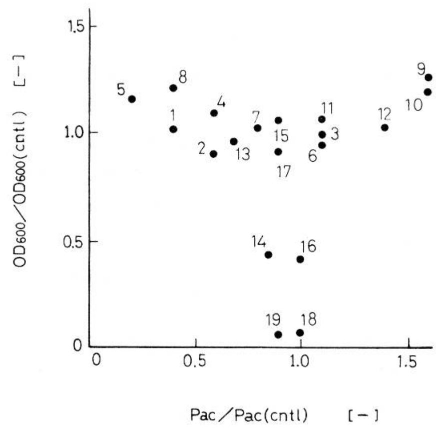

Fig. 2. Effects of addition of various flocculants on the flocculation and acetate production of *A. woodii*

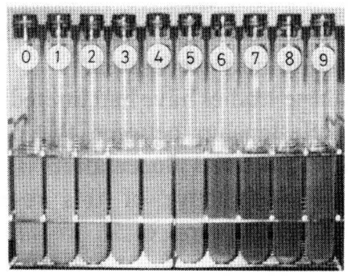

Fig. 3. Flocculation test of *A. woodii* by adding 2.0 g/L of various cationic polymeric flocculants (The numbers 1 to 9 indicate the flocculants in Table 2. The number 0 indicates the control.)

PA and MR, and MR and ε_{coh}. In such cases, one descriptor was discarded.

The best-fit equation found is:

$$OD_{600}/OD_{600}\ (\mathrm{cntl}) = -0.387C_{eq} - 0.09\varepsilon_{coh} + 2.1 \quad (1)$$
$$(n=9,\ r=0.972,\ s=0.075)$$

Table 2. Data table on which multiple regression analysis was performed to investigate the relation between the properties of polymeric flocculants (dosage: 2.0 g/L) and the flocculation of *A. woodii*

Flocculants	OD_{600}/OD_{600} (cntl)	Properties of flocculants					
		C_{eq}	MW	$MV^{a)}$	$PA^{b)}$	$MR^{c)}$	$\varepsilon_{coh}{}^{d)}$
ACCOFLOC							
1. C481	0.66	0.9	9.0	6.35	18.10	2.21	11.48
2. C483	0.76	1.6	7.0	5.29	14.60	1.74	7.34
3. C475	0.91	2.4	3.5	2.81	7.53	0.88	2.91
4. C485	0.83	2.4	6.0	4.82	12.90	1.51	4.98
5. C486	0.55	2.9	5.0	4.19	11.00	1.27	3.39
6. C488	0.38	3.9	4.0	3.60	9.08	1.03	1.58
7. C480	0.22	4.8	3.5	3.37	8.23	0.91	0.39
8. C470H	0.24	4.8	3.0	2.89	7.05	0.78	0.34
9. C470	0.19	4.8	2.5	2.41	5.88	0.65	0.28

a) Estimated based on Exner's method [Exner, 0., *Collect. Czec. Chem. Comm.*, **32**, 1 (1967)]; b) Estimted based on Sugden's method [Meissner, H. P., *Chem. Eng. Prog.*, **45**, 149 (1949)]; c) Estimated based on Eisenlohr's method [Meissner, H. P., *Chem. Eng. Prog.*, **45**, 149 (1949)]; d) Estimated based on the method of Van Krevelen and Hoftyzer [Van Krevelen, D. W. and Hoftyzer, P. J., Properties of Polymers: Their Estimation and Correlation with Chemical Structure, Elsevier, Amsterdam (1976)].

The equation using C_{eq} and MW as independent variables gives a lower correlation ($r=0.949$, $s=0.101$). Therefore, two physicochemical properties of the flocculants, colloid equivalent and cohesive energy density, were identified as key parameters affecting the formation of flocs of *A. woodii* under these experimental conditions. Further studies are underway to apply this approach to the continuous production of acetic acid in a bubble-column bioreactor.

Conclusion

Flocculation of *A. woodii* by a variety of flocculants was tested. Cationic synthetic polymeric flocculants having a strong positive charge were found to be highly effective in flocculating *A. woodii.* This approach will be valuable not only for simplifying the process, but also for utilizing the energy required for the separation of the cells from the liquid contents.

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Nomenclature

C_{eq}	=	colloid equivalent	[ml-0.0025N-PVSK/g]
n	=	number of data points in set	[—]
MR	=	molar refraction	[10^6 cm^3·mol^{-1}]
MV	=	liquid-state molar volume	[10^6 cm^3·mol^{-1}]
MW	=	molecular weight	[10^6]
OD_{600}	=	optical density at 600 nm	[—]
PA	=	parachor	[10^6 (cm^3·mol^{-1})(dyn·cm^{-1})$^{1/4}$]
P_{ac}	=	production rate of acetic acid	[g·L^{-1}·d^{-1}]
r	=	multiple correlation coefficient	[—]
s	=	standard deviation	[—]
ε_{coh}	=	cohesive energy density	[10^7 J·cm^{-3}]

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