The Korean Journal of Rheology,
Vol. 8, No. 3+4, December 1996 pp. 226-231

〈단 신〉

Shear Index로 표시된 *E. coli* Floc의 강도

김찬화 · 나초균*

고려대학교 생명공학원 생물공정 및 생물재료공학 연구실, *미국 MIT 생물재료공학 연구실
(1996년 9월 2일 접수)

Strength of *E. coli* Floc as Indicated by Shear Index

Chan-Wha Kim and ChoKyun Rha*

Graduate School of Biotechnology Bioprocessing and Biomaterials Engineering Laboratory
Korea University, Seoul 136-701, Korea
*Biomaterials Science and Engineering Laboratory Massachusetts Institute of Technology
Cambridge, Massachusetts 02139, USA
(Received September 2, 1996)

요 약

침강제에 의해 형성된 *E. coli* floc들의 강도를 측정하기 위해 floc의 shear index를 측정하였다. 형성된 *E. coli* floc은 10/sec 같이 낮은 shear rate에서도 분쇄되거나 변형되었다. 측정된 shear index의 감소에서 보듯이 *E. coli* floc의 강도는 염의 농도가 증가함에 따라 감소하였다. *E. coli* floc의 shear index는 NaCl의 농도가 0에서 100 mM로 증가함에 따라 0.47에서 0.09로 줄었다. 발효배지의 조성에서 형성된 *E. coli* floc들은(shear index=0.18-0.24 with BPA-1000, 0.13-0.22 with BPA-1050, and 0.37-0.42 with BPA-5020) 염이 없을 때 형성된 floc에(shear index=0.47 with BPA-1000 and 0.46 with BPA-1050) 비해 약하였다. 따라서 발효배지에서 형성된 floc은 생물공정 중 쉽게 shear에 의해 분쇄되거나 변형될 것이다.

Abstract—The strength of *E. coli* flocs formed with flocculants was quantified by measuring the shear index. *E. coli* flocs formed were disrupted or deformed at the shear rate even below 10/sec. The strength of the *E. coli* flocs decreased with increases in the concentration of salt as indicated by decreases in the shear index. The shear index of the *E. coli* flocs decreases from 0.47 to 0.09 when the concentration of NaCl increased from 0 to 100 mM. The *E. coli* flocs formed with various flocculants in fermentation media (shear index=0.18 to 0.24 with BPA-1000, 0.13 to 0.22 with BPA-1050, and 0.37 to 0.42 with BPA-5020) were weaker than those formed with flocculants in the absence of salt (shear index=0.47 with BPA-1000 and 0.46 with BPA-1050). Therefore, *E. coli* flocs formed with flocculants in fermentation media will be easily disrupted or deformed under shear during bioprocesses.
Keywords: Floc strength, shear index, flocculant, shear rate dependency, cell debris removal.

1. Introduction

The removal of cell and cell debris is the first step of the down-stream processes in the manufacturing of therapeutic proteins. The process inherently lacks the driving force required for the efficient solid/liquid separation due to the small cell and cell debris size. In order to increase the driving force the negative charges at the cell surfaces have been utilized. Positively charged par-

ticulates and water soluble polymers have been introduced to fermentation broth or cell lysate. Cell and cell debris are flocculated by the electrostatic adsorption on the flocculants. The adsorption of cells on the flocculants increases the size of particles, thus facilitates sedimentation and filtration [1].

The flocculation, however, is affected by the concentration of salt in the suspending medium. The size and the sedimentation rate of flocs were decreased with increases in the concentration of salt [2]. In addition, the flocs formed were weak and fragile. The floc strength, however, is one of the most important characteristics of flocs for the efficient removal of cell and cell debris because the filtration and centrifugation subject flocs to be under significant shear during the bioprocesses [3,4]. In consequence, the size of flocs becomes small and the removal of cell and cell debris is inefficient. Therefore, in this study the strength of flocs at the various concentrations of salt was evaluated by determining the shear index.

2. Experimental

2.1. Materials

Flocculants (BPA-1000, BPA-1050, and BPA-5020) provided by Rohm & Haas Co. (Spring House, PA) were diluted to 1% (w/v) with deionized water and used without further treatment. BPA-1000 is a polyacrylic particulate flocculant with a mean diameter of 0.1 μm, a charge density of 270 $\mu C/cm^2$, and quaternary amines as a functional group. BPA-1050 is a polyacrylic particulate flocculant with a mean diameter of 0.1 μm, charge density of 340 $\mu C/cm^2$, and tertiary amines as a functional group. BPA-5020 is a water soluble polymer with a molecular weight less than 200,000 dalton and tertiary amines as a funtional group.

E. coli (Strain B, Sigma Chemical Co., St. Louis, MO) was washed with deionized water and centrifuged for 1 hour at 700×g to remove water soluble materials. The washing was repeated three times. The dry cell weight (DCW) of the *E. coli* suspensions was determined by oven drying for 24 hours at 90°C.

2.2. Construction of Fermentation Media

Three typical *E. coli* fermentation media without carbon and nitrogen sources were prepared to evaluate the performance of flocculants in practical systems. Medium A contains 3 g/L KH_2PO_4, 6 g/L Na_2HPO_4, 2 g/L $(NH_4)_2SO_4$, 5 mg/L $FeSO_4$, 50 mg/L $MgSO_4$, and 3 g/L NaCl [5]. Medium B contains 3 g/L KH_2PO_4, 6 g/L Na_2HPO_4, 5 g/L NH_2Cl, 1.1 g/L Na_2SO_4, 0.1 g/L $MgSO_4$, 18 mg/L $ZnSO_4 \cdot 6H_2O$, 16 mg/L $CuSO_4 \cdot 5H_2O$, 0.5 mg/L $CaCl_2$, 16.7 mg/L $FeCl_2 \cdot 6H_2O$, 18 mg/L $CoCl_2 \cdot 6H_2O$, and 20 mg/L EDTA [6]. Medium C contains 5 g/L NaCl.

2.3. Preparation of *E. coli* Flocs

Twenty m*l* of *E. coli* suspensions (10 g DCW/L) in NaCl (0 to 100 mM) or fermentation media were prepared in 50 m*l* centrifuge tubes. The optimum concentrations of flocculants (0.4 g/L of BPA-1000, 1 g/L of BPA-1050, and 0.8 g/L of BPA-5020) which were determined in earlier study [5] were added into the cell suspensions. The mixtures were shaken for 10 minutes on a Thermolyne Speci-Mix (Thermolyne Co., Dubuque, IW) and used for the shear rate dependency study on *E. coli* flocs.

2.4. Shear Rate Dependency

The apparent viscosity and the shear stress of *E. coli* flocs were determined in the shear rate range of 1.2 to 180/sec at 25°C using a Bohlin Rheometer System with a concentric cylinder (C25) and a 0.22 gm spring (Lund,

Sweden). The viscosity reading was calibrated with a viscosity standard S60 (102.4 mPas at 25°C, Cannon Instrument Co., State College, PA). The apparent viscosity and shear stress were recorded during the course of increasing shear rate with a 5 second initial delay at the starting shear rate (1.2/sec), 2 second constant delay between each shear rate, and a 5 second integration at the given shear rate to read them.

3. Results and Discussion

Fig. 1 shows the shear rate dependency of *E. coli*/BPA-1000 flocs (10 g DCW *E. coli* and 0.4 g/L BPA-1000), *E. coli* (10 g DCW *E. coli*), and BPA-1000 (0.4 g/L BPA-1000) in deionized water. BPA-1000 is a positively charged particulate flocculant with quaternary amines. As the shear rate increases, the apparent viscosity of *E. coli*/BPA-1000 flocs decreases exponentially, while the apparent viscosity of *E. coli* (10 g DCW/L) and BPA-1000 (0.4 g/L) alone remains almost constant. The apparent viscosities of BPA-1050 and BPA-5020 in deionized water also do not decrease with increases in the shear rate, rather seem more likely to increase. The apparent viscosity of the *E. coli*/BPA-1000 flocs decreases from 42 mPas to 11 mPas when the shear rate increases from 1.2/sec to 9.2/sec. This suggests that *E. coli*/BPA flocs are reoriented or disrupted when they are sheared at the shear rate below 10/sec. In addition, the apparent viscosity of *E. coli*/BPA-1000 flocs is greatly higher than those of *E. coli* or BPA-1000 alone, especially within the low shear rate range (>40/sec). Therefore, flocculation of *E. coli* with flocculants increases greatly the viscosity of the cell suspension due to increases in the particle size.

The apparent viscosity of the *E. coli*/BPA-1000 flocs disrupted under shear increases with decreases in the shear rate as shown in a shear dependency hysteresis of *E. coli*/BPA-1000 flocs (Fig. 2). The flocs disrupted, however, did not reform completely as indicated by the lower apparent viscosity at the same shear rates.

The apparent viscosity of *E. coli*/BPA-1000 flocs was lower at the higher concentration of

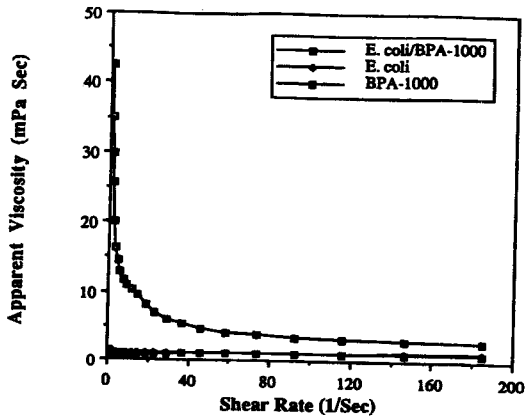

Fig. 1. Shear rate dependency of *E. coli*/BPA-1000 flocs, *E. coli* (10 g DCW/L), and BPA-1000 (0.4 g/L) in deionized water. As the shear rate increases, the apparent viscosity of *E. coli*/BPA-1000 flocs decreases exponentially, while *E. coli* and BPA-1000 remains almost constant.

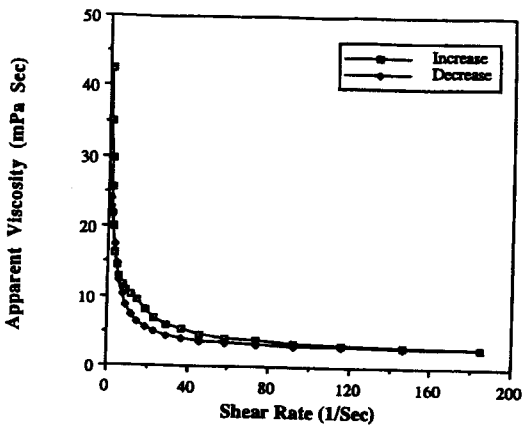

Fig. 2. Shear rate dependency hysteresis of *E. coli*/BPA-1000 flocs formed in deionized water (10 g DCW/L *E. coli* and 0.4 g/L BPA-1000). The shear rate was increased first up to 180/sec and then decreased to 1.2/sec.

NaCl at given shear rates (Fig. 3). The shear thinning effect on the flocs increases with increases in the concentration of NaCl. Therefore, the flocs formed at the higher concentrations of NaCl loose the floc strength. This agrees with the sedimentation results reported earlier [2].

Fig. 4 shows the shear rate dependency of the *E. coli*/BPA-1000 flocs formed in fermentation media. The apparent viscosity of the flocs formed in deionized water is always higher than those of the flocs formed in fermentation media. The shear thinning effect of the flocs formed in fermentation media is greater than that of the flocs formed in the deionized water. This suggests that the cell and cell debris flocs formed in practical fermentation media are weaker than that formed in deionized water, thus disrupted easily under shear. During the cell and cell debris removal process in continuous centrifuges, especially with disc bowl centrifuges, a significant shearing takes place at the interfaces between the light phase stream and the dense phase stream as well as the interface between the disc and the phase streams [3,4]. Therefore, cell and cell debris flocs formed with flocculants in the presence of salts would be easily broken in continuous disc bowl centrifuges.

The same results were obtained with BPA-1050 and BPA-5020. The shear thinning effect of the flocs formed in fermentation media was greater than that of the flocs formed in deionized water.

In order to quantify the shear thinning effect of salt, the shear stress can be analyzed using the power low equation as:

$$\text{Log}(\tau - \tau_o) = \text{Log } b + s \text{ Log } \gamma \quad (1)$$

where τ = shear stress

τ_o = yield stress

b = viscosity constant

s = shear index or flow behavior index

γ = shear rate

When the shear stress was extrapolated to 0 shear rate, it was close to 0 in most measurements. Therefore, the yield stress was assumed 0 (τ_o=0). Then, Eq. (1) simplifies as:

$$\text{Log } \tau = \text{Log } b + s \text{ Log } \gamma \quad (2)$$

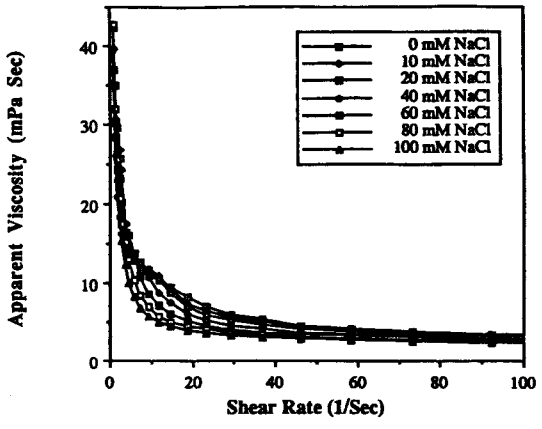

Fig. 3. Effects of NaCl on the shear rate dependency of *E. coli*/BPA-1000 flocs (10 g DCW/L *E. coli* and 0.4 g/L BPA-1000). The shear thinning effect on the flocs increases with increases in the concentration of NaCl.

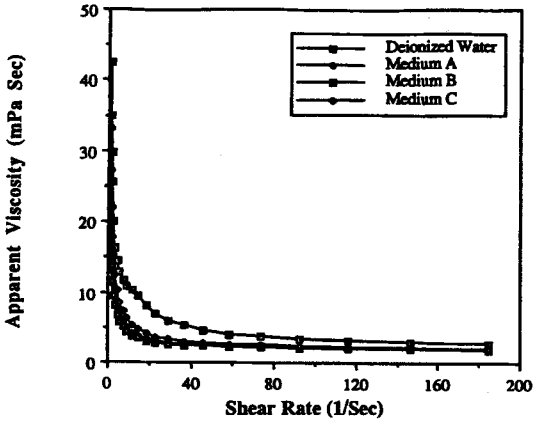

Fig. 4. Shear rate dependency of *E. coli*/BPA-1000 flocs formed in fermentation media (10 g DCW/L *E. coli* and 0.4 g/L BPA-1000). The shear thinning effect of the flocs formed in fermentation media is greater than that of the flocs formed in the deionized water.

The shear index can be determined from the slope of the plot Log τ vs. Log γ.

In the linear plot of Log τ vs. Log γ of *E. coli*/BPA-1000 flocs in various concentrations of NaCl, the slope was smaller than 1 and decreased with increases in the concentration of NaCl (Table 1). In the absence of NaCl, the shear index of the flocs is 0.47. In the presence of 100 mM NaCl, the shear index, however, decreases to 0.09. In all cases, the correlation coefficient was higher than 0.98. The shear index of the *E. coli*/BPA-1000 flocs formed in fermentation medium A, B, and C is 0.24, 0.23, and 0.18, respectively. They are about 50% of the shear index of the flocs formed in deionized water (s=0.47). The shear index of the *E. coli*/BPA-1000 flocs formed in fermentation (0.22 for medium A, 0.26 for medium B, 0.13 for medium C) is also smaller than that of the flocs formed in deionized water (s=0.46).

The shear index of the *E. coli*/BPA-5020 flocs formed in fermentation media (0.42 for medium A, 0.37 for medium B, and 0.40 for medium C) are also smaller than that of the *E. coli*/BPA-1000 flocs formed in deionized water (s=0.47). They, however, are larger than those of other *E. coli*/BPA flocs formed in fermentation media. This indicates that the linear flocculant (BPA-5020) is less susceptible to salts than the particulate flocculants (BPA-1000 and BPA-1050).

E. coli (10 g DCW/L), BPA-1000 (0.4 g/L), BPA-1050 (1 g/L), and BPA-5020 (0.8 g/L) suspensions behave as a Newtonian fluid. Their shear index are close to 1.

4. Conclusions

The strength of *E. coli* flocs formed with floc-

Table 1. Shear Index of the *E. coli* flocs formed with Flocculants

Flocculant and/or *E. coli*	Salt or Fermentation Medium	s (Shear Index)	Log b (Viscosity Constant)	Correlation Coefficient
E. coli	D.W.[a]	1.03	-0.03	1.00
BPA-1000	D.W.	1.06	-1.13	1.00
E. coli/BPA-1000	D.W.	0.47	1.58	0.99
	10 mM NaCl	0.47	1.58	0.99
	20 mM NaCl	0.46	1.54	0.99
	40 mM NaCl	0.45	1.50	0.99
	60 mM NaCl	0.33	1.59	0.99
	80 mM NaCl	0.19	1.63	0.98
	100 mM NaCl	0.09	1.61	0.99
	A	0.24	1.39	1.00
	B	0.23	1.34	1.00
	C	0.18	1.57	0.99
BPA-1050	D.W.	1.02	-0.01	1.00
E. coli/BPA-1050	D.W.	0.46	1.43	0.99
	A	0.22	1.30	0.99
	B	0.26	1.25	0.99
	C	0.13	1.53	0.96
BPA-5020	D.W.	0.98	0.16	1.00
E. coli/BPA-5020	D.W.	-	-	-
	A	0.42	1.34	1.00
	B	0.37	1.42	0.99
	C	0.40	1.32	1.00

[a]Deionized water

culants can be evaluated by measuring the shear index. The flocs formed are easily disrupted under shear even at the shear rate below 10/sec. *E. coli* flocs loose their strength in the presence of salt as indicated by decreases in the shear index. The shear index of the *E. coli*/BPA-1000 flocs decreases from 0.47 to 0.09 when the concentration of NaCl increases from 0 to 100 mM. The *E. coli* flocs formed with flocculants in fermentation media are weaker than those formed with flocculants in the absence of salt, thus deflocculate more under shear.

Acknowledgment

This study was supported in part by the research project of the Center for Advanced Food Science and Technology of Korea University, a center of recommendation for SRC of the Korea Science and Engineering Foundation (CAFST research paper No. 960014).

References

1. C.W. Kim, S.K. Kim, C.K. Rha and E. Robinson, "Flocculation in Biotechnology and Separation Systems," ed by Y.A. Attia, Elsevier Science Publishers B.V., Amsterdam, 1987, p.429.
2. C.W. Kim, *J. Inst. Biotechnol., Korea Univ.*, **7**, 53 (1995).
3. I.J. Krijgsman, "Product Recovery in Bioprocess Technology," Thomson Litho Ltd, East Kilbride, Scotland, 1992.
4. D.I.C. Wang, C.L. Cooney, A.L. Demain, P.Dunnill, A.E. Humphrey and M.D. Lilly, "Fermentation and Enzyme Technology," John Wiley & Sons, New York, 1979.
5. C.R. Craven, E. Steers, Jr., and C.B. Anfinsen, *J. Biol. Chem.* **240**, 2468 (1965).
6. A.D. Kelmers, C.W. Hancher, E.F. Phares, and G.D. Novelli, *Methods in Enzymology* **20**, 3 (1971).