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NOTE

Enhancement of the *Escherichia coli* Floc Strength with Water Soluble Polymers

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The floc strength of *Escherichia coli* was enhanced by adding water soluble polymer flocculants (BPA-5020 and BPA-5000) to the particulate flocculant (BPA-1000) as indicated by the increase in the shear index. The shear index of the *E. coli* flocs increased from 0.39 with the particulate flocculant alone to 0.94 with the particulate flocculant in conjunction with the water soluble polymer flocculant. In addition, the sedimentation rate of flocs was higher and the sedimented volume of flocs was smaller when the particulate flocculant was used with the water soluble polymer flocculant. When *E. coli* was flocculated first with the water soluble flocculant and the particulate flocculant was added later into the *E. coli* flocs formed, the sedimentation rate of the flocs was greater than that of any other combination. The shear index of the flocs was, however, independent of the sequence of the flocculant addition.

The removal of cells and cell debris is one of the most important solid/liquid separation processes in the downstream process of protein manufacturing. The removal process is usually accomplished by centrifugation or filtration. These individual operations, however, often suffer from a lack of sufficient driving force for complete separation due to the small size of cells and cell debris and the small density difference between cells and medium. Therefore, flocculants or filtration aids are often utilized to increase the separation efficiency (1, 2, 7, 9, 11).

Positively charged particulates or water soluble polymers have been introduced to flocculate cells and cell debris which have negatively charged groups at the surface (3-5, 12). The degree of flocculation by electrostatic interaction, however, is affected by the concentration of salt present in the medium (6). The size and the sedimentation rate of flocs is decreased with increases in the concentration of salt. In addition, the flocs formed became weak and fragile when the concentration of salt is increased (8). Floc strength, however, is one of the most important characteristics of flocs in determining the efficiency of the cells and cell debris removal process because flocs are under significant shear stress during the filtration and centrifugation processes (10, 13). Therefore, in this study the effects of water soluble polymer flocculants on the enhancement of the floc strength was evaluated by determining the shear index of flocs from the shear rate dependency tests.

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Materials

A particulate flocculant (BPA-1000) and two water soluble polymer flocculants provided by Rohm & Haas Co. (Spring House, PA, U.S.A.) were used for this study. The particulate flocculant (polyacrylic acid closslinked with divinylbenzene) has a mean diameter of 0.1 μm (surface areas of 55 m^2/g), quaternary amines as a functional group, and a charge density of 270 $\mu C/cm^2$. The water soluble flocculants (polyacrylic acid) have an average molecular weight of > 200,000 and have either tertiary amines (BPA-5020) or quaternary amines (BPA-5000) as a functional group. The flocculants were diluted to 1% (w/v) with deionized water and used without further treatment. *Escherichia coli* (Strain B, Sigma Chemical Co., St. Louis, MO, U.S.A.) was washed with deionized water and centrifuged for 30 min at $700\times g$ 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 h at 90°C.

Simulation of Salt Composition in Fermentation Medium

The salt composition of a typical rich fermentation medium for recombinant *E. coli* (2XTY) was simulated by adding 5 g/l NaCl to deionized water.

Sedimentation Test

Ten ml of *E. coli* suspensions (0.5% DCW) in the simulated medium were prepared in 10 ml graduated volumetric cylinders. The particulate flocculant was premixed with a tertiary amine water soluble polymer (BPA-5020) or a quaternary amine water soluble polymer (BPA-5000) for 1 h with Thermolyne Speci-Mix (Thermolyne Co., Dubugue, IW, U.S.A.). The premixed flocculant was added into the *E. coli* suspensions which were equilibrated with the simulated fermentation medium 2XTY. The mixtures were mixed for 10 min by hand and left standing to allow *E. coli* flocs to settle. Upon standing, the flocs formed with flocculants sedimented leaving clear supernatant. The volume of the sediment was measured as a function of time.

To evaluate the effect of the addition order in the flocculation procedure, one flocculant (BPA-1000 or BPA-5020 or BPA-5000) was added first into the cell suspensions and mixed for 5 min. After 5 min mixing, the other flocculant was added into the mixtures and the mixtures were mixed again for 5 min.

Shear Rate Dependency Test

The apparent viscosity and shear stress of *E. coli* flocs (1% DCW *E. coli*/l, 0 to 0.06% BPA-1000, and 0 to 0.12% BPA-5020) were determined in the shear rate range of 0.12 to 180 sec^{-1} at 25°C using a Bohlin Rheometer System with a concentric cylinder (C25) and a 1.41 gm spring (Bohlin Rheologi, Lund, Sweden). The viscosity reading was calibrated using a viscosity standard s60 (102.4 mPa.s at 25°C, Cannon Instrument Co., State College, PA, U.S.A.). The apparent viscosity and shear stress were recorded during the course of increasing shear rate with a 5 second initial delay at a starting shear rate of 0.12 sec^{-1}, with 2 second constant delays between each shear rate, and a 5 second integration at the given shear rate to read them.

The shear stress was expressed using the power law equation as:

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

where τ = shear stress

τ_o = yield stress

b = viscosity constant

s = shear index or flow behavior index

γ = shear rate

The yield stress was assumed to be 0 (τ_o=0) and the shear index was determined from the slope of the plot Log τ vs. Log γ.

Sedimentation of *E. coli* Flocs

Fig. 1 shows the sedimentation of *E. coli* flocs (0.5% DCW *E. coli*) formed with premixed flocculants (0 to 0.05% BPA-5020 with 0.02% BPA-1000). The treatment of the water soluble polymer flocculant BPA-5020 in conjunction with the particulate flocculant BPA-1000 facilitated the clarification of the cell suspensions. More than 50% of the total volume of the cell flocs (sedimented volume < 5 ml) sedimented within 20 min when the water soluble polymer flocculant was added at a level between 0.02 and 0.05%. However, when the water soluble polymer flocculant levels were below 0.01%, less than 5% of the cell suspension was clarified after allowing 20 min for sedimentation. The fastest sedimentation was obtained at 0.03% BPA-5020. This clearly demonstrates that the addition of a water soluble polymer flocculant enhances the sedimentation rate of *E. coli* flocs formed with the particulate flocculant.

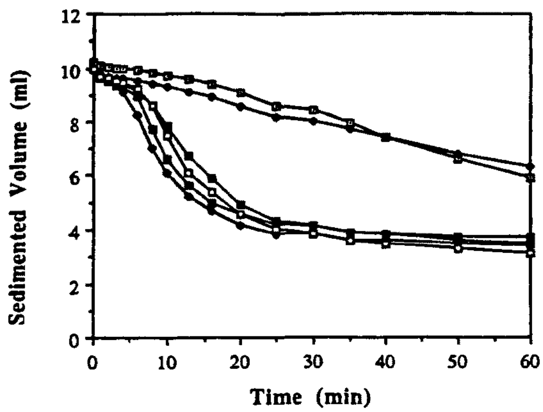

Fig. 1. Sedimentation of *E. coli* (0.5% DCW) treated with premixed particulate flocculant BPA-1000 (0.02%) and varying concentrations of water soluble polymer flocculant BPA-5020 (0 to 0.05%).
—□—, 0.00% BPA-5020; —◆—, 0.01% BPA-5020; —■—, 0.02% BPA-5020; —◇—, 0.03% BPA-5020; —□—, 0.04% BPA-5020; —□—, 0.05% BPA-5020.

In order to study the effect of the concentration of the particulate flocculant BPA-1000 at a given concentration of the water soluble polymer flocculant BPA-5020, 0.03% BPA-5020 was premixed with varying concentrations of BPA-1000 (0 to 0.03%). As shown in Fig. 2, although the sedimentation of the cell flocs was a little faster at 0.01% BPA-1000 than that without BPA-1000 (0.03% BPA-5020 alone), no significant improvement in the sedimentation velocity was observed by the premixing of the particulated flocculant with the water soluble polymer flocculant. Further increases in the concentration of BPA-1000 above 0.01% reduced the sedimentation velocity. The treatment of 0.005% BPA-1000 did not facilitate the sedimentation of the cell flocs, which suggests that the optimum concentration of premixed flocculants is 0.01% BPA-1000 with 0.03% BPA-5020.

Fig. 3 shows the effects of another water soluble polymer flocculant BPA-5000 premixed with the particulate flocculant BPA-1000 on the sedimentation of *E.*

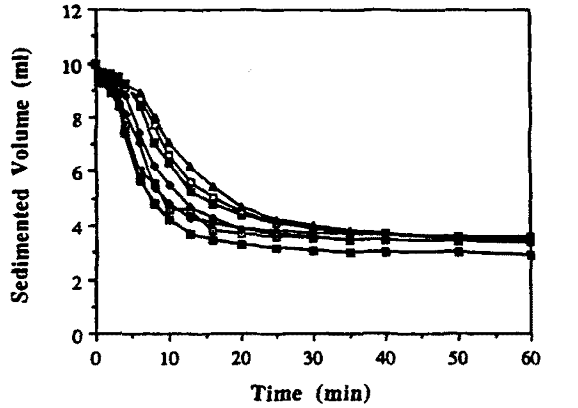

Fig. 2. Sedimentation of *E. coli* (0.5% DCW) treated with premixed water soluble polymer flocculant BPA-5020 (0.03%) and varying concentrations of particulate flocculant BPA-1000 (0 to 0.03%).
—□—, 0.00% BPA-1000; —◆—, 0.005% BPA-1000; —■—, 0.010% BPA-1000; —◇—, 0.015% BPA-1000; —⊟—, 0.020% BPA-1000; —□—, 0.025% BPA-1000; —▲—, 0.30% BPA-1000.

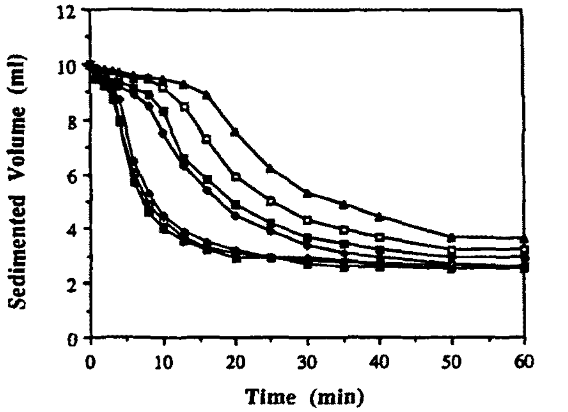

Fig. 4. Sedimentation of *E. coli* (0.5% DCW) treated with premixed water soluble polymer flocculant BPA-5000 (0.01%) and varying concentrations of particulate flocculant BPA-1000 (0 to 0.03%).
—□—, 0.00% BPA-1000; —◆—, 0.005% BPA-1000; —■—, 0.010% BPA-1000; —◇—, 0.015% BPA-1000; —⊟—, 0.020% BPA-1000; —□—, 0.025% BPA-1000; —▲—, 0.30% BPA-1000.

coli flocs (0.5% DCW *E. coli*). The treatment of BPA-5000 in conjunction with BPA-1000 also facilitated the clarification of the cell suspensions. The sedimentation was fastest at 0.01% BPA-5000 and became slower with increases in the concentration of BPA-5000 above 0.03%.

In order to determine the optimum concentration of the particulate flocculant BPA-1000 at a given concentration of the water soluble polymer flocculant BPA-5000, 0.01% BPA-5000 was premixed with varying concentrations of BPA-1000 (0 to 0.03%). As shown in Fig. 4, the sedimentation of the cell flocs was fastest at 0.01% BPA-1000, at which the sedimentation was slightly faster than that without BPA-1000 (0.01% BPA-5000 alone). Further increases in the concentration of the particulate flocculant BPA-1000 did not facilitate the sedimentation of the cell flocs, which suggests that the optimum concentration of premixed flocculants is 0.01% BPA-1000 and 0.01% BPA-5000.

Floc Strength

Fig. 5 shows the shear index of the *E. coli* (1% DCW) flocs formed with premixed particulate flocculant BPA-1000 (0.02%) and varying concentrations of the water soluble polymer flocculant BPA-5020 (0 to 0.12%). The shear index of the cell flocs increased with increases in the concentration of BPA-5020 up to 0.06%. The shear

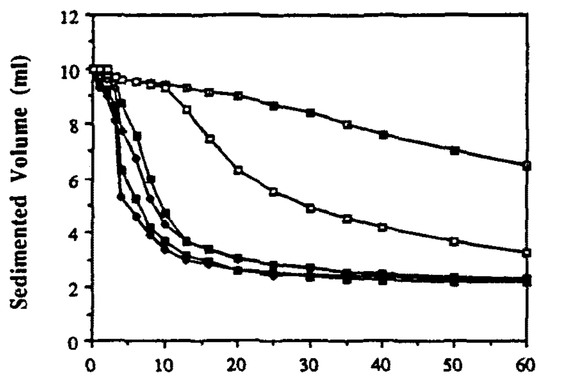

Fig. 3. Sedimentation of *E. coli* (0.5% DCW) treated with premixed particulate flocculant BPA-1000 (0.01%) and varying concentrations of water soluble polymer flocculant BPA-5000 (0 to 0.05%).
—□—, 0.00% BPA-5000; —◆—, 0.01% BPA-5000; —■—, 0.02% BPA-5000; —◇—, 0.03% BPA-5000; —⊟—, 0.04% BPA-5000; —□—, 0.05% BPA-5000.

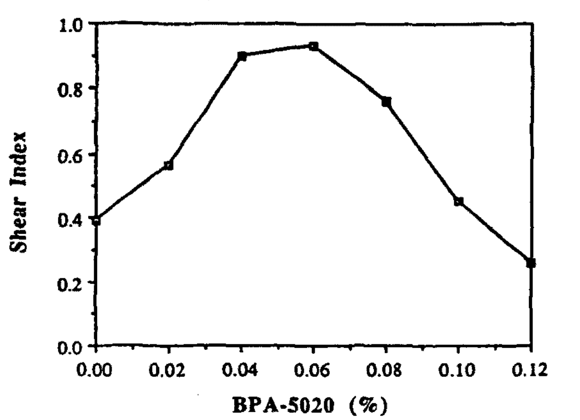

Fig. 5. Shear index of *E. coli* flocs (1% DCW) treated with premixed particulate flocculant BPA-1000 (0.02%) and varying concentrations of water soluble polymer flocculant BPA-5020 (0 to 0.12%).

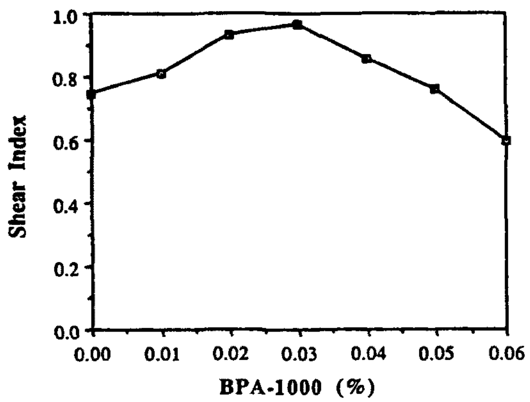

Fig. 6. Shear index of *E. coli* flocs (1% DCW) treated with premixed water soluble polymer flocculant BPA-5020 (0.06%) and varying concentrations of particulate flocculant BPA-1000 (0 to 0.06%).

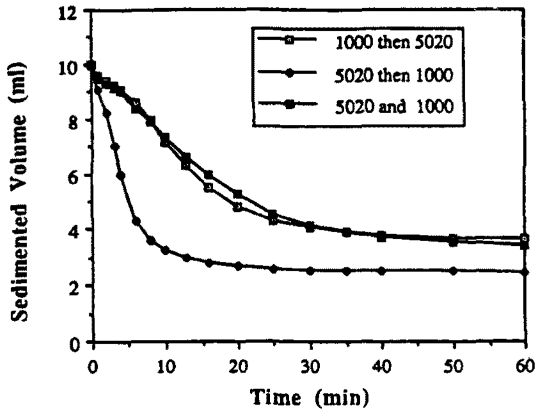

Fig. 7. Sedimentation of *E. coli* flocs (0.5% DCW) treated in different order with particulate flocculant BPA-1000 (0.01%) and water soluble polymer flocculant BPA-5020 (0.03%). —□—, BPA-1000 then BPA-5020; —◆—, BPA-5020 then BPA-1000; —■—, BPA-5020 and BPA-1000.

index was 0.39 without BPA-5020 and 0.93 with 0.06% BPA-5020. The shear index of 0.93 is even higher than that of *E. coli* flocs formed in deionized water with the particulate flocculant BPA-1000 (shear index=0.47) (8). This suggests that the use of a water soluble polymer flocculant enhances the strength of *E. coli* flocs formed with the particulate flocculant. The enhancement of the floc strength with the use of the water soluble polymer flocculant can be explained by the ability of the long chain water soluble polymer flocculant to entangle cells. The entanglement of cells by the water soluble polymer flocculant contributes the compactness of the flocs as well as the strength of the flocs. Therefore, the sedimentation rate was higher when the water soluble polymer flocculant was added.

Further increases in the concentration of BPA-5020 decreased the shear index (Fig. 5). The shear index decreased to 0.27 as the concentration of BPA-5020 increased to 0.12. This confirms that the optimum concentration of premixed flocculants is 0.02 g BPA-1000 and 0.06 g BPA-5020/g DCW *E. coli*.

Fig. 6 shows the shear index of the *E. coli* (1% DCW) flocs formed with premixed BPA-5020 (0.06%) and varying concentrations of BPA-1000 up to 0.03%. This again demonstrates that the use of the particulate flocculant in combination with the water soluble polymer flocculant improves the strength of the *E. coli* flocs. The enhancement of floc strength with the addition of the particulate flocculant may be due to charge neutralization.

Treatment Order of Flocculants

In order to test the effect of the addition order of the flocculants on sedimentation and the strength of the cell flocs, one flocculant (either BPA-1000 or BPA-5020) was added first into the *E. coli* suspensions and mixed for 5 min. After 5 min mixing, the other flocculant was added and mixed for another 5 min. For the premixing of flocculants, BPA-1000 and BPA-5020 were added together and mixed for 1 h prior to use. The premixed flocculants were added to the cell suspensions and mixed for 10 min.

As shown in Fig. 7, the sedimentation of the cell flocs was fastest when the water soluble polymer flocculant BPA-5020 was added first. The sedimentation velocity of the cell flocs treated with the premixed flocculant was about the same as that which was treated with particulate flocculant BPA-1000 first. Therefore, adding the water soluble polymer first could improve sedimentation of cell flocs.

Table 1 shows the shear index of the cell flocs treated with flocculants in different orders. The shear index was the same regardless of the flocculant treatment order. Therefore, the strength of the cell flocs is not affected by the order of flocculant addition.

Table 1. Shear index of *E. coli* flocs.

Treatment Order	Shear Index
BPA-1000 and BPA-5020	0.95±0.01
BPA-1000 then BPA-5020	0.94±0.03
BPA-5020 then BPA-1000	0.94±0.04

*1% *E. coli*, 0.02% BPA-1000, 0.06% BPA-5020 in 5 g/l NaCl.

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