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Effects of Ionic Strength on Bacterial Adhesion and Stability of Flocs in a Wastewater Activated Sludge System

ANNA ZITA AND MALTE HERMANSSON*

Department of General and Marine Microbiology, University of Göteborg, S-413 90 Göteborg, Sweden

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The success of biological wastewater treatment is to a large extent governed by the ability of bacteria to induce floc formation, thereby facilitating the separation of particles from the treated water. We performed studies on the dynamics of floc stability, the desorption of cells from the flocs, and the reflocculation of detached material. The floc stability was affected by the ionic strength of the medium in a way that strongly suggests that the interactions between the floc components can be explained by the theory of Derjaugin, Landau, Verwey, and Overbeek (DLVO theory). At increasing concentrations of electrolytes, the stability of the flocs increased. However, above an ionic strength of about 0.1 the floc stability decreased, and it seems that at this high electrolyte concentration the DLVO theory cannot be applied. The reversibility of the electrostatic double-layer effects was experimentally shown by treating the sludge repeatedly with a low-ionic-strength solution until parts of the flocs detached. When salt was added at this point, flocs re-form, resulting in a dramatic decrease in the turbidity of the supernatant liquid. Both reflocculation and detachment of floc material were seen with calcium as well as with potassium. This finding clearly indicates that the reflocculation and destabilization of flocs were due to changes in double-layer thickness rather than bridging effects of multivalent ions such as calcium. The results indicate that the ionic strength may well be an important factor for the floc stability in wastewater in situ.

One of the most important biotechnological applications of bacterial attachment and aggregate formation is the activated sludge process used in the treatment of wastewater (13). The success of biological wastewater treatment is to a large extent governed by the ability of bacteria to induce floc formation, thereby facilitating the separation of particles from the treated water. Activated sludge is a complex ecosystem consisting of bacteria, protozoa, viruses, and many other types of organisms (12).

Eriksson et al. suggested a model for the floc structure at different sludge age conditions (5). At low sludge ages, flocs form chain-type structures which may result in weak interactions, probably due to a relatively low production of polymers. The polymers bind cells together by forming bridges, and here the presence of multivalent cations is important in binding polymers and cells together. At higher sludge ages, cells are surrounded by polymers in more compact aggregates, predominantly in the central regions of the flocs, but in the outer parts, cells can still be attached in relatively weak structures that are shear sensitive (5). Activated sludge is characterized through a variety of parameters, for example, sludge volume index (SVI) and stirred sludge volume index (SSVI). The difference between these two indices depended on the shape of the flocs, which was represented as a shape factor S (4). There is an absence of uniformity in the floc structure which can be characterized by the concept of fractals (17). The floc has numerous internal water gaps of various sizes, which enable liquid flow through the floc.

Because bacteria are such a large and important component of activated sludge flocs, bacterial adhesion is a crucial mechanism of the flocculation. The adhesion process can be seen as a two-step event wherein the first step is a reversible phase which may be followed by an irreversible attachment. The reversible adhesion involves long-range forces which may be described by the theory of Derjaugin, Landau, Verwey, and Overbeek (DLVO theory) (3, 32). The DLVO theory can be used to calculate the interaction Gibbs energy between two surfaces as a function of the separation distance. The net interaction Gibbs energy is interpreted as a balance of attractive van der Waals interactions and an electrostatic factor, which is usually repulsive as a result of overlapping electrical double layers at negatively charged surfaces. Although bacteria differ in their outermost surfaces, as a result of variations in chemical and/or physical structure, basically all bacteria as well as other natural surfaces carry a net negative charge (14, 22). Three different situations can be distinguished, depending on the electrolyte concentration. (i) The net interaction is repulsive at all separation distances, and the two surfaces may not come into contact. This happens at low electrolyte concentrations, when the double layers are extensive. (ii) At an intermediate electrolyte concentration, a shallow secondary minimum may be formed at some separation distance (typically 5 to 15 nm), where the cell may be attached in a reversible manner. (iii) At high electrolyte concentrations, the net interaction is attractive at all separation distances and results in a strong, basically irreversible adhesion. The DLVO theory was originally described for colloidal particles but has been shown to explain bacterial attachment in various biological systems (6, 8, 18, 24, 26, 28, 30).

The irreversible adhesion which involves short-range interactions can be described by a thermodynamic approach (1, 23). This concept assumes the formation of a bacterium-surface interface. The contact may be mediated by cell surface structures (such as fimbriae or flagella) or less structured extracellular polymeric substances.

Many of the proposed mechanisms of bioflocculation in activated sludge involve the formation of extracellular polymers (16, 25, 27). The accumulation of polymeric material often increase with increasing culture age and was shown to be

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* Corresponding author. Mailing address: Department of General and Marine Microbiology, University of Göteborg, Medicinaregatan 9C, S-413 90 Göteborg, Sweden. Phone: 46 31 7732500. Fax: 46 31 7732599. Electronic mail address: Hermansson@gmm.gu.se.

TABLE 1. Background data on sludge characteristics from different sampling occasions

Sludge sample	Conductivity	BOD (mg of O_2/liter)	COD (mg of O_2/liter)	SS (mg/liter)	Settling index SV (ml)	Settling index SVI (ml/g)	Settling index SSVI (ml/g)	Shape factor, S
1	725	6	52	6	110	65	48	1.5
2	713	7	54	7	210	100	68	0.6
3	1,158	5	47	4	190	106	74	0.7
4	1,155	7	45	4	195	103	72	0.7
5	1,256	7	50	8	200	91	77	0.4
6	1,028	7	43	9	285	92	85	0
7	1,477	6	48	8	240	96	83	0.2
8	810	6	46	11	210	91	79	0.3
9	879	7	38	6	150	94	67	1.0
10	561	6	35	7	290	145	68	0.9
11	944	5	38	6	480	178	78	0.5
12	1,114	5	44	4	200	100	70	0.6
13	1,126	10	61	4	210	96	61	0.7
14	504	7	30	10	160	64	40	1.2
15	493	6	37	11	160	70	44	1.2
16	675	6	49	8	165	66	41	1.1
17	753	8	53	7	200	95		
18	769	9	53	8	205	93	65	0.6
19	820	12	54	11	180	82	55	0.8
20	976	12	54		180	69	22	2.9

directly correlated with the flocculation (27). Divalent cations are considered to be important bridging agents between negatively charged exopolymers and bacteria. Ca^{2+} seems to be the most important cation involved in flocculation in activated sludge (10, 15). It absorbs to the sludge surface and causes alterations in the bound-water content of the sludge (9). The absorption of other metal ions occur in different bacteria when extracellular gels are produced, but the metal-polymer interactions are not fully understood. However, it is apparent that this absorption affects the settling of the sludge (10).

We performed studies on the dynamics of floc stability, the desorption of cells from the flocs, and the reflocculation of detached material. Flocs from a wastewater treatment plant (WWTP) were treated with different ionic strengths and different ions in a simple model system to test whether the DLVO theory is applicable in such a complex ecosystem as the activated sludge system. We tested the effects of different ionic strengths of calcium and potassium as well as iron sulfate and also measured the release of Ca^{2+} from the flocs during the treatments to be able to distinguish between electrostatic double-layer effects and the possible bridging effect of Ca^{2+}.

MATERIALS AND METHODS

Rya WWTP. All experiments were performed on activated sludge taken from Ryaverket (Rya) WWTP, in Göteborg, Sweden. The plant receives wastewater from approximately 550,000 people and 220,000 equivalents of industry, mainly food (fat and milk) and paper pulp (20).

At present, the wastewater is treated by primary sedimentation followed by an activated sludge process with a solids retention of 3 to 4 days. The aeration tanks are of plug-flow type and are aerated by diffused air. Phosphorus is removed through simultaneous precipitation with an average dosage of 0.6 liter of ferrous sulfate per s, estimated from the total flow, which is 4 m^3/s (21). The sludge passes through the aeration and settling tanks in 3 and 2 h, respectively. The mean annual temperature of the inflowing water was 15°C, and the pH was 7. Low dissolved oxygen concentrations were not detected during the sampling periods.

Sludge characteristics. Analyses of influent water, activated sludge, and effluent water were performed at the Rya WWTP. The analyses included conductivity in the influent water (microsieverts per centimeter), biological oxygen demand (BOD; milligram of O_2 per liter), chemical oxygen demand (COD; milligrams of O_2 per liter), solids in suspension (SS; milligrams per liter), sludge volume (SV; milliliters), SVI (milliliters per gram), and SSVI (milliliters per gram). The shape factor, S, was defined as $S = SQI/1.16\ SSVI - 1$, where $SQI = (SV/3) + 200/SS$ (4). Table 1 shows data on the sludge characteristics at the different sampling occasions.

Floc stability tests. (i) Dissociation of flocs. Flocs were treated with different ionic strengths of KCl, $FeSO_4$ (data not shown), or $CaCl_2$ solutions or with effluent water from the WWTP. The sludge was gently mixed and divided into aliquots of 10 ml. After 15 min of sedimentation, 6 ml of the supernatant was removed and the turbidity was measured spectrophotometrically at 420 nm (LKB Novaspec). We then added KCl, $FeSO_4$, or $CaCl_2$ solutions or effluent water to the original volume. Subsequently, the samples were gently mixed and flocs were allowed to settle for 15 min. This procedure was repeated 10 times. The different series of ionic strengths (I) were $0.05 \cdot 10^{-3}$, $0.5 \cdot 10^{-3}$, $5 \cdot 10^{-3}$, $50 \cdot 10^{-3}$, and $500 \cdot 10^{-3}$ for KCl, $0.1 \cdot 10^{-3}$, $1 \cdot 10^{-3}$, and $10 \cdot 10^{-3}$ for $FeSO_4$, and $0.05 \cdot 10^{-3}$, $0.5 \cdot 10^{-3}$, $5 \cdot 10^{-3}$, $50 \cdot 10^{-3}$, $500 \cdot 10^{-3}$, $0.06 \cdot 10^{-3}$, $0.6 \cdot 10^{-3}$, $6 \cdot 10^{-3}$, and $60 \cdot 10^{-3}$ for $CaCl_2$.

Untreated portions of activated sludge were used as controls in the dissociation tests to measure the possibility of a disintegration of the sludge flocs as a result of the sample treatment. Equal amounts of activated sludge (10 ml) were allowed to settle for 15 min, and the turbidity in the supernatant was measured as described above. The samples were mixed and again allowed to settle without addition of salt solutions. This procedure was repeated throughout the time for the dissociation tests.

(ii) Reflocculation studies. In these experiments, flocs were pretreated three times with deionized water (MilliQ) until a strong dissociation of the flocs occurred; different ionic strengths of KCl or $CaCl_2$ were then added. The reflocculation of the dissociated flocs was measured as a turbidity change in the supernatant as described above.

Microscopical studies. Floc structure and shape were determined with a light microscope at a magnification of ×400. Observations were also made on the amount of filaments and ciliates, both attached and free-swimming.

Floc material from the supernatants in the dissociation and reflocculation experiments was studied by epifluorescence microscopy. Samples were stained with acridine orange and passed through a 0.2-μm-pore-size Nucleopore filter (prestained with Sudan black B). Filters were viewed at a magnification of ×1,250 in an Olympus epifluorescence microscope, using blue excitation light where λ = 390 to 490 nm (515-nm filter).

Sampling strategy, calculations, and statistical treatment. Activated sludge was sampled at Ryaverket, Göteborg, at different occasions to cover both seasonal variations, including dilution due to heavy rain fall, and fluctuations in the performance of the treatment plant. The sludge was taken from the end of the aeration tank and transported to the laboratory. All experiments were performed at ambient temperature, varying between 10 and 20°C, depending on seasonal changes. Samples were taken from the treatment plant during 1 year, giving 20 different sludges. Figures 1 and 2 show representative data from the floc dissociation tests.

The absorbance measured after each round of treatment was accumulated to show the total dissociation of the flocs up to each time point during the experiment.

The accumulated turbidity made it possible to calculate a dissociation coefficient. The dissociation coefficient for each treatment is defined as the slope of the calculated straight line (using standard linear regression) of the accumulated turbidities plotted against number of treatments. The dissociation coefficients were plotted against increasing ionic strength in the salt solutions.

Correlations between sludge characteristics (BOD, COD, SV, SVI, SSVI, and SS), conductivity or pH, and the dissociation of the flocs in deionized water were tested by statistical analysis. The analysis carried out was a stepwise selection *F* test (both backward and forward) where the *F* value for acceptance was set to 4.0. This corresponds to an approximate significance level of 5%.

The reflocculation experiments were performed on 10 different sludges (n = 5 for each datum point); standard deviations were calculated and are presented as bars in Fig. 8 and 9. All experiments showed the same trend, and representative data are presented.

Calcium measurements. Free Ca^{2+} was measured in the water for the treatment with an ionic strength of 0.1 for KCl, deionized water, and effluent water from the Rya WWTP. Before the Ca^{2+} measurements, the samples were centrifuged for 10 min at 10,000 rpm, and the supernatant was passed through a 0.2-μm-pore-size Nucleopore filter. Ca^{2+} was measured with a Perkin-Elmer atomic absorption spectrophotometer with an acetylene-air flame; 1% $LaCl_3$ was added to the samples to bind phosphates that can disturb the measurements.

RESULTS

In the treatments with KCl, $FeSO_4$ (data not shown), or $CaCl_2$, the turbidity in the supernatant was dependent on the ionic strength in the medium (Fig. 1 and 2). Low ionic strengths resulted in a steeper slope of the accumulated turbidity data for both KCl and $CaCl_2$ as a result of a release of floc fragments and free cells into the supernatant, which did not sediment during the incubation time. As we increased the ionic strength, the sedimentation improved because of less fragmentation of the sludge flocs. Also, the concentration of free cells decreased in the supernatant as we increased the ionic strength. These effects resulted in a less steep slope of the accumulated absorbance compared with the low-ionic-strength treatments. It is interesting that the accumulated turbidity data showed similar trends for both KCl and $CaCl_2$. For the control samples of untreated sludge, the turbidity in the supernatant was fairly constant, and no increase in turbidity could be detected during the time of the tests.

It is clear from the results that the conditions in the activated sludge (Table 1) affected the absolute values of the test results. Figure 3 shows the accumulated turbidity for the treatment with deionized water on various sludges. The absolute values for the dissociation of the sludges varied, but the relative results and the trends are general. All of the 20 different sludge samples showed the same trend despite the composition and shape of the sludge flocs. The statistical analysis showed that the observed variations could not be explained by any of the independent variables mentioned previously. Hence, the differences in the absolute turbidity values could not be explained

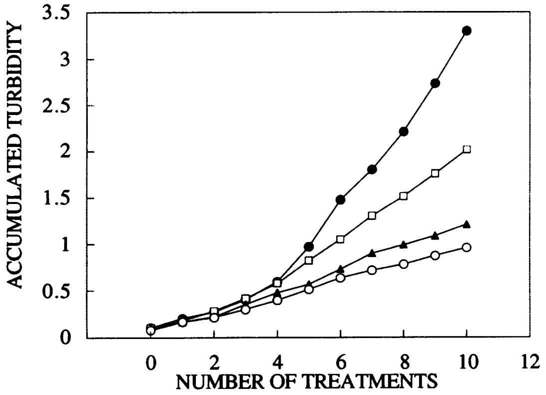

FIG. 1. Accumulated turbidity in the supernatant as a function of ionic strength. KCl solution was used for treating the flocs. The ionic strengths used were $0.05 \cdot 10^{-3}$ (●), $0.5 \cdot 10^{-3}$ (□), $5 \cdot 10^{-3}$ (▲), and $50 \cdot 10^{-3}$ (○).

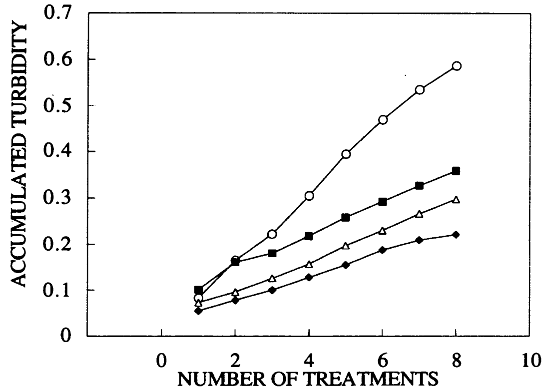

FIG. 2. Accumulated turbidity in the supernatant as a function of ionic strength. $CaCl_2$ solution was used for treating the sludge flocs. The ionic strengths used were $0.06 \cdot 10^{-3}$ (○), $0.6 \cdot 10^{-3}$ (■), $6 \cdot 10^{-3}$ (△), and $60 \cdot 10^{-3}$ (◆).

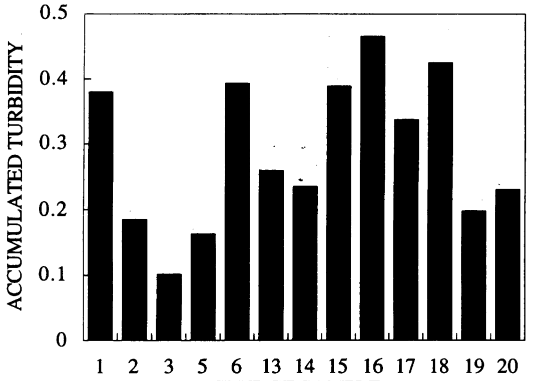

FIG. 3. Accumulated turbidity in the supernatant after treating various sludges with deionized water (three treatments).

by sludge characteristics (BOD, COD, SV, SVI, SSVI, and SS), conductivity, or pH.

The microscopic investigations of the sludge flocs showed that the dominant flocs were long and thin with various amounts of branches. The contents of filaments were usually low, but some sludges contained higher numbers of filamentous organisms. Various amounts of ciliates were found in the sludges and belonged to the subclasses *Peritrichia*, *Holotrichia*, and *Spirotrichia*. Typical genera of these subclasses were *Vorticella*, *Aspidisca*, and *Paramecium*. The amounts and types of ciliates in the sludges did not affect the general trends and results from the treatments with different ionic strengths.

We also included effluent water from the Rya WWTP as a reference (Fig. 4 and 5). Here we can note that the ionic strength of the effluent water from the Rya WWTP was within the range of the tested ionic strengths and seemed to follow the general trend of the KCl and $CaCl_2$ treatments.

Tests with higher ionic strengths were also performed. In the treatments with an ionic strength of 0.5, the turbidity increased dramatically. In contrast, the treatments below 0.05 followed the predicted trend.

The effects of the treatments with different ionic strengths were also studied by epifluorescence microscopy (Fig. 6). The increase in absorbance when the ionic strength was decreased was shown to be due to a release of small pinpoint flocs and free cells during treatment (Fig. 6A and B). However, the increase in turbidity during treatment with I = 0.5 for KCl and $CaCl_2$ was shown, almost exclusively, to be the result of the release of free cells and not of floc fragments (Fig. 6C).

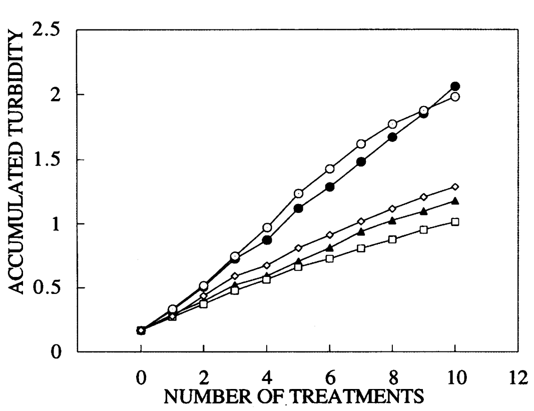

FIG. 4. Treatment with KCl solution and effluent water from the treatment plant. The ionic strengths used were $0.5 \cdot 10^{-3}$ (●), $5 \cdot 10^{-3}$ (▲), $50 \cdot 10^{-3}$ (□), and $500 \cdot 10^{-3}$ (⊙). The effluent water at the Rya WWTP had an ionic strength of approximately $5 \cdot 10^{-3}$, as judged from the conductivity for KCl (◇).

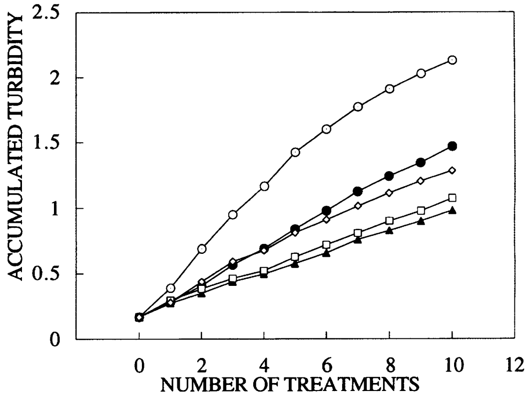

FIG. 5. Treatment with $CaCl_2$ and effluent water from the Rya WWTP. The ionic strengths used were $0.5 \cdot 10^{-3}$ (●), $5 \cdot 10^{-3}$ (▲), $50 \cdot 10^{-3}$ (□), and $500 \cdot 10^{-3}$ (⊙). The effluent water had an ionic strength of approximately $5 \cdot 10^{-3}$, as judged from the conductivity for KCl (◇).

The dissociation coefficient (see Materials and Methods) is a convenient way to illustrate the stability of the flocs in different ionic strengths (Fig. 7). At low ionic strengths, the dissociation coefficient is high as a result of a substantial fragmentation of the sludge flocs. The dissociation of the flocs decreases as the ionic strength increases, resulting in a minimum of floc degradation between 0.005 and 0.05. If the electrolyte concentration is increased to 0.5, the dissociation coefficient increases again, presumably as a result of factors other than electrical double-layer effects.

In the reflocculation experiments, the reversible nature of the electrolyte effects was tested. In the control with deionized water, the flocs continued to disperse (Fig. 8 and 9). When salt was added, a rebuilding of the flocs occurred, which resulted in an improved sedimentation. In some cases, the turbidity was even lower after the treatment than before the treatment. The trend from the dissociation experiments was followed such that the best reflocculation occurred at an ionic strength of between $0.5 \cdot 10^{-3}$ and $50 \cdot 10^{-3}$, and the treatment with I = 0.5 showed a slightly lower reflocculation (Fig. 8 and 9).

Measurements of free Ca^{2+} in the water with atomic absorption spectrophotometry showed that there was a very small release of Ca^{2+} in the treatments with an ionic strength of 0.1 for KCl and deionized water. A total of 2 μM Ca^{2+} was released after 10 treatments with deionized water. For the treatment with I = 0.1 for KCl, the total release of Ca^{2+} was 3 μM (Fig. 10).

DISCUSSION

The detachment of material from the flocs was affected by the ionic strength of the medium in a way that strongly suggests that the interactions between the floc components can be explained by the general DLVO theory (3, 19, 22, 32). That is, at increasing concentrations of electrolytes, which results in a compression of the electrical double layers around all surfaces, the stability of the flocs increased. Below an ionic strength of 0.05, the strength of the flocs increased with increasing ionic strength. This was shown to be the case both for the mono- and

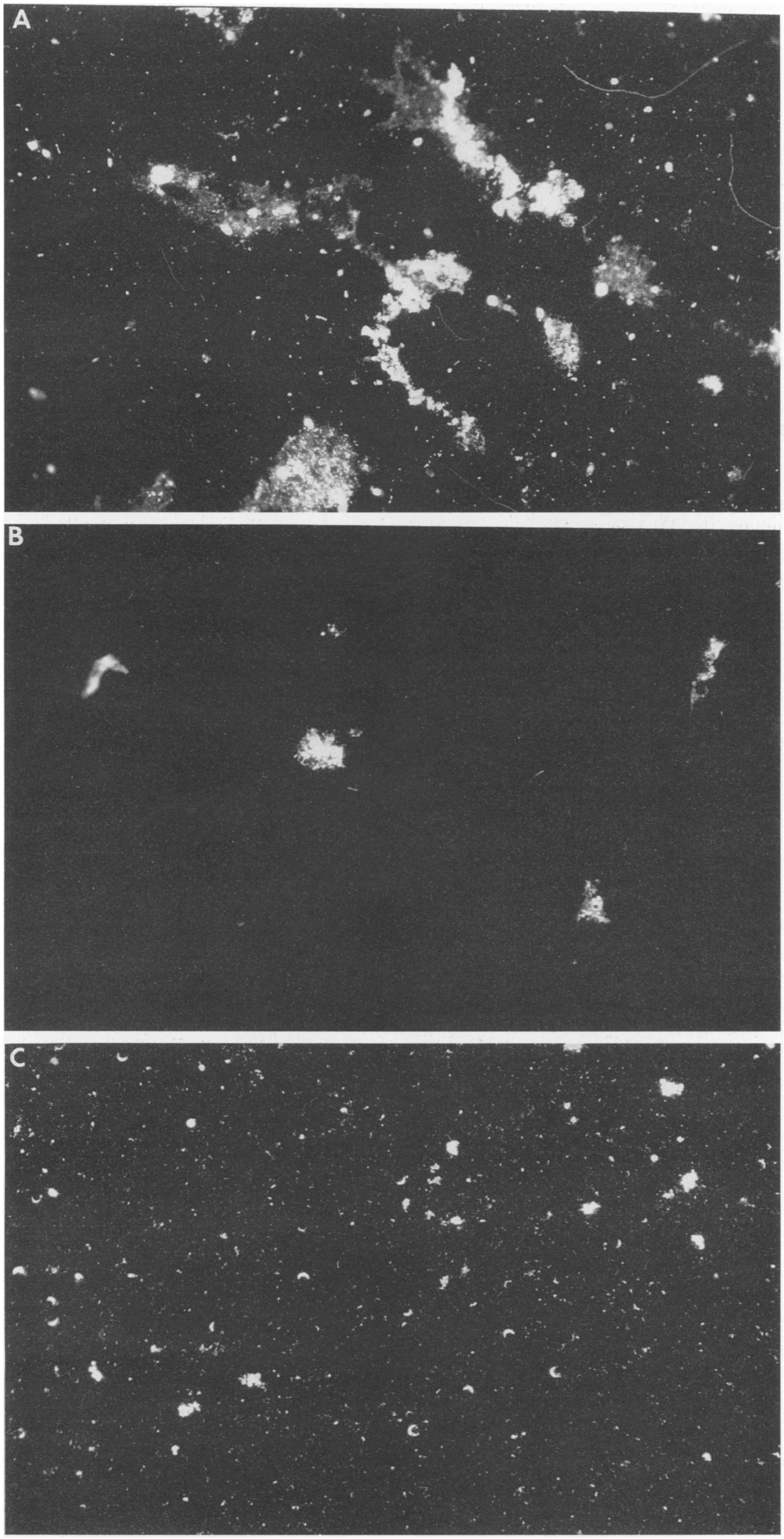

FIG. 6. Micrographs from epifluorescence microscopy examinations of supernatants in the dissociation experiments. Magnification was ×1,138 for all micrographs. (A) $I = 0.05 \cdot 10^{-3}$ to $0.5 \cdot 10^{-3}$; (B) $I = 5 \cdot 10^{-3}$ to $50 \cdot 10^{-3}$; (C) $I = 0.5$.

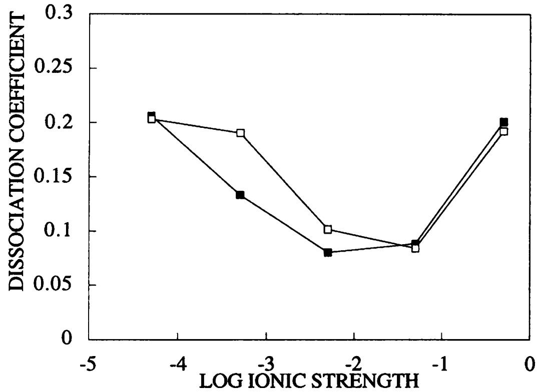

FIG. 7. Dissociation coefficient for activated sludge as a function of ionic strength. □, KCl; ■, CaCl$_2$.

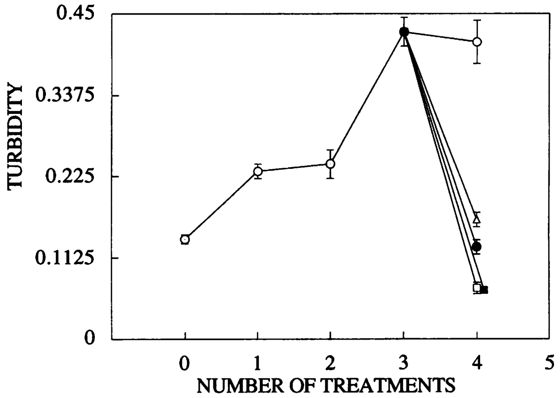

FIG. 9. Reflocculation of degraded activated sludge with CaCl$_2$ solution after three treatments with deionized water ($I = 0$). The ionic strengths in the final solutions were 0 (⊙), $0.5 \cdot 10^{-3}$ (△), $5 \cdot 10^{-3}$ (□), $50 \cdot 10^{-3}$ (■), and $500 \cdot 10^{-3}$ (●).

divalent cations. The ambient ionic strength in the wastewater was within the range where the DLVO theory is applicable, and therefore the stability of the flocs could well be affected by differences in the ionic strength of the influent wastewater in situ.

In the intermediate interval, approximately 0.005 to 0.05, the stability of the flocs is constant and changes in ionic strength do not affect them particularly. This could be the result of the small change in double-layer thickness. Loosdrecht et al. (19) showed that the reversible adhesion of bacteria to a surface was increased with increasing electrolyte concentration and finally reached a saturation value in the interval of 10 to 100 mM. This finding agrees well with our results, indicating that there are similar mechanisms involved in the maintenance of the flocs.

We are of course aware of the highly complex situation in wastewater, with various compositions and structures of the sludge flocs, through fluctuations in one WWTP but also between different plants, and we do not expect that the DLVO theory can explain all interactions within the flocs. It is probable that the outer parts of the flocs are held together in a reversible manner and that this is the part that could be affected by the differences in the double-layer thickness, rather than the inner parts where the polymer matrix is extensively developed (4). The polymer matrix in the central parts mediate a close contact between cells, a situation that is not well described by the DLVO theory.

Surface charge density, surface potential, and ionic composition of the solution (through its effect on K, where $1/K$ is the Debye length or the double-layer thickness) are interrelated (29). However, the surface charge is affected only by the adsorption of potential-determining ions. Therefore, if inert electrolyte is added, $1/K$ will decrease and the surface potential will decrease without any change in the surface charge density. An increase in the ionic strength, therefore, has a double effect on the interaction between two surfaces: a decrease both in the thickness of the double layer and of the surface potential (29).

By measuring SSVI and calculating the shape factor, S, it was possible to predict the structure of the sludge flocs. These predictions were verified by comparison with the microscopic investigations of the floc structure. Sludges 1, 14, 15, and 20 have high S values and low SSVI indices. These sludges have extremely long and thin flocs. This chain-type structure is found at low sludge ages and is weak because of the low number of cell-cell contacts and a limited amount of polymers. In these types of flocs, the consequences of changes in the electrolytic composition in the surrounding medium should be of importance, and this is probably a reason why the flocs reacted so obviously upon the changes in ionic strength.

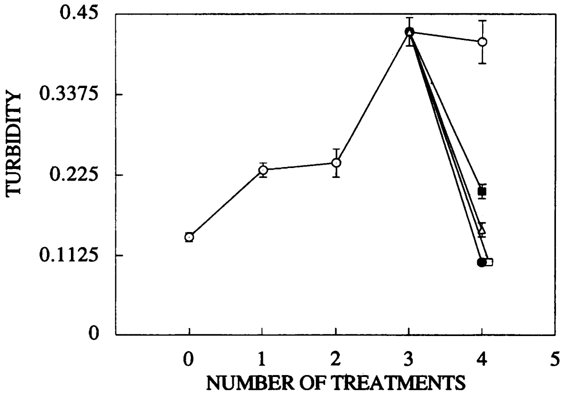

FIG. 8. Reflocculation of degraded activated sludge with KCl solution after three treatments with deionized water ($I = 0$). The ionic strengths in the final solutions were 0 (⊙), $0.5 \cdot 10^{-3}$ (■), $5 \cdot 10^{-3}$ (□), $50 \cdot 10^{-3}$ (●), and $500 \cdot 10^{-3}$ (△).

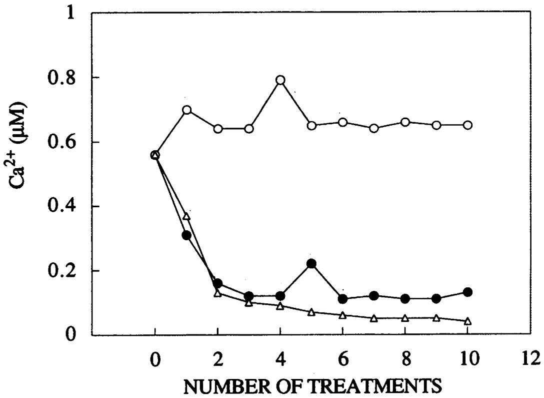

FIG. 10. Measurement of free Ca^{2+} with atomic absorption spectrophotometry. ○, treatment with effluent water from the WWTP; ●, deionized water; △, KCl solution with an ionic strength of 0.1.

Sludges 6 and 7 had low S values and intermediate SSVI indices. These flocs are built as a three-dimensional network with short filaments holding the flocs together. Also, flocs of this type were affected by the ionic strength in the medium. In fact, there were no obvious differences in the dissociation of different sludge types.

It should be stressed that 20 different samples were taken during 1 year, and although we cannot expect to find all kinds of floc types in one treatment plant, all samples showed the same qualitative result. The differences in absolute values (Fig. 3) between samplings could not be explained by any of the sludge parameters listed in Table 1.

The DLVO theory can be said to function in a reversible situation when there is not yet contact between the two surfaces (19). The reversibility of the floc system was experimentally shown simply by treating the sludge repeatedly with a low ionic strength until parts of the flocs were detaching. When salt was added at this point, flocs were re-forming, resulting in a dramatic decrease in the turbidity of the supernatant (Fig. 8 and 9).

The phenomena of reversible flocculation and dispersion have been shown earlier indirectly for a *Flavobacterium* isolate found as a predominating species in activated sludge (31). Flocculation was independent of the production of polymers but was induced by both mono- and divalent cations. The flocs disintegrated to a uniformly dispersed cell suspension by suspending the flocs in deionized water. The dispersed cells flocculated again by the addition of cations. Even if the cells were killed by heat or treated by trypsin, they did not lose their ability to form flocs upon addition of mineral salts. The electric charge of the bacterial cell surface was negative, and the reversible nature of the flocculation process indicated that the mechanism was physicochemical rather than physiological (31). These results from pure culture experiments agree well with our findings in the more complex floc system.

An important mechanism in floc formation is the action of multi- or divalent bridging cations (2, 15). These ions increase flocculation by forming bridges between positively charged groups on polymers or cells. Bruus et al. (2) reported that addition of cations (Na^+, K^+, and Mg^{2+}) to thickened sludge resulted in a release of Ca^{2+} from the polymer structure. This release gave an increase in turbidity in the water and was correlated with the amounts of added cations. In our experiments, however, the disintegration as well as the reflocculation could be seen with both calcium and potassium. If bridging ions such as calcium had been removed by our treatments, dissociation of flocs should have been higher for KCl than for $CaCl_2$. Also, reflocculation should have been poorer with KCl than with $CaCl_2$. Measurements of release of calcium from the flocs by atomic absorption spectrophotometry showed that there was very little release of Ca^{2+} in the treatments with an ionic strength of 0.1 for KCl or deionized water. This finding clearly indicates that the effects are due to changes in the double layer rather than a removal of bridging ions such as calcium. Again, the differences in observations may also depend on the structure and composition of the sludge flocs.

When the ionic strength was increased to 0.5, a different kind of mechanism seemed to affect the floc structure. A sudden release of free cells into the water occurred at this high ionic strength. According to the DLVO theory, the interparticle distance in the suspension is fairly constant at these high salt concentrations and should not affect the adhesion. The observation that the mechanism for bacterial attachment differs when the electrolyte concentration is increased over 100 mM has been reported previously in studies of bacterial adhesion to solid surfaces (11). From our data, this appears to be the case also for bacterial aggregates. This behavior has been proposed to have several causes. Some of the suggestions are salting-out effects (11), ion-exchange mechanisms (2), and changes in adhesive polymer-substratum interactions (7).

A salting-out effect (11) as a result of bacterial coagulation at high ionic strengths cannot explain our results. Such an effect should stimulate the sedimentation via further aggregation of the cells. This was quite the contrary to our observation that there was a release of free cells into the water.

As discussed above, the bridging capacities of Ca^{2+} in the polymer network could be destroyed by the ion-exchange mechanism of K^+ and other cations. Our observations showed that high concentrations of K^+ resulted in an increasing turbidity, but this was not due to a release of Ca^{2+} into the water, as measured by atomic absorption spectrophotometry. The turbidity increase at very high ionic strengths was also achieved for $CaCl_2$, which could not be explained merely by ion-exchange mechanisms.

The initial adhesive polymers in freshwater bacteria are shown to be affected by changes in electrolyte concentration by interference reflection microscopy (7). Changes in ionic strength resulted in a contraction or expansion of the polymers but not in a reproducible manner. The distance at which the cell was attached to the surface therefore varied as a result of the salt concentration. A direct effect of the ionic strength on the polymers might be part of the explanation in our experiments. In summary, presently we are not able to explain the massive release of free cells from the flocs at high ionic strengths.

The results show that the ambient ionic strength in the effluent wastewater was within the range where the DLVO theory was applicable and that the wastewater followed the general trend of the KCl and $CaCl_2$ treatments. Therefore, the stability of the flocs in wastewater in situ could well be affected by differences in the ionic strength of the influent wastewater.

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