STUDY OF FLOCCULATION MECHANISMS BY OBSERVING EFFECTS OF A COMPLEXING AGENT ON ACTIVATED SLUDGE PROPERTIES

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ABSTRACT

Electrostatic interactions between bacterial surfaces, extracellular polymers (ECP) and polyvalent metal ions are important in activated sludge flocculation. An indirect study of these mechanisms was done by adding different concentrations of EDTA to activated sludge samples from 6 Swedish wastewater treatment plants. The effects on sludge properties were studied with sedimentation and filtration tests as well as analysis of released extracellular polymers.

EDTA had a significant effect on sedimentation velocity in all investigated sludges. This shows that charged polymers are important for the properties of the floc surfaces and in building up the sludge macroflocs. The effect on filtration resistance where the bulk properties of the primary flocs are more important varied considerably for the different sludges. Thus, both electrostatic and other interactions are involved to a varying extent in building up the primary flocs in the sludges investigated. Variations in sedimentation velocity, residual turbidity, filtration resistance and release of ECP with variations in EDTA concentrations could be explained by effects of polyvalent metal ions on ECP binding and conformation.

KEYWORDS

Activated sludge, flocculation, extracellular polymers, biopolymers, sedimentation, filtration resistance, floc strength, complexing agents, polyvalent metal ions.

INTRODUCTION

The function of the activated sludge process depends on the fact that certain microorganisms are capable of forming well settling flocs. It is well understood that this flocculation is caused by extracellular microbial polymers.

At neutral pH the microorganism/biopolymer matrix has a net negative charge. In order for flocs to be formed, attractive polymer-surface and polymer-polymer interactions must come into play. The nature of these interactions may be hydrophobic, hydrogen bonding, physical enmeshment and electrostatic. It is usually found that polyvalent metal ions (e.g. Ca and Mg) are necessary for microbial flocculation to occur (Endo et al., 1976). The metal ions serve as ionic bridges between negatively charged sites on surfaces and polymers. It has also been found that strong complexing agents such as EDTA may have a
dispersing effect on microbial flocs. However, in mixed activated sludge cultures deflocculation is normally not obtained.

Activated sludges can have very different floc structures and separation properties. This can to a large extent be traced back to the flocculation mechanisms. In this investigation we have tried to get information on the relative importance of the electrostatic metal ion/polymer/surface interactions for these mechanisms. This was done by studying the effect of different additions of EDTA on sludges with different properties.

MATERIALS AND METHODS

Sludge samples were taken from the outlets of the aeration basins at the Eskilstuna, Margretelund, Käppala, Himmersfjärden, Henriksdal and Rya wastewater treatment plants. To 1380 ml sludge 120 ml EDTA solutions were added to give the final EDTA concentrations 0, 5, 10, 20, 30, 50, 100, 200, 400 and 800 mg/l. The samples were mixed for 20 min whereafter settling curves in 1 l measuring cylinders were recorded.

Samples were also taken for analysis of released extracellular carbohydrates and proteins. The analyses were made in the supernatant after 30 min centrifugation at 4000 r/min of sludge samples treated for 2 min in a Waring blender. Carbohydrates were analysed with the anthron method (Scott and Melvin, 1953) and proteins with the dye method according to Bradford (1976) as well as the Folin phenol reagent (Lowry et al., 1951)

After settling for 60 min residual turbidities were measured at the 800 ml level and capillary suction times (CST) of the thickened sludges were measured after 10, 20, 50 and 110 sec stirring at 1000 r/min. The slopes of CST against stirring time were taken as a measure of the floc breakdown.

RESULTS AND DISCUSSION

Table 1 shows results for the investigated sludges without addition of EDTA. The two different protein analysis methods were used in order to estimate the content of humic acids which have been suggested as important components of the ECP (Peter and Wuhrman, 1970). Since a phenol reagent is used in the Lowry method it was assumed that the results with this method when corrected for the protein content obtained with the Bradford method would give the amount of humic acids.

<table>
<thead>
<tr>
<th>Sludge nr</th>
<th>Treatment plant</th>
<th>SVI (ml/g)</th>
<th>CST (sec)</th>
<th>Slope CST versus stirring time</th>
<th>Carbohydrate (mg/g SS)</th>
<th>Protein (mg/g SS)</th>
<th>Humic acids (mg/g SS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Margretelund</td>
<td>203</td>
<td>125</td>
<td>0.34</td>
<td>11.5</td>
<td>35.0</td>
<td>17.0</td>
</tr>
<tr>
<td>2</td>
<td>Käppala</td>
<td>168</td>
<td>180</td>
<td>0.92</td>
<td>10.3</td>
<td>29.4</td>
<td>18.9</td>
</tr>
<tr>
<td>3</td>
<td>Rya</td>
<td>103</td>
<td>114</td>
<td>0.28</td>
<td>6.5</td>
<td>16.7</td>
<td>16.4</td>
</tr>
<tr>
<td>4</td>
<td>Käppala</td>
<td>165</td>
<td>73</td>
<td>0.072</td>
<td>13.1</td>
<td>29.2</td>
<td>14.4</td>
</tr>
<tr>
<td>5</td>
<td>Eskilstuna</td>
<td>127</td>
<td>54</td>
<td>0.073</td>
<td>6.0</td>
<td>16.6</td>
<td>13.3</td>
</tr>
<tr>
<td>6</td>
<td>Henriksdal</td>
<td>138</td>
<td>28</td>
<td>0.0035</td>
<td>3.8</td>
<td>10.4</td>
<td>6.5</td>
</tr>
<tr>
<td>7</td>
<td>Himmersfjärden</td>
<td>99</td>
<td>14</td>
<td>0.012</td>
<td>2.7</td>
<td>8.6</td>
<td>4.4</td>
</tr>
</tbody>
</table>

It is clear that an increase in floc strength and filterability correlates to a decrease in amount of ECP. This has indeed been observed earlier (Eriksson and Hårdin, 1984; Ericsson and Eriksson, 1988). The correlation occurs for all three components with some deviations in the carbohydrates and proteins.
Novak and coworkers (1977, 1978, 1980, 1982) have claimed that an increase in amount of dissolved ECP is the reason for increased filtration resistance. On the other hand, Karr and Keinath (1978) showed that filterability was mainly determined by the number of particles in the supracolloidal size range. However, large amounts of dissolved and easily extractable polymers indicate a badly flocculated sludge and an increase in these two fractions should coincide. Eriksson and Härdin (1984) and Eriksson (1987) also suggested on the basis of electron microscopy studies and sludge conditioning tests that well flocculated sludges contain large amounts of binding polymers that will not be extracted with the method used here. This was proved by Kajornatiyudh (1986) who analysed ECP in sludges with different flocculation degrees after a mild (centrifugation) as well as a very strong (rising pH to 11) extraction method. The extraction method used in this study is supposed to be relatively mild compared to the high pH method.

The sludges in Table 1 are roughly ordered after increasing sludge age. The first sludges had strongly irregularly shaped flocs while the last ones had more compact, spherical flocs. The total filament length seemed to be within normal limits except in sludge 1, where it was somewhat high, and in sludge 2, where it was very low.

The results after addition of EDTA at different concentrations are shown in Figs. 1-3. Many of the curves show some considerable variations at low EDTA concentrations. Since the reproducibility of these variations has not been investigated for all the sludges they will not be analysed in detail here. However, all of the sludges have some common features, e.g. the decrease in CST between 50 and 100 mg/l EDTA. Moreover, repeated investigations on sludges from the Margretelund plant indicate that the observed variations are real. One of the plants (Rya) has a water hardness that is approximately twice that of the others. Although not evident with the available data there are some indications that characteristic features of the curves from the Rya plant are shifted towards higher EDTA concentrations compared with curves from the other plants.

![Fig. 1. a) Sedimentation velocity times suspended solids concentration and b) residual turbidity after 60 min sedimentation as function of EDTA concentration.](https://iwaponline.com/wst/article-pdf/24/7/21/102362/21.pdf)
Examples of solids flux results are given in Fig. 1 a. The other four sludges gave results similar to those with sludge 5 and 6, although sludge 4 gave smaller variations than the others.

It is evident that the relatively weak interactions between the flocs in the early stage of the settling process strongly depend on EDTA-sensitive molecular interactions. This was also observed in sedimentation of pure E.Coli bacteria (Eriksson and Axberg, 1981). The explanation proposed was that polyvalent metal ions strongly affect ECP conformation and binding to surfaces and/or other polymers. A concentration of CaCl₂ could be found (~1 mM) that gave a minimum in the flocculation rate. Since activated sludge contains different organisms and different polymers a complicated interaction and thus settling behaviour would be expected when increasing amounts of EDTA compete with the sludge matrix for polyvalent metal ions.

The settling tests indicate that the polymers responsible for building up the large macroflocs from primary flocs interact via metal ion bridges in all of the investigated sludges. This also implies that such polymers are located at the outer parts of the primary flocs or are dissolved. The residual turbidities shown in Fig. 1 b increase with increasing EDTA concentrations except for sludge 1 where only a slight shift between 30 and 50 mg/l occurs. This indicates that dispersed cells are released from the floc surfaces in sludges 2-7 while in sludge 1 the flocs might be divided into smaller but still settling parts. The variations in turbidity at low EDTA concentrations also illustrate the complicated floc matrix - metal ion interactions in the same way as the settling velocities.

The results of the CST-tests where the flocs are subjected to larger forces by stirring at 1000 r/min are shown in Fig. 2. With respect to this property the sludges with low CST values (and good floc strength) are relatively unaffected by the EDTA treatment whereas the effects on high CST sludges are strong.

This implies that the primary flocs in the readily dewatered sludges are built up to a large extent by other polymer interaction mechanisms than electrostatic. Microscopic examination revealed that sludge 7 was built up by compact spherical
cal primary flocs with a smaller amount of material with different appearance in between. On the other hand, sludge 1 had irregular flocs consisting of, as it appeared, a single type of material. Sludges 2 and 3 had very long-shaped primary flocs and a high activity of larger rod-shaped dispersed and adhering bacteria could be seen especially at the ends of the flocs. This illustrates some of the different types of floc structures in different stages of development proposed by us earlier (Eriksson and Hardin, 1984). The irregular and long-shaped flocs, typical in a young sludge, were supposed to be formed by a polymer bridging mechanism. According to the present study binding via metal ions should be important. The proposed reason for the elongated floc structure was that the electrostatic repulsion between rod-shaped particles is smallest at the end-to-end configuration. Thus, bridging with polymers that have a limited size would be most probable in that configuration.

The round flocs were proposed to be older and built up by polymer complexes, for example enmeshed polysaccharide fibrils. The latter type of interaction was indicated in studies by Friedman et al., (1968, 1969) and Deinema and Zevenhuisen (1971). Steiner et al., (1976) observed the presence of two different groups of polymers, one dissolved containing carboxyl groups and one bound to the floc matrix containing hydroxyl groups.

An interesting observation is that the filterability after 110 sec stirring of sludges 2 and 3 is significantly improved after addition of about 50 mg/l EDTA. This is an indication that the combination of mechanical and chemical treatment removes weakly flocculated structures from the surface of the flocs (compare Fig. 1 b). This should expose material in the primary flocs with stronger binding ability. Microscopic observation on a sample of sludge 3 with 200 ppm EDTA revealed that the flocs were much larger and more compact after 100 sec stirring than the original flocs but also that the amount of dispersed cells was very high.

The release of polymers is shown in Fig. 3. It is clear that the effect of increased EDTA concentrations on polymer release is stronger for the humic acids than for the proteins and carbohydrates. It should also be noted that carbohydrates and proteins generally increase at low EDTA concentrations and then decrease in the same manner as the CST values at about 50 mg/l. A reasonable quantitative interpretation of these results can be made if it is assumed that they reflect an interplay of complexation and electrostatic effects. One would expect the primary effect to be removal of the polyvalent ions from the flocs by complexation with EDTA. In sludge without EDTA the polymers are in a partly precipitated or strongly complexed state (Fletcher and Floodgate, 1976; Novak and Haugan, 1978). Upon removal of polyvalent metal ions the precipitates/complexes may dissolve and the polymers be in a strongly coiled conformation due to internal site-site binding. This will facilitate their diffusion through the floc and thus removal. When further metal ions are complexed with EDTA the polymers will stretch out due to segment-segment repulsion and their diffusion through the floc will be hindered. During these events the floc matrix still exists due to binding by other than electrostatic mechanisms. In an elongated conformation also the charged polymers can co-operate in the flocculation by these other mechanisms. Since there are several kinds of polymers with different metal complexing abilities this procedure may be repeated or overlapped at higher EDTA concentrations. This may eventually lead to considerable deflocculation if the fraction of charged and nonfibrillar polymers is large as it evidently is in sludge 1.

Sludge 1 shows a different behaviour than the others with respect to release of humic acids. It increases sharply at low EDTA concentrations but at higher it decreases while the release of proteins is strong. This indicates that the flocs are opened up and degraded to a larger extent than in the other sludges. In such a case metal ions would be extracted more easily from proteins and carbohydrates than from humic acids which are very strong complexing agents. Also an exchange of ions from proteins/carbohydrates to humic acids seems likely when the sludge matrix begins to degrade. This partial deflocculation occurs at EDTA concentrations higher than 50-100 mg/l and explains the observed strong increase in CST, especially at the longer stirring time.
Fig. 3 Concentrations of released a) carbohydrates, b) proteins and c) humic acids as function of EDTA concentrations for different sludges.

From the above results it is clear that activated sludges are flocculated by at least two different groups of polymers. One interacts via charged groups and metal ions and the second by other mechanisms. In well flocculated sludges the primary flocs seem generally to be built up by these other mechanisms. The weaker long-shaped primary flocs are susceptible to EDTA and they are proposed...
Flocculation mechanisms

(Eriksson and Härthin, 1984) and observed (Ericsson and Eriksson, 1988) to be the first stage in the floc formation events. It must therefore be concluded that polymer/metal ion bridging is the primary step in activated sludge flocculation. This is followed by a second step probably involving formation of a polysaccharide fibril matrix when production of such complex polymer structures has been possible which will take some time. This confirms the findings of Forster (1976) who also suggested a two-step flocculation process. Actually the effect of charged polymers in building macroflocs may be considered as a third step in which also the action of filamentous organisms is very important.

With respect to residual turbidity after sedimentation a floc form intermediate between strongly elongated and spherical seems to be optimal. This type of floc is normally obtained at medium sludge ages.

CONCLUSIONS

1. Activated sludge is flocculated by different polymers of which one group interacts via charged groups and polyvalent metal ions and one group by other mechanisms.

2. Charged polymers are involved in the primary flocculation step leading to long-shaped primary flocs.

3. Polymer network formation probably by enmeshed polysaccharide fibrils is the second flocculation step in which the primary flocs become more spherical and strong.

4. Formation of macroflocs from primary flocs may be considered as a third step in which charged polymers and, if present, filamentous organisms play an important role.

5. Repeated changes between positive and negative effects on settling and filtration resistance when increased amounts of EDTA is added, is due to the fact that polyvalent metal ions affect both conformation and binding of charged extracellular polymers and that there are different polymers with different complexing abilities.

ACKNOWLEDGEMENT

The authors would like to thank Prof. P. Stenius for helpful suggestions regarding the manuscript. The investigations were sponsored by the Swedish Board for Technical Development (STU).

REFERENCES


