Activated sludge flocculation: direct determination of the effect of calcium ions

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Abstract The effect of calcium on activated sludge flocculation dynamics is investigated using a unique experimental technique. The technique allows on-line analysis of the size of activated sludge flocs during flocculation and provides valuable insight into the mechanisms of flocculation. Activated sludge samples were firstly sonicated for 3 minutes at 50W and then stirred at 100 rpm. The floc size was subsequently measured on-line using a Malvern Mastersizer/E. For concentrations of calcium less than 4 meq/L no significant increase in final floc size was observed even though an increase in the initial rate of change of floc size could be seen. Addition of calcium greater than 4 meq/L resulted in a dramatic increase in floc size. Results from this investigation support the theory that cations are involved in flocculation through cationic bridging, and will be used in ongoing investigations to model the flocculation process.

Keywords Activated sludge; calcium; cationic bridging; flocculation

Introduction
The aim of this paper is to quantify the effect of calcium ions on the dynamics of activated sludge flocculation. This will be achieved with a unique experimental method, which allows on-line analysis of the size of activated sludge flocs during flocculation and provides valuable insight into the mechanisms of flocculation. This is important for modelling activated sludge flocculation and ultimately improving the wastewater treatment process.

Cations have been shown to have a significant effect on the bulk properties of activated sludge. Novak and co-workers (Higgins and Novak, 1997a; Higgins and Novak, 1997b, and Novak et al., 1998), showed that for both lab-scale and full-scale wastewater treatment systems, sludge settling and dewatering properties could be improved by the addition of cations to the influent wastewater. In each case, settling properties were improved with the addition of calcium or magnesium. Batch addition of cations to activated sludge also showed improvement in the sludge settling characteristics (Higgins and Novak, 1997a).

Activated sludge can be considered as a medium for ion exchange. The replacement of divalent ions in the sludge with monovalent ions leads to weaker polymer bonds and therefore poor sludge characteristics (Novak et al., 1998). This was also demonstrated by Higgins and Novak (1997b) where the addition of sodium deteriorated sludge characteristics.

Zita and Hermansson (1994) described the role of cations in floc formation in terms of the Derjaugin, Landau, Verwey and Overbeek (DLVO) theory. Using this theory, the presence of cations reduces the separation distance between negatively charged bacteria promoting flocculation. They found that both monovalent and divalent cations had the same capacity to enhance flocculation and no ion exchange was observed. Contradictory to this model, Higgins and Novak (1997a) proposed that cations were involved in flocculation through ionic bridging. In this case, the negatively charged sites of extracellular polymers are bridged by cations. Extracellular polymers are the third main component of activated sludge flocs (Li and Ganczarczyk, 1990). Higgins and Novak (1997a) also maintained that according to the DLVO theory, settling and dewatering should be improved with sodium at any concentration. This was experimentally shown not to be the case. The cationic bridging model was also proposed by Kakii et al. (1985) and Eriksson and Alm (1991) who found the
removal of calcium from sludge with a chelating agent (ethylenediaminetetraacetic acid (EDTA)), resulted in reduced settling and dewatering characteristics.

Therefore, significant work by Novak and co-workers has been directed at investigating the effect of cations on bulk sludge characteristics such as settling (Novak et al., 1998) and dewatering (Higgins and Novak, 1997a, b). Recent work by Cousin and Ganczarczyk (1999) has investigated the effect of calcium on specific floc properties such as size and density. It was found that the addition of calcium (> 4 meq/L) resulted in an increase in floc size and a decrease in porosity. A minimum addition of 4 meq/L was thought to be necessary to overcome the ion exchange between competing ions, such as sodium. The increase in floc size was then speculated to be due to the formation of calcium bridges among microbial aggregates and particles.

Whilst the effect of cations has been investigated for bulk settling properties, the effect of cations on activated sludge flocculation and floc properties is still not fully understood. This is largely due to an inability to directly measure the flocculation mechanisms. A customised technique has been developed (Biggs and Lant, 2000) to monitor activated sludge flocculation. This technique is based on a method developed by Spicer et al. (1998) for inorganic particles.

On-line analysis of floc size was measured during the flocculation of activated sludge (Biggs and Lant, 2000). The flocculation dynamics were described in terms of two key mechanisms, aggregation and breakage. Initially, aggregation was controlling, resulting in a rapid increase in floc size. Breakage became more dominant as the floc size increased. Eventually, equilibrium was reached between aggregation and breakage and a steady-state floc size was maintained.

The technique developed by Biggs and Lant (2000) was used to directly determine the effect of calcium on the dynamics of activated sludge flocculation. Specific results from this technique, such as the effect of cations on the steady state floc size will then be used in on going research to model flocculation.

**Materials and method**

**Wastewater collection**

For all experiments, activated sludge was collected from a full-scale continuous biological nutrient removal (BNR) wastewater treatment plant at Wacol, Brisbane, Australia. Grab samples of effluent were also collected. After collection, the samples were returned to the laboratory (within 1 hour) and stored at 4°C. All samples were kept for a maximum of four days.

**Cation analysis**

Calcium used in this investigation was added as chloride salts. Analysis of cations, Ca$^{2+}$, Na$^+$ and Mg$^{2+}$ (in meq/L) was carried out on a Varian SpectraAA – 30 Atomic Absorption Spectrometer.

**Floc size analysis**

A Malvern Mastersizer/E instrument was used to measure the floc size. This instrument is a light scattering instrument that operates on the principle of Fraunhofer Diffraction Theory. Dilute samples of activated sludge were introduced into the path of a 2 mW He–Ne laser. Scattered light from the diluted sample is detected on the custom designed detector. The size distribution is based on volume and the average size is quoted as the mass mean based on volume equivalent diameter.

**Flocculation technique**

The flocculation technique is based on a technique developed by Spicer et al. (1998). The flocculation technique is described in this section. A more detailed development of the technique can be found in Biggs and Lant (2000).
Disruption of the activated sludge flocs was achieved with a Branson 450 sonifier. The purpose of sonication is to disrupt the sludge into consistently smaller flocs with minimum cell lysis. The re-flocculation of activated sludge may then be observed in a controlled environment. Minimum cell lysis is required to conserve the sludge samples as much as possible in order to study the representative flocculation mechanisms.

Li and Ganczarczyk (1990) and Snidaro et al. (1997) found that the structure of flocs consisted of three levels. The first is made up of bacteria tightly bound together by a gel matrix to form the second level of structure called microcolonies. These microcolonies were found to have a median diameter of approximately 13 \( \mu \text{m} \) and are further linked by polymers to make up the final activated sludge flocs. Even though the primary particles of activated sludge flocs are bacteria, Snidaro et al. (1997) found that it would be difficult to totally disrupt the sludge without causing significant cell lysis.

Therefore, the sonication protocol for the flocculation experiments involved placing 100 mL of activated sludge in an iced water bath and sonicating for 3 minutes at an output level of 50 W. This was found to produce consistently small flocs with a median diameter of approximately 15 \( \mu \text{m} \) (mass mean diameter of approximately 30 ± 5.7 \( \mu \text{m} \)) without causing significant cell lysis (Biggs and Lant, 2000).

After sonication, 60 mL of activated sludge was added with 1.135 L of filtered effluent (0.45 \( \mu \text{m} \) Millipore filters) to a 1.2 L baffled batch reactor and mixed with a flat six blade impeller. Unless otherwise stated the experiments were mixed at 100 rpm. This was the minimum shear needed to keep the solution in suspension. The dimensions of the baffled tank and impeller were designed according to the standard tank configuration (Holland and Chapman, 1966). The ratio of sonicated sludge to effluent was chosen for optimal sizing conditions in the Malvern Mastersizer/E. The activated sludge suspension was then continuously recycled from the stirred batch reactor through the sample cell of the Malvern Mastersizer/E with a peristaltic pump (Masterflex). The pump flowrate was set at 3 mL/s. This flowrate was observed to cause minimum shear on the flocs whilst still providing a representative sample of sludge to the Malvern Mastersizer/E (Biggs and Lant, 2000). Figure 1 is a photo of the experimental setup.

![Figure 1 Experimental setup](https://iwaponline.com/wst/article-pdf/43/11/75/428927/75.pdf)
Results and discussion

Two separate experiments were conducted to investigate the effect of calcium concentration on the dynamics of activated sludge flocculation. Experiments were conducted on the day of collection. Prior to the addition of sonicated sludge to the baffled batch reactor, different concentrations of calcium (as CaCl₂) were added to the effluent solution and mixed at 100rpm. Samples of effluent with and without calcium addition were analysed before the flocculation experiments. After flocculation, sludge samples were filtered (0.45 µm Millipore filters) and measured for cation concentration.

The first experiment investigated the calcium concentrations of 0, 2, 4, 8 and 16 meq/L. The second experiment investigated calcium concentrations of 0, 8, 16 and 32 meq/L and was performed one week after the first experiment. The cation concentration is presented in meq/L for ease of comparison between other studies performed in the literature. To demonstrate the effect of calcium on the flocculation dynamics, the results from both experiments are shown in Figure 2 and Figure 3 as the increase in mass mean size with time for different calcium concentrations.

The experimental technique provides valuable information about the dynamics of activated sludge flocculation that has not been investigated before. From Figure 2 and Figure 3 it can be seen that in each case, the floc size increases rapidly until a steady state floc size is reached. Initially the rapid increase in floc size is controlled by the rate of aggregation. As the floc size increases the rate of breakage becomes more dominant. Eventually,

![Figure 2](https://iwaponline.com/wst/article-pdf/43/11/75/428927/75.pdf)

**Figure 2** Change in floc size with addition of different calcium concentrations (Experiment 1)

![Figure 3](https://iwaponline.com/wst/article-pdf/43/11/75/428927/75.pdf)

**Figure 3** Change in floc size with addition of different calcium concentrations (Experiment 2)
equilibrium is reached between the rate of aggregation and breakage resulting in the observed steady state floc size.

Whilst the initial floc sizes for both Experiment 1 and Experiment 2 at 0 meq/L of calcium are not significantly different, this is not the case for the final steady state floc size. The final steady state floc sizes for Experiment 1 and Experiment 2 are 136 ± 6 µm and 111 ± 6 µm respectively. Since activated sludge is a heterogeneous biological system it is not surprising that a small difference occurs between sludge collected from a full-scale wastewater treatment plant on different days. Despite this small difference the flocculation dynamics are the same.

Several interesting features can be observed for the change in floc size with calcium concentration for Experiment 1 as seen in Figure 2. Firstly, compared to the case of 0 meq/L calcium added, the addition of calcium did not significantly increase the final floc size until 8 meq/L of calcium was added. After this point a dramatic increase in final floc size was observed. Secondly, for the case where 16 meq/L of calcium was added, the change in floc size followed the profile of the 8 meq/L experiment and then dropped off at the end of the experiment. For Experiment 2, the final floc size increased with calcium concentration until 32 meq/L was added. The change in floc size for this experiment followed the profile of the case where 16 meq/L of calcium was added.

Visual observations showed that at the higher concentrations of calcium (that is >16 meq/L), large flocs were formed and sludge had settled on the bottom of the stirred batch reactor at the mixing speed (100 rpm). Due to the position of the sample port of the reactor and the settling of the suspension, it is conceivable that the large flocs were not recycled through the Malvern Mastersizer/E for sizing during the flocculation experiment. This could explain the decrease in final floc size for the highest calcium concentrations (for example, the addition of 32 meq/L of calcium as shown in Figure 3).

Mixing is known to have a significant effect on the flocculation dynamics of activated sludge and was therefore chosen at a speed to minimise shear effects on the sludge, whilst keeping the solution in suspension (Biggs and Lant, 2000). Therefore, despite the settling of sludge, mixing speed was not increased, as this would have made it difficult to decouple the effect of calcium and shear on the flocculation dynamics. Settling in the mixing vessel was not observed at the lower concentrations of calcium. However, at the higher calcium concentrations, the characteristics of the sludge have changed such that the mixing speed of 100 rpm is no longer suitable. This confirms the findings of Higgins and Novak (1997a) that the settling velocity of the sludge increased with calcium addition.

Novak et al. (1998) investigated the uptake of calcium during batch tests and observed that activated sludge was a medium for ion exchange. This was also observed for Experiments 1 and 2. The uptake was measured as the difference in calcium concentration before and after flocculation. Table 1 shows the uptake of calcium for each flocculation experiment. Sodium and magnesium ions were also measured before and after the flocculation experiments. These results are also presented in Table 1.

For Experiment 1 a significant uptake of calcium was observed for the case where 16 meq/L of calcium was added compared to the other experiments. However, even though a greater uptake in calcium was observed the change in floc size over time closely followed the case where 8 meq/L of calcium was added. Settling of sludge was also observed for the case where 16 meq/L of calcium was added. This indicates that the density of the flocs had increased even though there were no visible signs of increase in final floc size compared to the addition of 8 meq/L of calcium. Higgins and Novak (1997a) and Cousin and Ganczarczyk (1999) also found an increase in floc density and decrease in floc porosity with the addition of polyvalent cations. A similar result was also observed for Experiment 2 where 32 meq/L of calcium was added.
The concentration of magnesium and sodium ions in the solution after flocculation was generally greater than the initial concentration for each experiment, which is represented as a positive release of magnesium and sodium ions in Table 1. This indicates that an exchange of ions was occurring during the flocculation process resulting in a release of sodium and, to a lesser extent magnesium, with an uptake of calcium ions. This confirms that sludge is a medium for ion exchange and that cations are used in the formation of activated sludge flocs as proposed by Higgins and Novak (1997a).

From Figure 2, it can be seen that even though the final floc size is not statistically different for calcium additions of 0, 2 and 4 meq/L, the initial rate of increase in floc size increases with calcium concentration. This rate of increase in floc size is the net effect of both the rates of aggregation and breakage and is calculated as the rate of change in floc size for the first five minutes. The errors associated with the initial rate are calculated using linear regression analysis. The initial rate of increase in floc size, as a function of calcium concentration, for both experiments is shown in Figure 4.

The initial rate of change of floc size increases initially with the addition of calcium concentration and then approaches a steady state rate at the higher concentrations. This suggests that at higher calcium concentrations, saturation of the floc has occurred, and the rate of change of floc size is independent of calcium concentration. It is also apparent that for low calcium concentrations, the rate of aggregation is more dependent on the calcium concentration.

### Table 1  Ion concentration changes in solution during experiments

<table>
<thead>
<tr>
<th>Calcium concentration added (meq/L)</th>
<th>Change in Ca$^{2+}$ concentration (meq/L)</th>
<th>Change in Mg$^{2+}$ concentration (meq/L)</th>
<th>Change in Na$^{+}$ concentration (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>$-0.2 \pm 0.1$</td>
<td>$0.008 \pm 0.04$</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>$-0.4 \pm 0.2$</td>
<td>$0.02 \pm 0.04$</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>$-0.5 \pm 0.4$</td>
<td>$0.03 \pm 0.04$</td>
<td>$0.04 \pm 0.2$</td>
</tr>
<tr>
<td>16</td>
<td>$-2.0 \pm 0.7$</td>
<td>0</td>
<td>$0.09 \pm 0.2$</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>$-0.8 \pm 0.3$</td>
<td>$0.05 \pm 0.03$</td>
<td>$0.22 \pm 0.1$</td>
</tr>
<tr>
<td>16</td>
<td>$-0.55 \pm 0.6$</td>
<td>$0.06 \pm 0.03$</td>
<td>$0.70 \pm 0.1$</td>
</tr>
<tr>
<td>32</td>
<td>$-2.75 \pm 0.9$</td>
<td>$0.11 \pm 0.04$</td>
<td>$2.2 \pm 0.2$</td>
</tr>
</tbody>
</table>

![Figure 4](https://iwaponline.com/wst/article-pdf/43/11/75/428927/75.pdf)  
**Figure 4**  Effect of calcium concentration on initial rate of change of floc size
concentration than the rate of breakage. This is shown by the increase in the initial rate of change of floc size since it is the net effect of both aggregation and breakage.

The results from this investigation support the model that cations are involved in flocculation through cationic bridging. Uptake of calcium and release of sodium was observed, confirming the occurrence of ion exchange. Further experiments need to be conducted however, to investigate the effect of other cations such as sodium and magnesium on activated sludge flocculation to further support the model proposed by Higgins and Novak (1997a).

Conclusions
This paper has presented an experimental technique developed to directly monitor activated sludge flocculation and to quantify the effect of calcium on the floc size. The technique enabled the dynamics of flocculation to be observed and quantified. Initially the floc size increased rapidly until a steady state floc size was reached. The steady state floc size is due to the dynamic equilibrium between the rates of aggregation and breakage.

For concentrations less than 8 meq/L of calcium, no significant increase in floc size was observed even though an increase in the initial rate of change of floc size was seen. Addition of calcium greater than 8 meq/L resulted in a dramatic increase in floc size. The floc density also increased as indicated by the observed settling of activated sludge in the batch reactor. Ion exchange was observed with the uptake of calcium and the release of sodium and to a lesser extent magnesium. The results from this investigation support the role of cations in flocculation as cationic bridging.

Using this experimental technique has provided valuable quantitative information about the effect of calcium on activated sludge flocculation and will be used as part of ongoing research to model the flocculation dynamics.

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References