Key parameters in sludge dewatering: testing for the shear sensitivity and EPS content

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Abstract The fraction of extractable extracellular polymeric substances (EPS) and the shear sensitivity ($k_{SS}$) are key parameters with respect to sludge dewatering, affecting the dry matter content of dewatered sludge and the dewatering rate and conditioner demand, respectively. Methods are described for determination of the two key parameters by use of the same laboratory test reactor. The implications of such characterisation with respect to dewatering are discussed based on examples of application to sludge processing and novel process development for sludge minimisation.

Keywords Adhesion energy; dewatering; EPS content; floc strength; shear sensitivity

Introduction

In current wastewater treatment operation, sludge dewatering and disposal are very costly topics, contributing significantly to the operation cost of the entire treatment process. In recent years, these costs related to sludge management have continued to increase, mainly due to growing concerns regarding feasibility of sludge reuse due to potential risks to human health. Current emphasis on improvement of modern wastewater operation is therefore to a great extent concerned with the potential for process optimisation or introduction of novel processes with respect to sludge minimisation.

For such development, suitable tools for sludge characterisation with respect to dewatering are necessary. Not only must novel sludge treatment technologies be evaluated by their effect on sludge dewatering and sludge quality for reuse; traditional treatment plant operation may also be developed further, bearing in mind the potential for improvement of sludge properties and therefore reduction of total operation costs.

A missing link to such process improvement has been fundamental sludge characteristics for determining impact on sludge dewatering. Some of this uncertainty may have been clarified, as it appears that sludge dewatering properties may be characterised to a large extent by only two key parameters for dewatering: the shear sensitivity ($k_{SS}$) and the content of extractable extracellular polymeric substances (EPS), even when considering different types of sludge as primary, activated or anaerobically digested sludge (Mikkelsen and Keiding, 2002a). The dry matter content of dewatered sludge was shown to be related to EPS content in an inverse manner, i.e. a high EPS content of sludge was related to low cake dry matter content. This is assumed to be due to the osmotic pressure of counter ions for EPS charge neutralisation as described by Keiding et al. (2001). The shear sensitivity on the other hand, related to the colloid content (CC) of sludge, has a detrimental effect on the dewatering rate (Mikkelsen and Keiding, 2002a). In practice, high dewatering rates must be retained, and high CC is therefore expected to cause increased demand for conditioning polymers, as demonstrated also by Mikkelsen and Keiding (2001a). It is important to note, that the colloid content with relevance to dewatering should be measured at the point of sludge conditioning, since erosion of sludge colloids may occur in pipes etc. As the systems are sealed at this point, this is usually not possible. Instead, the shear sensitivity parameter...
k_{SS} can be used, being a descriptor of the colloid fraction of sludge when exposed to erosion in a high shear hydraulic environment (Mikkelsen et al., 2001).

It is therefore desirable to improve sludge properties for dewatering by optimisation of both wastewater treatment processes and novel sludge treatment processes with respect to the content of EPS and colloids (shear sensitivity). This requires tools for monitoring of these parameters in full scale treatment operation and in process development. This paper provides a methodology for sludge characterisation with respect to these two key parameters. A discussion is given of the implications of such characterisation based on a fundamental understanding of sludge structure, and examples of application are given.

**Sludge floc structure**

A sludge floc is considered to be a collection of particles (e.g. bacteria) embedded in a matrix of extracellular polymeric substances (EPS). The importance of EPS for the integrity of such aggregates has been demonstrated in many studies. The EPS is considered to make a tangled network of cross-linked polymer – a gel – in which cells and other particles may be entrapped. Also, particles may be attached to the floc surface by chemical or physical bonds, rendering a fraction of the sludge particles vulnerable to erosion when exposed to hydraulic forces. For high shear rates, cells and other colloidal particles are eroded from the floc surface (e.g. Parker et al., 1970), while colloid adhesion – or reflocculation – occurs during quiescent shear conditions (e.g. Wahlberg et al., 1994). The presence of a balance between erosion and adhesion of colloids is consistent with observations that the particle size distribution of activated sludge is bimodal, containing large flocs and smaller sized single dispersed cells (e.g. Li and Ganczarczyk, 1991; Parker et al., 1970). The fraction of colloids is assumed to depend on the bond energy of the colloids adhered to the surface, i.e. strong bonds correspond to few colloids (e.g. Mikkelsen and Nielsen, 2001).

**Colloid content**

The adhesion-erosion model (AE-model) quantifies the colloid content due to erosion in turbulent shear flow (Mikkelsen and Keiding, 1999). The model incorporates the adhesion enthalpy, which is equivalent to the bond energy of reversibly attached colloids. This bond energy is the sum of DLVO forces, in which van der Waal’s forces and electrostatic forces affect inter-particle attraction and non-DLVO forces as hydrophobic interaction, steric forces, and polymer entanglement – forces which have been found important also by e.g. Hermansson (1999), Gregory (1989) and Unz (1987).

The erosion of colloids increases with solids content, an effect related to the crowding of flocs at high concentrations, and thereby related to sludge rheology (Mikkelsen, 2001). For a given solids content, the effect of turbulent shear is given by Mikkelsen and Keiding (1999):

\[
m_{d,\infty} = e^{\frac{\Delta H_G}{G}} - e^{q_m}
\]

where \(m_{d,\infty}\) is the dispersed (colloid) mass concentration at equilibrium at shear rate \(G\), \(\Delta H_G\) is the adhesion “enthalpy”, which is an expression of the bond energy, \(G\) is the root-mean-square velocity gradient as described by Camp and Stein (1943) for characterisation of turbulent shear, and \(q_m\) is a constant for a given solids concentration. The value of \(\Delta H_G\) determines the domain of shear rates at which erosion dominates, while \(q_m\) quantifies the extent of erosion and relates to the dispersible fraction (DF) of a sludge (Figure 1). From Eq. (1) follows for DF as the shear rate approaches infinity:

\[
DF = \frac{m_{d,\infty}}{m_T} = \frac{e^{q_m}}{m_T}
\]
The shear sensitivity \( k_{SS} \) was defined by Mikkelsen (1999) and Mikkelsen and Keiding (2002b) based on a standardised shear test experiment with \( G = 800 \text{ s}^{-1} \) and solids content in the range 3.5–4.0 g SS/l:

\[
k_{SS} = \frac{m_{f,\infty}}{m_T}
\]

The shear sensitivity reflects the colloid fraction under the standard test conditions and may thus serve as an indicator of changes in bond energy of colloids to floc surfaces – for example when exposing a given sludge to changes in chemical or biological conditions. From Figure 1 follows, that \( k_{SS} \) (measured at \( G = 800 \text{ s}^{-1} \)) will increase with increasing DF and with decreasing \( \Delta H_G \).

**EPS effect on cake dry matter**

The floc EPS is considered to exist in a cross-linked matrix, giving some gel-like properties to the sludge. In other words, the EPS is considered to have a “water-holding capacity”, a term introduced to cover both osmotic water as well as water physically trapped within the polymer network (Mikkelsen and Keiding, 2002a). A relation was found in the same study between EPS and the cake dry matter content (CDM) of the dewatered sludge cake after filtration in terms of a model:

\[
CDM = \frac{100\%}{1 + k \cdot C_{EPS} + q}
\]

Where \( C_{EPS} \) is an EPS factor, \( k \) is a water holding constant for the EPS, and \( q \) is an EPS-independent constant water content [g/g SS]. Total EPS content, EPS protein content and EPS charge density were all correlated. Thus, any of these three parameters may be used in Eq. (4); the choice would merely be reflected in the value of \( k \). Assuming osmosis to be the main source of water retention as argued by Keiding et al. (2001), EPS charge density may be theoretically preferable. However, such an assumption is not necessary for the use of Eq. (4) when only being concerned with predictions of cake dry matter of dewatered sludge.

**Materials and methods**

**Shear reactor**

The extraction of EPS and erosion of colloids are both sensitive to the applied shear rate, which must therefore be controlled and known. A 110-mm PVC reactor with four vertical 11-mm baffles (Appendix) was used with a 12-mm by 50-mm single bladed paddle placed...
in the reactor centre, approximately 3–4 cm above the bottom and controlled by a Heidolph electronic mixer for precise mixing speed control.

The shear rate of a fully turbulent reactor may be estimated by the expression:

\[
G = \sqrt{\frac{P}{\mu V}}
\]  
(5)

where \( P \) is the added power [W] in a sample volume \( V \) [m\(^3\)], and \( \mu \) is the liquid viscosity [kg/m\( \cdot \)s].

For the standard reaction chamber and paddle, \( P \) may be calculated by \( P = k \cdot \text{rpm}^3 \), where rpm is the mixer speed [min\(^{-1}\)] and \( k = 8.87 \times 10^{-10} \text{ W} \cdot \text{min}^3 \). This gives Table 1 for the standard test.

### Shear sensitivity

1,000 ml sludge samples were sheared in the standard reactor in an iced water bath (ca. 4°C) with a shear rate of \( G = 800 \text{ s}^{-1} \), and solids content ca. 3.5–4.0 g SS/l, as suggested by Mikkelsen (1999, 2001). The erosion of colloids was characterised from supernatant turbidity versus shearing time, measured as absorbance (650 nm) of supernatant after 2 min. centrifugation at 2,200 rpm. The absorbance was converted to Formazine Turbidity Units (FTU) by a standard curve. CC was estimated by use of the turbidity to mass concentration conversion factor of 1.2 mg SS/l/FTU reported by Wahlberg et al. (1994). The colloid content at equilibrium for a given shear test, was estimated by fitting to the expression used by Mikkelsen and Keiding (1999):

\[
m_{d,t} = m_{d,\infty} + (m_{d,0} - m_{d,\infty}) \cdot \frac{6}{\pi^2} \sum_{N=1}^{9} \frac{1}{N^2} e^{-N^2Dt}
\]  
(6)

where \( m_{d,t} \) is the colloid mass concentration at time \( t \), \( m_{d,0} \) and \( m_{d,\infty} \) are colloid mass concentration initially and at equilibrium, respectively, \( N \) is an integer and \( D \) is an effective diffusion constant. This fitting procedure gives estimates of \( D \), \( m_{d,0} \) and \( m_{d,\infty} \). The shear sensitivity was subsequently estimated by Eq. (4).

### Extended shear testing

For studying underlying phenomena of colloid erosion, some sludges were exposed to the same shear test procedure with controlled turbulent shear rate in the range \( G = 125–1,700 \text{ s}^{-1} \). Equilibrium colloid mass concentrations were estimated as described above. The adhesion enthalpy \( \Delta H_G \) and the constant \( q_m \) for given \( m_T \) were estimated from Eq. (1) by linear regression of \( \ln m_{d,\infty} \) versus \( 1/G \) plots for a series of shear tests with varying \( G \). With these estimates of \( \Delta H_G \) and \( q_m \), the dispersible sludge mass was estimated from Eq. (2).

### EPS fraction

EPS was extracted from sludge by the combined action of a cation exchange resin (CER) and high turbulent shear rate as described by Frølund et al. (1996). A 300 ml sludge sample

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**Table 1** The turbulent shear rate (G) for different stirring speeds (rpm) using the standard reactor and paddle from the Appendix. Expressions derived from Eq. (5) are \( G = 0.0284 \text{rpm}^{3/2} \) for ca. 4–8°C and \( G = 0.0349 \text{rpm}^{3/2} \) for ca. 20°C (taking the change of viscosity with temperature into account).

<table>
<thead>
<tr>
<th>Shear rate [s(^{-1})]</th>
<th>125</th>
<th>200</th>
<th>350</th>
<th>500</th>
<th>800</th>
<th>1,100</th>
<th>1,400</th>
<th>1,700</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V = 1,000 \text{ ml} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td>269</td>
<td>367</td>
<td>533</td>
<td>677</td>
<td>926</td>
<td>1,145</td>
<td>1,344</td>
<td>1,530</td>
</tr>
<tr>
<td>20°C</td>
<td>234</td>
<td>320</td>
<td>465</td>
<td>590</td>
<td>807</td>
<td>998</td>
<td>1,172</td>
<td>1,334</td>
</tr>
<tr>
<td>( V = 600 \text{ ml} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td>227</td>
<td>310</td>
<td>450</td>
<td>571</td>
<td>781</td>
<td>966</td>
<td>1,134</td>
<td>1,291</td>
</tr>
<tr>
<td>20°C</td>
<td>197</td>
<td>270</td>
<td>392</td>
<td>498</td>
<td>681</td>
<td>842</td>
<td>988</td>
<td>1,125</td>
</tr>
</tbody>
</table>
with a suspended solids content of 8 gSS/l was obtained by thickening or dilution with tap water, depending on the solids content. This sample was centrifuged immediately at 2,000 g for 15 minutes at 4°C. The pellet was resuspended to 300 ml in phosphate buffer (2 mM Na₃PO₄, 4 mM NaH₂PO₄, 9 mM NaCl, 1 mM KCl at pH 7) to minimise cell lysis during extraction. The sample was then placed in the shear reactor in an iced water bath, and 180 g washed Dowex cation exchange resin (Silhorko HCR-S, sodium-charged) was added, corresponding to an excess CER dose (above approximately 75 g/gVSS). The sample was stirred with the single bladed paddle at 900 rpm (G = 1,400 s⁻¹) for two hours. Solids were subsequently removed by 3 times 15 minutes centrifugation with 12,000g at 4°C. In between the centrifugation steps, supernatant was poured into a clean centrifugation tube. The supernatant containing the extracted EPS was analysed for protein and humics by the modified Lowry method described by Frølund et al. (1995) with BSA and Humic acid (Jansen Chemica) as respective standards, and for polysaccharides by the anthrone method modified by Raunkjær et al. (1994) with glucose as standard. The EPS fraction was estimated as the total mass of these components relative to the sludge suspended solids (SS) and in some cases to the volatile suspended solids (VSS).

Using this extraction procedure, a (small) fraction of EPS will remain unextracted. This could e.g. be a hydrophobic fraction. To increase the extraction efficiency to include these polymers would likely cause cell lysis, which would give misleading EPS extraction results. From a dewatering perspective, the osmotic effect of hydrophobic polymers is likely to be small due to relatively low charge densities. On the other hand, use of CER is necessary for extraction of the charged polymer fractions contributing to the osmotic properties of the gel. This extraction methods thus gives an intermediate result that seems meaningful in relation to sludge properties, as indicated also by Eq. (4).

Sludge samples
Activated sludge and anaerobically digested sludge was collected from full scale WWTPs treating combined domestic and industrial wastewater (Mikkelsen and Keiding, 2002a). For laboratory scale studies, activated sludge from Aalborg East WWTP (sludge age approximately 30 days) was used. The sludge was fresh or stored overnight at 4°C.

Results and discussion
In WWTP operation, some processes affect floc EPS content, some affect the shear sensitivity, and some affect both. Ideally, processes should be optimised in a manner by which EPS is reduced while maintaining low shear sensitivity (and thus low colloid content). Typically, EPS changes occur in long term plant operation, whereas the colloid content and shear sensitivity may vary in both long- and short terms. Therefore, we may expect sludge EPS content and properties to be determined mainly by process design, while colloid content may vary with factors such as sludge SS, shear rates occurring in various parts of the system, and also with changes in surface chemistry by addition or removal of specific compounds or by biological chock effects. The following section gives some examples of such variation and the application of the presented sludge characteristics for studying effects of process parameters on sludge properties.

Anaerobic digestion
In current process development for sludge treatment, the trend is to aim for sludge minimisation due to the increasing costs related to sludge disposal or reuse. In Denmark, this has recently been achieved predominantly by a growing use of anaerobic digestion, and in particular more frequent use of thermophilic anaerobic digestion for increased sludge mineralisation compared to mesophilic digestion. Unfortunately, the thermophilic process usually
causes a high demand for conditioners; in at least one case severe enough for the thermophilic process to be abandoned.

When looking at the sludge key characteristics for different types of sludge from full-scale wastewater treatment operation (Table 2), this effect may be understood from the large shear sensitivity and colloid content observed for the thermophilic sludge. This is somewhat expected, since EPS degradation is enhanced in thermophilic processing (Table 2). Because EPS generally is observed to improve floc stability – often considered as “floc glue” – due to the creation of particle-embedding polymer-gel networks, it is easily conceivable that EPS reduction causes increase in shear sensitivity. The dispersible fraction (DF) for the relatively EPS-rich activated sludge is usually in the region 2–10% (Mikkelsen and Nielsen, 2001). The higher shear sensitivity for the anaerobic sludge indicates that DF is higher for anaerobic sludge. Also, the actual colloid content is relatively high for anaerobic sludge, indicating that erosion occurs at low shear rates. This would correspond to weak adhesion bonds, i.e. low adhesion enthalpy $\Delta H_G$. Although not directly comparable to continuously fed systems, we get from Figure 2 and Table 3 an indication that anaerobic processes do tend to increase the dispersible fraction and reduce the bond energy with increasing effect with higher temperatures (Table 3). These changes are the reason behind the change in shear sensitivity (Table 3) and may be due to EPS degradation, changes in specific bacterial types, etc.

Lowering the EPS fraction not only reduces total sludge mass for disposal, but simultaneously permits higher cake dry mater content to be achieved due to reduced water retention (e.g. Mikkelsen and Keiding, 2002a) and is therefore desirable for sludge processing. The simultaneous increase in shear sensitivity is however an unfortunate side-effect. It seems we cannot have it all – but we can try! The latter suggestion originates from the observation of data presented in Table 4. It appears that the high shear sensitivity of thermophilic sludge is not solely determined by high EPS turnover. It was demonstrated by

<table>
<thead>
<tr>
<th>Activated (8 samples)</th>
<th>Mesophilic anaerobically digested (3 samples)</th>
<th>Thermophilic anaerobically digested (5 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractable EPS [mg/g SS]</td>
<td>130 (65)</td>
<td>78 (49)</td>
</tr>
<tr>
<td>Colloid content (CC) [%]</td>
<td>0.40 (0.40)</td>
<td>8.6 (3.6)</td>
</tr>
<tr>
<td>Shear sensitivity ($k_{SS}$) [%]</td>
<td>6.2 (4.9)</td>
<td>24 (1.6)</td>
</tr>
</tbody>
</table>

**Table 2** Average and standard deviation () of extracted EPS content, actual sludge colloid content, and shear sensitivity of different types of sludge samples from full scale operation. From Mikkelsen and Keiding (2000a)

Figure 2 Equilibrium colloid content versus turbulent shear rate (G) for untreated activated sludge ( ), and after batch storage for 11–15 days at 20°C ( ), 37°C ( ) and 53°C ( )

Figure 3 Turbidity versus time data for shear tests of sodiumhypochlorite modified activated sludge. Doses [g NaOCl/kg SS]: 0.0 ( ), 5.0 ( ), 15.0 ( ), 30 ( )
continuously fed laboratory scale anaerobic digestion, that introducing a pre-treatment of thermal hydrolysis gives similar EPS degradation by both mesophilic and thermophilic digestion. However, the shear sensitivity of mesophilic sludge remains lower than for the thermophilic sludge, indicating a tendency for thermophilic processes to cause higher dispersion – possibly due to specific thermophilic bacterial populations. In other words: there seems to be a possibility of having the best of both worlds. High EPS degradation (for low water retention in dewatering, and thus achievement of high dry matter cake) with a simultaneous reasonably low colloid content and shear sensitivity (for high dewatering rate and low conditioner demand). One way of achieving this would be to use a two-step process, in which the first step ensures hydrolysis, while the actual organic matter turnover is performed in the second step. Such operations already exist today, but so far this has not been based on fundamental understanding of the effect on sludge dewatering characteristics. It would therefore be valuable to introduce the methods for EPS- and colloid content and shear sensitivity to such systems in order to demonstrate their mode of function.

Excess sludge storage

For practicality in plant operation, sludge dewatering is often performed during weekdays only. However, it is often encountered, that sludge dewatering properties are poorer on a Monday (e.g. Rasmussen et al., 1994). This could be related to processes occurring unintentionally during the storage. Sometimes, some extent of surface mixing or aeration exists in order to maintain the sludge aerobic to aid this problem. However, sometimes this mixing is not sufficient for ensuring aerobic conditions.

Table 5 shows the combined effect of shearing and oxygen conditions on shear sensitivity of activated sludge stored for 14 days. During anaerobic storage a certain deflocculation always takes place due to lack of aerobic microbial activity and due to anaerobic processes such as microbial Fe(III) reduction and sulphide formation (Wilén et al., 2000a; Mikkelsen et al., 2001). Sulphide in particular has been demonstrated to affect the erosion of sludge colloids, i.e. the dispersible fraction is increased with sulphide present, probably due to reduction in chemical cross-linking of gel-networks, as ferric iron is reduced to ferrous iron bound mainly as iron sulphide (Nielsen and Keiding, 1998; Mikkelsen and Nielsen, 2001). Shear sensitivity determination in this case reveals an important practical result: mixing for maintaining aerobic conditions helps to maintain low shear sensitivity during storage, as
The presence of oxygen is more important than the shear rate. Therefore, we expect insufficient mixing to be worse than no mixing at all! Furthermore, it is important to note that activated sludge cannot be completely reflocculated again once it has been deflocculated by anaerobic conditions (Wilén et al., 2000b).

**Chlorination for filament reduction**

Chlorination is an example of a process introduced in aeration basin process control (in order to reduce the amount of filamentous bacteria and their effect on sludge settling). Chlorination improves settling due to its toxicity to most filaments. However, some toxic effect may occur also to floc forming bacteria and thus cause floc disintegration. If such an effect were to take place, this could be interpreted in terms of increased shear sensitivity, as more cells are eroded — and the effect may be even more severe when exposed to higher shear rates as in the dewatering line. An undesired side-effect of chlorination could thus be higher colloid content and therefore higher conditioner demand during dewatering. A way of testing this is by laboratory addition of chlorine to sludge for subsequent shear sensitivity testing.

Figure 3 shows results of such a study. It is seen that more colloids are eroded in the shear sensitivity test for the chlorinated sludge. The effect is not very dramatic for low doses, but increasing with higher dosing, indicating that conditioning and deterioration of dewatering may arise at higher doses. This is yet an example of application of the shear sensitivity test for laboratory evaluation of the impact of process tank operation on effluent quality and subsequent sludge dewatering properties.

**Conclusions**

It has been shown before that only a part of sewage sludge flocs is dispersible by means of erosion, i.e. only this fraction (DF) may take part in adhesion–erosion processes. The DF increases with reduction in EPS content, probably due to the floc stabilising effect of EPS. On the other hand, EPS causes sludge water retention in dewatering, and EPS reduction is therefore desirable with respect to the achievement of high solids content in dewatering.

Process development with the goal of decreasing sludge EPS content while maintaining low shear sensitivity is therefore desirable. In this paper, methodologies are provided for characterisation of these two key parameters by use of the same shear reactor. This is a practical advantage, as control of the hydraulic shear rate in the tests is of utmost importance to the results. In fact, any test of these parameters only provides meaningful information, if the shear rate is controlled — and known! Therefore, the use of the standard reactor described herein — which has been adopted also in past studies by others — is recommended.

Given the appropriate attention to the methodologies, key sludge parameters with respect to efficient dewatering are found. The shear sensitivity being an indicator of the sensitivity for colloid erosion due to weak adhesion bonds affects dewatering rate or alternatively causes increased conditioner demand. This parameter is measured at a fairly high shear rate, and is therefore likely to represent the extent of colloid erosion that may occur

**Table 5** Shear sensitivity of stored (14 days) activated sludge with different storage shear rates and oxygen conditions

<table>
<thead>
<tr>
<th>Mixing shear rate (G) [s⁻¹]</th>
<th>Oxygen conditions</th>
<th>kₜ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Anaerobic</td>
<td>4.0 (0.1)</td>
</tr>
<tr>
<td>100</td>
<td>Anaerobic</td>
<td>3.6 (0.06)</td>
</tr>
<tr>
<td>200</td>
<td>Aerobic</td>
<td>1.3 (0.06)</td>
</tr>
<tr>
<td>300</td>
<td>Aerobic</td>
<td>1.7 (0.09)</td>
</tr>
</tbody>
</table>
during sludge pumping etc. The EPS content affects sludge water retention, and thus affects the dry matter content of dewatered sludge. Monitoring of sludge in full scale or laboratory scale process development for sludge minimisation may be characterised by these two parameters.

**Acknowledgements**

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**References**


