The role of carbohydrate and protein parts of extracellular polymeric substances on the dewaterability of biological sludges

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Abstract There are many factors affecting the biological sludge dewaterability such as particle size distribution, floc structure, extracellular polymeric substances (EPS), etc. In this research, the role of the protein and carbohydrate parts of EPS (EPS_{carbohydrate} and EPS_{protein}) on the dewaterability of biological sludges was investigated. The sludge EPS composition was altered by feeding the sludges of same origin, in different reactors, with synthetic media having carbon to nitrogen (C/N) ratios of 8, 19 and 30 (in terms of COD/NH₃-N), respectively. EPS in sludge samples were extracted by a cation exchange resin (CER). The characteristics of EPS were investigated by analytical methods and by using FT-IR spectroscopy. The dewaterability of the sludges was determined in terms of filterability and compactibility. Filterability, as filterability constant (X), and compactibility, as cake solids concentration, of sludges were determined by using the capillary suction time (CST) test and the centrifugation, respectively. The floc structure of sludge samples was also observed microscopically. Filterability and compactibility of the sludge samples were improved considerably with the increasing carbohydrate part and the decreasing protein part of the sludge EPS. EPS_{protein} was inversely related to the cake solids concentration, which might be explained by the water holding capacity of EPS_{protein}. Filterability and compactibility of sludges improved by the increase of the size and strength of the flocs.

Keywords Biological sludge; compactibility; extracellular polymeric substances; filterability; sludge dewaterability

Introduction

Sludge dewaterability characteristics take an important role in the selection of the optimal dewatering method. Many characteristics are reported to influence the dewaterability of sludges such as particle size distribution, bound water content, viscosity, structure, and composition of the flocs, in relation to the extracellular polymeric substances (EPS), etc. Bioflocculation, which is highly affected by EPS, should be well understood for the determination of the physical properties of sludges such as dewaterability.

Microbial EPS, being the third major component of sludge flocs, are organic polymers produced by bacterial cells and can be expected to have an influence on sludge dewaterability through the high level of hydration of the polymer surrounding bacterial cells and its role in flocculation (Houghton et al., 2001). These substances have a very heterogeneous chemical composition mainly containing protein, carbohydrate, lipids, humic acids, and nucleic acids. Protein and carbohydrate are usually found as the major components of sludge EPS, but there is not a certain opinion on which of these components has a higher portion.

The EPS components in the different sludges show variations because of the differences in sludge treatment methods, bacterial activities, feed compositions or extraction processes. EPS components are usually determined by analytical measurement methods and the accuracy of these methods is discussed by many researchers (Frølund et al., 1996;
Durmaz and Sanin, 2001). Analytical measurement can be supported by instrumental methods, such as Fourier Transformation Infrared Spectroscopy (FT-IR), C-NMR and H-NMR. FT-IR, which is also used in this study, is commonly used for the spectrophotometric identification of organic compounds or complex molecules (Zhou et al., 2001; Bura et al., 1998).

Many researchers use specific resistance to filtration (SRF) and capillary suction time (CST) for the measurement of sludge dewaterability. However, these parameters are the indicators of filterability. Sludge filterability tests are inadequate alone to determine the grade of separation of sludge solids from the water. On the other hand, compactibility tests involve the liquid-solid separation of sludge by centrifugation, which is one of the most used dewatering processes before the disposal of sludges. Sludge compactibility can be expressed in terms of cake solids content or the volume of compacted sludge cake, only if the sludge samples have same initial solids concentration. Sludge filterability and compactibility should be considered together, in order to determine the dewaterability characteristics of sludges (Erdincler and Vesilind, 2000) and the most appropriate dewatering method (Emir, 2002).

There are many opinions about the effect of EPS composition, carbohydrate and protein parts of EPS, on the dewaterability of biological sludges. However, there is still not a precise conclusion on this topic in the literature. Although some researchers found a strong relationship between the components of EPS and dewaterability of sludges in relation to the effect of protein fraction on bioflocculation and dewaterability (Wu et al., 1982; Nielsen et al., 1996; Higgins and Novak, 1997; Bura et al., 1998; Mikkelsen and Keiding, 2002), some others did not observe a strong relationship.

This study investigates the role of protein and carbohydrate parts of extracellular polymeric substances on the dewaterability of biological sludges. The ratio of EPS_{carbohydrate} to EPS_{protein} was altered by changing the influent C/N ratio. The amounts and characteristics of the EPS components were determined by using both analytical and instrumental methods after the extraction with a cation exchange resin. The filterability of sludge samples was measured by using CST test. Compactibility was determined by centrifuging the sludge samples and measuring their cake solids concentrations.

**Methods**

In this research, a biological sludge obtained from the aeration tank of a pharmaceutical raw material industry was used as microbial seed in three sets of semi-continuously fed batch reactors having volume of four litres each. Each set had duplicate reactors operated at room temperature in the laboratory. The reactor sets were fed synthetically, with the media having C/N ratios of 8 (Reactors 1 and 2), 19 (Reactors 3 and 4) and 30 (Reactors 5 and 6), respectively to change the ratio of EPS_{carbohydrate} to EPS_{protein} in the sludge samples. The characteristics of sludges in the reactors changed depending on the C/N ratio of synthetic feed media. The mean cell residence time (MCRT) of the reactors was adjusted to 10 days. Steady state conditions were followed in each reactor by measuring the effluent COD, pH, TS, MLSS and MLVSS concentrations of the sludges according to Standard Methods (1975). Additionally, TKN, ammonia-N and pH of the feed media were followed. Polymer extraction operations and dewaterability experiments were conducted after reaching the steady state conditions. The composition of sludge EPS was altered by operating the reactor sets in different carbon to nitrogen ratios measured in terms of chemical oxygen demand to ammonia-N (COD/NH\textsubscript{3}-N) in the medium.

Extraction of EPS was carried out similarly to the description of Frølund et al. (1996) by using CER (DOWEX 50 * 8, 20–50 mesh in the sodium form) at a dosage of 75 g/g volatile suspended solids (VSS). The 300 mL of sludge samples were centrifuged at 2,000 g for 20
minutes. Supernatants were discarded and sludge pellets were resuspended to their original volume by using phosphate buffer solution (PBS), before CER was added to each sample. Samples were stirred at 150 rpm for seven hours. After the extraction, sludge samples were first settled by gravity and then the supernatant was centrifuged 3 times at 3,650 g for 10 min at 4°C, in order to separate EPS/sludge suspension from CER.

After the extraction process, the amounts of extracted \( \text{EPS}_{\text{protein}} \) and \( \text{EPS}_{\text{carbohydrate}} \) in the supernatants of the sludges were measured by using Lowry (Lowry et al., 1951) and Phenol-Sulfuric Acid (Rao and Pattabiraman, 1989) methods, respectively. The characteristics of EPS were also investigated by using FT-IR spectroscopy. After the extraction process, a precipitation-drying method by using ethanol was applied to the supernatants containing sludge EPS (Sanin and Vesilind, 1994). Dried EPS of sludge samples from Reactors 2, 3, and 6 were mixed with drier KBr with a ratio of 1:50 (EPS:KBr) and then pelletized under a pressure of 10 tonnes for 1 min (Bura et al., 1998). Fourier Transformation Infra-Red Spectroscopy (FT-IR) (Perkin Elmer, Model 1600) was used to determine the functional groups of bacterial EPS in the 4,000–400 cm\(^{-1}\) range of frequency for the sludges in Reactors 2, 3 and 6.

The floc structures of sludge samples were also observed microscopically by an epifluorescence microscope (Olympus BX-50 with Olympus 40 water immersion lenses with a \( \times10 \) eyepiece). The dewatering characteristics of the sludge samples were determined by using filterability and compactibility. Filterability was measured by CST test. The sludge filterability constant, \( X \), described by Vesilind (1988), was calculated to correct the CST measurements for the effect of solids concentration and temperature (viscosity) so that the measurements among different samples could be compared. \( X \), using a common instrument and paper, is calculated by this equation:

\[
X = [\varnothing \cdot (\mu \times C/t)]
\]  

where: \( X \): filterability constant (kg\(^2\)/m\(^4\).s\(^2\)),
\( \varnothing \): a dimensionless constant characteristic of the CST apparatus and paper used,
\( \varnothing = 0.118 \) (obtained from the manufacturer instructions),
\( \mu \): viscosity of water at the temperature of the sludge sample used in the CST test (kg/s.m) (\( \mu_{\text{water}} = 0.890 \times 10^{-3} \) kg/s.m at 25°C),
\( C \): sludge total solids (kg/m\(^3\)),
\( t \): capillary suction time (s).

The results of CST tests were used to calculate filterability constant with Eq. (1). Compactibility of sludges was measured in terms of cake solids concentration. Compaction tests were carried out by using a centrifuge instrument (Hettich Universal 16A) as described by Erdincler and Vesilind (2000). 10 mL samples were centrifuged at 2,800 g for 30 minutes. The heights of compacted sludge layer (pellet) were measured. The compactibility of sludges, in terms of cake solids concentration, was calculated as:

\[
C_k = C_0 \times \left( \frac{H_0}{H_k} \right)
\]  

where: \( C_k \): cake solids concentration of sludge sample after centrifugation (%),
\( C_0 \): suspended solids concentration of sludge sample before centrifugation (%),
\( H_0 \): initial pellet height before centrifugation (in this study, \( H_0 = 10 \) mL),
\( H_k \): final pellet height after centrifugation (mL) (Vesilind, 1978).
Results and discussions
The characteristics of sludges in the reactors changed depending on the C/N ratio of synthetic feed media (Table 1). Sludges in parallel reactors showed almost the same characteristics. High COD loading in the feed led to high COD concentrations in all of the reactors. However, COD removal rates in sludges were efficient enough. The flocculation of the sludge samples also seemed to be quite good in all of the reactors, as supported by visual and microscopic observations.

Characterization of EPS by analytical methods and FT-IR analysis
Figures 1 and 2 demonstrate the functional groups of sludge EPS in Reactors 2 and 6 obtained by FT-IR spectroscopy, between the wave numbers of 4,000–400 cm\(^{-1}\). The evaluation of FT-IR spectra supported the results obtained by the measurement of \(\text{EPS}_{\text{protein}}\) and \(\text{EPS}_{\text{carbohydrate}}\) concentrations by analytical methods. Since FT-IR spectroscopy gives qualitative data, the height ratios of carbohydrate and protein peaks were given as low, medium and high in Table 2.

The main absorption bands were almost the same for three EPS samples and the observed bands were: a broad band near 3,500 cm\(^{-1}\) (stretching of H- bonded OH), a sharp peak at 1,660 to 1,636 cm\(^{-1}\) (stretching C=O of proteins), a sharp peak near 1,380 to 1,390 cm\(^{-1}\) (NH band of proteins), and a peak at 1,100 to 1,080 cm\(^{-1}\) (C-O band of carbohydrates). The peak evaluations were made based on the literature (Silverstein et al., 1991; Theophanides, 1984). The changes in the \(\text{EPS}_{\text{carbohydrate}}\) to \(\text{EPS}_{\text{protein}}\) ratios were evaluated by the peak heights of two reference peaks in the spectra obtained from FT-IR instrument for each sample. Peak height was defined as the distance of the center point of the bottom of

### Table 1 Average characteristics of sludges after steady-state conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C/N = 8</th>
<th>C/N = 19</th>
<th>C/N = 30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactor 1</td>
<td>Reactor 2</td>
<td>Reactor 3</td>
</tr>
<tr>
<td>pH</td>
<td>6.8</td>
<td>6.6</td>
<td>7.4</td>
</tr>
<tr>
<td>TS (mg/L)</td>
<td>2,380</td>
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<td>MLSS (mg/L)</td>
<td>890</td>
<td>1,155</td>
<td>2,410</td>
</tr>
<tr>
<td>MLVSS (mg/L)</td>
<td>790</td>
<td>1,090</td>
<td>2,160</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>124</td>
<td>115</td>
<td>104</td>
</tr>
<tr>
<td>COD Removal Rate (%)</td>
<td>72</td>
<td>74</td>
<td>90</td>
</tr>
</tbody>
</table>

Figure 1 FT-IR spectrum of EPS of sludge sample taken from Reactor 2 (C/N ratio = 8)
the peak from the baseline of the spectrum. The ratio between the heights of peaks at 1,100 to 1,080 cm\(^{-1}\) (presenting C-O band of carbohydrates) and 1,380 to 1,390 cm\(^{-1}\) (presenting NH band of proteins) were compared in order to have a carbohydrate/protein ratio of EPS of sludge samples.

The FT-IR spectra of sludge EPS samples from reactors having different C/N ratios demonstrated that the carbohydrate to protein ratio was highly affected by the change in the C/N ratios of feed media. The peak height ratios increased with increasing C/N ratios. The lowest ratio between carbohydrate and protein parts of sludge EPS was observed in Reactor 2 containing the sludge with the C/N ratio of 8. On the other hand, EPS of sludge having the C/N ratio of 30, in reactor 6, had the highest carbohydrate to protein ratio among the others. Besides, EPS of sludge in Reactor 2 had more proteins than other sludge samples. On the other hand, EPS from the sludge sample of Reactor 6 had more carbohydrate than the other sludge samples.

The amounts of extracted EPS\(_{protein}\) and EPS\(_{carbohydrate}\) in the supernatants of the sludges were also determined by Lowry and Phenol-Sulfuric Acid methods after the extraction process, respectively. Optimal EPS extraction time was selected as 5 hours by applying time dependent extraction tests. After 5 hours, no significant change was observed in the concentrations of EPS components. According to the results of analytical measurements given in Table 3, the protein content of extracted EPS increased as the C/N ratio decreased. Therefore, the highest amount of EPS\(_{protein}\) was released in the sludges of Reactor 1 and Reactor 2 having C/N ratio of 8, indicating the carbon-limited conditions. On the other hand, when C/N ratio increased, the carbohydrate content of extracted EPS increased considerably. The highest amount of extracted EPS\(_{carbohydrate}\) was observed in the sludges of Reactor 5 and Reactor 6, with the C/N ratio of 30. The results agree with that of Bura and co-workers (1998) and Durmaz and Sanin (2001).
The relationship between the components of EPS and sludge dewaterability

Table 4 summarizes the effect of protein and carbohydrate parts of sludge EPS on filterability and compactibility of the sludge.

The results of filterability tests showed that the filterability of sludge increased considerably with the increasing concentrations of EPS carbohydrate and the decreasing concentrations of EPS protein (low nitrogen conditions) (Figure 3). This result is in contradiction with the results of the study by Wu et al. (1982). In another study, Houghton and Stephenson (2002) stated that there was not an evident effect of EPS composition on the dewaterability of sludge, in terms of filterability. However, they found that after the digestion of sludges the protein content of extracted EPS increased and carbohydrate part decreased leading to a decrease in the sludge filterability. Their result agrees with the filterability results of this study, indicating that the sludge filterability, in terms of filterability constant, decreases with the increasing EPS protein and increases with increasing EPS carbohydrate.

The compactibility of sludges also increased with the increasing carbohydrate part and the decreasing protein part of the EPS (Figure 4). An inverse relationship was observed.

<table>
<thead>
<tr>
<th>Reactor No.</th>
<th>C/N Ratio</th>
<th>TS (kg/m³)</th>
<th>SS (%)</th>
<th>Compactibility as cake solids concentration (%)</th>
<th>Filterability as filterability constant, X, (kg²/m⁴.s²)(×10⁻⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>2.38</td>
<td>0.089</td>
<td>3.6</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>2.49</td>
<td>0.116</td>
<td>3.9</td>
<td>2.9</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>3.84</td>
<td>0.24</td>
<td>4.8</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>3.95</td>
<td>0.26</td>
<td>4.7</td>
<td>3.8</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>4.90</td>
<td>0.35</td>
<td>5.8</td>
<td>4.1</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>5.66</td>
<td>0.38</td>
<td>5.8</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Table 3 The extracted EPS protein to EPS carbohydrate ratios of sludge samples

<table>
<thead>
<tr>
<th>Ratio</th>
<th>C/N = 8</th>
<th>C/N = 19</th>
<th>C/N = 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPS of sludge in Reactor 1</td>
<td>EPS of sludge in Reactor 2</td>
<td>EPS of sludge in Reactor 3</td>
<td>EPS of sludge in Reactor 4</td>
</tr>
<tr>
<td>EPS carbohydrate</td>
<td>0.36</td>
<td>1.76</td>
<td>2.88</td>
</tr>
<tr>
<td>EPS protein</td>
<td>0.44</td>
<td>1.76</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3 The relationship among the amounts of protein and carbohydrate parts of EPS and filterability constants of sludge samples

Figure 4 The relationship among the amounts of protein and carbohydrate parts of EPS and cake solids concentrations of sludge samples
between the cake solids concentration and the amount of $\text{EPS}_{\text{protein}}$. This can be explained with the water-holding capacity of the protein part of EPS. In this study, contrary to the results of the study by Mikkelsen and Keiding (2002), both the filterability and the compactibility of the sludge samples followed the same trend. Mikkelsen and Keiding (2002) stated that there is a negative correlation between the cake dry matter content and the filterability (in terms of SRF) of sludges.

Figure 5 shows the microscopic photographs of Reactors 2, 3, and 6, having the different C/N ratios, before the extraction process. The photographs indicated that the activated sludge flocs became larger and stronger with the increasing C/N ratio. This led to an improvement in the filterability and compactibility of sludges. The flocs in the sludge having C/N ratio of 8, in the Reactor 2, were smaller and weaker than that of the sludge in Reactor 6. The sludge in reactor 6, with the C/N ratio of 30, had larger and stronger flocs. This can be explained with the higher solids concentration of the sludge. The protein fraction of EPS is known to affect the sludge floc structure. According to the results of this study, the increase in the carbohydrate fraction of EPS may also have a stabilizing effect on floc structure. The observations in this study correlate with that of Wu et al. (1982) reporting that the sludge filtering properties become excellent when the activated sludge floc is large and strong. Goodwin and Forster (1985) also stated that the increase in the polysaccharide fraction of EPS improved the settling properties of the sludge due to the improvement in the bioflocculation.

**Conclusions**

Based on the results of the research the following conclusions can be drawn.

- The C/N ratio of sludge affected its dewaterability, in terms of filterability and compactibility, by changing the EPS composition, carbohydrate and protein ratio of EPS in the sludge. $\text{EPS}_{\text{protein}}$ decreased and $\text{EPS}_{\text{carbohydrate}}$ increased with increasing C/N ratio. The results of FT-IR analysis supported the results of analytical tests and showed that the carbohydrate to protein ratio was highly affected by the change in the C/N ratios of sludges.

- Dewaterability of sludge, both filterability and compactibility, improved with increasing carbohydrate part, $\text{EPS}_{\text{carbohydrate}}$, and decreasing protein part, $\text{EPS}_{\text{protein}}$, of the EPS in the sludge.

- An inverse relationship was observed between the cake solids concentration and the protein part of the EPS. This can be attributed to the water-holding capacity of proteinaceous part of EPS.

- The activated sludge flocs became larger and stronger with the increase of C/N ratio. Filterability and compactibility of sludges improved by the increase of the size and strength of the flocs. The increase in the carbohydrate fraction of EPS may have a stabilizing effect on floc structure and further investigations may be useful about the effective part of EPS on the floc strength.
References


