Evaluation of different shape parameters to distinguish between flocs and filaments in activated sludge images

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Abstract The ratio of flocs to filaments in activated sludge waste water treatment plants is of extreme importance for the overall performance of the plant. In order to control this ratio the individual concentrations of flocs and filaments need to be measurable. However, no sensors which can measure these concentrations are currently available. It is proposed that by means of image analysis techniques the ratio of flocs to filaments can be determined. Combination of this ratio with the total biomass concentration results in the individual floc and filament concentration. This contribution focuses on the last step of the image analysis procedure, i.e., the classification of objects as either floc or filament. Five different shape parameters, i.e., aspect ratio, roundness, form factor, fractal dimension and reduced radius of gyration, are evaluated and compared. The results indicate that the form factor is the least suitable and the reduced radius of gyration the most suitable shape parameter to accurately classify flocs and filaments in activated sludge images.

Keywords Biological waste water treatment; filamentous bacteria; filamentous bulking; floc-forming bacteria; image analysis; mixed cultures

Introduction

The activated sludge process is most widely used for biological treatment of waste water and consists of two steps: biodegradation and sedimentation. In the biodegradation step the activated sludge, a mixed culture of microorganisms, oxidizes the organic waste in the water to carbon dioxide and water. Next, the activated sludge is separated from the purified water and thickened by means of gravitation. The thickened sludge is recycled to the biodegradation tank in order to maintain high biomass concentrations. This stresses the necessity of good settling properties of the activated sludge.

Good settling and thickening of activated sludge requires the formation of large and firm sludge flocs. The filamentous backbone theory of activated sludge flocs assumes that the structure of the activated sludge floc is formed at two levels (Sezgin et al., 1978). The first level, termed micro-structure, depends on the bioflocculation of floc-forming bacteria. Micro-structure flocs are small, spherical and compact, but mechanically rather weak. Large activated sludge flocs exhibit a macro-structure provided by filamentous microorganisms. It is postulated that filamentous microorganisms form a backbone within the floc, to which floc-formers are firmly attached by their extracellular polymers. Therefore, an optimal ratio of floc-forming to filamentous bacteria in the biodegradation tank is required. If this ratio is too large, small and weak flocs are formed with poor settling properties. If, on the other hand, this ratio is too small, filaments will protrude from the flocs and thickening is hindered.

In order to monitor and control the activated sludge process, microscopic observation of the sludge is of utmost importance. Sezgin (1982) found that the Sludge Volume Index (SVI), an often used parameter to characterize the settling properties of the activated sludge, is influenced by the floc size and the presence of filamentous organisms. In practice, the floc size and total filament length are manually determined using a counting chamber and the flocs and filaments are grouped in different size ranges.
Although this information is very valuable, the direct microscope observation of activated sludge strongly depends on the individual approach of the investigator. In order to have a quantitative determination of the floc size, Li and Ganczarczyk (1986) developed an image analysis system. A light pen was used to indicate the boundaries of the flocs and the following geometric characteristics of the flocs were determined: longest dimension, breadth, equivalent diameter, cross-sectional area, perimeter, elongation, and circularity. Filamentous microorganism abundance was still qualitatively estimated by microscopic observation of free filaments and filaments extending from the flocs.

Grijspeerdt and Verstraete (1997) used image analysis to estimate the settling properties of activated sludge. Four parameters, which quantify the shape of the flocs, were measured: form factor, three-dimensional aspect ratio, roundness and fractal dimension. They found that there is a statistical correlation between the mean form factor of sludge flocs and the diluted sludge volume index. This information can be exploited in control schemes incorporating the settleability of the sludge, because it acts as an early warning system. They also investigated the possibility to determine the sludge concentration by means of image analysis.

The objective of this research is to develop an automated image analysis system to measure the individual floc and filament concentrations in the activated sludge. On the one hand, these concentrations will be used in an early warning system, and on the other, in a model-based control strategy for activated sludge plants. In Cenens et al. (2000) a prototype mathematical model, which describes the competition between flocs and filaments on one limiting substrate, is proposed. In order to use this model in a control strategy, the individual concentrations of flocs and filaments need to be measurable.

It is proposed that the combination of image analysis techniques with on-line biomass measurements, e.g., turbidity, enables the on-line measurement of the individual concentrations of flocs and filaments. With images of the activated sludge the ratio of flocs to filaments is determined by means of image analysis, and the total biomass concentration can be divided into the individual concentrations of flocs and filaments.

Image analysis generally consists of five distinct stages that follow each other logically, namely, display, image enhancement, segmentation, mathematical morphology and extraction of quantitative information (Glasbey and Horgan, 1995). In the third step, segmentation, the objects of interest are isolated from the background. Afterwards, the quality of the segmented image can be improved with mathematical morphology enabling the extraction of reliable quantitative information which is the ultimate goal.

This contribution focuses on the last step of the image analysis procedure, i.e., the extraction of quantitative information. In order to obtain the ratio of flocs to filaments, the objects in an activated sludge image need to be classified as either floc or filament. Five simple shape parameters, i.e., aspect ratio, roundness, form factor, fractal dimension and reduced radius of gyration, are evaluated for their ability to classify the different objects as floc or filament. This is done using computer generated and real flocs and filaments respectively.

Material and methods

Image capturing system

Images (magnification 10×) of activated sludge from a brewery waste water treatment plant were taken using a light microscope with phase contrast illumination (Leica DMLB) equipped with a CCD video camera (Leica DC200). The digitized images (768 × 576 pixels) were processed by means of the MATLAB Image Processing Toolbox (The Mathworks, Inc.).
Definition of parameters

In this section a definition of the five different shape parameters is provided. First four size parameters, used to calculate some shape parameters, are defined.

Length. There is no unique definition for the length of an object. For this application the length is defined as the major axis length, which is the longest axis of an ellipse with the same second order moment as the object itself (Haralick and Shapiro, 1992).

Breadth. By analogy with the length, the breadth of an object is defined as the minor axis length, the shortest axis of an ellipse with the same second order moment as the object itself (Haralick and Shapiro, 1992).

Area ($A$). An object’s area can simply be estimated by counting the pixels which the object consists of.

Perimeter. The perimeter of an object is the length of the pixel line which forms the boundary between the object and the background. In this application the perimeter is estimated by adding the distances between the pixel centres of 8-connected line segments. In an 8-connected region the pixels have horizontally, vertically or diagonally adjacent neighbours.

Aspect ratio ($AR$). The aspect ratio is the ratio of the length to the breadth of an object. This parameter is dimensionless and provides a measure of how elongated the feature is (Russ, 1990). A circle has got an equal sized length and breadth and consequently an aspect ratio of 1. The more elongated the object, the larger its aspect ratio.

Form factor ($FF$). The form factor is defined as the ratio of the object’s area to the area of a circle with the same perimeter as the object. The form factor of a circle is equal to 1 and it decreases for objects deviating from a circle.

Roundness ($R$). This parameter is also a measure of how elongated an object is and is defined as the ratio of the object’s area to the area of a circle with a diameter equal to the length of the object. The roundness of a circle is equal to 1 and it decreases for objects deviating from a circle. The roundness is similar to the form factor but instead of the perimeter, it uses the length of a object, which makes it more sensitive to how elongated the object is, rather than how irregular its outline is.

Fractal dimension ($FD$). The British mathematician Richardson observed that the perimeter of a object is dependent on the measurement distance that is used to estimate this perimeter (Russ, 1990). The fractal dimension is defined as the factor by which the perimeter decreases in relation to an increasing measurement distance and is used as a measure for the irregularity of the perimeter. An object perimeter with a fractal dimension approaching 2 would cover the entire plane, while one close to 1 would remain nearly a line (Russ, 1990). The fractal dimension can be estimated from a Richardson plot, which is obtained by plotting the length of the measured perimeter versus the length of the measuring distance, both in logarithmic scale. The fractal dimension equals 1 minus the slope of the linear portion of the plot. In this work the mosaic amalgamation algorithm is used to construct this plot. The perimeter is measured as the image resolution is progressively coarsened. The coarsening is begun by replacing each block of 4 pixels by one solid block. With coarsening rules it is decided if the block will be white (background) or black (object). This results in an image with a more blocky appearance and a shorter perimeter, but on the
average, the same area. For details about this algorithm, reference is made to Russ (1990).

Reduced radius of gyration (RG). This global characteristic is based on the moments of an object (Pons and Vivier, 2000). The reduced radius of gyration quantifies how dispersed the pixels in an object are from their centroid. For a disc the reduced radius of gyration is equal to 0.707, while it is higher for objects deviating from a disc. The reduced radius of gyration is calculated as follows:

\[ RG = \frac{\sqrt{M_{xx} + M_{yy}}}{D_{eq}/2} \]

with

\[ M_{xx} = \frac{\sum_{i=1}^{N}(x_i - x_g)^2}{N}, \quad M_{yy} = \frac{\sum_{i=1}^{N}(y_i - y_g)^2}{N}, \quad x_g = \frac{\sum_{i=1}^{N}x_i}{N}, \quad y_g = \frac{\sum_{i=1}^{N}y_i}{N} \]

\( (x_i, y_i) \) defines the position of pixel \( i \) of an object of \( N \) pixels according to the columns and lines of the image. \( D_{eq} \) is the equivalent diameter of the object.

Results and discussion

Robustness of shape parameters

To examine how robust the shape parameters are, and to gain more insight into the effect of changes in shape on their values, the five parameters are calculated for computer generated objects (Figure 1).

To examine whether the parameters are calculated correctly, the first of eight objects is a disc. It can be seen from Table 1 that the calculated values correspond to what can be expected from the parameter’s definition, which means that their estimation can be considered reliable. Concerning the effect of changes in shape on the calculated value (Table 1), a number of conclusions can be drawn.

- Comparing the aspect ratio of a smooth versus a ragged floc (objects 2 and 3) on the one hand, and a smooth floc versus one full of holes (objects 2 and 4) on the other, demonstrates that there is no considerable change in parameter values under the influence of irregularities and holes. However, the aspect ratio is very sensitive to curvature and branching of filaments, as can be seen from objects 5, 6 and 7. This can result in misclassification of curved and branched filaments.

- The roundness exhibits a slight decrease under the influence of ragged boundaries and holes because the area decreases. Curvature and branching conversely bring about a slight increase of the roundness as a result of a larger area. Furthermore, the discrepancy between values of flocs and filaments appears to be of significant magnitude to allow classification.

- A floc with ragged boundaries (object 3) has a small form factor in comparison with a smooth floc (object 2) because of its larger perimeter. A floc with holes (object 4) also has a lower value, but this is due to a smaller area. When looking at filaments, the increase of the area in the case of curvature and branching cannot compensate for the simultaneous increase of the perimeter, resulting in a slight decrease in form factor values.

- The fractal dimension of an irregular floc (object 3) or a floc with holes (object 4) is larger than that of a smooth one (object 2), while curvature and branching have no influence on the fractal dimension of filaments. However, between the parameter values of flocs and filaments appears to be hardly any difference, and this may cause a problem when using this parameter to classify objects as either floc or filament.
The values of the reduced radius of gyration are relatively stable within the group of flocs and filaments respectively. Moreover, comparing values for flocs and filaments show that there is a significant difference between both types of objects.

Identification of most suitable shape parameter

To identify the most suitable shape parameter for classifying flocs and filaments in an activated sludge image, it is necessary to calculate the five parameters for a large number of objects. From binary images of activated sludge, 100 objects are identified as flocs and 100 objects are identified as filaments by means of human observation. The five shape parameters are calculated for those 200 objects. For each parameter the best discriminating level, the value which determines if an object is classified as a floc or as a filament, is determined. This is done by selecting different discriminating values for each parameter and computing for each value the percentage of wrongly classified objects. A wrongly classified object is either a floc classified as a filament or vice versa. Table 2 shows the values of the shape parameters corresponding to the smallest amount of wrongly classified objects.

This table can be converted into a graph, which allows a better comparison (Figure 2). The first thing that meets the eye is the rather large minimal total error of nearly 30% when using the form factor or the fractal dimension. The aspect ratio and the roundness have both a minimal total error around 15%. Finally, the reduced radius of gyration has a total error of only 3%.

Table 1  Parameter values of the computer generated objects represented in Figure 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Object 1</th>
<th>Object 2</th>
<th>Object 3</th>
<th>Object 4</th>
<th>Object 5</th>
<th>Object 6</th>
<th>Object 7</th>
<th>Object 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspect ratio</td>
<td>1.00</td>
<td>1.40</td>
<td>1.45</td>
<td>1.42</td>
<td>63.8</td>
<td>7.73</td>
<td>3.66</td>
<td>1.33</td>
</tr>
<tr>
<td>Roundness</td>
<td>1.00</td>
<td>0.729</td>
<td>0.685</td>
<td>0.589</td>
<td>0.0190</td>
<td>0.0301</td>
<td>0.0431</td>
<td>0.0762</td>
</tr>
<tr>
<td>Form factor</td>
<td>0.92</td>
<td>0.711</td>
<td>0.239</td>
<td>0.179</td>
<td>0.0616</td>
<td>0.0525</td>
<td>0.0205</td>
<td>0.0348</td>
</tr>
<tr>
<td>Fractal dimension</td>
<td>1.02</td>
<td>1.07</td>
<td>1.29</td>
<td>1.51</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Red. radius of gyration</td>
<td>0.707</td>
<td>0.737</td>
<td>0.751</td>
<td>0.816</td>
<td>3.63</td>
<td>2.92</td>
<td>2.53</td>
<td>2.31</td>
</tr>
</tbody>
</table>

Figure 1  Computer generated objects used to evaluate the five shape parameters

Table 2  Discriminating values for the five shape parameters and the number of wrongly classified flocs and filaments

<table>
<thead>
<tr>
<th>Shape parameter</th>
<th>Value</th>
<th>Flocs (%)</th>
<th>Filaments (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspect ratio</td>
<td>2.44</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Roundness</td>
<td>0.350</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>Form factor</td>
<td>0.315</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td>Fractal dimension</td>
<td>1.16</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>Red. radius of gyration</td>
<td>1.10</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
All these findings indicate that the form factor is the least suitable, and the reduced radius of gyration the most suitable shape parameter to classify flocs and filaments. To illustrate this, objects in activated sludge images are classified as either floc or filament based on their reduced radius of gyration (Figure 3). The discriminating value which is used is the value presented in Table 2.

Conclusions
The aim of this research is to obtain the ratio of flocs to filaments in activated sludge samples. Therefore, images of activated sludge are taken and the digitized images are...
processed by means of image analysis techniques. This work focuses on the last step in the image analysis procedure, i.e., the classification of objects as either floc or filament. Five shape parameters, i.e., aspect ratio, roundness, form factor, fractal dimension and reduced radius of gyration, are evaluated and compared. By means of computer generated objects the robustness of the different parameters is evaluated. Afterwards, the best parameter to classify flocs and filaments is identified. The five parameters are calculated for 100 flocs and 100 filaments and the best discriminating value is determined. This is the parameter value by which the least objects are wrongly classified. It can be concluded that the form factor is the least suitable and the reduced radius of gyration the most suitable parameter to classify flocs and filaments. To illustrate the utility of the reduced radius of gyration, objects in activated sludge images are classified as either floc or filament.

The preceding image analysis steps, especially the segmentation step, are of utmost importance for the quality of the final step. The development of an automatic segmentation procedure followed by morphological operations is the subject of ongoing research.

Afterwards, the developed automatic image analysis procedure combined with a measurement of the total biomass concentration, will be applied to quantify flocs and filaments in activated sludge, the ultimate goal of this research.

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