Quantitative image analysis for sludge volume index and total suspended solids prediction in activated sludge system disturbances

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The settling phase is a critical step of the activated sludge process in biological wastewater treatment plants (WWTPs). The sludge settling ability is mainly dependent on the structure and nature of the activated sludge flocs, and these properties can change drastically depending on the operational conditions in WWTPs [1]. Filamentous bacteria are also present in activated sludge flocs providing a backbone for the overall floc structure [2]. The success of a given activated sludge system depends on the ecosystem balance among floc-forming bacteria and filamentous bacteria [3,4]. However, a variety of bulking phenomena can affect the normal operation of the plant.

To guarantee the success of a given activated sludge system, a global examination of the sludge must be performed. This characterization is based on the biomass morphological characteristics, which have a considerable variation in wastewater treatment systems. Recent developments on floc structure analysis associated to microscopy techniques give access to an in deep analysis of structural information [5]. The characteristics of bioaggregates, including their internal structure, chemical composition and microbial ecology, determine the transport properties and chemical reaction rates, and affect the overall performance of treatment processes involving aggregates [6]. A variety of other factors are also known to affect settling rates due to flocculation and deflocculation processes [7]. In the last few years, quantitative image analysis approaches, coupled to multivariate statistical analysis (partial least squares – PLS), have been increasingly used to clarify filamentous bulking detection and monitoring in activated sludge processes [8,9].

The present study focuses on predicting the Sludge Volume Index (SVI) and Total Suspended Solids (TSS) for different types of conditions affecting an activated sludge system. Four experiments were conducted simulating filamentous bulking, viscous bulking, pinpoint floc formation, and normal conditions. Alongside the SVI and TSS determination, the aggregated and filamentous biomass contents and morphology was studied, as well as the biomass Gram and viability status. Upon the determination of the image analysis data, regression analysis and partial least squares were used to reduce the dataset and model each studied condition.

Experimental Section:
Experimental data was obtained from a lab-scale activated sludge system based on a 17 L aerated tank with suspended biomass, followed by a 2.5 L cylindrical clarifier. The study of the four above-mentioned conditions (filamentous bulking, pinpoint flocs, viscous bulking, and normal conditions) was sequentially conducted. Between each experiment, the system was recharged with biomass to guarantee a rapid establishment of the new condition. During each experiment, TSS measurements were conducted by weighing. The biomass settling ability was measured through the determination of the SVI in a 1 L Imhoff cone, with the sludge height variation monitored for 30 min.

The microbial community was observed by means of an Olympus BX51 (Olympus, Tokyo, Japan) optical microscope at 100x magnification, coupled with an Olympus DP25 (Olympus, Tokyo, Japan) digital camera. Images were acquired at 1360 × 1024 pixels and 8-bit format by the commercial software Cell^B (Olympus, Tokyo, Japan).

The Live/Dead® BacLight™ bacterial viability kit was used to differentiate viable and damaged bacteria [10]. The Live BacLight™ bacterial Gram stain allowed to easily classify bacteria as Gram-positive or Gram-negative without using fixatives [11]. Slides with stained sludge samples were observed in an Olympus BX51 epifluorescence microscope (Olympus, Tokyo, Japan) at 200x magnification.
magnification. Two long pass filters were used, one in the green wavelength range with an excitation bandpass of 470-490 nm and emission at 516 nm, and the second filter in the red wavelength range with an excitation bandpass of 530-550 nm and emission at 591 nm. Images were acquired at 1360 × 1024 pixels, and 24-bit RGB format by the commercial software Cell^B (Olympus, Tokyo, Japan).

A total of 79 parameters were determined by image analysis, with 55 parameters referring to the morphological analysis and 24 parameters to the viability and Gram status analysis. A cross-correlation analysis between the collected data was then performed in order to reduce the dataset, leading to the exclusion of one variable for each pair presenting a correlation factor above 90%. The PLS was then conducted to extract linear combinations of the essential features of the original X data while modeling the Y (SVI and TSS) data dependence on the work set.

Results:
The results obtained for the PLS analysis are presented in Table 1 where 79 parameters were analysed combining the biomass contents and morphology with Gram and viability status. After a parameters reduction analysis preformed by cross-correlation and VIP (variable importance in the projection) selection, good prediction abilities of SVI and TSS were attained (R² > 0.9). These results showed that the association between image analysis information and multivariate statistical analysis (PLS) revealed good results for activated sludge monitoring and control in different operating conditions.

Table 1. PLS modeling results for SVI and TSS for each condition studied using the biomass contents, morphology, Gram and viability status.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Filamentous bulking</th>
<th>Pinpoint flocs</th>
<th>Viscous bulking</th>
<th>Normal conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SVI</td>
<td>TSS</td>
<td>SVI</td>
<td>TSS</td>
</tr>
<tr>
<td>Number of parameters</td>
<td>7</td>
<td>19</td>
<td>25</td>
<td>17</td>
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<tr>
<td>Parameters reduction (%)</td>
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<td>75.95</td>
<td>68.35</td>
<td>78.48</td>
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<td>Latent variables</td>
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<td>2</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>R² (training + validation)</td>
<td>0.98</td>
<td>0.96</td>
<td>0.92</td>
<td>0.99</td>
</tr>
</tbody>
</table>

References: