Evaluation of activated sludge systems by image analysis procedures

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Abstract
Biomass inspection under optical microscopy coupled to automated image analysis methodologies is, nowadays, increasingly used. Image analysis is presently considered a powerful tool to identify and quantify biomass morphological and physiological changes. In this work, an image analysis program was developed in *Matlab* environment, allowing the identification and characterization of microbial aggregates and protruding filaments in six different wastewater treatment plants. The results showed that the developed image analysis methodology proved to be a feasible method for a continuous monitoring of the activated sludge contents both in terms of aggregated biomass and filamentous bacteria, comparing the results with operating parameters. Furthermore, the results obtained during the monitoring period indicate that automated image analysis can help clarifying the nature of the events within the aeration tanks when the system is submitted to disturbances.

Keywords
Activated sludge; image analysis; aggregates; filaments; settling ability

INTRODUCTION

Wastewater treatment plants are frequently operated with activated sludge systems. In a general way, this system consists in two steps: an aerated tank based on suspended solids containing different groups of microorganisms, organic, and inorganic particles comprising the biochemical stage; and thereafter, the biomass separation in a secondary clarifier, named settling phase. It is well established that an adequate balance between the different types of bacteria is essential to form aggregates with acceptable properties of structure, size and density allowing an effective settling ability of the sludge and therefore, low suspended solids effluent levels (Jenkins *et al.*, 2003). As a matter of fact, these characteristics are a function of the variable operating conditions within wastewater treatment plants (Wilén and Balmér, 1999), affecting the sludge settling ability as shown by the sludge volume index (SVI) (Schuler and Jang, 2007a,b).
Monitoring activated sludge processes by microscopic observation and image analysis information is a well established technique with the utmost importance for the scientific community. After the manual counting, evaluation and control developed by Sezgin *et al.*, (1978), image acquisition and inspection successfully advanced with several researches over the last decades. Sezgin (1982) found that the sludge volume index, the most suited parameter to characterize sludge settling properties, is strongly influenced by the aggregates size and the filamentous bacteria presence. In other studies comprising image processing methodologies (Ganczarczyk, 1994; Grijspeerdt and Verstraete, 1997) it was found that the microorganism's morphology in biological systems can be related to the sludge settling properties. Over the last years, automated image analysis applications in activated sludge has largely increased, such as the assessment of biomass morphological changes (Jenné *et al.*, 2003), and image acquisition methodologies, both for phase-contrast (Cenens *et al.*, 2002) and bright field microscopy (Amaral and Ferreira, 2005). Another current field of study centers on the balance between floc forming and filamentous bacteria, namely on the identification of imbalance occurrences and consequent bulking events on activated sludge systems, that may induce biomass washout, filamentous bulking or foaming problems (da Motta *et al.*, 2001a,b; Jenné *et al.*, 2004, 2007; Amaral and Ferreira, 2005).

The present work aims to survey the filamentous bacteria and aggregates contents and morphology of the biomass collected from six wastewater treatment plants during a period of several months. For that purpose, an image processing and analysis program was used for bright field microscopy, providing the necessary data to monitor the biological system. The results based on this image analysis procedure were furthermore related to the operating conditions.

MATERIAL AND METHODS

The biomass used in this study was collected from the aeration basins of six WWTP (identified as 1 to 6), treating domestic effluents, located in the North of Portugal. Samples were taken to perform physical measurements, on one hand, and microscopic observations, on the other, in order to estimate the contents and morphology of the microbial aggregates and protruding filamentous bacteria by image acquisition and analysis. For each sample, the biomass settling ability was measured in a cylindrical column with the sludge height variation monitored with time for 30 min in a 10L settling cylinder. Total suspended solids (TSS) were determined by weight, and used to determine the SVI parameter (APHA *et al.*, 1989).

Image Acquisition

For image acquisition, a volume of 25µL was placed on a slide and covered with a 20x20 mm cover slip for visualization and image acquisition. Around 200 images were obtained in bright field microscopy to obtain representative information about the sludge contents. All the images were acquired in a *Leitz Laborlux S* optic microscope *(Leitz, Wetzlar)*, with 100x magnification, coupled to a *Zeiss AxioCam HRc (Zeiss, Oberkochen)* camera. The image acquisition was performed in 1300x1030 pixels and 8-bit format through the Axion Vision 3.1 *(Zeiss, Oberkochen)* software.

Image Processing and Analysis Methodology

The image processing and analysis program for aggregates and filaments, was developed in *Matlab* 7.3 (*The Mathworks, Inc., Natick*) language, adapting a previous version developed by Amaral and Ferreira (2005). Primarily, the image processing step determined the binary images from the aggregated biomass and the protruding filamentous bacteria and thereafter, the morphological parameters were determined. Figure 1 shows an example of the original, binary and labeled images resulting of the main steps of the program, comprising the image pre-treatment, segmentation, and debris elimination whereas the image analysis program was oriented to the aggregates and filaments contents determination.

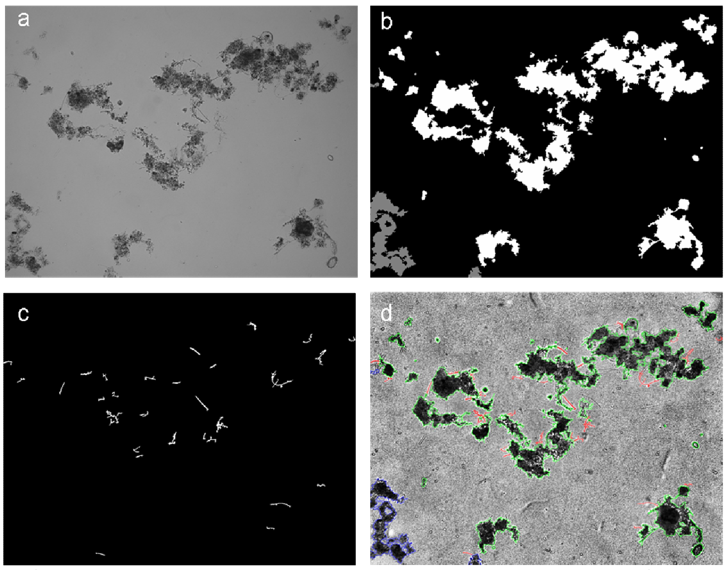

Figure 1. Original image from the activated sludge system with 100x magnification (a), binary aggregates image (b), binary filaments image (c) and final labeled image (d).

Image Analysis Parameters

The image analysis program allowed the determination of both the aggregated biomass and protruding filamentous bacteria contents. As the total protruding filaments length per total suspended solids (TL/TSS) and per total aggregated area ratio (TL/TA) ratios characterize the filaments dynamics, these parameters were also included.

Furthermore, the aggregates morphological data was subdivided into 3 classes: larger aggregates with an equivalent diameter above 0.25 mm, intermediate aggregates with an equivalent diameter ranging from 0.025 to 0.25 mm, and smaller aggregates (pin-point flocs) with an equivalent diameter below 0.025 mm.

RESULTS AND DISCUSSION

Standard biomass contents and settling ability parameters

The microbial aggregates structure in activated sludge and effluent quality are crucial in solid-liquid separation processes, as well as TSS and SVI providing significant information on the biomass contents and sludge settling ability. The TSS and SVI values monitored throughout the survey are presented in Figure 2. This data is, furthermore, quite relevant to relate with the image analysis data during the monitoring period.

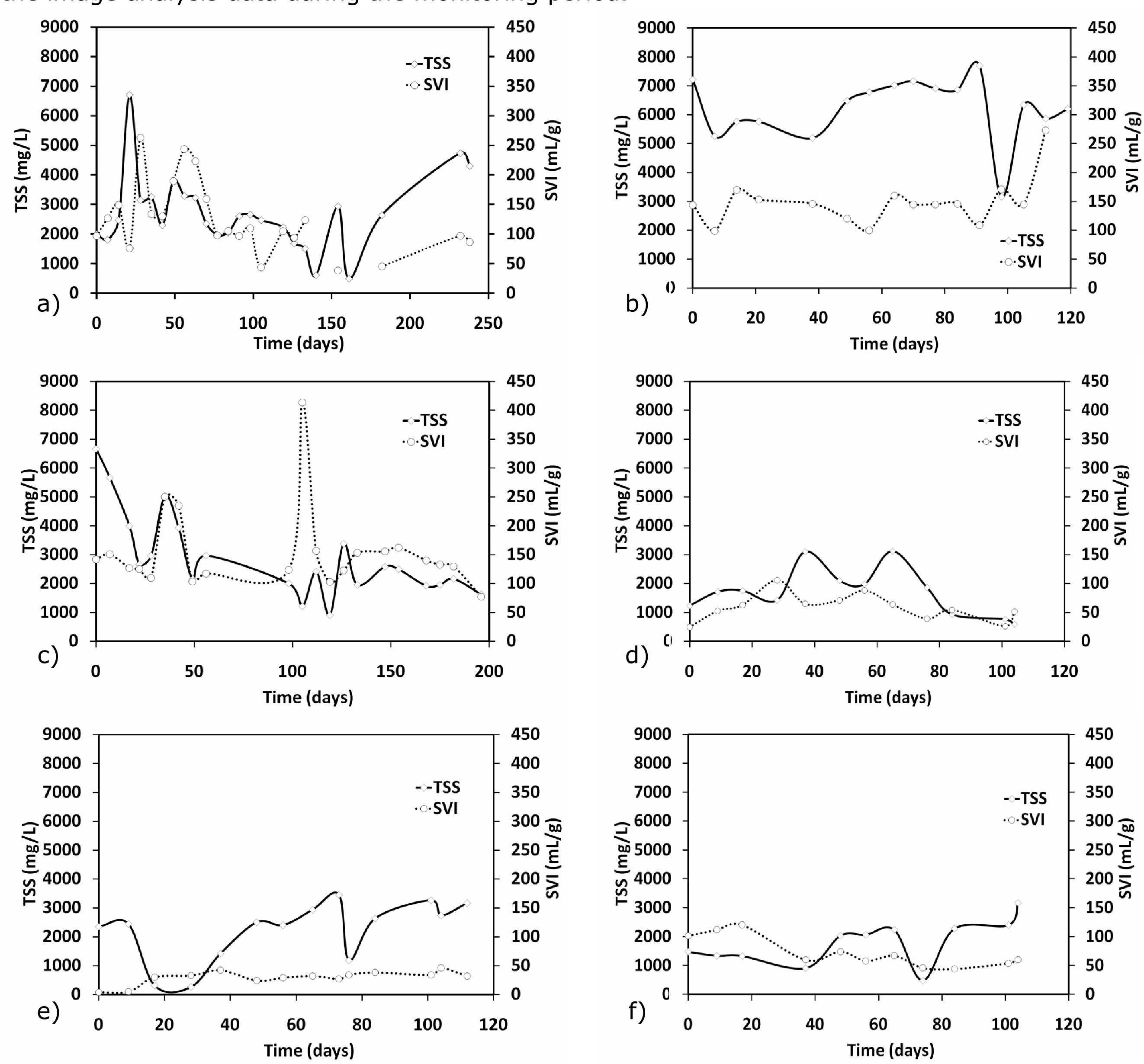

Figure 2. TSS and SVI values from the six WWTP aerated tanks.

From Figure 2, it can be withdrawn that the six aerated tanks have different operating conditions, sometimes with strong fluctuations in terms of biomass contents or SVI values.
For the first WWTP, the TSS results, here depicted on the Figure 2a, presented mainly values ranging from 2000 mg/L to 5000 mg/L, throughout the survey period, indicating a normal operation of the biological system. However, day 21 (highest value), 140 and 161 (lower values), presented different solids contents, showing a disturbance on the plant. As a matter of fact, it could be considered that there was a biomass boost on day 21 followed by a steep decrease up until day 42 and a washout phenomenon in days 140 and 161 (TSS lower than 1000 mg/L). Following the washout in day 161 the biomass contents steadily increase up until the end of the survey attaining TSS values just short of 5000 mg/L. With respect to the sludge volume index (SVI), the plant presented values mainly below the threshold limit for bulking events (150 mL/g), based on the previous study of Jenkins *et al*. (2003). Only five days (days 28, 49, 56, 63, and 70) presented SVI values higher than 150 mL/g (bulking phenomenon), ranging from 160 to 265 mL/g, and during this period the TSS were within normal values. The nature of the observed bulking was established by the filaments dynamic data, and is described within the discussion of the image analysis parameters presented below.
TSS concentrations above 5000 mg/L were attained for the 2nd WWTP studied (Figure 2b), showing that this plant operated with higher biomass contents than WWTP 1. Furthermore, the SVI values were always on the verge of configuring a bulking phenomenon and in days 14, 21, 64, 98 and 112 were, in fact, higher than 150 mL/g. Also, a sharp decrease was detected on TSS concentration for day 98, indicating a disturbance on the activated sludge system. However, right after this disorder, the TSS rapidly recovered normal operational conditions. Furthermore, in the last monitored day, the settling properties of the biomass suffered a sudden deterioration with an SVI value of 273 mL/g. It was, therefore, found that the WWTP 2 was nearly always working with mediocre settling ability conditions (see SVI results) and strong biomass contents. With respect to the nature of the low sludge settling ability it was found imperious to compare these results with the image analysis information described in the following section.
The aerated tank behavior of the WWTP 3 is depicted on Figure 2c, showing TSS concentrations ranging from around 1000 mg/L to 7000 mg/L. From the beginning of the survey until day 22, and again from day 35 until day 49, the system suffered a sharp biomass contents decrease, with a recovery period between days 22 and 35. From day 49 onwards until the end of the survey the TSS values oscillated around an average value of 2000 mg/L although a washout event seems to have taken place in day 119 (TSS of 900 mg/L). Therefore, apart from the beginning of the survey until day 17 when the activated sludge system presented high biomass contents and from day 119, the biomass contents was within normal operating conditions. Regarding the sludge settling ability, the plant presented values mainly below the threshold limit for bulking events although such was not the case for a few periods of the survey. In fact, for day 7, day 112 and from day 133 to 154 the plant presented mild bulking phenomena, and for days 35 to 42 and day 105 severe bulking problems. The nature of the bulking events will be further discussed when related with the image analysis information.
WWTP 4, 5 and 6 (Figure 2d, e and f, respectively) operated in general with biomass concentrations below 4000 mg/L. Regarding WWTP 4 (Figure 2d), low biomass contents and SVI values predominated throughout the survey. With respect to the TSS, this WWTP operated with low biomass contents, further aggravated from day 65 until the end of the survey. As a matter of fact, it could be established that, from day 84 onwards, TSS values were lower than 1000 mg/L configuring washout phenomena. The SVI behavior remained below the bulking threshold (150 mL/g) during all the monitoring period. Such was also the case of WWTP 5 (below 50 mL/g) and 6 (between 40 and 120 mL/g), depicted on Figure 2e and Figure 2f, respectively. Moreover, the biomass contents of these plants were mediocre, ranging from 250 to 3500 mg/L for WWTP 5 and from 500 to 3000 mg/L in WWTP 6. Considering WWTP 5 (Figure 2e), the TSS values pointed to a heavy biomass loss on day 76 and washout events on days 17 and 28. For the 6th WWTP (Figure 2f), days 37 and 74, with biomass concentrations lower than 1000 mg/L, suggested the same phenomena within the aerated tank.

Image analysis based biomass characterization and settling ability

Taking into consideration the Amaral and Ferreira (2005) study, the filaments length per total aggregates area (TL/TA) was determined due to the usefulness to relate the free filamentous bacteria and the aggregates presence. A parameter originated from the filaments contents versus solids contents (TL/TSS) was also included in this analysis, due to its importance on the prediction of sludge settling ability, taking into consideration Sezgin (1982) and Schuler and Jassby (2007c) approaches. From the analysis of this parameter, the most significant information withdrawn was the determination of the relationship between the protruding filamentous bacteria and the aggregates presence. Figure 3 presents the TL/TSS and TL/TA ratios for the 6 surveyed WWTP.

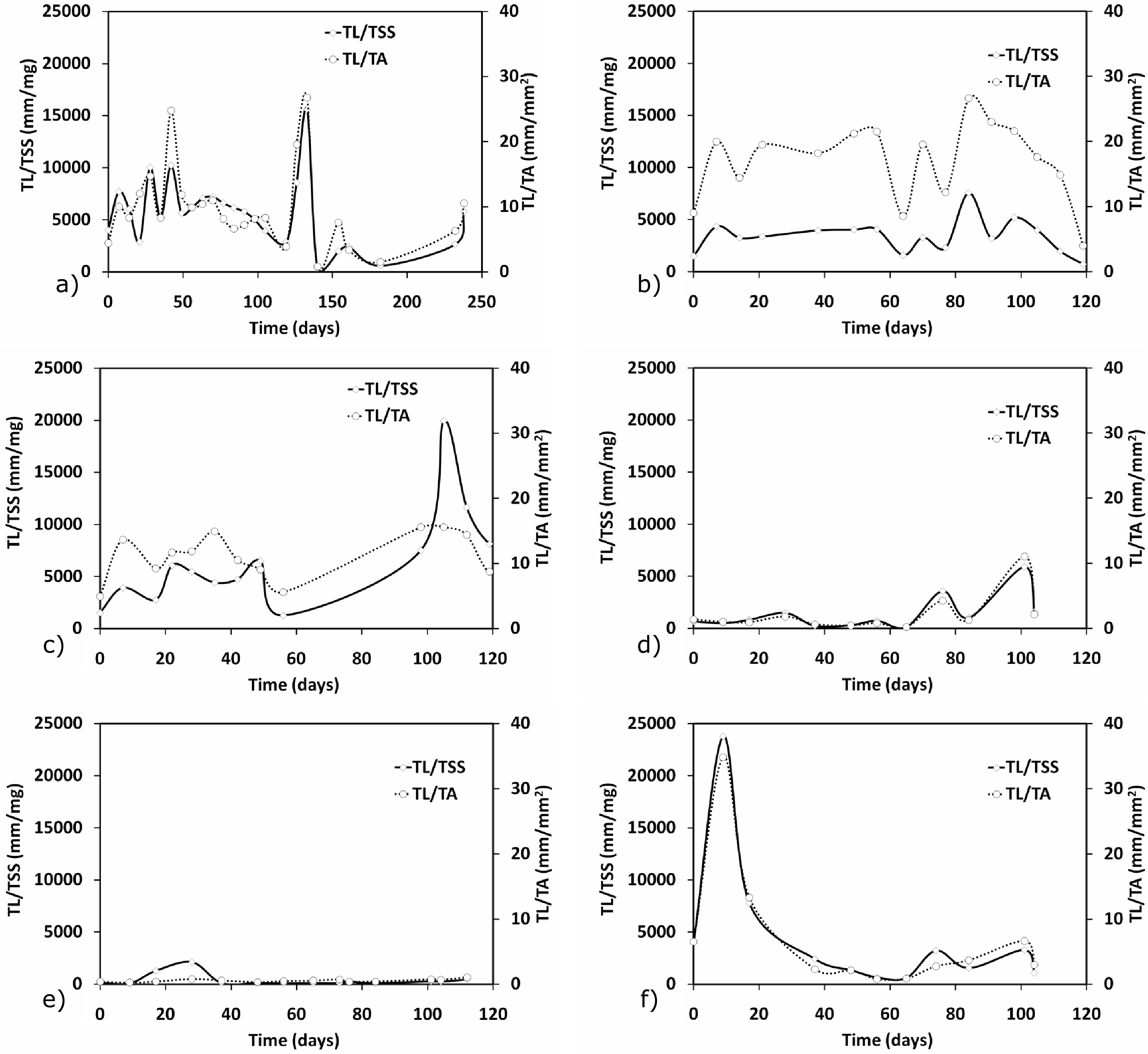

Figure 3. TL/TA and TL/TSS behavior from the six activated sludge systems considered for this study.

From Figure 3, it can be withdrawn that the six aerated tanks have different behaviors during the survey. However, it is also clear a similar trend between the TL/TSS and TL/TA parameters as these parameters are related. As the SVI test is the settled volume normalized by biomass content, it seems more appropriate, in the case being, to use primarily the TL/TSS parameter complemented with the TL/TA for further analysis. However if dilutions were made, the TL/TA parameter could be used instead, due to its larger robustness to the dilution effects.

As already stated, the filaments contents per solids contents ratio (TL/TSS) is essential for this investigation taking into consideration Sezgin (1982) approaches, due to its importance on the prediction of the sludge settling ability. However, cautions are advisable during the evaluation of the system and a balance with physical properties is desirable. According to Amaral and Ferreira (2005), SVI and TL/TSS parameters are highly dependent on biomass contents, and its determination is important in order to establish the true nature of the phenomena occurring within the biological system. The TL/TSS parameter was also studied by Amaral and Ferreira (2005), revealing a strong relationship with the SVI, and pointing towards the existence of a filamentous bulking phenomenon. Furthermore, in that study, the authors concluded that the TL/TSS parameter may be used, at some extent, to monitor the SVI behavior in a plant. Moreover, recent studies (submitted) of the same authors have determined a filamentous bulking threshold limit for the TL/TA ratio of 15 mm/mm^2.

For the first WWTP, the TL/TSS was normally bellow 10000 mm/mg, which according to Sezgin (1982) is the threshold limiting the existence of a filamentous bulking problem. Furthermore, as the TL/TA remained normally below 15 mm/mm^2, no filamentous bulking was pointed by these parameters except for days 126 and 133. At those days, the TL/TSS values of 8573 and 15569 mm/mg and TL/TA values of 19.54 and 26.74 mm/mm^2, respectively, pointed to filamentous bulking, which however, was not confirmed by the observed SVI values. The analysis of the images from those days allowed establishing that the protruding filamentous bacteria were short in terms of their length and the aggregates presented no linked structure. As the biomass contents on those days were quite low (1690 and 1510 mg/L), the high TL/TSS and TL/TA ratio values may not reflect a real strong filamentous presence, and, therefore, not configuring an actual bulking event. The bulking phenomenon of the 28th day (SVI value slightly above 150 mL/g) was found to be not only derived of filamentous nature (TL/TSS of 10019 mm/mg and TL/TA of 14.71 mm/mm^2) but also due to the high amounts (above 60% in area) of pin-point flocs. However, such was not the case of the bulking nature of the days 49, 56, 63, and 70, with the TL/TSS well below 10000 mm/mg, and TL/TA below 15 mm/mm^2, proving that it was not of filamentous origin. Furthermore, the predominance (40 to 50%) of pin-point flocs from day 21 to 70 (data not shown) revealed the pin-point nature of the bulking between days 49 and 70.

For the 2nd surveyed WWTP (Figure 3b), whereas the TL/TSS data (values lower than 10000 mm/mg) indicated no filamentous bulking events within the aerated tank, such was not the case for the TL/TA values (majority of values above 15 mm/mm^2). As discussed above, the SVI values were, throughout the survey, near the bulking limit and, for days 14, 21, 64, 98 and 112 seemed to indicate a mild bulking problem. The data from the aggregates size distribution showed predominance (70 to 80%) of intermediate aggregates, thus dismissing both pin-point (smaller aggregates around 20%) and zoogleal bulking (larger aggregates below 10%) (data not shown). The most logical explanation to the near bulking behavior throughout the survey was, therefore, a combination of some filamentous bulking (attested by the high TL/TA values) and the high biomass contents interfering with the normal settling of the sludge.

WWTP 3 (Figure 3c) exhibited TL/TSS and TL/TA results below bulking phenomena threshold limit, from the beginning of the monitoring period and up until day 98. The high TL/TSS value obtained for the day 105 (19945 mm/mg), together with a TL/TA value of 15.55 mm/mm^2, indicates a severe filamentous bulking phenomenon, confirmed by the high peak depicted on Figure 2c. Filamentous nature seemed to have been also the cause of the mild bulking events of day 112 (TL/TSS of 11637 mm/mg and TL/TA of 14.38 mm/mm^2) and of the period between days 133 to 154, presenting TL/TSS values above or around 10000 mm/mg and TL/TA above or around 15 mm/mm^2. With respect to the bulking problems of day 7, and 35 to 42 (TL/TSS below 5000 mm/mg and TL/TA between 10 and 15 mm/mm^2), the aggregates size distribution showed a predominance of intermediate aggregates (70% to 80%), thus dismissing both pin-point and zoogleal bulking (data not shown). Whereas the mild bulking event of day 7 could be explained by the high biomass contents interfering with the normal settling of the sludge, the bulking events from days 35 to 42, although appearing to be associated with the sudden increase on the biomass contents, need further investigation.

For the remaining (4th, 5th and 6th) surveyed WWTP (Figure 3d, 3e and 3f), no filamentous bulking events took place within the aerated tank, with the TL/TSS values remaining below 10000 mm/mg and TL/TA below 15 mm/mm^2. The only exception was noticed on day 9 of WWTP 6 presenting values of TL/TSS of 23770 mm/mg and TL/TA of 34.84 mm/mm^2, denoting possible filamentous bulking events that was not supported by the observed SVI. The analysis of the images from that day allowed establishing that the protruding filamentous bacteria were short in terms of their length and the aggregates presented no linked structure. As this WWTP operated throughout all the survey with low biomass contents (below 2500 mg/L), the high TL/TSS and TL/TA ratio values may not reflect a real strong filamentous presence. Those being the case, the obtained high TL/TSS and TL/TA values do not configure an actual bulking event.

CONCLUSIONS

The results showed that the developed image analysis methodology proved to be adequate for continuous examination of microbial communities in terms of protruding filaments and biomass contents and morphology. Furthermore, this methodology was able to follow the disturbances within the aerated tank of different plants. This study also emphasized the advantages of combining SVI determination with image analysis parameters, in order to establish the true nature of the phenomena occurring within the biological system. In conclusion, it was found that the proposed image analysis methodology has provided relevant information concerning the monitoring period events that could not have been determined by conventional survey methods.

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REFERENCES

Amaral A.L. and Ferreira E.C. (2005). Activated sludge monitoring of a wastewater treatment plant using image analysis and partial least squares regression. Anal. Chim. Acta, **544**, 246-253.

APHA, AWWA, WPCF. (1989). Standard Methods for the Examination of Water and Wastewater. 17th Ed., American Public Health Association, Washington D.C.

Cenens C., Jenné R. and Van Impe J.F. (2002). Evaluation of different shape parameters to distinguish between flocs and filaments in activated sludge images. Wat. Sci. Tech., **45**(4-5), 85-91.

da Motta M., Amaral A.L., Casellas M., Pons M.N., Dagot C., Roche N., Ferreira E.C. and Vivier H. (2001a). Characterisation of activated sludge by automated image analysis: validation on full-scale plants. IFAC Computer Applications in Biotechnology, Québec City, Canada, 427-431.

da Motta M., Pons M.N. and Roche N. (2001b). Automated monitoring of activated sludge in a pilot plant using image analysis. Wat. Sci. Tech., **43**(7), 91–96.

Ganczarczyk J.J. (1994). Microbial Aggregates in Wastewater Treatment. Wat. Sci. Tech., **30**, 87-95.

Grijspeerdt K. and Verstraete W. (1997). Image analysis to estimate the settleability and concentration of activated sludge. Water Res., **31**, 1126–1134.

Jenkins D., Richard M.G., Daigger G. (2003). Manual on the causes and control of activated sludge bulking, foaming and other solids separation problems. Lewis publishing, Boca Raton, FL.

Jenné R., Banadda E.N., Philips N. and Van Impe J.F. (2003). Image Analysis as a Monitoring Tool for Activated Sludge Properties in Lab-Scale Installations. J. Environ. Sci. Health Part A - Toxic/Hazardous Subs. & Environ. Engineering, **38**(10), 2009–2018.

Jenné R., Banadda E.N., Smets I.Y., Deurinck J. and Van Impe J.F. (2007). Detection of Filamentous Bulking Problems: Developing an Image Analysis System for Sludge Composition Monitoring. Micros. Microanal., **13**, 36-41.

Jenné R., Banadda E.N., Smets I.Y. and Van Impe J.F. (2004). Monitoring activated sludge settling properties using image analysis. Wat. Sci. Tech., **50**(7), 281-285.

Schuler A.J. and Jang H. (2007a). Causes of variable biomass density and its effects on settling in full scale biological wastewater treatment systems. Environ. Sci. Technol. **41**(5), 1675-1681.
Schuler, A.J. and Jang, H. (2007b). Density effects on activated sludge zone settling velocities. Water Res., **41**(8), 1814-1822.
Schuler A.J. and Jassby D. (2007c). Filament content threshold for activated sludge bulking: Artifact or reality? Water Res., **41**, 4349-4356.
Sezgin M., Jenkins D. and Parker D. (1978). A unified theory of filamentous activated sludge bulking. J. Water Pollut. Control Fed., **50**, 362-381.
Sezgin M. (1982). Variation of Sludge Volume Index with Activated Sludge Characteristics. Water Res., **16**, 83-88.
Wilén B.M. and Balmér P. (1999). The effect of dissolved oxygen concentration on the structure, size and size distribution of activated sludge flocs. Water Res., **33**(2), 391-400.