Simultaneous determination of activated sludge floc size distribution by different techniques

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Abstract The activated sludge floc size distribution (FSD) is investigated by using different techniques and the results are compared against each other in order to gain insight into the FSD characteristics, as well as to detect the limitations of each measurement technique. The experimental set-up consisted of three devices coupled in series: a MastersizerS, an automated image analysis system (IMAN) and a CIS-100. The latter instrument has two measurement channels, based on time of transition (TOT), and image analysis (SHAPE) principles. In order to minimise the variability between successive measurements, the activated sludge samples collected from a pilot-scale SBR were flocculated until steady state was achieved. The results show that the MastersizerS and SHAPE devices yield similar volume weighted FSD. In contrast, the IMAN overestimated the floc size and TOT frequently showed a bimodal distribution. The number distributions from TOT and SHAPE were in agreement, while those generated by the MastersizerS were mainly located in the submicron range and those of IMAN corresponded to larger sizes. The experimental distributions show a good fit to the log-normal model. It is shown that the measurement principle is of utmost importance and results transformation may lead to data misinterpretation.

Keywords Activated sludge; floc size distribution; measurement techniques; particle sizing

Introduction

In the activated sludge process, the separation of the bioflocs from the purified effluent is influenced by the activated sludge structural properties. Detailed knowledge of floc size and size distribution is required for more effective control of process performance. Activated sludge flocs represent a heterogeneous mixture of different micro-organisms, dead cells, particulate organic and inorganic material, and extracellular polymeric substances (EPS) with a variety of different sizes, pore spaces and water channels. The morphology of the floc cross section and floc structure profiles are often described to be self-similar, suggesting that the floc can be characterized by fractal concepts (Li and Ganczarczyk, 1990).

Various methods have been established to measure the size of activated sludge flocs. The most commonly used approach is microscopy (Barbusinski and Koscielniak, 1995). It represents an excellent technique for directly examining the flocs. However, for manual microscopy, elaborate sample preparation is necessary and only a few particles can be examined. More recently, by connecting the microscope to automated image analysis software, a faster evaluation of activated sludge floc properties became possible (Grijspeerdt and Verstraete, 1997). Another technique used for characterising the activated sludge floc size and size distribution is the Coulter Counter (Andreadakis, 1993). This technique requires sample suspension in an electrolyte, which can create structural disturbance on biological flocs or might cause clogging of the aperture during the measurement of the large size particles. Due to these limitations, the Coulter technique is preferred only for small particles size and in stable environments (Li and Ganczarczyk, 1991). Hiligardt and Hoffmann (1997) used a CIS I device for floc size characterisation. A CCD TV microscope

incorporated into the basic unit allowed them to observe the settling properties and floc shape, which together with size measurements helped to optimise the solid flux in a secondary clarifier.

A laser light diffraction technique was used for on-line determination of the changes in floc structure such as fractal dimension (Waite *et al.*, 1998) or direct size distribution (Biggs, 2000). This method represents a fast and reliable technique for determining the size of flocs, which cover a relatively wide floc size range. It was demonstrated that the laser diffraction technique is able to follow the flocculation dynamics and the data can be used for modelling the activated sludge flocculation/deflocculation process by using a population balance approach (Biggs, 2000; Nopens *et al.*, 2002). Furthermore, a focus beam reflectance method (FBRM) was successfully used to measure *in-situ* the floc size distribution in a secondary clarifier of a wastewater treatment plant (De Clercq *et al.*, 2002). The authors demonstrated the devices' applicability for a wide range of solids concentrations, which actually represents the major drawback of all other alternative sizing devices.

The floc size and size distribution have been often reported in literature as outcomes of a particular measurement technique and less importance has been given to the influence of the measurement technique on the results. Since operation of various devices is based on a broad range of measurement principles, it is expected that different results are obtained. Moreover, for the case of activated sludge, due to the biological fragile and irregular structure of the flocs, the results may often lead to a misinterpretation of the data. Therefore, in this paper, a systematic investigation of the activated sludge FSD obtained from several instruments with different working principles is performed. The results are compared and the performance of each technique is critically assessed.

Methods and practical considerations of sizing devices

Three devices were coupled in series: a MastersizerS (Malvern,UK), a CIS-100 (Ankersmid, Belgium) and an automated image analysis system (IMAN) based on LabView software (NI, USA). These devices have been selected based on their different measurement principles, flow-through capabilities and because of the highly similar sample dilution requirements in sample preparation.

MastersizerS

The MastersizerS is a particle size analyser based on low-angle laser light scattering (LALLS). A 300RF lens was used in experiments corresponding to a particle size range of 0.05–900 μm. The MastersizerS software generates a volume-weighted FSD. Dilution of the
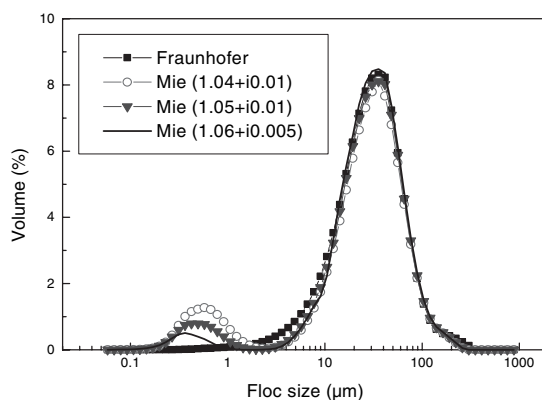
Figure 1 Comparison of the Fraunhofer and Mie theories for different real and imaginary refractive index (n+in' – where n is real refractive index and n' the imaginary refractive index)

sample prior to analysis is required, since too concentrated samples leads to multiple scattering, biassing the FSD measurements. For small particles (lower than 10 μm), the angular scattered light intensity largely depends on the optical properties of the particles and suspension medium. The refractive index dependence becomes significant because at such small sizes the light irradiated onto the particle is not completely absorbed and can emerge as a refracted ray. In this case, the Mie theory should be used instead of the Fraunhofer theory, which does not take into account the optical properties of the analysed particles. When examining the activated sludge floc size, the optical polydisperse properties are difficult to be characterized and the Fraunhofer theory has to be used (Biggs, 2000). From this viewpoint, the users may already expect errors in small particle size measurements. Figure 1 shows an example obtained for an activated sludge sample by using the two optical models. The real refractive index values were selected based on the consideration that the refractive index of the biological solids is very near to those of water (n<1.05) (Waite *et al.*, 1998).

CIS-100

The CIS-100 combines size analysis based on the time-of-transition (TOT) principle with a dynamic size and shape characterization method based on image analysis (SHAPE).

The TOT measurement covers a size range of 0.5–3,600 μm in 300 discrete size intervals, depending on the lens used. In this work, a size range of between 2–600 μm has been chosen. The device has a relatively high resolution since the measurements are made on individual particles. The results are not dependent on the optical properties of the particle and consequently, the knowledge of the refractive index of the particles is not required. For this device, it is important to correctly select the sample characteristics by using so called "*sample modes*" in order to make the size determination independent of the particle opacity. Tests performed by using different *sample modes* available in the CIS-100 software showed that for the case of the activated sludge the "Special" mode was best suited. Even then, special attention should be paid to the interpretation of the volume-based results where in certain conditions the "Special" mode may interpret two very close opaque particles as one transparent particle producing a second peak in the displayed data. When using the "Super Regular" mode, the detection algorithm does not perform the transparency check, which can be an important issue in view of the activated sludge structure. When working in "Regular" mode, small and transparent particles can be rejected.

By using the video channel of the CIS-100 (SHAPE), size and shape characterisation can be performed by acquiring images of moving particles and analysing them with the included image analysis software. The video channel is connected to a CCD video camera microscope. Acquired images are passed to a frame grabber card for analysis and are then visualised on a screen. Features such as rejection of out-of-focus particles, separation of touching particles and automatic light correction enable optimisation of the sample measurement. Software algorithms allow for calculation of a variety of parameters including Ferets diameter, area, perimeter, shape factor, and aspect ratio. The device also allows selection of different lenses with varying objective magnifications. In these experiments, a lens with a size detection range between 10–600 μm was used.

IMAN

The second image analysis system (IMAN), developed in house (Govoreanu *et al.*, 2002), permits an automated investigation of the particle size and shape parameters. Images of activated sludge samples are taken using a CX40 optical microscope (Olympus, Japan) connected to a ICD-46E CCD camera (Ikegami Electronics Inc., USA) and digitised with a frame grabber PCI – 1411 (NI, USA). The digitised images are processed on a PC, by means of specific software developed in LabView 6i (NI, USA). A 4×-magnification lens is

used in measurements and in order to enlarge the image view on the PC a 0.35× C mount adapter (Olympus, Japan) was placed between the CCD camera and the microscope resulting in an image window of size 4.5 mm × 3.5 mm. In this way, a relatively large number of particles can be observed in a single picture. The major drawback is related to the detection of small size particles, due to the limited resolution (1 pixel = 5.81 µm). IMAQ Vision Builder (NI, USA) was used for prototyping and testing the image processing algorithms. After prototyping, the algorithm was implemented in LabView, allowing us to perform real-time acquisition, analysis and storage of the images.

To perform the automatic measurements, the sample flow is directed through a rectangular cell (40 mm × 10 mm × 1 mm) fixed to the microscope. Two valves controlled by LabView allow automatic flow control, stopping the flow through cell when an image is acquired. In order to permit simultaneous measurements by the other devices, the flow is directed via a bifurcation to a tube that is not directly connected to the microscope (Figure 2).

Activated sludge samples

The activated sludge samples have been collected from a pilot-scale SBR. Three experiments have been performed over a period of 4 months. During this period the sludge characteristics changed as observed microscopically and by the measurements of floc size and settling properties. For the latest, the sludge volume index (SVI) showed values of 469 ml/g; 131 ml/g and respectively 256 ml/g. Moreover, as described by Govoreanu *et al.* (2003) a very dynamic microbial community was observed in the SBR sludge samples. The analysed samples will be further referred to as S1, S2, S3.

The set-up

For analysis, a small volume of sludge (20–30 ml) was diluted into 800 ml of filtered effluent (0.45 µm filter). The sludge concentration was controlled by fixing the obscuration level at 15–20% for the MastersizerS, which was found to correspond to an optimal or near optimal dilution ratio for all the devices used. The experimental set-up used is schematically drawn in Figure 2.

The experiments have been conducted by using an MSX17 (Malvern, UK) automated wet sample dispersion unit as reaction vessel. Preliminary experiments demonstrated that a flow rate of 3 mL/s (Biggs, 2000) and a mixing speed of 210 rpm corresponded to the optimal conditions for maintaining the flocs in suspension (data not shown). From the MSX17, the samples were passed to the MastersizerS, CIS-100 and IMAN to finally return to the reaction vessel. In this way, the variations in size that can occur due to sample preparation and manipulation were avoided.

Allen (1997) concluded that the Mastersizer had the highest reproducibility from a set of more than 25 devices investigated by using standard quartz powders. Consequently, the
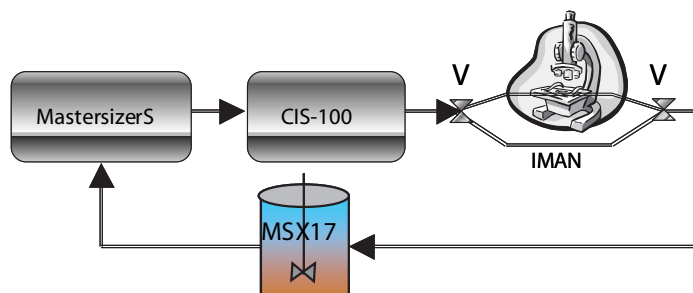

Figure 2 The experimental set-up. (V- two-way valve controlling the flow through the microscope)

experiments have been started when a stable FSD was recorded with the MastersizerS for more than 10 minutes. During this time the sludge sample was recirculated through the devices' flow cells. For each sample two experiments were performed. In the first experiment the sample was analysed for 15 minutes and every 30 seconds a MastersizerS measurement was recorded, 10,000 particles were analysed with SHAPE and every 15 seconds one image was recorded and analysed by using IMAN. The second experiment was run by maintaining the same sample in the reaction vessel and under identical conditions, except for the CIS-100 device, which was switched to the TOT channel and for which the same time sequence as for the MastersizerS was used.

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Results and discussion

An important fact to consider when comparing the results of the different techniques is that IMAN, SHAPE and TOT are all counting techniques, primarily yielding *number* distributions, whereas laser diffraction is an ensemble technique that is most sensitive to the volume contribution of each size class, and as such primarily yields *volume* distributions. Consequently, the obtained number- and volume-based distributions are the subject of the present investigations. The comparison starts with an interpretation of the observed results as they can be obtained directly from the devices and their transformations in number and volume distributions, based on a spherical shape assumption. Finally, statistical data analysis and comparison are carried out.

The measurements performed over the four months period showed a rather consistent trend of the results given by the devices even if the sludge structure changed. Particularly,
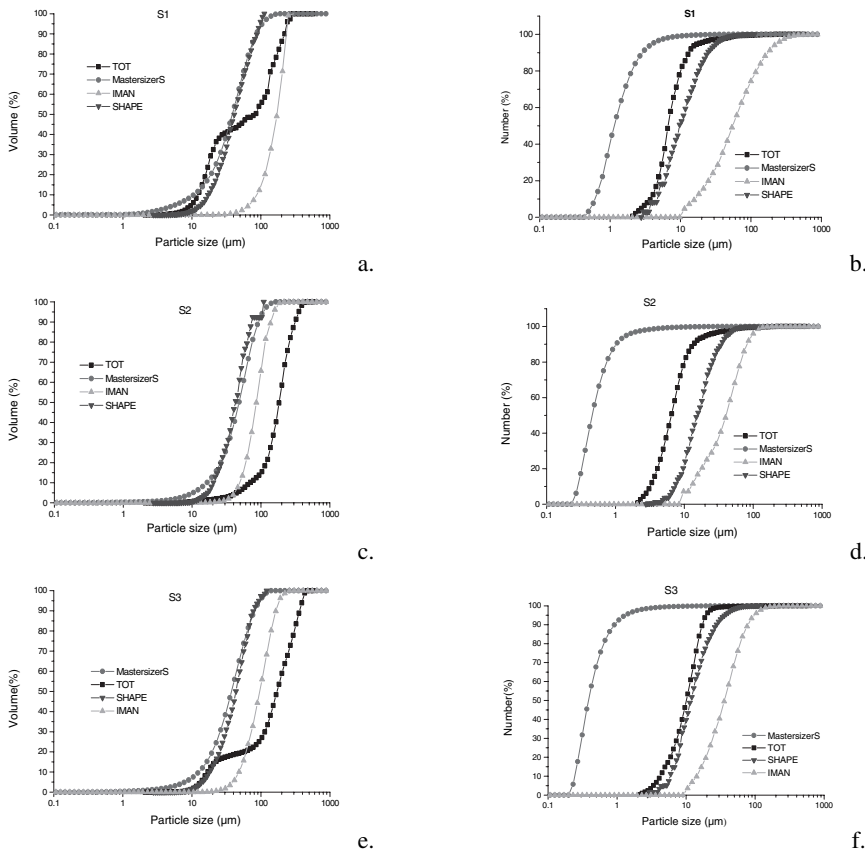
Figure 3 Comparison between volume (a,c,e) and number (b,d,f) cumulative distributions obtained from different devices for the three activated sludge samples (S1, S2, S3)

the MastersizerS and SHAPE devices yielded similar volume FSD (Figure 3.a,c,e). IMAN had the tendency to overestimate the floc size, whereas TOT showed a bimodal distribution. The latter may be due to the used measurement method, which, as discussed before, may consider two very close opaque particles as a bigger transparent one. By looking at the number distributions obtained from TOT measurements it was observed that only a few particles have been detected in that higher range (Figure 3.b,d,f) and the results were in agreement with those obtained from SHAPE measurements. The number distributions generated by the MastersizerS were mainly located in the submicrometre region. These results are believed to be unrealistic and caused by the non-linear conversion from the excessively broadened volume-weighted distribution. This is a well known feature of all ensemble techniques. On the other hand, the distributions generated by IMAN were shifted towards larger sizes, which can be explained by the limited size detection range of this method. For this reason, the IMAN technique is more suitable for analysing particles larger than 15–20 µm. In order to decrease the detection range a higher magnification lens should be used. However, this introduces a limitation in the measurement time, since the smaller image window means that fewer particles are counted at a time and a longer time is needed to obtain a reliable statistical estimate of the distributions.

The activated sludge FSD is reported to be described well by a log-normal distribution (Li and Ganczarczyk, 1990). Accordingly, this model was selected in the present work to fit the data. To allow for an easy comparison, the measured frequency distributions were first normalized. The log-normal distribution used for fitting the experimental distributions is given by:

$$f(x) = \frac{1}{x\sqrt{2\pi}\ln\sigma_g} \exp\left[-\frac{\left(\ln\left(x/x_g\right)\right)^2}{2\ln^2\sigma_g}\right]$$

(1)

where: x is the particle size, $f(x)$ is the probability density function, x_g is the geometric mean of the distribution and σ_g is the geometric standard deviation.

Most of the experimental distributions fitted well to this log-normal distribution curve. However, a known problem when fitting the log-normal distribution to the whole size range is that it is difficult to choose the minimum and maximum size limits (Allen, 1997). Consequently, fitting with a log-normal distribution will give good agreement with the real data in the middle of the distribution, whilst it may fail at each of the tails. In addition, due to the already existing differences between the size detection range of each device, fitting of the middle of the distribution is a good compromise for comparing the data. In most cases, the distributions were monomodal, which allowed fitting them to log-normal curves. However, the relatively broadened distributions observed especially for the MastersizerS are an indication of a possible bimodal distribution originating from monomodal distributions with largely overlapping tails. This fact also can be observed from the standard deviations obtained after fitting a log-normal distribution to the data. The geometric standard deviations from fitting the IMAN, SHAPE and TOT data were mainly between 0.35 µm and 0.60 µm, whereas those of the MastersizerS device were between 0.87 µm and 1.29 µm. Comparable geometric means have been obtained when fitting SHAPE and TOT number distributions and SHAPE, TOT and MastersizerS volume distributions (Table 1).

Since the geometric mean and the median of a log-normal distribution coincide, the distribution can be completely characterized by calculating its parameters according to Allen (1997):

$$x_g = x_{50}$$

(2)

$$\log\sigma_g = \log x_{84} - \log x_{50} = \log x_{50} - \log x_{16} = \log\sqrt{\frac{x_{84}}{x_{16}}}$$

(3)

Table 1 The geometric mean obtained from fitting the results with the log-normal distribution (N = number based distribution, V = volume based distribution)

	x_g N/x_gV (μm) (MastersizerS)	x_g N/x_gV (μm) (TOT)	x_g N/x_gV (μm) (Shape)	x_g N/x_gV (μm) (IMAN)
S1	1.15/51.45	6.61/–	9.58/43.41	45.32/188.5
S2	0.45/54.8	7.09/–	15.46/43.63	37.62/91.38
S3	0.38/43.49	10.98/–	11.75/44.36	35.45/101.07

Table 2 The geometric mean and geometric standard deviation obtained from the transformation based on log-normal distribution

Sample	MastersizerS		TOT		Shape		IMAN	
	σ_g (μm)	x_g N(μm)	σ_g (μm)	x_g V(μm)	σ_g (μm)	x_g V(μm)	σ_g (μm)	x_g V(μm)
S1	2.02	8.53	1.58	12.56	1.99	40.35	2.52	220.20
S2	1.89	14.13	1.71	15.53	1.78	41.47	2.15	238.15
S3	2.15	6.66	1.72	24.78	1.79	32.81	1.90	137.15

where x_{16}, x_{50} and x_{84} are the particle sizes at 16%, 50% and 84%, respectively and are extracted from the cumulative floc size distributions.

If a number distribution fits a log-normal distribution, then its transformation to volume distribution will result in another log-normal distribution (Allen, 1997), characterized by:

$$\sigma_g V = \sigma_g N \tag{4}$$

$$ln\, x_g V = ln\, x_g N + 3 ln^2 \sigma_g \tag{5}$$

Eqs (4), (5) were applied to transform the CIS-100 and IMAN fitted log-normal number distributions into log-normal volume distributions, which were then compared with original MastersizerS volume-based distributions. Similarly, the fitted log-normal volume distributions from the MastersizerS were transformed into log-normal number distributions and compared with the number-based distributions measured by using the counting techniques. By performing these transformations, new values for the geometric standard deviation and geometric mean have been obtained (Table 2).

It was observed that for all analysed samples the volume distribution given by the Malvern correlated well with the transformed CIS-100 distributions (Figure 4a). This is mainly due to the fact that the few big size particles detected by the TOT method are not accounted for any more in the log-normal volume distribution when this is calculated back from the fitted log-normal number distribution. The log-normal volume distribution calculated from the IMAN fitted log-normal number distribution showed a similar shape to the
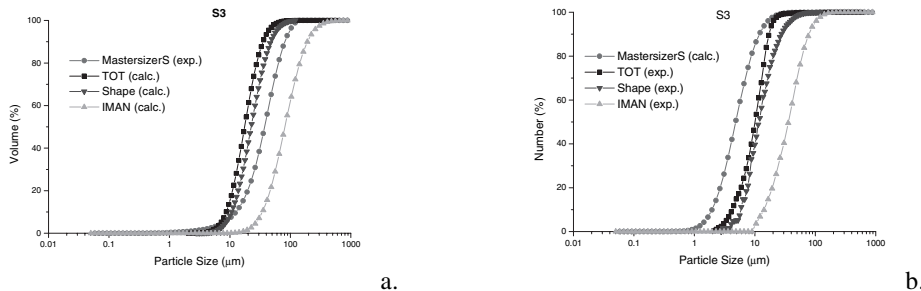
Figure 4 Log-normal volume (a) and number (b) distributions calculated from fitted log-normal number (a) and volume (b) distributions

IMAN volume distribution calculated from the experimental data based on a spherical shape assumption.

For the case in which the experimental number distributions of CIS-100 and IMAN are compared with the calculated log-normal number distributions of the MastersizerS, the latter does not show the very large number in small particles any more and tends to agree better with the results obtained with the CIS-100 device (Figure 4b).

Conclusions

By coupling three sizing devices it was possible to comparatively measure the activated sludge floc size distributions, while eliminating variations in time due to sample preparation and manipulation. All techniques used turned out to be fast and reliable methods to quantify the floc size distribution under steady state conditions. The measurement results were often in agreement, and the methods complemented each other in terms of size range. However, when the focus is on monitoring flocculation dynamics, the techniques based on laser light scattering are more suited to follow the fast changes that may occur in floc size during the process. The counting devices suffer from the longer acquisition times needed. Since devices like the MastersizerS do not usually offer visual information, coupling them to an image analysis system allows a direct visual inspection of the process evolution. The counting techniques are more accurate when the number distributions are of interest. From this viewpoint, it is concluded that the measurement principle (number or volume based) is of high importance for correct interpretation of the data. Conversion of the results may be misleading and should be used with precaution, especially for ensemble techniques.

Acknowledgements

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