A methodological approach for direct quantification of the activated sludge floc size distribution by using different techniques

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ABSTRACT

The activated sludge floc size distribution (FSD) is investigated by using different measurement techniques in order to gain insight in FSD assessment as well as to detect the strengths and limitations of each technique. A second objective was to determine the experimental conditions that allow a representative and accurate measurement of activated sludge floc size distributions. Laser diffraction, Time Of Transition (TOT) and Dynamic Image Analysis (DIA) devices were connected in series. The sample dilution liquid, the dilution factor and hydraulic flow conditions avoiding flocculation proved to be important. All methods had certain advantages and limitations. The MastersizerS has a broader dynamic size range and provides accurate results at high concentrations. However, it suffers from an imprecise evaluation of small size flocs and is susceptible to particle shape effects. TOT suffers less from size overestimation for non-spherical particles. However, care should be taken with the settings of the transparency check. Being primarily a counting technique, DIA suffers from a limited size detection range but is an excellent technique for process visualization. All evaluated techniques turned out to be reliable methods to quantify the floc size distribution. Selection of a certain method depends on the purpose of the measurement.

Key words | activated sludge, dynamic image analysis, floc size distribution, sizing techniques

INTRODUCTION

In the activated sludge process, bioflocculated microbial aggregates are essential components of the system. The activated sludge floc size and size distribution are the result of a dynamic equilibrium state between formation, transformation and breakage of the microbial aggregates moving about in the system. It represents one of the most important parameters to characterize the process performance, as it is very closely related to biological processes (e.g. simultaneous nitrification/denitrification) and physical processes like settling, compressibility and dewatering properties of the sludge. Accordingly, floc size and size distribution evaluation prove to be very useful tools to understand the influence of changes in process conditions such as substrate loading (Barbusinski & Koscielniak 1995), shear forces (Galil et al. 1991), sludge age (Andreadakis 1993) and dissolved oxygen concentration (Wilen & Balmer 1999). In spite of its practical importance, the mechanisms and mathematical modeling of activated sludge flocculation have not been well established, mainly due to the limitations in correct measurement and evaluation of floc size (Nopens et al. 2005). Due to the floc’s biological nature, the very complex and fragile structure and heterogeneous
composition, the size measurement procedure itself often influences the experimental outcome and affects the interpretation of the activated sludge floc size distribution (FSD), leading to contradictory results.

Optical microscopy is considered as an excellent technique for direct examination of the individual floc size, shape and structural properties. However, manual microscopy requires elaborate sample preparation and only few particles can be examined at once. Recently, on-line measurement techniques were developed based on automated image analysis (Grijspeerdt & Verstraete 1997; Govoreanu et al. 2004). These techniques are suitable for a faster estimation of the activated sludge floc size distribution. One of the main drawbacks of image analysis is the size detection range, which is limited to the magnification of the lens used.

Andreadakis (1993) determined the floc size distribution by using the electrical sensing zone principle. A drawback of this technique is the requirement to suspend the sample in an electrolyte, which can create a structural disturbance of biological flocs. Moreover, for larger particle sizes this is a very slow method and clogging of the aperture during the measurement might occur. Due to this limitation, the electrical sensing zone technique is used only for small particle sizes and in stable environments (Li & Ganczarczyk 1991).

Recently, a laser light diffraction technique has been used for on-line determination of the changes in floc structure expressed as fractal dimension (Guan et al. 1998) or observed directly from the size distribution (Biggs & Lant 2000). This method represents a fast and reliable technique for determining the size of the flocs and covers a relatively wide size range allowing determination of flocculation dynamics. The main drawbacks consist of the requirement for sample dilution, the assumption of sphericity and the difficulty to determine the optical properties of the biological flocs (especially in the small floc size range).

Furthermore, a focus beam reflectance method (FBRM) has been used successfully to measure the floc chord length distribution in situ in a secondary clarifier of a wastewater treatment plant (De Clercq et al. 2004). The authors demonstrated the applicability of the device for a wide range of solids concentrations (up to 50 g/l). Nevertheless, for reliable results the device needs careful manipulation with special attention to the location of the focal point of the laser beam (by changing its position different size distributions were obtained) and to the particles’ velocity in the suspension, which should be high enough to avoid particles to reside on the sensor window. Also, one needs to be careful when interpreting the obtained distributions (Pons et al. 2006).

In contrast to microscopy and image analysis, the latter techniques are indirect measurements making it inherently more prone to error introduction due to conversion of a raw signal to the eventual distribution.

Even if various techniques have been applied for sizing the activated sludge flocs, less importance seems to be allocated to the measurement errors likely to occur due to the device measurement principle or due to sample preparation and manipulation. In performing particle size measurements, most of the sizing devices start from the approximation of spherically shaped particles. Due to this, the size measurement of particles with irregular shape can be subject to measurement errors.

The aim of this paper is to investigate the activated sludge floc size distribution by using different measurement techniques connected in series and to compare the results in order to gain insight in FSD assessment as well as to detect the limitations of each measurement technique. Emphasis will be on the assessment of the effect of floc shape on the obtained size distributions. For a better quantification and evaluation of the measurement capabilities of the used devices, the experiments performed with activated sludge samples are compared with measurements of other less complex particulate systems starting from spherical glass beads and irregularly shaped sand particles.

MATERIALS AND METHODS

Equipment

A laser diffraction device type MastersizerS (Malvern, UK) and a CIS-100 (Ankersmid, Belgium) have been connected in series allowing a simultaneous measurement of the same sample. These devices have been selected based on their different measurement principle, flow-through measurement capabilities allowing on-line analysis and similar sample dilution requirements for sample preparation.
The MastersizerS device is a particle size analyzer based on the low-angle laser light scattering (LALLS) principle. The MastersizerS uses a low power He-Ne laser with a wavelength of 632.8 nm to form a collimated and monochromatic beam of light. A 300RF lens (detection range 0.05–900 μm) was used to form the far field diffraction pattern based on the principle that particles in a laser beam scatter laser light at angles that are inversely proportional to the size of the particles. In this way large particles scatter at small forward angles, whereas small particles scatter light at larger angles.

The TOT measurement principle is based on a He–Ne (wavelength 632.8 nm) rotating laser beam that scans single particles within its focus. The diameter of a particle is directly correlated to the time during which the laser beam is obscured by the particle. The TOT measurement covers a size range of 0.1 – 3,600 μm by using two interchangeable lenses.

For on-line analysis, a liquid flow cell GCM-104A (Ankersmid, Belgium) with a cross section of 1 cm x 1 cm was used.

The video channel of the CIS-100 (DIA) is an alternative analysis channel that allows for size and shape characterisation by acquiring images of moving particles and analysing them with the provided image analysis software. A special macro consisting of image acquisition, background extraction, threshold setting and selection of the focus level was applied to count and analyse around 10,000 particles during each measurement.

Sample description

Glass beads

Solid glass beads were selected for evaluating the size distributions obtained from spherical particles and for establishing dilution requirements. Two samples were analysed corresponding to a size range, as measured by sieve analysis, of 44 μm and less (sample AQ 313) and 149 to 250 μm (sample AC) respectively (Microperl®, Sovitec-Glaverbel, Belgium). The particles had a real refractive index of 1.515.

Sand particles

Two types of irregular sand particles, namely Sikron® M400 (volume median diameter $D_{50} = 7 \mu m$) and Millisil® M4 ($D_{50} = 49 \mu m$) (Sibelco, Belgium) were used for evaluating the shape effect on the measured particle size distribution. According to the distributor the median diameters were determined by laser diffraction. The particles consist of a selected silica sand (SiO₂). The real refractive index of the particles is 1.55.

Activated sludge

The activated sludge samples have been collected from a pilot-scale Sequencing Batch Reactor (SBR). The SBR performance was monitored according to a methodology described by Govoreanu et al. (2003). The sludge samples (S1) were characterised by filamentous bulking with a sludge volume index (SVI) of 469 ml/g.

Experimental set-up

For the experiments, the sizing devices were connected in series as shown in Figure 1. The experiments were conducted by using an MSX17 (Malvern, UK) automated
wet sample dispersion unit as reaction vessel. A flow rate of 3 ml/s and a mixing speed of 210 rpm were used in all experiments.

**Experimental procedure**

For each analysed sample two experiments were performed in order to investigate the two available measurement channels of CIS-100. In the first experiment, the on-line size distributions were recorded sequentially by MastersizerS and DIA. By maintaining the same sample in the dispersion unit MSX17 (938 ± 13 ml volume), a second experimental run was performed by recording the size distributions from MastersizerS and TOT.

**Sample dilution**

The devices’ dilution requirement was evaluated by using different concentrations of glass beads (AQ 313 sample) dispersed in 1 l deionized water. Different amounts of glass beads were selected on a logarithmic scale in order to obtain 10 different concentrations between 0.2 g/l and 20 g/l. The analysis sequence and device settings are presented in Table 1.

**Particle shape effect evaluation**

Sample concentrations corresponded to an obscuration level of the MastersizerS laser beam of 10 to 15%, which are within the acceptable range. The dilution was performed by using deionised water except for the case of activated sludge in which filtered effluent (0.45 μm) was used to avoid deflocculation. The experiments started when a steady state size distribution was recorded for more than 10 min. The results were evaluated as averages of the measured steady state distributions over a period of 15 min.

**RESULTS AND DISCUSSION**

**Influence of dilution medium and required dilution level**

The size distribution of raw activated sludge samples cannot be measured by any of the proposed techniques without prior dilution. The dilution step aims to enable a proper size measurement so that all particles can be measured individually without disturbing influences from neighbouring particles. At the same time, this necessary dilution operation must not lead to a modification of the size properties by e.g. flocculation or disintegration of existing flocs, which obviously affects the measurement accuracy.

By diluting activated sludge samples with deionised water, a deflocculation effect, characterized by a shift of the floc size distribution to a smaller floc size range occurred (as measured by the MastersizerS). The latter did not happen in the experiment where filtered effluent was used as the dilution medium (Figure 2 top). Subsequently, the sludge samples were subjected to sonication (10 min. at 35 W) followed by a reflocculation step under the original conditions. It was observed that for the sludge diluted with deionised water sonication had less impact on the floc size and only minor reflocculation occurred compared to the sample diluted with filtered effluent (Figure 2 bottom). The latter sample was more affected by sonication and exhibited a larger portion of reflocculation. In agreement with this, Mikkelsen et al. (1996) demonstrated that diluting the sludge with deionised water led to an increased shear sensitivity due to the desorption of cations and the reduction of the ionic strength, resulting in weaker flocs that were more susceptible to agitation, and an increased negative surface potential, thus hampering reflocculation. It is therefore concluded that the dilution medium is of high importance for a good interpretation of the results. Changing the sludge aqueous environment will lead to

<table>
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<th>Table 1</th>
<th>The analysis sequence and devices settings</th>
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<td><strong>Device</strong></td>
<td><strong>Lens/size range (μm)</strong></td>
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<tr>
<td>MastersizerS</td>
<td>500RF/0.05–900</td>
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<tr>
<td>CIS-100 (DIA)</td>
<td>CW/2–150</td>
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<td>CIS-100 (TOT)</td>
<td>A/0.1–300</td>
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destabilization of the flocs and to a misinterpretation of the flocculation process. Hence, it was decided to use filtered effluent as the only suitable dilution liquid for sludge samples in order to prevent floc breakage. When following the latter dilution procedure, the nearly constant mass mean diameter values in the first part of Figure 2 (bottom) clearly indicate that floc size measurements were very reproducible under conditions of constant mixing and pumping speeds. Moreover, measurements of the floc size distribution over a 36 day period showed high long term reproducibility, taking into account the natural variations that occur in sludge biology and physiology (Govoreanu 2004). This makes sludge floc size distribution measurement a usable tool for many applications.

In order to investigate the necessary dilution factor and to find a suitable dilution level for in-line measurements with all three measurement principles, it was decided to use glass beads, thus avoiding all possible particle size modifications during the full course of the experiment. The MastersizerS produced similar particle size distribution results for a concentration range of 0.5 to 5 g/l glass beads. At higher concentrations the distributions exhibited an increased tendency to underestimate the size of the particles, probably due to multiple scattering phenomena suggested by a high obscuration of more than the maximum level of 30% (as recommended by the operating instructions for the device). A detailed overview of the glas beads measurements at different concentrations is given in Saveyn et al. (2006). TOT measurements yielded similar results as MastersizerS up to a concentration of about 2 g/l glass beads. At higher concentrations, an additional mode of large particles appeared that increased with increasing concentration. This is considered to be an artifact, originating from the device’s measurement principle whereby two neighbouring particles at short distance are measured as one larger particle (Govoreanu et al. 2004; Saveyn et al. 2006). DIA presented consistent results within the range from 0.2 to 2 g/l of glass beads. At higher concentrations the image analysis could no longer be applied since the light was completely obscured by particles. From the findings that TOT and DIA need low particle concentrations and that MastersizerS requires relatively high particle concentrations, it may be concluded that small range concentrations are preferred to carry out simultaneous measurements with all devices. As such, it was observed that an obscuration level between 10–15% for the MastersizerS gave a suitable sample concentration for all considered devices, which was then used for the comparative measurements. This obscuration level delivered satisfying results for all samples, regardless of the nature and size distribution of the material.

**Effect of particle shape on particle size distribution**

The effect of particle shape on the obtained particle size distributions is discussed for the experiments performed with glass beads, sand particles and activated sludge. In order to circumvent data noisiness on differential graphs, arising from rescaling the different measurement outputs to a single set of size classes, cumulative size distributions will be used for the sake of clarity.
Spherical glass beads particle size distributions

A good agreement between the volume-based cumulative distributions measured by all devices was found for spherical glass beads (Sample AC, Figure 3) indicating that for the case of spherically shaped particles the devices generate very similar size distributions.

Irregular sand particle size distributions

For both investigated irregular sand particle samples, some differences occurred in the measured size distribution. However, a similar trend in the results was observed (Figure 4). The MastersizerS results showed a broader distribution as compared to the other two techniques. At the lower end of the diameter spectrum, this apparent broadening by the MastersizerS may be due to the detection limit of the TOT and DIA principles, which are not able to measure submicron particles because of hardware limitations. At the upper end of the diameter range, this broadening of the distribution can be explained by a preference of orientation. When a non-spherical particle is dragged along with the liquid flow, the longest side tends to line up with the direction of the flow, in order to reduce the drag force. This flow direction is perpendicular to the incident laser beam in the MastersizerS, and therefore diffraction occurs at the dimension with the largest size. As a result, the laser diffraction method might yield an overestimation of large particles. The distributions measured by using TOT and DIA exhibited great similarity and showed fewer large particles than in the case of MastersizerS, especially for the M4 sample. For DIA, the optical 2D projection allows a good interpretation of the true particle shape, minimizing the risk of size overestimation for non-spherical particles. For TOT, an overestimation of large particles is less likely to occur as well, since here the time of obscuration by a rotating laser is measured. As such, particles lined up with the liquid flow along the dimension with the largest size will therefore cause the rotating laser beam to intersect at a dimension with a smaller size. As a consequence, the smallest dimensions of the particles are likely to be favoured by TOT, leading to a small shift of the distribution to smaller sizes, as compared to DIA.
Activated sludge floc size distributions

The shape effect of the activated sludge flocs on the obtained size distributions was evaluated after reaching a steady state. The volume distributions obtained from the sizing techniques showed a similar trend as those obtained for the sand particles (Figure 5).

The MastersizerS showed a less broad distribution as compared with the one observed for the sand particles. One of the reasons for this is the absence of submicron particles, which are below the detection limit for TOT and DIA, in the MastersizerS measurement. Another reason was provided by detailed microscopic image analysis, which showed that the circularity of the sludge flocs was higher than that of the M4 sand particles. As such, overestimation of the presence of large particles was reduced in the case of activated sludge.

Under some circumstances, TOT yielded a bimodal volume based distribution, as depicted in Figure 5. This is due to a setting in its transparency checking feature resulting in the measurement of two close particles as one big particle. This was extensively discussed in previous work (Saveyn et al. 2006). As shown in Govoreanu et al. (2004), this phenomenon has an especially large influence on the calculated volume distributions of the activated sludge flocs. However, this second peak represents just an artefact and therefore it may be considered that its occurrence is not due to the floc shape influence. This was confirmed by measuring perfectly spherical particles with the same transparency checking settings, where a similar bimodal distribution could be obtained, which disappeared upon changing the settings from the so called ‘special mode’ to ‘regular mode’ or ‘super regular mode’.

Shape effect on particle size distribution–quantitative comparison

The term ‘broadness’ or ‘width’ of a particle size distribution can be criticized as a somewhat subjective criterion. In order to quantify the distribution width and to allow a general comparison of the results obtained for differently shaped particles as measured by the considered devices, the span function was used on a series of particle size distribution measurements. The span factor gives an indication of the distribution width, independent of the median size, and may be calculated by using the following formula:

\[
\text{Span} = \frac{D(v, 90) - D(v, 10)}{D(v, 50)}
\]

where \(D(v,10)\), \(D(v,50)\) and \(D(v,90)\) are the volume-based percentile diameters which represent 10, 50 and 90% of the particle size, respectively.

As shown in Figure 6 all devices showed narrow distributions and rather similar span values when glass beads (AQ313 and AC) were analysed. For irregular sand particles (M400 and M4), MastersizerS showed a wider distribution as compared with the other devices, in agreement with the results from Figure 4. The high span
values encountered at the MastersizerS measurement for the sand particles suggest again a very irregular particle shape, especially for the M4 sample. The more narrow distributions and low span values obtained for the technique based on image analysis (DIA) may indicate less influence of the particle shape on the distributions generated by calculation of the equivalent spherical diameter. This may be a virtue of the measurement principle of the image analysis system, which allows a full 2D visualisation of the particles and therefore yields more relevant diameter values. As such, the latter are based on the information obtained by the full projection of a particle surface, rather than just coming from a random laser beam intersection (TOT) or the diffraction at the edges of a particle (MastersizerS).

A somewhat broader distribution was measured by TOT for sand particles as compared with image analysis (DIA). Referring to the data in Figure 4, it can be concluded that this is mostly likely due to the somewhat smaller $D_{50}$ measured by the TOT method than by DIA, for the reasons stated above.

For this comparison, a new measurement of activated sludge was made as well, thereby avoiding the bimodal peak generation by TOT due to a wrong setting of the transparency check. It is clear from Figure 6 that this change was efficient in reducing the width of the distribution, as demonstrated by the low span values for TOT, which are in agreement with the ones by MastersizerS and DIA.

**Effect of operating conditions on particle size distribution**

Referring again to Figure 4, it is noted that the measured volume median diameter corresponds well with the provided value for the M400 sample (provided $D_{50} = 7 \mu m$), but not for the M4 sample (provided $D_{50} = 49 \mu m$). In the latter case, a lower median value is measured by all three techniques (measured $D_{50}$ ranging from 12 to 22 $\mu m$). Sedimentation effects, causing particles to accumulate at the bottom of the dispersion unit rather than being pumped through the system, could be a possible explanation for this discrepancy. However, the measurements in Figure 3 with glass beads of a similar density and even higher diameters, performed under the same circumstances, showed that sedimentation of large particles did not occur with the methodology from this work. A possible explanation might be that the particle size measurements by the supplier were performed in a different way (e.g. dry measurements), where other effects could have occurred (e.g. segregation of particles). This stresses the importance of selecting operating conditions that allow a representative measurement of the full sample. Therefore, pumping and mixing conditions should be selected that allow for homogeneous flow-through of the full sample, but that avoid at the same time aggregation or deflocculation. This will often be a “trial and error” procedure, where care has to be taken that the particle size distribution remains steady in time, as can be seen in Figure 2 (bottom), and that large particles don’t disappear due to sedimentation when pumping speeds are decreased.

**Optical properties for laser diffraction measurements and measurement range**

When performing static laser scattering measurements on large particles (10 $\mu m$ and more), the scattering pattern is largely independent of the optical properties of the material or the suspension medium and is only caused by diffraction at the particle edges. In the small particle range (i.e. for particle diameters smaller than 20 times the wavelength of the incident light beam, which is about 13 $\mu m$), the refractive index dependence becomes significant, given that at such small sizes the light inciding the particle is not completely absorbed and can emerge as a refracted ray. For these particles, the Fraunhofer theory being an approximation of the Mie scattering theory solely based on diffraction, no longer holds. Hence, for these small sizes the full Mie theory needs to be used for fitting spectral data to particle size distributions. However, the latter action requires the optical properties to be known. Unfortunately, for the particular case of activated sludge flocs the optical properties are difficult to determine due to their complex and heterogeneous chemical composition. To illustrate the differences that may occur due to the selection of a certain theory and associated parameters, different FSD’s were generated for one single measurement.
by using different real and imaginary refractive index values. In agreement with this, Saveyn et al. (2002) stressed the necessity to have correct refractive index information for an accurate prediction of the particle size distribution in the small size range. However, for the case of activated sludge the determination of the floc’s optical properties is not straightforward and may vary with floc composition. Therefore, the Mie theory does not offer any clear advantage in the case of sludge flocs and only increases the calculation time. Hence, the Fraunhofer theory might be best suited for activated sludge but attention should be paid to the interpretation of the small size classes’ results. This might be the case for instance when using the data to explain pore clogging of a membrane bioreactor by submicron particles or when simulating flocculation dynamics. It should be noted that none of the three measurement techniques discussed in this work are well suited for determining submicron particles and therefore other techniques will be necessary for a correct estimation of the nanoparticle fraction. A variety of techniques is available for this, such as e.g. image analysis by electron microscopy, dynamic light scattering or microscopic tracking analysis.

For TOT and DIA, the lower size range was determined by hardware limitations. For TOT, the width of the rotating laser and the electronics did not allow reliable measurements in the submicron range. For DIA, the optical properties of the lenses and the electronic properties of the CCD (0.7 μm per pixel) did not allow reliable measurements below 5 μm.

Regarding the upper size of the different measurement ranges, all techniques are well suited to measure FSD’s. By installing different lenses, TOT, DIA and Mastersizer S can measure particles up to 3,600, 3,600 and 3,500 μm, respectively, more than an order of magnitude larger than the average sludge floc size.

With respect to the broadness of the size range, the Mastersizer S offers the broadest measurement range without the need to swap lenses (theoretical ranges of 0.05–900 and 4–3,500 μm), followed by TOT (0.5–300, 2–600 and 10–3,600 μm theoretical ranges) and DIA (2–150, 10–600 and 20–3,600 μm ranges). Also, the time needed to set up the instrument after changing the lens was the shortest for the Mastersizer S instrument.
Influence of measurement principle on activated sludge floc size distributions

The DIA and TOT methods are counting techniques, primarily yielding number distributions, whereas laser diffraction is an ensemble technique that is most sensitive towards the volume contribution of each size class and as such primarily yields volume distributions. The effect of transforming the obtained FSD as measured by the devices into volume distributions (DIA, TOT) or number distributions (MastersizerS), assuming a spherical shape, was evaluated and statistically analysed by Govoreanu et al. (2004) for activated sludge samples presenting different structural properties. The results showed that the measurement principle is of high importance for the correct interpretation of the data and the conversion of the results may be misleading and should be used with caution. Special attention needs to be paid to the number distributions calculated from laser light scattering devices. As shown in the previous section, an erroneous assumption of the optical properties when using the Mie theory can lead to an overestimation of the presence of small particles. This overestimation will be magnified when transforming volume distributions to number distributions, since for the smallest particles even a minor volume contribution can result in a large number contribution.

CONCLUSIONS

From this study the following conclusions can be drawn with regard to proper measurement of particle size distribution.

- During sample preparation, the dilution level needs to be set properly so as to avoid measurement errors due to interactions between neighbouring particles. Size modifications or segregation of the sample should be avoided by selecting the right hydraulic flow conditions and by opting for a dilution medium that does not affect the surface charge properties of the sludge particles.
- The selection of the measurement method may depend on the goal of the measurement and the nature of the material. All three methods used in this study have certain advantages and limitations.
- The MastersizerS (laser diffraction) has a broader dynamic size range and can provide accurate results at higher concentrations as compared to the other methods. However, laser diffraction suffers from an imprecise evaluation of small size flocs and it is the technique most susceptible to particle shape effects producing a broader distribution for irregular, non-spherical particles.
- TOT (time-of-transition) represents a valuable alternative for floc size measurement and suffers less from size overestimation in the case of non-spherical particles. However, the TOT measurement accuracy depends on the settings of the transparency check, which can lead to artefacts in the form of bimodal distributions when not properly adjusted. Being primarily a counting technique, TOT delivers more correct information on the small particle range than the MastersizerS, which is an ensemble technique characterized by a pronounced sensitivity for large volume contributions from big particles.
- DIA (dynamic image analysis) suffers from a limited size detection range but it is an excellent technique for process visualization. Since laser diffraction devices do not usually offer visual information, coupling them to an image analysis system allows a direct visual inspection of the process evolution.

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