Journal of Chemical Technology and Biotechnology *J Chem Technol Biotechnol* **79**:610–618 (online: 2004)
DOI: 10.1002/jctb.1028

Focused beam reflectance technique for *in situ* particle sizing in wastewater treatment settling tanks

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Abstract: Due to the complexities involved with measuring activated sludge floc size distributions, this parameter has largely been ignored by wastewater researchers and practitioners. One of the major reasons has been that instruments able to measure particle size distributions were complex, expensive and only provided off-line measurements. The Focused Beam Reflectance Method (FBRM) is one of the rare techniques able to measure the particle size distribution *in situ*. This paper introduces the technique for monitoring wastewater treatment systems and compares its performance with other sizing techniques. The issue of the optimal focal point is discussed, and similar conclusions as found in the literature for other particulate systems are drawn. The study also demonstrates the capabilities of the FBRM in evaluating the performance of settling tanks. Interestingly, the floc size distributions did not vary with position inside the settling tank flocculator. This was an unexpected finding, and seriously questioned the need for a flocculator in the settling tank. It is conjectured that the invariable size distributions were caused by the unique combination of high solids concentration, low shear and zeolite dosing.
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Keywords: Focused Beam Reflectance Method (FBRM); particle size distribution; settling tank; flocculator

NOTATION

FBRM	Focused Beam Reflectance Method
PSD	Particle Size Distribution
RGB	Red, Green and Blue

INTRODUCTION

The activated sludge process is the most popular method used for biologically treating wastewater. Bacteria, essential for the process, remove the soluble and insoluble pollutants by using them as substrates for metabolism. Bacteria exist in the system as aggregates called flocs. These flocs are a heterogeneous flocculated mass of bacteria, organic and inorganic material collectively called activated sludge. Flocs typically vary in size from 10 to 600 μm.[1] The formation of flocs is essential for the operation and efficiency of the treatment process. Typically, the separation of bacteria occurs by gravitational solid–liquid separation. If flocculation fails to occur, active biomass essential for the operation of the process is lost from the system. Not only does this reduce the process efficiency but may result in excess solids being discharged into the environment.

Floc aggregation and disaggregation occur in the secondary settling tank and are due to both the floc properties and the local system hydrodynamics. Indeed, agitation may tear flocs apart but it may also bring particles together in order to aggregate.[2] For that reason, a flocculator is often built at the inlet of settling tanks and designed in such a way that the local hydrodynamic conditions enhance aggregation.

The Particle Size Distribution (PSD) of the flocs is one of the key parameters influencing the performance of the settling tank, and thus the effluent quality. The determination and evaluation of the floc size distribution are the focus of this paper.

Several sizing techniques are available to determine the PSD of activated sludge. Methods such as image analysis,[3,4] Coulter counter[5] and laser diffraction[6] are available. Unfortunately, in general, these sizing techniques cannot be applied *in situ* and thus require off-line analysis. This is very important, because the

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Contract/grant sponsor: Fund for Scientific Research — Flanders (FWO)
Contract/grant sponsor: Ghent University Research Fund
Contract/grant sponsor: Australian Research Council Discovery Grant Scheme; contract/grant number: A00104943
(*Received 22 October 2003; revised version received 11 December 2003; accepted 15 December 2003*)
Published online 16 April 2004

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sampling, sample transport and preparation prior to analysis may significantly affect the measured PSD. Laser reflection has shown the influence of fluvial sediment particle settling during transport.[7] One hour of settling, followed by re-suspension, resulted in an increase of 14% of the volume-weighted mean size. The longer the sample was allowed to settle, the larger the increase in volume-weighted mean size. Even 25% changes of volume-weighted mean sizes due to sample transport have been reported.[8] Pumping samples to the measuring device causes floc fragmentation in the tubing, thus affecting the observed PSD.[6,9,10] Another study[11] revealed that syringes used for sampling have a similar impact on the PSD. Pipettes altered the PSD more dramatically and significantly smaller particles could be observed after transfer with a pipette.

To avoid any biological effect on the PSD, samples for image analysis can be prepared by agar solidification.[5] The function of agar is to both dilute the sample and stabilise the floc by stopping the nutrient and oxygen supply to the microorganisms. Phenol has been used to quickly kill the microorganisms in the flocs and, therefore, stop any further microbial activities that may lead to changes in floc structure.[5]

Many errors are clearly introduced when determining the PSD of an activated sludge sample *ex situ*. Several waterproof laser diffraction instruments are available on the market.[9,12–15] These *in situ* laser diffraction instruments only operate at very low concentrations, which is relevant to open water systems, but restricts their use for activated sludge settling tanks. Other *in situ* particle sizing techniques are rather rare. Spectroscopy of an acoustic wave reflected on the particles can reveal information on the PSD.[16] However, this technique is still in its infancy. Alternatively, the Focused Beam Reflectance Method (FBRM) can be applied. This technique can handle very high solids concentrations, up to 50 kg m^{-3}. Applications can be found in the fields of chemical reactor technology,[17] rivers[7,18] and radioactive slurry transport,[8] but to the knowledge of the authors, no applications yet exist in wastewater treatment.

The aims of this paper are to:

(i) Provide an overview of the FBRM measurement principle, highlighting the importance of its focal point position.
(ii) Evaluate the impact of the measurement technique on the resulting PSD. Measurements from the FBRM are compared with laser diffraction and image analysis.
(iii) Demonstrate the applicability of the FBRM for wastewater treatment applications. Steady-state PSD profiling was carried out inside a secondary settling tank, providing a unique insight into its operation, and raising questions about the design of settlers, in particular the function of the flocculator.

MATERIALS AND METHODS

Focused beam reflectance method

The FBRM probe used in this research was the Lasentec FBRM M500 (Lasentec, Redmond, Washington, USA). It consists of a laser that is focused in a focal plane outside its sapphire window (Fig 1(a)). The laser rotates at a high fixed speed, ie 2 m s^{-1}, and describes a circle of 8.4 mm in diameter; particle motion is therefore insignificant to the measurement. As particles pass the focal plane, the focused beam intersects the edge of a particle and begins to backscatter the laser light. The backscatter continues until the focused beam reaches the particle's opposite edge. This backscatter is collected by the FBRM optics and is converted into an electronic signal.

The FBRM uses a discrimination circuit to isolate the time period of backscatter from one edge of an individual particle to its opposite edge. This time period is multiplied by the scan speed and the result is a distance, ie the chord length (Fig 1(b)).

The FBRM hardware stores data in 1324 channels according to the particle size and ranging between 0 and 1024 µm on a linear scale. This scale is split into two ranges, 0–100 µm and 100–1024 µm. The 400 channels in the 0–100 µm range provide finer, 0.25-µm resolution, whereas the upper 924 channels provide a coarser, 1-µm resolution, covering a wider micron range (100–1024 µm).

Data processing of the detector responses interprets both the strength and the slope of the reflected light pulse. Current instrument data processing is adjustable through the use of either F-(fine) or C-(coarse) electronics modules, the main difference being the way the backscattered signals are interpreted. When a 'continuous' surface is scanned, a single chord will be produced. Instead, a loosely packed floc will show signal interruptions; a significant decrease in the backscattered signal relative to the signal from the continuous portion of the floc surface is observed.

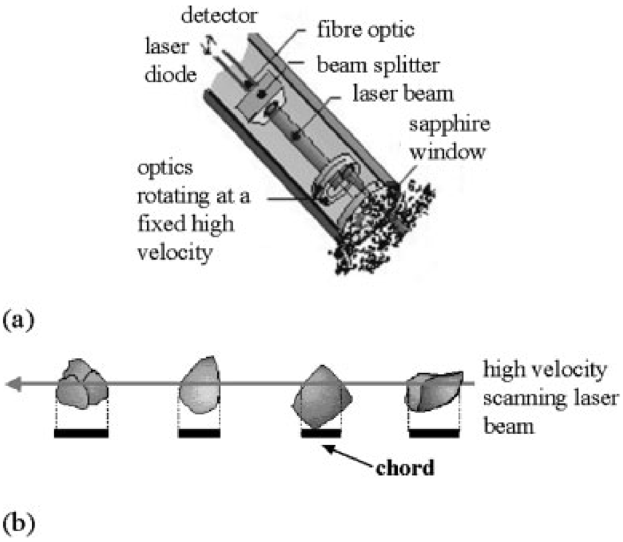

Figure 1. Principle of FBRM; (a) probe layout and (b) definition of chord length (Lasentec).

The F-electronics are more sensitive to these signal interruptions, hence small particles travelling closely together are more easily detected. Instead, the C-electronics allow the FBRM to track larger loosely packed flocs or large particles with extreme surface structures. The F-electronics are typically used for systems where the particles are primarily between 0.1 and 350 µm. The C-electronics are mainly used in applications where there are very rough surface features (larger than 20 µm). Compared with the F-electronics, the C-electronics are significantly less sensitive to changes below 20 µm.[19]

From preliminary experiments with activated sludge, it was observed that small particles may stick to the window and as a result are detected repeatedly with each scan of the laser. Such high-count frequencies at particular sizes make the PSD 'spiky', and is not a true representation of the 'real' PSD. This phenomenon was observed during *in situ* measurements in settling tanks, where the shear rate is very low ($<<5\,s^{-1}$). However, the literature[19] also mentions river sediment particles sticking to the sapphire window at shear rates up to $65\,s^{-1}$. In this work, data filtering was used to account for these undesirable effects. To investigate whether particles stick to the window, the number frequency is expressed as counts per second. Theoretically, immobile particles will be detected with the same frequency as the rotation frequency of the laser (75 Hz for Lasentec FBRM M500). Perfect immobility does not occur though and slight deviations from this 75 Hz are observed. The presence of sticking particles typically results in very spiky PSDs instead of smooth distributions obtained from representative sampling. These peaks typically occur at a few data chord lengths. An example is given in Fig 2. Due to the easily identifiable peaks, low-pass filtering is proposed to remove the outliers. In this way, statistical parameters of the PSD can be calculated more accurately.

Image analysis and laser diffraction

Two other particle sizing techniques are utilised in this work; laser diffraction and image analysis. All three techniques were applied to size inorganic particles, and only laser reflection and image analysis were used for activated sludge. For the comparative studies the experiments were conducted in a 1.2 dm^3 baffled batch reactor,[6] and the slurry was stirred with a Heidolph RZR 2020 mixer at 500 rpm.

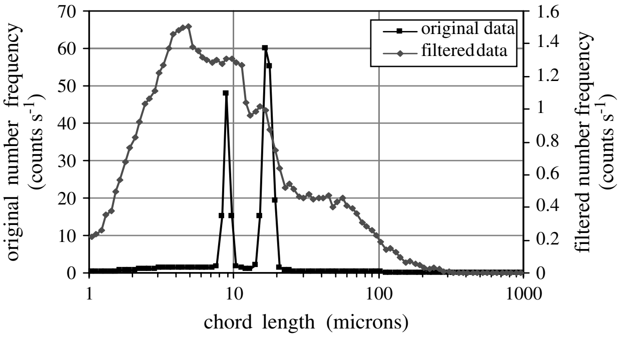

Figure 2. Filtering of PSD measured *in situ* in a secondary settling tank, eliminating the effect of sticking particles.

Particle sizing by laser diffraction was performed with the Malvern Mastersizer/E, which measures particle sizes between 1.2 and 600 µm. Because a considerable fraction of flocs in activated sludge may exceed this size range, it was decided to exclude laser diffraction from the comparative study on activated sludge. Identical concentrations were used for all subsequent experiments with other sizing devices; sample-dependent dilution was necessary to detect enough scattering with the Malvern and to avoid multiple inter-particle scattering (ie setting the right obscuration level). In total, 4000 sweeps (ie snapshots of PSDs) were recorded in one measurement. A Masterflex peristaltic pump with constant speed performed a continuous recycle of the suspension through the Malvern. The speed was chosen such that the particles did not settle in the tubes.

The micrographs used for the image analysis work were obtained with a Nikon Microphot-FXA microscope. The objectives used in this study covered magnifications of 4× and 10×. Pictures of flocs were taken with a SPOT (SP100) camera (Diagnostic Instruments Inc, Sterling Heights, USA), mounted on top of the microscope. This digital Kodak KAF-1400 camera had a CCD resolution of 1315 × 1035 pixels. At a magnification of 4× and 10×, one millimetre corresponded to 424 and 1332 pixels respectively. Further, three images were taken, ie one each in red, green and blue (RGB). The software subsequently combined the images in one 36-bit RGB colour image. To optimise the picture quality, the white balance (without particles) was computed. The optimal exposure time was determined with the software, and thus the shutter time could be specified. For all pictures white light was used, together with an ND32-filter. The flocs were mainly illuminated from above, and light from below the slides was minimised in order to achieve a black background. This approach gave the best result for determining the particle boundaries, and it is comparable to dark-field lighting.[20] Three slides were analysed in all cases. Since a submersion lens was not available, cover slips of 22 × 64 mm were used. A couple of drops were applied by means of a syringe. Because bioflocs are highly irregular, 3000–6000 particles were needed to set up a representative PSD. In contrast, only 150–350 inorganic particles were counted.

The images were analysed in ImageJ v1.24. It is freeware and can be downloaded at http://rsb.info.nih.gov/ij. The images were first converted from colour to an 8-bit grey scale. By means of a user-definable threshold set, the boundary of particles was defined. A common threshold value was adopted for the inorganic particles. However, every image of activated sludge was individually manipulated to 'optimise' the threshold. This was essential because the flocs did not show any crisp boundaries and the threshold may be different from one image to the other. The surface area was subsequently computed by means of a calibration ruler relating the number of pixels to a length scale. The surfaces and number frequencies obtained were converted to lengths and volumes with the assumption of a spherical floc geometry.

Investigated wastewater treatment plant

The FBRM was applied to a full-scale municipal wastewater treatment plant located in Brisbane, Australia. The circular settling tank had a central feed, a peripheral overflow weir, a central conical sludge hopper and blade scraper. It had a maximum depth of 5.4 m and a surface area of 308 m^2. A cross-section of the tank is shown in Fig 3. The inlet flow rate was characterised by a diurnal pattern and, during the measurement campaign, the inlet solids concentration ranged between 1800 and 2200 $g\,m^{-3}$. ZELflocc (Zeolite Australia, Australia) was dosed in the underflow line at a mass fraction of 40% in order to improve the settleability of the activated sludge. The ZELflocc (comprised of zeolite particles with an approximate mean particle size of 20 μm) is incorporated into the bioflocs, thus increasing the density and the settling velocity of the flocs.

The FBRM was deployed from the scraper bridge at different radial locations and depths.

RESULTS AND DISCUSSION

The experimental work was divided into three main tasks:

(i) In order to gain some insight into the FBRM's response to inorganic particles and biological sludge, a comparative study with other sizing techniques was first performed.
(ii) Subsequently, the issue of an optimal focal point for the laser beam was addressed prior to the full-scale PSD investigation.
(iii) The FBRM was utilised to study the behaviour of the settling tank with respect to particulate transport and flocculation.

These are presented in the following sections.

Comparative study of FBRM, laser diffraction and image analysis

Many comparative studies of the FBRM with other sizing techniques can be found in the literature. Compared with other optical methods, laser reflection is said to oversize particles smaller than 150 μm, while undersizing particles above 500 μm.[21,22]

Due to its relative novelty, the FBRM technique will be compared with other sizing methods in this study as well. The aim of the study was not to define calibration rules or 'mappings' between different techniques, but solely to increase the understanding of the behaviour of the FBRM instrument.

Two kinds of inorganic particles have been used in this study, ie glass beads and coric vinyl particles with a nominal mean diameter of 32.2 and 158.2 μm respectively (measured by laser diffraction). For these particles, the FBRM was operated under its standard settings.[23,24] Figure 4 shows the volume-based PSDs obtained from laser diffraction, image analysis and FBRM. The three distributions were very similar for coric vinyl particles and the volume-based mean sizes recorded with the different measurement techniques did not show any discrepancy. For the glass beads, however, laser diffraction and image analysis gave similar results, while the FBRM oversized the beads. This is in accordance with previous work[22] that confirmed the oversizing by the FBRM for particles smaller than 150 μm. Similar behaviour was seen in the distribution of the mixture of the latter two. The three distributions overlap each other at sizes corresponding to the coric vinyl particles. Instead, the FBRM again oversized the particles in the lower size range. In general, it can be noted that the distributions originating from image analysis were very similar to those from laser diffraction, although they measure surface-based and volume-based distributions respectively.

Biological sludge sampled from the underflow was investigated with the FBRM and image analysis method as well (data not shown). The size distributions of diluted, sonified and 'salted' sludge were measured. Table 1 summarises the solids concentrations applied to every experimental case. For sonication, a Branson Sonifier model 450 (Branson Ultrasonics Co, USA) equipped with a horn tip was used. Sonication

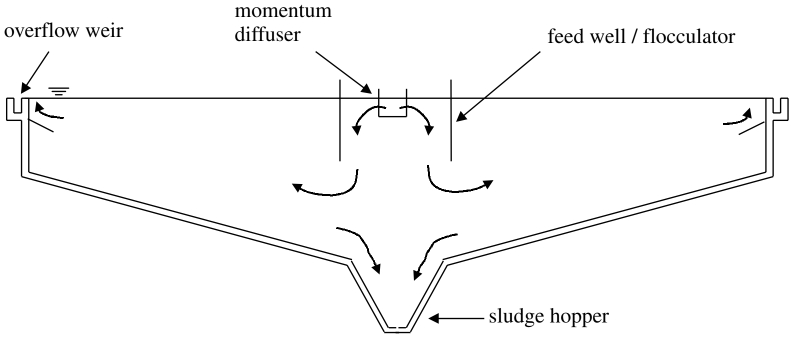

Figure 3. Cross-section of the investigated settling tank.

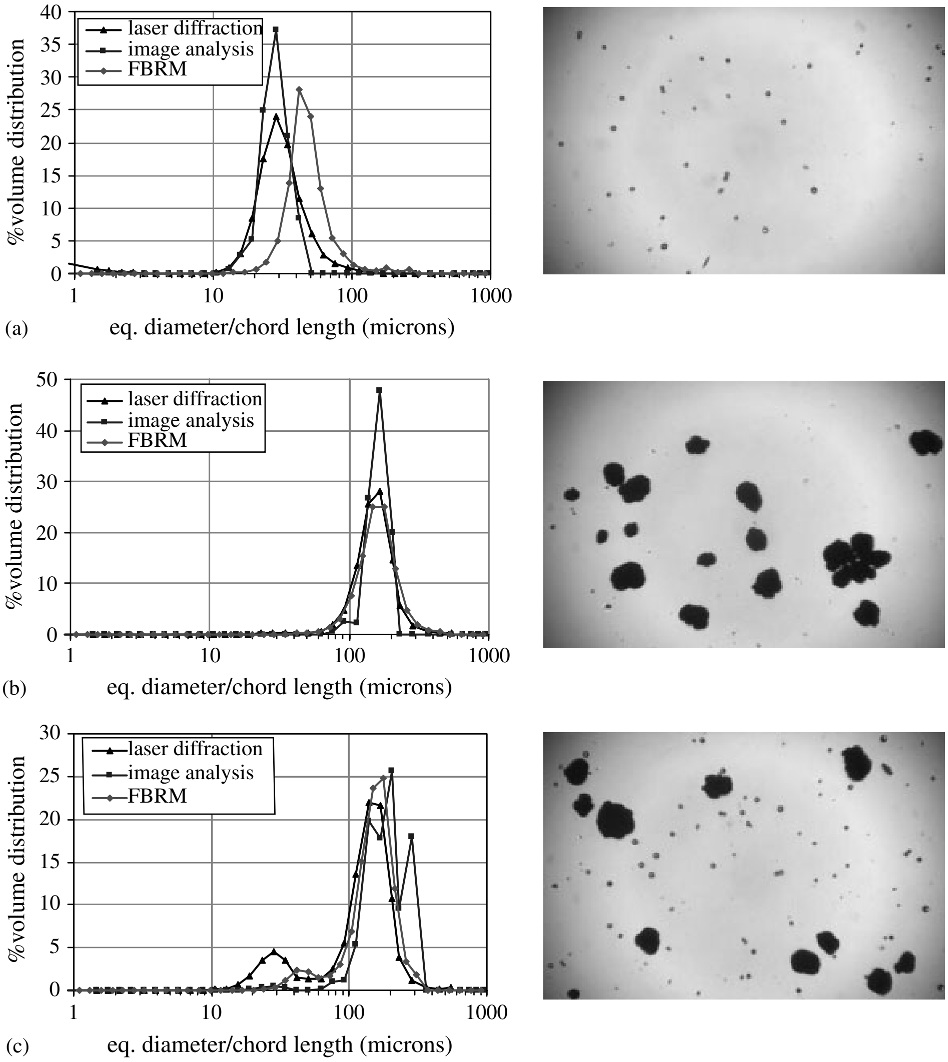

Figure 4. Volume distributions of three different systems measured with laser diffraction, FBRM and image analysis; (a) glass beads, (b) coric vinyl particles, and (c) a mixture.

of sludge was applied for 2 mins in continuous mode with a power of 65 W to a mixed liquor volume of 100 cm^3. The 'salted' sludge was prepared with 50 g NaCl dm^{-3}.

In this way, the sludges exhibited different particulate behaviours. Whereas the inorganic particles have the advantage of crisp boundaries, sludge consists of bioflocs that are extremely irregular and have diffuse boundaries. Size distributions measured by the FBRM and image analysis showed significant discrepancies and no consistent relationship could be found. It appears that no universal calibration rules between different sizing techniques exist, even though other researchers[22] have attempted to define these for multiple materials. It is concluded that a quantitative comparison between several techniques is impossible due to (i) the different measurement principles, and (ii) the natural variance inherent to biological sludges.

Table 1. Sludge concentrations used in the comparative study of measurement techniques

Sludge treatment	Solids concentration (g m^{-3})
Diluted	580
Salted	2120
Sonified	400

Optimisation of FBRM focal point position

Before starting FBRM measurements, it is essential to optimise the focal point of the FBRM probe.[21–23] This is especially true for highly irregular and large flocs (Worlitschek, private communication). The optimal focal point corresponds to the point with the 'maximum' reflectance. Several researchers[23,24] retain the standard setting for the probe, ie 20 µm inside the sapphire window. According to the manufacturer, this generally results in a maximal signal-to-noise ratio. On the other hand, others focus the beam on the window itself[17,25,26] or optimise the focal point position in order to have a maximum counts per second.[7,18,21,22,27]

The focal point was optimised in this study because bioflocs have a significantly different geometry compared with previously investigated particles. To this end, three different sludge samples were investigated that were thought to exhibit different laser reflectance responses due to their expected difference in PSD. Samples from the inlet, outlet and underflow of the Brisbane full-scale secondary settling tank were considered. The outlet only showed a solids concentration of $7\,\mathrm{g\,m^{-3}}$, whereas the samples from the inlet and the underflow had a concentration of 2500 and $5140\,\mathrm{g\,m^{-3}}$ respectively. In a gently stirred beaker, the focal plane of the laser was changed from the window to 3 mm into the suspension and one-minute countings were performed.

The results for the underflow sludge and effluent are shown in Fig 5. The results for the inlet sludge are very similar to those for the underflow sludge. From Fig 5(a) it is clear that at high solids concentrations the signal is increasingly shadowed with increasing focal distance; ie the number of counts per second decreases. This signal shadowing is mainly caused by the relatively high surface-to-volume ratio of small particles. On the other hand, the effluent solids concentration is so dilute that the reflectance signal is always detected by the FBRM optics with only a slight attenuation as focal distance increases. This behaviour is also seen in Fig 5(b) where the number-weighted mean size is shown as a function of the focal point distance. In the highly concentrated underflow sample, small particles are no longer detected (due to the smaller reflectance signal) and the size of large particles becomes underestimated. This agrees with other fundamental studies.[28] Hence, in concentrated suspensions, the focal point has to be close to the sapphire window. In this work, the optimal focal point position was selected on the basis of the maximum reflectance response.[7,18,21,22,27] Whereas an optimal focal point of 280 µm was obtained for the underflow and inlet samples, 55 µm was selected for effluent samples. Two focal points were therefore used for *in situ* measurements in the full-scale secondary settling tank: one for measurements above the solids blanket (55 µm), and one for below the blanket (280 µm).

Particle sizing in secondary settling tanks

This section investigates the use of the FBRM to determine the spatial evolution of PSDs in settling tanks. Particular attention is given to the flocculator, which is designed to provide increased floc size prior to settling.

PSD profiling was performed by sampling at different locations around the flocculator well. Measurements were conducted at afternoon inlet flow rates ($530 \pm 43\,\mathrm{m^3\,h^{-1}}$), which were the most stable that could be obtained. The underflow rate was kept constant at $201\,\mathrm{m^3\,h^{-1}}$.

Five PSDs were recorded at increasing depths inside the flocculator. Measurements outside the well and at different depths were performed as well. Whereas the total chord count frequency largely depends on the solids concentration, the local flow velocity determines the number of different particles measured and, hence, the statistical significance of a PSD. All locations were counted over a 10-min interval, except for the four upper locations outside the flocculator where 30 mins of counting was adopted. These counting times were determined in preliminary experiments. The total number of counts satisfactorily ranged between 0.4 and 4 million. Although past research[29] showed that statistically acceptable PSDs with the FBRM demand for 2000 or more particles to be sampled per second, the total number of scanned particles is considered to be appropriate here.

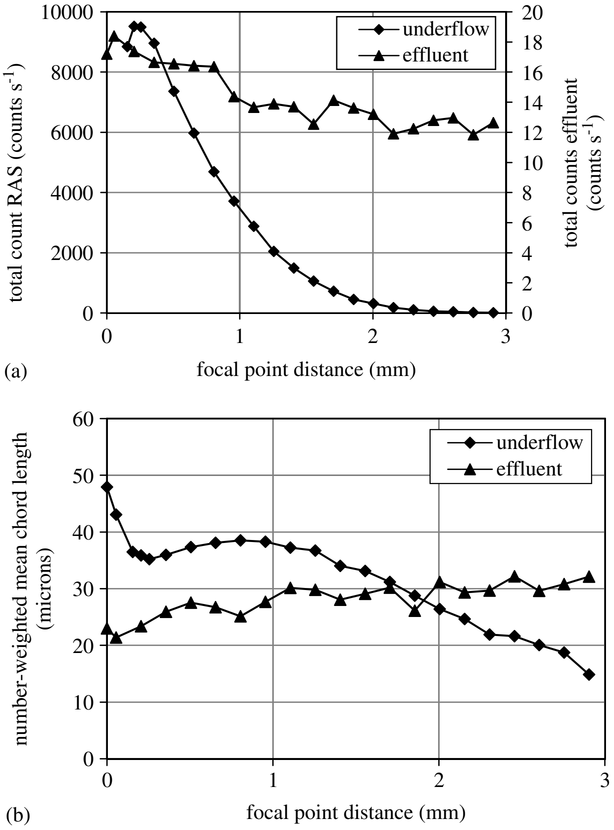

Figure 5. Influence of focal point position on (a) the total number of counts per second and (b) the number-weighted mean chord length for both underflow and effluent (0 mm represents the sapphire window).

Figure 6 shows the PSD measurements inside and outside the flocculator well. All PSDs obtained at high solids concentration (ie within the solids blanket, see Fig 6) show high counting frequencies and were very similar. Hence, they were grouped and represented by a single representative PSD in Fig 6. The number distribution shows a slight tri-modal shape. Above the solids blanket and outside the well counting frequencies are significantly lower. Therefore, the effect of solids concentration on the PSD can be easily observed by the counting frequency. Even after low-pass filtering, peaks in the number distributions still occur leading to considerable gradients in frequency. In view of these spikes, it should be mentioned that the FBRM probe was employed with the F-electronics, ie the instrument is more sensitive to the highly irregularly shaped flocs.

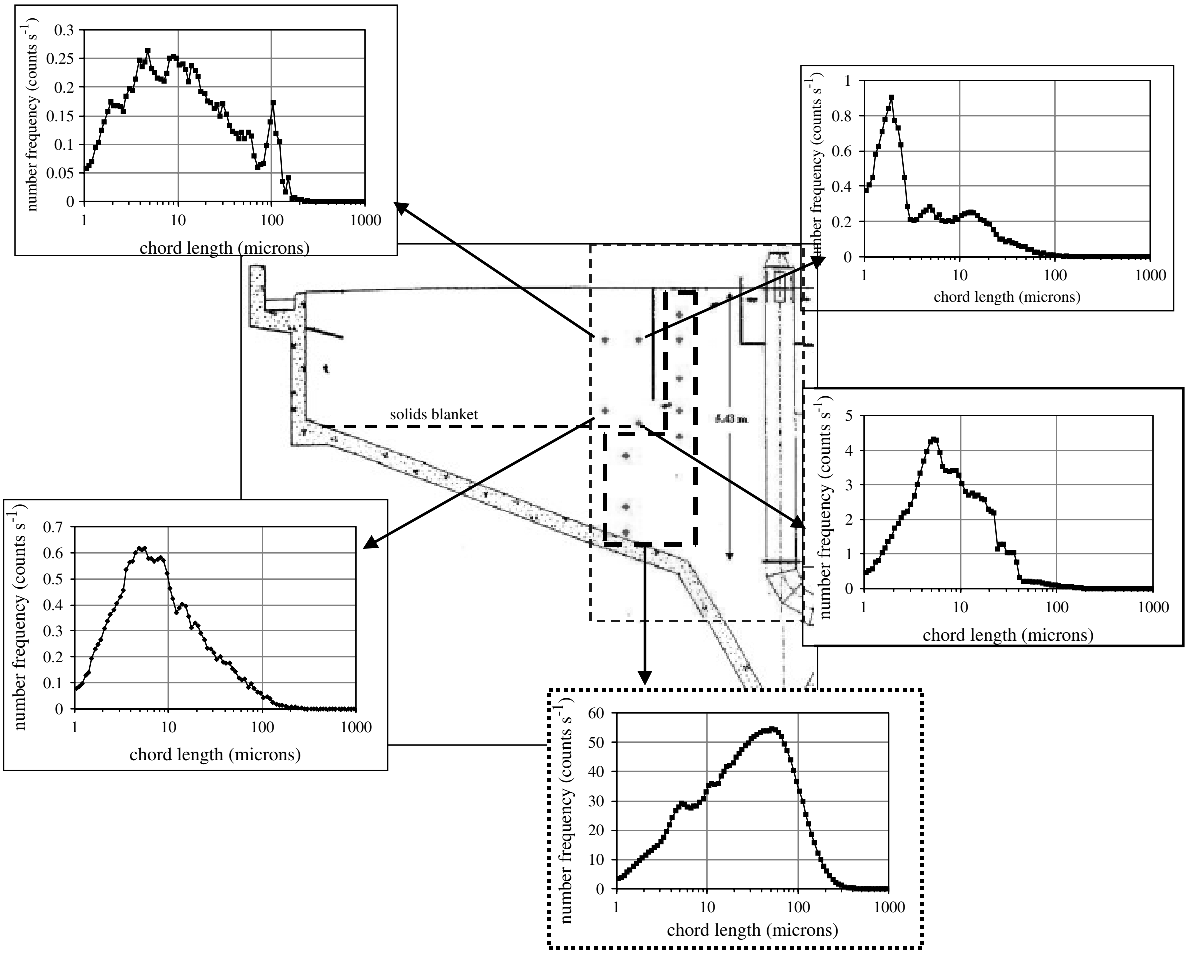

Figure 6. Measured PSDs at pseudo-constant inflow rates. The diamond symbols indicate the sampling locations in the settling tank. The dotted line delineates the region with identical PSDs.

Compared with the PSDs collected at high concentrations, it is clear that at low concentrations the PSDs are shifted to the lower chord length range. The fraction of particles larger than 100 µm is obviously significantly smaller than at locations inside the solids blanket and the flocculator well. For the most upper measurement location and next to the well this feature is most obvious (see Fig 6). A strong peak exists at small chord lengths indicating that at these locations large flocs have been removed by gravity. This is what would be expected, as the larger flocs are able to settle due to the low local fluid velocity. In contrast, the small flocs remain in suspension due to local turbulence, and are thus still observed in the PSD.

The invariable PSD inside the flocculator well is an interesting finding, and questions the role of the flocculator in this secondary settler. Three operational conditions specific to this particular settling tank may be the reason for this unexpected observation of invariable PSDs inside both the flocculator well and solids blanket.

Firstly, the zeolite dosing, intended to enhance the solids settling by increasing the floc density, may have produced 'strong' flocs with a mineral core, which may minimise floc break-up and thus re-flocculation. Secondly, high solids concentrations result in fast aggregation dynamics when shear is present,[17] such as near the inlet structure. This may mean that all flocculation has already occurred by the time the particles have reached the first sampling location in the flocculator. Thirdly, low shear rates prevailing away from the inlet structure and inside the solids blanket may result in a negligible particle collision frequency and, thus, no aggregation would occur. From 2D numerical simulations[30] the highest shear rates observed, up to 5 s^{-1}, occur near the inlet of the settling tank. This is in accordance with previous work[4,31] that reports shear rates in flocculator wells varying between 5 and 40 s^{-1}. However, shears may go down well below 1 s^{-1} inside the blanket.[30,32] Whereas high solids concentrations stimulate aggregation near the inlet structure, the extremely low shears inside the blanket would lead to negligible aggregation dynamics. The combination of these three operational conditions is assumed to result in the invariable PSDs as observed inside the solids blanket and flocculator well.

Although the floc aggregation capacity of the studied flocculator may be overestimated, the well is still important to help avoid short-circuit flows between inlet and outlet. If the well is properly designed, it also might serve to prevent damaging density currents.[33]

CONCLUSIONS

To the knowledge of the authors, this research is the first attempt to measure activated sludge floc size distributions *in situ* in a secondary settling tank of a wastewater treatment plant. Several interesting observations were made. Comparison of PSDs above and below the solids blanket clearly demonstrated the removal of the large-sized particle fractions by sedimentation. Interestingly, there was no spatial variability in PSDs inside the flocculator well, suggesting that no significant flocculation was occurring in the well. This was an unexpected observation, and several reasons have been conjectured for why this may have occurred. This study cannot draw conclusions about the flocculator design or secondary settler operation. This was not the objective. The study does, however, clearly illustrate the increased insight, which can be achieved by measuring the floc PSD *in situ*.

This research also focused on the optimisation of the focal point in order to perform statistically significant measurements in the quickest way. Different focal points resulted in different PSDs. Focal points must be located close to the sapphire window (in this study at 55 and 280 µm for low and high solids concentrations respectively).

A comparative study with laser diffraction and image analysis has also been performed. Although the measurement principles are completely different, PSDs with number-weighted mean diameters above 150 µm were similar for all measurement techniques. Below 150 µm particle sizes were oversized by the FBRM. This was in accordance with literature. The very irregularly shaped sludge flocs with diffuse boundaries however confirmed that no universal calibration rules can be set up between the FBRM and image analysis.

The FBRM has proved that it is able to measure PSDs *in situ* for a wide range of solids concentration. Most alternative particle sizers are more limited in working conditions.

ACKNOWLEDGEMENTS

The authors would like to thank Keith Barr of Brisbane Water (Queensland, Australia) for allowing the study to be carried out at one of their wastewater treatment plants. This research is financially supported by the Fund for Scientific Research—Flanders (FWO), the Ghent University Research Fund, and the Australian Research Council Discovery Grant Scheme (Project A00104943).

REFERENCES

1 Jorand F, Zartarian F, Thomas F, Block JC, Bottero JY, Villemin G, Urbain V and Manem J, Chemical and structural (2D) linkage between bacteria within activated sludge flocs. *Wat Res* **29**:1639–1647 (1995).

2 Parker DS, Characteristics of biological flocs in turbulent regimes. PhD thesis, University of California, Berkeley, USA, pp 156 (1970).

3 Nopens I, Biggs CA, De Clercq B, Govoreanu R, Wilén B-M, Lant P and Vanrolleghem PA, Modelling the activated sludge flocculation process combining laser light diffraction particle sizing and population balance modelling (PBM). *Wat Sci Tech* **45**:41–49 (2002).

4 Spicer PT and Pratsinis SE, Shear-induced flocculation: the evolution of floc structure and the shape of the size distribution at steady state. *Wat Res* **30**:1049–1056 (1996).

5 Li D and Ganczarczyk J, Size distribution of activated sludge flocs. *Research Journal WPCF* **63**:806–814 (1991).

6 Biggs CA, Activated sludge flocculation: investigating the effect of shear rate and cation concentration on flocculation dynamics. PhD thesis, University of Queensland, St Lucia, Australia pp 127 (2000).

7 Phillips JM and Walling DE, Measurement *in situ* of the effective particle-size characteristics of fluvial suspended sediment by means of a field-portable laser backscatter probe: some preliminary results. *Mar Freshwater Res* **46**:349–357 (1995).

8 Daymo EA, Hylton TD and May TH, Acceptance testing of the Lasentec focused beam reflectance measurement (FBRM) monitor for slurry transfer applications at Hanford and Oak Ridge, in *Proc SPIE Nuclear Waste Instrumentation Engineering*, 4–5 November, Boston, USA pp 82–92 (1999).

9 Bale AJ and Morris AW, *In situ* measurement of particle size in estuarine waters. *Estuarine, Coastal and Shelf Science* **24**:253–263 (1987).

10 Govoreanu R, Seghers D, Nopens I, De Clercq B, Saveyn H, Capalozza C, Van der Meeren P, Verstraete W, Top E and Vanrolleghem PA, Linking floc structure and settling properties to activated sludge population dynamics in an SBR. *Wat Sci Tech* **47**:9–19 (2003).

11 Spicer PT, Pratsinis SE, Raper J, Amal R, Bushell G and Meesters G, Effect of shear schedule on particle size, density, and structure during flocculation in stirred tanks. *Powder Technol* **97**:26–34 (1998).

12 Bale AJ, Barrett CD, West JR and Oduyemi KOK, Use of in-situ laser diffraction particle sizing for particle transport studies in estuaries, in *Developments in Estuarine and Coastal Study Techniques*, ed by McManus J and Elliott M. International Symposium Series, Olsen & Olsen, Fredensborg, Denmark pp 133–138 (1989).

13 Dyer KR and Manning AJ, Observation of the size, settling velocity and effective density of flocs, and their fractal dimensions. *J Sea Res* **41**:87–95 (1999).

14 Agrawal YC and Pottsmith HC, Instruments for particle size and settling velocity observations in sediment transport. *Mar Geol* **168**:89–114 (2000).

15 Serra T, Colomer J, Cristina XP, Vila X, Arellano JB and Casamitjana X, Evaluation of laser *in situ* scattering instrument for measuring concentration of phytoplacton, purple sulfur bacteria, and suspended inorganic sediments in lakes. *J Environ Engrg* **127**:1023–1030 (2001).

16 Reichel G and Nachtnebel HP, Suspended sediment monitoring in a fluvial environment: advantages and limitations applying an acoustic Doppler current profiler. *Wat Res* **28**:751–761 (1994).

17 Peng SJ and Williams RA, Control and optimisation of mineral flocculation and transport processes using on-line particle size analysis. *Mineral Engineering* **6**:133–153 (1993).

18 Phillips JM and Walling DE, An assessment of the effects of sample collection, storage and resuspension on the representativeness of measurements of the effective particle size distribution of fluvial suspended sediment. *Wat Res* **29**:2498–2508 (1995).

19 Neumann LE and Howes T, On-line monitoring of estuarine sediment flocculation using laser reflection, in *Proc 7th International Conference on Nearshore and Estuarine Cohesive Sediment Transport Processes (CD-ROM)*, 1–4 October, Glaucester Point, Virginia, USA (2003).

20 Grijspeerdt K and Verstraete W, A sensor for the secondary clarifier based on image analysis. *Wat Sci Tech* **33**:61–70 (1996).

21 Monnier O, Klein J-P, Hoff C and Ratsimba B, Particle size determination by laser reflection: methodology and problems. *Part Part Syst Charact* **13**:10–17 (1996).

22 Law DJ, Bale AJ and Jones SE, Adaptation of focused beam reflectance measurement to in-situ particle sizing in estuaries and coastal waters. *Marine Geology* **140**:47–59 (1997).

23 Barrett P and Glennon B, In-line FBRM monitoring of particle size in dilute agitated suspensions. *Part Part Syst Charact* **16**:207–211 (1999).

24 Dowding PJ, Goodwin JW and Vincent B, Factors governing emulsion droplet and solid particle size measurements performed using the focused beam reflectance technique. *Colloids and Surfaces A: Physicochem Eng Aspects* **192**:5–13 (2001).

25 Williams RA, Peng SJ and Naylor A, In situ measurement of particle aggregation and breakage kinetics in a concentrated suspension. *Powder Technol* **73**:75–83 (1992).

26 Heath AR, Fawell PD, Bahri PA and Swift JD, Estimating average particle size by focused beam reflectance measurement. *Part Part Syst Charact* **19**:84–95 (2002).

27 Richmond WR, Jones RL and Fawell PD, The relationship between particle aggregation and rheology in mixed silica–titania suspensions. *Chem Eng Sci* **71**:67–75 (1998).

28 Worlitschek J and Mazzotti M, Choice of the focal point position using Lasentec FBRM. *Part Part Syst Charact* **20**:12–17 (2001) (submitted).

29 Michael Y, Snowden MJ, Chowdhry BZ, Ashurst IC, Davies-Cutting CJ and Riley T, Characterisation of the aggregation behaviour in a salmeterol and fluticasone proprionate inhalation aerosol system. *Int J Pharmaceutics* **221**:165–174 (2001).

30 De Clercq B, Computational fluid dynamics of settling tanks: development of experiments and rheological, settling, and scraper submodels. PhD thesis, Ghent University, Belgium, pp 324 (2003).

31 Ekama GA, Barnard JL, Günthert FW, Krebs P, McCorquodale JA, Parker DS and Wahlberg EJ, *Secondary settling tanks: theory, modelling and operation*. IAWQ Scientific and Technical Report No 6 (1997).

32 Kinnear DJ and Deines K, Acoustic Doppler current profiler: clarifier velocity measurement, in: *Proc* 74th *Annual WEF Conference and Exposition on Water Quality and Wastewater Treatment (CD-ROM)*, 13–17 October, Atlanta, USA (2001).

33 Parker DS, Kinnear DJ and Wahlberg EJ, Review of folklore in design and operation of secondary clarifiers. *J Environ Engrg* **127**:476–484 (2001).