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# Modelling the Impact of Filamentous Bacteria Abundance in a Secondary Settling Tank: CFD Sub-models Optimization Using Long-term Experimental Data

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#### Abstract

The objective of this work was to assess the impact of filamentous bacteria on the settling velocity and rheological behaviour of activated sludge. We then identified the relevant settling and rheological model parameters to account for the impact of filamentous bulking on the prediction of sludge mixing and transport in secondary settling tanks by a computational fluid dynamics (CFD) model. We identified the relevant settling velocity and rheology model parameters influenced by the filamentous bacteria content of activated sludge. The hindered, transient, and settling parameters of the settling velocity model proposed in our previous study were estimated using measurements from batch settling tests with a novel column setup. Additionally, the rheological measurements from experiments with a rotational viscometer were used to calibrate the Herschel-Bulkley rheology model including the rheology correlations with the sludge concentration obtained in our previous study. Both settling and rheological tests were performed with sludge samples collected biweekly from the Lundtofte wastewater treatment plant in a four-month measurement campaign. Quantitative fluorescent in-situ hybridisation (qFISH) analysis was carried out on the sludge samples to quantify the volume fraction of filamentous bacteria. Based on the correlations of settling and rheological model parameter values with the volume fraction of filamentous bacteria, we identify the significant impact of filamentous bacteria on the hindered settling of activated sludge. However, no significant impact on the transient and compression settling model parameters was observed. This study also finds that microbial filaments residing inside the microbial flocs can significantly alter the rheological behaviour of activated sludge. A two-dimensional, axi-symmetrical CFD was used to assess the impact of calibration scenarios for settling and rheology under low and high abundance of filamentous bacteria on the CFD predictions. Results obtained suggest that the influence of filamentous bulking on the settling and rheology of activated sludge can affect the solids distribution and transport in the SSTs.

#### Keywords

Activated sludge; filamentous bulking; compression settling; computational fluid dynamics; rheology; secondary settling tank

## **INTRODUCTION**

Secondary settling tanks (SSTs) are located after the biological reactors in wastewater treatment plants (WWTPs) to separate the treated water from the microbial mass by means of gravity

sedimentation. The clarification and thickening performance of SSTs depend on their hydraulic features as well as the settleability of activated sludge. A malfunctioning SST with a poor quality effluent in terms of suspended solids and insufficiently thickened sludge for recycling to the reactors, impacts the sludge retention time (SRT) in the system, and potentially deteriorates the performance efficiency of the biological processes. Moreover, SSTs are the hydraulic bottlenecks of WWTPs. The efficiency of SSTs can limit the maximum flow rate entering the WWTPs under wetweather conditions.

A common operational problem in SSTs is the poor settling of activated sludge resulting from the excessive growth of filamentous bacteria, which prevents the formation of well-settling sludge (Wanner, 1994). Activated sludge flocs have a very heterogeneous structure, which consists of a variety of microorganisms as well as organic and inorganic particles and dead cells surrounded by extracellular polymeric substances (Wilén et al., 2008). The operational and seasonal variations in activated sludge units, such as dissolved oxygen concentration, nutrient deficiency and substrate limiting conditions, influence the structure of the growing flocs in bioreactors (Comas et al., 2008). However, the exact cause of filamentous bulking can be very diverse (Jenkins et al., 1993), and is not fully understood (Mielczarek et al., 2012). A common approach to identify filamentous bulking is to detect and quantify the content of filamentous bacteria in activated sludge samples by performing quantitative fluorescent *in-situ* hybridisation (qFISH) analysis (Nielsen et al., 2009).

In WWTP modelling, conventionally, the influence of filamentous bulking is accounted for by modifying the hindered settling parameters in the settling velocity formulation in the SST models (Ekama et al., 1997). Several studies have shown the relation between the morphology of bulking sludge and settling parameters (Grijspeerdt and Verstraete, 1997; Jin et al., 2003; Wilén et al., 2008). However, the question arises whether filamentous bulking can also affect the transient and compression settling as well as the rheology of activated sludge and how these effects influence the sludge distribution in the SSTs.

Computational Fluid Dynamic (CFD) models have been used to predict the internal flow and solids transport in SSTs (Deininger et al., 1998; Lakehal et al., 1999; De Clercq, 2003; Weiss et al., 2007). CFD models are computationally heavy, and they are thus mainly used for the purpose of design of new SSTs, or optimization and trouble shooting of existing SSTs. However, validated CFD models can replace expensive field experiments to calibrate and validate one-dimensional models (De Clercq, 2003; Plósz et al., 2007). The non-Newtonian behaviour of activated sludge as well as its hindered and compression settling behaviour have significant impacts on the overall solids transport in the SSTs (Ekama et al., 1997). Thus, the accurate CFD prediction of hydrodynamics and solids distribution in the tank requires inclusion of optimized setting and rheology models.

In this study, we used a validated two-dimensional, axi-symmetrical CFD model with settling velocity and rheology models developed in our previous study (Ramin et al., 2014) to simulate the sludge distribution in the SST at Lundtofte WWTP. Additionally, we used the long-term settling and rheological measurements, as well as qFISH analysis performed by Wágner et al. (2014) on the sludge samples from Lundtofte WWTP.

The main objectives of this study are (i) to assess the impact of filamentous bacteria on hindered, transient and compression settling as well as the rheological behaviour of activated sludge based on the measurements with activated sludge of varying filamentous bacteria abundance, (ii) to identify the relevant settling and rheological model parameters, and finally (iii) to investigate how the effect of filamentous bulking on the model parameters can influence the prediction of sludge mixing and transport in SSTs by the CFD model.



## MATERIAL AND METHODS

## Laboratory experiments

In this section, the settling and rheology experiments, as well as qFISH analysis (Fig. 1) performed by Wágner et al. (2014) are briefly described.



Figure 1. The laboratory set-ups for the settling (a), rheology (b), and qFISH (c) experiments performed by Wágner et al. (2014).

*Sampling*. Activated sludge samples were collected biweekly for four months from the combined recycle flow at Lundtofte WWTP (Lyngby, Denmark). The samples were used on the same day of collection for settling experiments, and some were stored in 4 °C for rheology experiments on the next day. The concentration of sampled sludge was determined using method 2540 D of Standard Methods (APHA, 1995). Additionally, some sludge was pre-treated and fixed with 4 % paraformaldehyde to preserve its initial state, and then stored at -20 °C until the qFISH analysis.

Settling tests. Settling tests were performed using the newly developed settling column set-up (Ramin et al., 2014), consisting of a large settling column (diameter = 20 cm, Height = 80 cm) with a total suspended solids (TSS) sensor (Solitax®, Hach Lange, Germany) installed at the bottom of the settling column (Fig. 1a). Prior to each settling test, the sludge sample was diluted with the SST effluent in the settling column and homogenized with coarse–bubble aeration. During each 60-minute settling test, the evolution of sludge blanket height (*SBH*) and the sludge concentration at the bottom ( $X_b$ ) were recorder. Next, the settled sludge was re-homogenized and diluted to a lower concentration. Overall, the settling tests were performed at sludge concentration in the range 1.5–4.5 g/l.

*Rheology measurements.* The rheological experiments were performed using a standard rotational rheometer (TA Instruments AR2000, USA) with a conical single-gap cylindrical geometry (Fig. 1b). The experiments were performed on sludge samples diluted with SST effluent over the concentration range of 5-12.8 g/l under shear-stress controlled conditions to obtain shear rates in the range of 0.001-250 s-1. The shear stress was applied from high to low values to minimize the sludge settling problem in the sample during the tests.

FISH analysis. The qFISH procedure was conducted using 1  $\mu$ l of fixed sample taken from the activated sludge used in the settling and rheological experiments. The total amount of bacteria and the specific filamentous bacteria were targeted with two different fluorescently-labelled probes (MPA mix: MPA 645, MPA 223, MPA 60). A confocal laser scanning microscope (LEICA SP5®, Leica, Germany) was used to assess the samples with 20x magnification and 2x zoom (Fig. 1c). 15-20 randomly chosen images were taken using the confocal microscope (Nielsen et al., 2009). The images were analysed using the *daime* (digital image analysis in microbial ecology) software (Daims et al., 2006). We note that, in the study of Wágner et al. (2014), the two dominant microbial species namely *Chloroflexi spp*. (CFX) and *Microthrix parvicella* (MPA) were identified in the activated sludge samples. In the present paper, we only present results obtained on the impact of the MPA volume fraction on the settling and rheological behaviour of activated sludge. For further information on the study, readers are kindly referred to Wágner et al. (2014).

## Numerical modelling

*Description of the SST*. The SST under study is part of the Lundtofte WWTP (Lyngby, Denmark). It is a circular centre-feed conical tank with a diameter of 24.5 m and an average depth of 4 m.

*CFD simulations of the SST.* The CFD simulations of the SST was executed in OpenFOAM CFD toolbox (OpenCFD, 2012) and using the settlingFoam solver (Brennan, 2001). The physics of the solver is based on the average Eulerian two-phase flow combined with the modified k- $\varepsilon$  model, accounting for density stratification. To predict the distribution of solids, a convection-diffusion equation derived from the continuity equation for the solid phase (drift flux model) is coupled with the momentum and turbulence equations in the solver.

To reduce the computation effort, the flow in the SST is assumed to be axi-symmetric, and only a radial segment of the tank is considered for CFD modelling. The geometry is discretised with around 6000 polyhedral grids in depth and radial directions (Fig. 2). The imposed boundary conditions are as follows. The water-surface is modelled as a symmetry-plane, i.e. normal gradients to the surface are zero. The inclined bottom is considered as a frictionless boundary to simulate the effect of an ideal scraper facilitating the sludge flow to the hopper by overcoming the wall stress, as proposed by Deininger et al. (1998). The rest of the walls were considered as no-slip with standard wall-functions to approximate the mean velocity near the wall.



**Figure 2.** The 2-D axi-symmetric mesh with about 6000 polyhedral grids generated in STAR-CCM+® and implemented in OpenFOAM for CFD simulations of the circular SST at Lundtofte WWTP.



## THE SETTLING VELOCITY AND RHEOLOGICAL MODEL

## The settling velocity model

The settling velocity model developed by Ramin et al. (2014) accounts for hindered, transient and compression settling regimes that are typically observed in the activated sludge batch settling tests. This model was developed based on an evaluation of state-of-the-art settling velocity models with measurements from the simple, novel batch settling experimental set-up explained in the previous section.

The widely used double-exponential hindered settling velocity model developed by Takács et al. (1991) and the mechanistic compression settling velocity model based on phenomenological sedimentation-consolidation theory (Bürger, 2000; Kinnear, 2002; De Clercq, 2006) with the empirical effective solids stress formulation developed by De Clercq et al. (2008) were evaluated based on their predictions of the *SBH* and  $X_b$  measurements. To evaluate these settling velocity models, the different models were implemented in a dynamic 1-D model of the settling column, developed based on a modified form of the 1-D SST model by Plósz et al. (2007), i.e. using a discretisation level of 60 layers and the numerical fluxes treated with the Godunov scheme (Bürger et al., 2011). The differential mass conservation equation is

$$\frac{\partial X}{\partial t} - \frac{\partial (v_s X)}{\partial z} = 0 \tag{1}$$

where X is the sludge concentration, t denotes time,  $v_s$  is the settling velocity model, and z is the depth in the column. We note that using the compression formulation in Eq. 1 yields a second order partial differential equation.

Results obtained by Ramin et al. (2014) show that, using Takács hindered settling velocity model with the hindered parameter ( $v_0$ , and  $r_H$ , Vesilind, 1968) estimated directly from the *SBH* measurements the predictions were shown to diverge from the *SBH* and  $X_b$  measurements during the transient and compression regime. Furthermore, including the mechanistic compression settling velocity model with the effective solids stress formulation of De Clercq et al. (2008) was shown to over-predict the  $X_b$  data when it was calibrated to the *SBH* data only. Consequently, a new power formulation for the effective solids stress was developed to improve the predictions of  $X_b$ . Finally, by applying an exponential transition formulation in the compression zone, the best prediction of  $X_b$  data could be achieved. The formulation of the settling velocity model is

$$v_{s} = \begin{cases} v_{0}e^{-r_{H}.X} - v_{0}e^{-r_{P}.X} & X < X_{c} \\ v_{0,t}e^{-r_{t}X} \left(1 - \frac{\rho_{s}}{(\rho_{s} - \rho_{f})gX} \left(\frac{X - X_{c}}{C_{1}}\right)^{C_{2}}\frac{dX}{dz}\right) & X \ge X_{c} \end{cases}$$
(2)

where  $v_0$  is the maximum settling velocity;  $r_H$  and  $r_P$  are the hindered and low concentration indices, respectively;  $v_{0,t}$  and  $r_t$  are the transient settling parameters;  $C_1$  and  $C_2$  are parameters in the compression settling model;  $\rho_s$  and  $\rho_f$  are the sludge and water density, respectively; g denotes the gravity constant;  $C_1$  and  $C_2$  are compression parameters; and  $X_C$  is the threshold compression concentration. Fig. 3 illustrates the prediction of the sludge profile in the settling column by simultanously calibrating it to the *SBH* and  $X_b$  measurements using the adaptive Markov Chain Monte Carlo (MCMC) Bayesian global optimization method DREAM<sub>(ZS)</sub> (Laloy and Vrugt, 2012). Using the DREAM<sub>(ZS)</sub> optimization algorithm, the prediction uncertainty of the settling velocity model for the estimated parameters can be obtained from the posterior parameter distributions. Fig. 4 shows the prediction accuracy of the settling velocity model for the measurements with sludge samples taken from two WWTPs in Denmark, Lundtofte (PE= 135.000, SRT = 31 d) and Lynetten (PE= 750.000, SRT = 29 d).



**Figure 3.** Prediction of the settling velocity model (Ramin et al., 2014) calibrated to the *SBH* and  $X_b$  measurements by implementing it in a 1-D settling column model (with 60 layers discretization). The lines correspond to the simulated evolution of sludge concentration in each layer.



**Figure 4.** Predictive uncertainty (95% confidence intervals of the model prediction due to parameter uncertainty) of the settling velocity model calibrated to the measurements with the Lundtofte and Lynetten WWTP sludge using the DREAM<sub>(ZS)</sub> optimization algorithm.



## The Rheological model

The rheological measurements can very accurately described with the yield-pseudoplastic type Herschel-Bulkley rheology model (e.g., Ratkovich et al., 2013):

$$\eta = \frac{\tau_0}{\gamma} + K\gamma^{n-1} \tag{3}$$

where  $\eta$  is the apparent viscosity,  $\tau_0$  is the yield stress,  $\gamma$  is the shear rate, *K* is the consistency index, and *n* is the flow behaviour index. Fig. 5 shows the predictions of the Herschel-Bulkley model for one set of measurements with different sludge concentrations. A constraint of maximum viscosity was set for the Herschel-Bulkley model for the shear rates of below  $0.01s^{-1}$  to avoid unrealistic prediction of apparent viscosity values at very low shear rate conditions.



**Figure 5.** An example on the prediction of activated sludge apparent viscosity with the Herschel-Bulkley model (Eq. 3), shown for one set of the rheological measurements (out of eight) with different sludge concentrations.

The estimated parameters of the Herschel-Bulkley model ( $\tau_0$ , *K*, and *n*) were correlated to the sludge concentration with the correlations presented in our previous study (Ramin et al., 2014), e.g. for yield stress, the following power formulation was used:

$$\tau_0 = A X^B \tag{4}$$

where A and B are the yield stress correlation parameters.

## RESULTS

## Impact of filamentous bulking on settling

*Hindered settling*. Fig. 6a illustrates the relation between the ratio of hindered settling parameters  $(v_0/r_H)$  estimated from the settling measurement sets, and the volume fraction of *Microthrix parvicella* (MPA) filamentous bacteria. The decreasing trend of  $v_0/r_H$  with increasing filament volume fraction (i.e. development of filamentous bulking sludge) is in line with conventional theory (Ekama et al., 1997).



Figure 6. Correlation of the ratio between the hindered settling parameters with the volume fraction of MPA filamentous bacteria in the activated sludge ( $\mathbf{a}$ ), and dependency of the compression settling parameter on the initial sludge concentration in each settling test ( $\mathbf{b}$ ). The hindered parameters were estimated for the eight measurement sets (one outlier), and the compression settling parameter were estimated for each settling test (3 to 4 tests for each measurement set).

Transient and compression settling. We further assessed the influence of filamentous bacteria on the transient and compression settling processes, characterised by parameters  $r_t$  and  $C_2$ , respectively (Eq. 2). No clear relation between these parameters and the volume fraction of filamentous bacteria was observed (data shown by Wágner et al., 2014). The estimated values of  $r_t$  were obtained in a narrow range (0.6–1.0 l/g) regardless of the sludge concentration and the filamentous bacteria content. On the other hand, the estimated values of  $C_2$  were scattered in a wider range (0.1–0.8, dimensionless). The dependency of  $C_2$  on the sludge concentration is further investigated in Fig. 3b, showing  $C_2$  as a function of the initial sludge concentration in the settling column tests.

Based on Fig. 3b, no effective correlation between  $C_2$  and X or distinct tendencies under bulking and no-bulking conditions (defined based on the MPA volume fraction – cut-off value: 1.5%) can be observed. The 95% confidence interval (defined by the dashed lines in Fig. 4) shows a relatively high uncertainty in estimating the value of  $C_2$  based on the initial sludge concentration of the settling tests. For the CFD model (Ramin et al., 2014), the value of  $C_2$  is determined based on the SST feed concentration (3 g/l). Therefore, later in this paper it is investigated if the variability of  $C_2$ parameter values (see Fig. 3b) causes any significant variation in the CFD simulation results of an SST.

## Impact of filamentous bulking on rheology

We investigated how the rheological behaviour of activated sludge is influenced by the presence of filamentous bacteria. The Herschel-Bulkley rheology model (Eq. 3) was calibrated to the eight rheology measurement sets (each set includes measurements with four to five different sludge concentrations). The estimated rheology parameters ( $\tau_0$ , K, and n) were then correlated to the volume fraction of MPA filamentous bacteria in the sludge. Among the three parameters, only the estimated yield stress values showed a higher degree of association with the volume fraction of



MPA (see Fig. 7a). No significant influence on the behaviour index (n) and consistency index (K) was observed (data not shown). Fig. 7a shows a decreasing tendency in the value of yield stress with the increase in MPA volume fraction at high sludge concentrations (only the concentration range of 7.5–9.5 g/l is shown here).



**Figure 7.** Dependency of yield stress of activated sludge on (a) the MPA filamentous bacteria (MPA) volume fraction for the concentration range of 7.5–9.5 g/l.

The reasoning behind the correlations shown in Fig. 7a can be explained as follows. The increase in the content of MPA filament, residing inside the flocs as the backbone of the flocs, influences the bulkiness of the flocs. As stated by Eshtiaghi et al. (2013), the increase of sludge water content can decrease the sludge viscosity. Since MPA resides in the floc, a high abundance of this filament can result in high bound water content, which would explain the observed decrease of yield stress. These results suggest that the filaments residing in the flocs mixture can impact the rheology of activated sludge.

Based on Fig. 7b, the estimated yield stress values shows an overall dependency on the sludge concentration (solid line in Fig. 7b), which confirms the proposed correlation (Eq. 4) in our previous study. However, the values are more scattered with the increase in the sludge concentration. Consequently, two power formulations are fitted to the lower and upper data points to determine the interval of the estimated yield stress values (the two dash lines in Fig. 7b). Based on Fig. 7b, good settling (or no bulking) condition, characterised with low MPA filament presence (<1.5 %) can increase the yield stress, which then corresponds to values closer to the upper curve (dashed-dotted line in Fig. 7b). On the other hand, bulking conditions (MPA volume fraction > 1.5 %) can decrease yield stress, which corresponds to values closer to the lower curve (dashed line in Fig. 7b).

## **CFD** simulations

Based on the above observations, it is investigated here how the influence of filaments filamentous organisms can impact on the hindered settling and yield stress of sludge, and thus the sludge distribution and hydrodynamics in the SST can influence the using CFD scenario simulations. Moreover the influence of uncertainty in estimating the compression parameter ( $C_2$ ) on the variation of the CFD simulation results is assessed. Table 1 summarizes the CFD simulation cases and

includes only the values of the parameters under investigation that were obtained from Figures 6a, 6b and 7b. In the first case, the impact of  $C_2$  is assessed by keeping the hindered and rheology parameters to the average values, and changing  $C_2$  to the minimum and maximum values at the feed flow sludge concentration to the SST ( $X_F = 3$  g/l) based on Fig. 6b. In the second case, the no bulking and bulking conditions are imposed to the CFD model by applying the minimum (0.1 % MPA) and maximum (extrapolated to 9% MPA) values of the hindered settling velocity parameter values based on Fig. 6a and the correlations of  $\tau_0$  to sludge concentration with the upper and lower curves in Fig. 7b.

**Table 1.** The CFD simulation cases and the values of the settling and rheological model parameters under investigation.





*Case I.* Fig. 8 illustrates the impact of the uncertainty in correlating the compression settling parameter  $C_2$  with the initial sludge concentration (Fig. 6b) on the CFD simulation results. The uncertainty of  $C_2$  is shown to result in about 10-30% variation in the pre-diction of sludge blanket height (Fig. 8a) and up to 50% in the maximum radial velocity in the density current (Fig. 8b). These results imply that the uncertainty in estimating  $C_2$  needs to be reduced probably by performing additional settling measurements.



**Figure 8.** The variation in the prediction of the vertical profiles of sludge distribution (**a**), and radial velocity (**b**) by the CFD model due to uncertainty in the compression parameter ( $C_2$ ). The profiles with solid lines are predicted with  $C_{2, max}$ , and the profiles with dashed lines are predicted with  $C_{2, max}$ . Profiles are shown with normalized height at four different radial distances from the centre of the tank.

*Case II.* To assess the impact of yield stress and hindered settling on CFD model prediction, the value of  $C_2$  is set constant to an average value of 0.34. Fig. 9 illustrates the CFD simulations based on the impact of filamentous bulking on the hindered settling parameters and yield stress. The rise in the sludge blanket height is up to 20% under the bulking condition (dashed lines in Fig. 9). Moreover, the slight increase in the flow of thickened sludge over the inclined bottom to the hopper can be observed in Fig. 9b due to the reduced yield stress under bulking condition. In general, the impact of hindered settling velocity parameter values and yield stress under bulking is not straight forward. This is because these parameters influence the complex interaction between the sludge distribution and hydrodynamics of the tank.



**Figure 9.** The predicted sludge distribution (**a**), and radial velocity (**b**) with the CFD model considering the influence of bulking (dashed line) and no bulking (solid line) on the estimated yield stress and hindered settling parameters. For more information see caption of Fig. 8.

## **CONCLUDING REMARKS**

This study investigated whether the volume fraction of *Microthrix parvicella* (MPA) filamentous bacteria, as quantified with qFISH analysis, can influence the settling and rheology of activated sludge as characterized by settling and rheology measurements. The activated sludge was sampled biweekly, during a period of four months from Lundtofte WWTP. The model parameters were estimated for the settling velocity model (Eq. 2) including hindered, transient and compression settling, and rheology (Hershel-Bulkley model, Eq. 3). Results obtained suggest that the abundance of MPA – identified as a species residing predominantly inside the microbial flocs – associates with hindered settling velocity and yield stress parameters. The obtained compression and transient settling parameters show high and low variability, respectively, in the four-month period; and, the filamentous bacteria are found not to directly relate to any of these parameters. The generality of the observations made in this study may be limited by the variability of the abundance of the filamentous bacteria during the four-month period. Therefore, future research on the association of microbial structure with functional characteristics will require higher filamentous bacteria levels than those shown in this contribution. Additionally, the impact of model structure and functionality of the events on the estimation of sludge retention time in the system should be evaluated in the future. Numerical simulations were performed using a validated CFD model from our previous study with full scale profile measurements under normal operational conditions. To further improve the prediction of the filamentous bulking effect on the sludge distribution of SSTs with the CFD model, full-scale profile measurements on the SST under filamentous bulking conditions could possibly yield more insight into the solids mixing and transport in SSTs, and would therefore be desirable to further investigate in the future.



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