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## Towards a biomolecular sensor for hindered settling velocity

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**Abstract:** We aim to answer whether it is possible to predict activated settling velocity based on biomolecular observations. Laboratory- and full-scale experimental activated sludge data retrieved combined information on the relative abundance of putative settling determinant *Cand. Microthrix parvicella*, *Chloroflexi* and *Thiothrix* as well as the associated hindered settling velocity parameter  $v_0/r_H$ . A new predictive tool for settling velocity was developed using laboratory- and full-scale measurements. Validation of the simulation model was carried out using a long-term performance and settling simulation of the Kloten-Opfikon activated sludge water resource reclamation facility (WRRF), Zürich, Switzerland. Experimental observations combined with dynamic simulation results indicate that *Microthrix* abundance can directly associate with hindered settling velocity. We also demonstrate that *Cand. Microthrix* is an ideal candidate to represent filamentous microbial populations, additionally including *Chloroflexi* and *Thiothrix* at significantly higher relative abundances than *Cand. Microthrix*.

**Keywords:** Activated sludge settling; hindered settling velocity; *Cand. Microthrix parvicella*; filamentous bulking; Benchmark Simulation Model 1; dynamic settling properties

### Introduction

Failure in activated sludge secondary settling is a major bottleneck in mitigating climate change impacts on centralised, biological wastewater treatment plants (WRRFs). Conventionally, activated sludge settling velocity parameters are assumed to exhibit irreducible parameter variability, thus requiring periodic settling tests to be undertaken. Consequently, sensor technology has been developed (Derlon et al., 2017; Ramin et al., 2014; Valverde-Pérez et al., 2017) to provide data for settling velocity parameter estimation.

Predictive tools for deteriorating hindered sludge settling velocity as a result of filamentous bulking have also been developed (Comas et al., 2008; Flores-Alsina et al., 2009). The models - based on a knowledge-based decision tree - predict the risk as a function of operating conditions conducive to the development of filamentous bulking sludge.

Activated sludge settling velocity can be modelled by accounting for hindered and compression settling regimes (Torfs et al., 2017; Plósz et al., 2018). For hindered settling, the  $v_0/r_H$  parameter ratio - with  $v_0$  set as constant ( $0.0025, \text{m d}^{-1}$ ) - can result in improve parameter identifiability (Plósz et al., 2018). Values of  $v_0/r_H$  can also be linked, notably, to the degree of sludge bulking (Wágner et al., 2015) that is the case to a much lesser extent for compression settling parameters.

A recent study (Wágner et al., 2015) - combining biomolecular and settling velocity measurements - show that not all filamentous organisms influence hindered

settling velocity to the same extent. The relative abundance of the lipid consuming filamentous microorganisms, *Cand. Microthrix* appears to exhibit the most significant impact on the hindered settling velocity. This was not the case for *Chloroflexi*, other filamentous organisms that were encountered in the same samples in more than double the relative abundance of *Cand. Microthrix*. The compression settling parameters showed no significant association with filamentous abundance. Hence, we postulate that *Cand. Microthrix* can be used as a surrogate microbial community to predict the  $v_0/r_H$  parameter ratio.

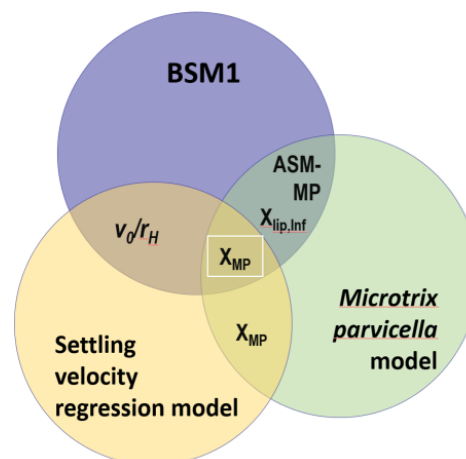
A process model of growth kinetics of *Cand. Microthrix* (ASM-MP) on an influent wastewater fractionated to include lipids was presented by Hug et al. (2006). ASM-MP could predict the measured seasonal variations of *Cand. Microthrix* abundance, which varied over two orders of magnitude, over a three-year duration (Kloten-Opfikon WRRF, Switzerland – Hug et al., 2006).

The BSM1 is a simulation model platform for implementing, analysing and evaluating impacts and performance of existing and novel control strategies applied to WRRFs (Alex et al., 2008). BSM1 includes information on long-term operational conditions, including WRRF influent characteristics, water temperature and solids retention time.

The main aims of this contribution comprise (a) quantifying the relative abundance of *Cand. Microthrix* and the hindered settling velocity parameters in samples collected in full-scale and laboratory-scale activated sludge systems (b) developing a simulation model on the BSM1 platform; and (c) carrying out scenario simulations and global sensitivity analysis.

## Material and Methods

The research layout in terms of simulation model development is shown in Fig. 1.



**Figure 1** Structure of simulation model development.

*Sampling for assessing the correlation of microbial abundance and hindered settling velocity.* Full-scale activated sludge samples were collected from the Lundtofte activated sludge WRRF (Lyngby, Denmark; design capacity: 135000 PE, SRT=31 days, carbon/nutrient removal) in a period of January-May (Wágner et al., 2015). Microbial abundance and hindered settling velocity data is shown in Fig. 2. Average hydraulic loading and solid concentration to the SSTs were 173 m<sup>3</sup>/h/SST and 5.49 g/l, respectively. Laboratory-scale experiments were carried out in an EBPR

resource recovery system operated as a continuous flow system, including, in a sequential order, 2 anaerobic completely stirred tank reactors (total volume of anaerobic CSTRs,  $V=1.25$  L), 1 settler, an aerobic CSTR (total volume: 5.8 L) and a final settler, operated in continuous-flow mode at HRT=18 h and SRT= 8 days; Valverde-Pérez, 2015). Batch settling tests were carried out by employing a column sensor prototype (Ramin et al. (2014), providing measured sludge blanket height (SBH) and TSS concentration data – the latter measured in the bottom of the column using a TSS SOLITAX (Hach, USA) infrared sensor.

*Sampling and experimental data collection for WRRF modelling.* Long-term full-scale activated sludge samples and operational data were collected in the Kloten-Opfikon WRRF (Switzerland) – insofar unpublished data by Hug (2006). Microbial abundance data is shown in Fig. 3.

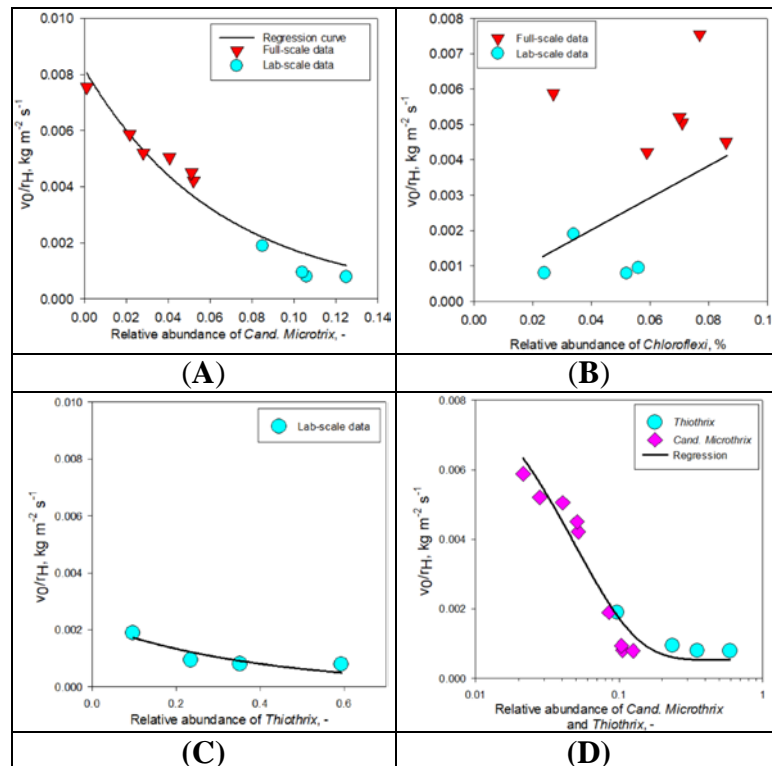
*Biomolecular analysis.* Quantitative fluorescence *in situ* hybridization (qFISH) was performed according to Nielsen et al. (2009).

*Regression analysis and global sensitivity and uncertainty analysis (GSUA).* Values of  $v_0/r_H$  are predicted in the simulation model using a two parameter exponential regression equation obtained using SigmaPlot 13 (Fig. 2a). GSUA was carried out on the BSM1 platform using linear regression of Monte Carlo simulation results (Saltelli et al., 2008).

*The simulation model platform.* The simulation model for the Kloten-Opfikon WRRF was developed using the BSM1 platform. The process arrangement comprises a primary clarifier, a nitrogen removal activated sludge unit, secondary settling tank (SST) and a sludge digestion unit. The biokinetic processes are described using the Activated Sludge Model No. 1 (ASM1) and the *Cand. Microthrix* growth kinetic model by Hug et al. (2006). Additionally, the simulation model also included an interface between ASM1 and ADM1, accounting for lipids and *Cand. Microthrix* growth and decay. The fractionation of total influent COD concentration data was carried out based on Roeleveld and van Loosdrecht (2002). To predict *Cand. Microthrix* concentration ( $X_{MP}$ ), the influent fractionation was extended by introducing particulate lipid concentration ( $X_{Lip}$ ) – the latter being 27.3, 32.8 and 36.4% of the total influent  $X_S$  (Hug et al., 2006) for three scenario simulations. Additional two state-variables and associated reaction rate equations were implemented, including soluble lipid ( $S_{Lip}$ ) and stored lipid ( $X_{StoLip}$ ). Hindered and compression settling as well as other transport phenomena in the SST were modelled using the one-dimensional advection-dispersion model by Plósz et al. (2018) implemented on the iCFD platform (Guyonvarch et al., 2016). The simulation model was implemented in Matlab-Simulink (Matworks, USA).

## Results and Discussion

Despite the diversity of activated sludge systems sample origins, values of  $v_0/r_H$  plotted as a function of the relative abundance of *Cand. Microthrix* show a strong correlation ( $R^2=0.97$ , Fig. 2a).



**Figure 2** Relative microbial abundance versus hindered settling parameter,  $v_0/r_H$ , and relative abundance of (A) *Cand. Microthrix*, (B) *Chloroflexi*, (C) *Thiothrix* and (D) combined *Cand. Microthrix* and *Thiothrix*. Solid lines represent regression curves.

The relative abundance of *Chloroflexi* – with data obtained from laboratory- and full-scale samples – shows a weak correlation with hindered settling ( $R^2=0.51$ ). The positive slope is contradictory to general theory, which states that higher filamentous abundance corresponds to lower hindered settling velocity. *Thiothrix* – found above the limit of detection only in the laboratory-scale system – shows a very broad relative abundance range (10–60%) that also effectively correlates with hindered settling velocity ( $R^2=0.87$ ). The hindered settling velocity range obtained in the laboratory-scale experiments is comparably narrow, and limited to severe filamentous bulking conditions with lowest  $v_0/r_H \sim 0.005\text{--}0.008$  [ $\text{kg m}^{-2} \text{s}^{-1}$ ]. Hence, both *Cand. Microthrix* and *Thiothrix* can be associated with hindered settling velocity (Fig. 2a and 2c). Additionally, the regression analysis on the combined relative abundances of *Cand. Microthrix* and *Thiothrix* (Fig. 2d) indicates  $v_0/r_H$  becoming independent of filamentous abundance above  $\sim 10\%$ , irrespective of the filamentous microbial community composition.

To postulate a constitutive relation, the following aspects of filamentous abundance and its potential impacts on hindered settling velocity are summarized:

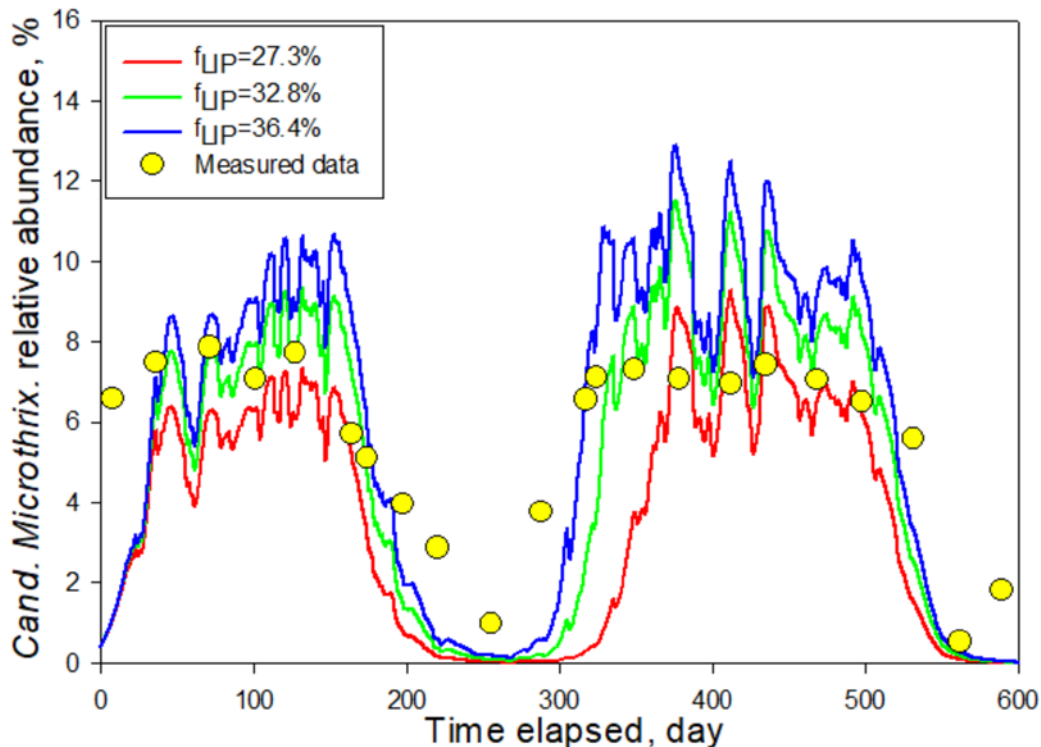
- It may not be a combined effect exhibited by all the filamentous bacteria, and may not translate into a proportionally large effect on settling velocity – see full-scale data obtained for *Chloroflexi* compared to *Cand. Microthrix*;
- The effect may be more specific to the prevalence of selected microorganisms – see data (Fig. 2a) obtained in very diverse systems, i.e. in full- and laboratory scale operated under different SRT conditions. Considering the above, we propose *Cand. Microthrix* as an ideal proxy for the filamentous microbial community fraction and predictor for  $v_0/r_H$  – see the overlapping *Cand. Microthrix* and *Thiothrix* abundance and settleability data at ~9 % relative abundance (Fig. 2d), whilst, above this, *Thiothrix* is up to 60% - a trend also predictable based on the *Cand. Microthrix* abundance data obtained below ~9 %.

To test the validity of this hypothesis, scenario simulations of the Klotten-Opfikon WRRF (Fig. 3) were carried out using the regression model identified (Fig. 2d,  $R^2=0.97$ ), i.e.

$$\frac{v_0}{r_H} = y_0 + \alpha \cdot e^{(-\beta \cdot f_{MP})}. \quad \text{Eq. 1}$$

Considering constant  $v_0=0.0025$  in the settling velocity model by Plósz et al. (2018),  $r_H$  is calculated as

$$r_H = y_0 + \frac{v_0}{\gamma \cdot e^{(\beta \cdot f_{MP})}}. \quad \text{Eq. 2}$$

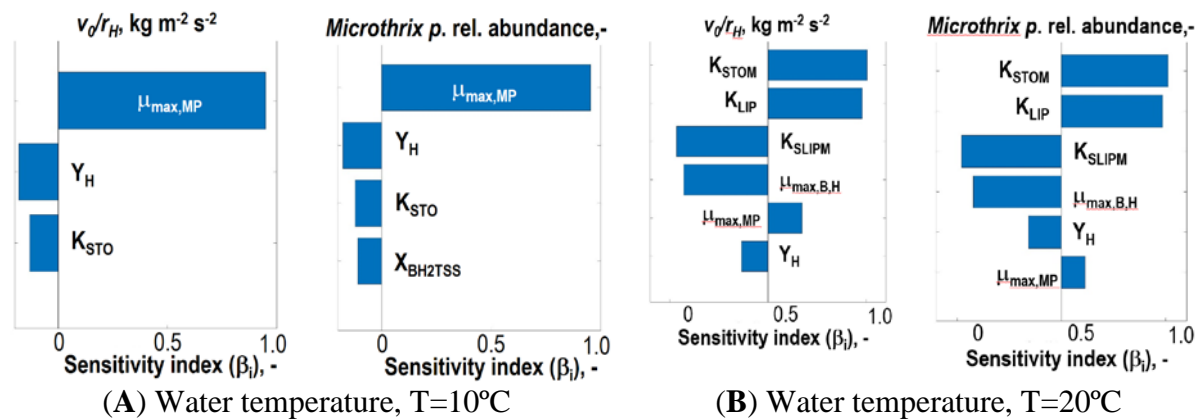


**Figure 3** Predicting *Cand. Microthrix* abundance (%) in the Klotten-Opfikon full-scale activated sludge WRRF – (A) Measured and scenario simulation results for relative *Cand. Microthrix* abundance (%); (B) Global uncertainty analysis – measured and simulated (95% confidence interval) results.

Long-term predicted *Cand. Microthrix* abundance closely agrees with measured abundance data in full-scale, thereby supporting the main hypothesis. The model predictions are influenced by six independent information sets, including lipid

fractionation in influent wastewater (Hug et al., 2006), microbial growth kinetics model (BSM1: Alex et al., 2008; ASM-MP: Hug et al., 2006), SRT (Kloten-Opfikon WRRF operation data), sludge settleability (regression model, Fig. 2d) and SST simulation model (Plósz et al., 2018).

As expected, prediction of *Cand. Microthrix* abundance, and prediction of  $v_0/r_H$  values display the same parameter sensitivity (Fig. 4). Additionally, parameter sensitivity rankings are dependent on water temperature (Fig. 4). At  $T=20^\circ\text{C}$ , settling velocity is mostly impacted by the lipid storage, whereas at  $T=10^\circ\text{C}$ , the maximum specific growth rate of *Cand. Microthrix* is the most dominant.



**Figure 4** Parameter sensitivity rankings for the WRRF simulation model outputs relative abundance of *Cand. Microthrix* and hindered settling velocity parameter obtained under cold (A,  $T=10^\circ\text{C}$ ) and warm (B,  $T=20^\circ\text{C}$ ) water temperatures. Sensitivity indices obtained in GSA using BSM2. Only the significant parameters ( $\beta_i \geq 0.1$ ) cases are shown.

## Conclusions

Experimental observations combined with simulation results indicate, for the first time, that *Cand. Microthrix* can serve as an ideal surrogate microbial population for the filamentous community fraction and can directly relate to hindered settling velocity. From the perspective of technology development, this outcome is significant because it indicates the feasibility of developing an innovative biomolecular sensor for direct prediction of settleability. In conjunction with the quantification of the WRRF influent lipid fraction, this method can offer a robust method for predicting hindered settling velocity parameters in model-based decision support systems used in activated sludge treatment.

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