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# Soft-sensing of oxygen and $N_2O$ gas mass-transfer using biomass particle characteristics

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**Abstract:** Methods for high-frequency monitoring of aeration efficiency – presently a major knowledge gap – can offer considerable advantages to develop efficient aeration control in the future. Such advanced control architecture for reactor aeration cannot only increase the energy efficiency of water resource recovery facilities, but can also be used to reduce $N_2O$ greenhouse gas emission. This study assess the relative contribution of particles propensities on gas mass-transfer – a significant knowledge gap – in terms of microbial floc/aggregate size, density (impacted by e.g., polyphosphate storage) and compactness (impacted by the relative abundances of filamentous vs. floc-forming communities). Mechanical aerators exhibit bubbles, forming in the proximity of surface paddles under relatively high-energy density relative to gas mass-transfer over bubbles carried over to the bulk liquid (low energy density conditions). This hydrodynamic behaviour effectively creates a spatial variability of aeration efficiency and alpha factor ($\alpha$) - a phenomenon assessed here, for the first time. Additionally, factors influencing the prediction of $N_2O$ stripping are evaluated to develop online soft-sensors for aeration and greenhouse gas emission control.

**Keywords**: Activated sludge treatment; fine-bubble aeration; alpha factor; biomass particle characterisation; filamentous bacteria *Ca.* Microthrix and *Thiothrix*; Meta-modelling.

## INTRODUCTION

Control of aeration processes can pose the highest impact on greenhouse gas emission potential of WRRF via increased energy intensity of treatment ($CO_2$) and through $N_2O$ emission. Conventionally, factors impacting gas mass-transfer and aeration efficiency, include surfactants, dissolved COD, the MLSS concentration, apparent sludge viscosity, and solids retention time (SRT). More recently, the interaction between filamentous bacteria and flow in the boundary film around bubbles, and its negative impacts on $O_2$ and $N_2O$ aeration efficiency ($\alpha$-factor) were demonstrated (Qiu et al., 2024a). WRRFs with anoxic and/or anaerobic selectors can effectively remove the bulk of soluble COD and surfactants, thus reducing their adverse impacts on $\alpha$ in the aerated reactors. In turn, it is primarily biomass-related effects that are hypothesised to impact $\alpha$.
The behaviour of biomass particles and their interactions with filaments can change as a function of solid density (compactness). As a result, aerobic granular sludge can accommodate high relative abundance of filamentous organisms (e.g., *Thiothrix*) without causing bulking – an effect observed under lower solids density, i.e., activated sludge (de Graaff et al., 2020). This raises the questions as to how floc/aggregate compactness can impact gas mass-transfer – a hypothesis test defined in this study. This physical-chemical effect can cascade into a metabolic impact via the resulting in-rector DO concentration through aerobic filamentous microbial growth kinetics. In fact, this is demonstrated to be a self-invigorating cycle (Qiu et al., 2024b). That is, the higher the relative filamentous abundance, the lower the aeration efficiency and DO concentration are. The latter of which can selectively favour filamentous bacteria growth through high relative oxygen affinity (Jenkins et al. 2004). These in-reactor conditions can potentially enhance biogeochemical $N_2O$ production pathways. The combined effects of increased aeration intensity and greenhouse gas emission can then lead to the excessive carbon-footprint of WRRF operations.
The main objectives of this study include (A) the assessment of gas mass transfer under high and low aeration energy density using laboratory-scale observation data; (B) characterisation of biomass floc particles (microscopy–image analysis) and settling velocity; (C) identification of dynamic meta-models for $\alpha$-factors to predict oxygen and $N_2O$ gas mass-transfer processes.

**RESULTS**

To demonstrate the variability of aeration intensity, here we draw from our research on surface aerated activated sludge reactors (Qiu et al., 2024a). The value of α can reach 1 (Fig. 1) when the observation point is close to the surface aeration rotor. This result agrees with theory (Rosso & Stenstrom, 2006), i.e., as a result of very high energy densities under advective interfacial transport flow regime, the high shear rate can significantly reduce the thickness of the laminar film. The spatial variability of energy density, and thus α, is a characteristic of aerated reactors – similar to e.g., bubble size distribution.

Laboratory-scale experiments (more on the setup in Bakos et al., 2025) were carried out to assess floc biomass (Fig. 2a) and settling velocity properties (Fig 2b). The progressive increase in the relative abundance of filamentous bacteria (*Thiothrix*) resulted in reduced hindered settling velocity ($v_0/r_H$) that is hypothesised to impact aeration intensity. Values of α in municipal wastewater were measured (Fig. 3a) under high- and low energy density. Subsequently, α values were converted to hyper-parameters *a* and *b* (Fig. 3b,c) using the meta-model identification method (Qiu et al., 2024b). The impacts of settling velocity and biomass concentration on α characteristically differ as a function of aeration energy density. Under high energy density and biomass concentration (orange data, Fig. 3b,c) no clear relationship between $v_0/r_H$ and α (*a* and *b*) can be established. Filamentous organisms can interfere with the oxygen gas mass-transfer through the laminar layer (Qiu et al., 2024b, 2025). The relative impact of floc biomass properties (Fig. 2) on α – quantified in a sensitivity analysis using standardised regression coefficient values ($\beta_j$). Firm- and compact as well as less-compact floc fractions are found as the most influential factors that can increase α (positive $\beta_j$). It is hypothesised that such floc particles can contribute to the break-up of larger bubbles and can increase the effective diffusive transport over the laminar boundary layer by increasing the exchange of liquid around bubbles. These two particle fractions exhibit high levels under well-settling conditions (Fig. 2a), and are reduced at the expense of increased filamentous abundance. Hindered settling velocity under bulking – associated with low biomass concentration in the surface aerated reactor – have an equally strong but negative impact on α. In fact, results suggest that $v_0/r_H$ has a higher impact on α than MLSS. The negative impact of settleability is additionally supported by the high negative $\beta_j$ values for SVI, DSVI and total filament abundance. MLSS concentration, as expected (Baquero-Rodríguez et al., 2018; Bencsik et al., 2022), can increase or decrease α at low and high concentration levels, respectively (Fig. 3d). Importantly, the relative impact of MLSS concentration is found to be lower than that for firm-compact, less-compact particle fractions and $v_0/r_H$.

To explain the uncertainties in and accurately predict α, the meta-model complexity is assessed. Besides the dependence on $v_0/r_H$ and MLSS concentration (Model 1), the impact of four different biomass particle propensities on α are assessed. Under high aeration energy density, besides MLSS concentration and $v_0/r_H$, accounting for the impact of firm/compact (Model 2) and less-compact pin-point flocs (Model 3) and weak pin-point flocs (Model 4) can significantly increase the model predictive accuracy and precision under both well-settling (Days 0-26) and filamentous bulking (Days 26 – 37). Similarly, under low aeration energy density, Model 4 can ensure model predictive accuracy under both well-settling and filamentous bulking conditions. $N_2O$ gas mass-transfer is modelled according to (Qiu et al., 2024b; Bakos et al., 2025). Dissolved oxygen and $N_2O$ concentrations in the lab-scale reactor (Fig. 4c,d) are predicted using the meta-model for α combined with the biokinetic soft-sensor, employing *Thiothrix* and Ca. Microthrix microbial communities (Bakos et al., 2025).

## FIGURES

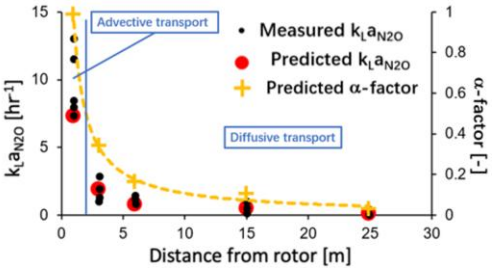


**Figure 1** Values of estimated α-factor and $K_La_{N2O}$ values in the Lynetten WRRF, Denmark – (full-scale measurements – black dots).

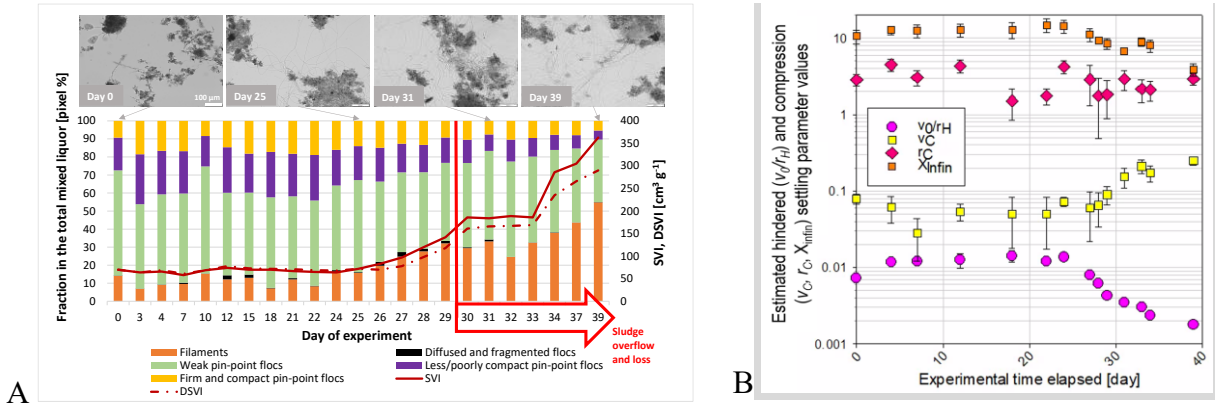


**Figure 2** Characterisation of biomass flocs – (A) microscopy-image analysis, sludge volume index (SVI), diluted sludge volume index (DSVI); (B) estimated settling velocity parameters.

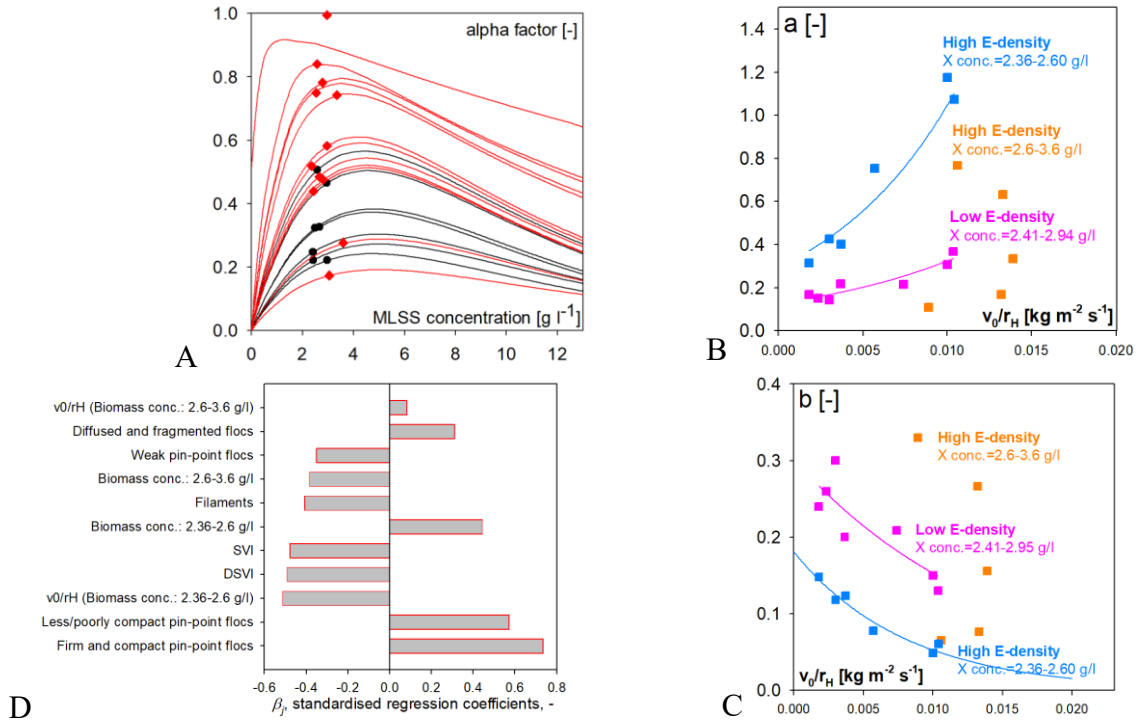


**Figure 3** Meta-model identification – (A) measured α values under high (red) and low (black) aeration energy density used to estimate meta-model hyper parameters (*a*, *b*); (B) Impact of $v_0/r_H$ on the alpha factor hyper parameters (*a*, *b*) – correlation assessment; (C) Sensitivity analysis using standardized regression – factors influencing the α; Note: High filamentous bacteria abundance corresponds to low $v_0/r_H$ values.

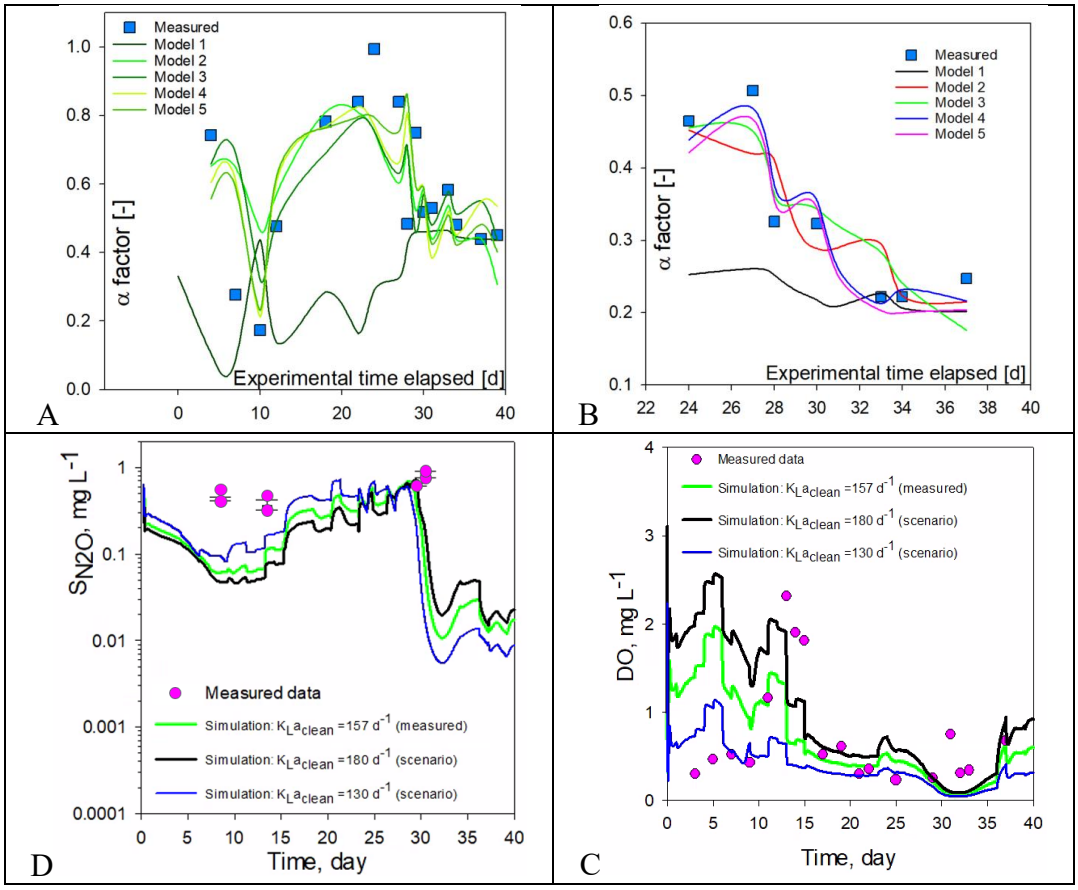


**Figure 4** Measured and predicted α values plotted as a function of experimental time obtained under high- (A) and low (B) aeration energy density. Experimental and simulated results for dissolved oxygen (C) nitrous oxide (D) concentrations.

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