

Understanding the effects of bulk mixing on the determination of the affinity index: consequences for process operation and design

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ABSTRACT

The main objective of this study is to demonstrate the importance of mixing conditions as a source of inconsistencies between half-saturation indices in comparable systems (e.g. conventional activated sludge, membrane bioreactor) when operated at different conditions or different scales. As proof-of-principle, an exemplary system consisting of the second vessel of a hybrid respirometer has been studied. The system has been modeled both using an integrated computational fluid dynamics (CFD)-biokinetic model (assumed to represent the physical system) and a tanks-in-series, completely stirred tank reactor biokinetic model (representing the applied model). The results show that different mixing conditions cause deviations in the half-saturation indices calculated when matching the applied model to the physical system performance. Additionally, sensor location has been shown to impact the calculation of half-saturation indices in the respirometric system. This will only become more pronounced at larger scales. Thus, mixing conditions clearly affect operation and design of wastewater treatment reactors operated at low substrate concentrations. Both operation and design can be improved with the development and application of integrated CFD-biokinetic or compartmental models.

Key words | activated sludge modeling, affinity constant, half-saturation constant, kinetic constants, Monod function, wastewater treatment

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INTRODUCTION

The Monod equation (Monod 1942) has been used extensively for modeling the majority of biological treatment processes in the wastewater treatment field (Henze *et al.* 2000). Essentially, the Monod equation describes microbial growth through two kinetic parameters: the maximum specific growth rate and the so-called 'affinity constant' (often termed the 'half-saturation constant'). Given that the affinity constant will mainly affect the description of the treatment performance at low substrate conditions and hitherto high-rate activated sludge processes (with high substrate concentrations) have been predominant, its importance on process evaluation and design has not been relevant. However, as effluent regulations are becoming more stringent and emerging wastewater treatment processes that operate with low substrate concentrations (e.g. simultaneous nitrification-denitrification (SND), partial nitrification, Anammox processes) are increasingly

implemented at full-scale, there is a growing interest in determining the values of affinity constants for different substrates, biological reactions and organisms. Given the high variability in the values reported for affinity constants in different studies for similar processes (Arnaldos *et al.* 2015), research on this area has only partially improved the understanding of wastewater treatment systems. The reported variability has been pointed out to be due to the inherent limitations of the affinity-constant concept. In order to reflect the real nature of this concept, the more appropriate terminology of 'half-saturation index' has been adopted (Arnaldos *et al.* 2015). The novel terminology reflects the fact that the 'affinity constant' is in fact not a constant, but rather an index influenced by numerous and heterogeneous phenomena. In fact, three distinct categories of phenomena influencing half-saturation indices have been identified: advective, diffusional and biological. Arnaldos

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et al. (2015) concluded that the variety of factors influencing the half-saturation indices has caused the – seemingly – conflicting evidence when determining their values for different treatment systems. A factor that was pointed out to potentially hold more importance than it is accredited with when determining half-saturation indices in certain systems is bulk mixing conditions (pertaining to advective phenomena). This factor and its impact on the half-saturation indices have not been systematically addressed, unlike other diffusional and biological factors, which have been granted significantly more attention by the wastewater treatment research community. However, it could be argued that, from a logical perspective, macroscopic factors should be analyzed in the first place before descending to microscopic phenomena.

Furthermore, direct and indirect evidence that mixing conditions are important when determining half-saturation indices can be found in the published literature. For instance, Munch *et al.* (1996) studied an SND system and found that the half-saturation index for oxygen ($K_{O,A}$) in ammonia-oxidizing bacteria (AOB) of 4.5 mg O₂/L represented the behaviour of the system appropriately. This high value has been suggested to result from limitations in oxygen diffusion through the activated sludge flocs (Daigger *et al.* 2007). In biofilm systems, however, maximum $K_{O,A}$ values for AOB of 2 mg O₂/L are commonly considered (Lackner & Smets 2012); it is therefore logical to assume that values significantly higher than 2 mg O₂/L are not due to diffusional limitations only but include other effects as well. One of these effects could be hypothesized to be mixing conditions (advective limitations) in the bulk liquid. This is consistent with SND being reported in many oxidation ditches, systems known to suffer from mixing limitations (Zhang & Qi 2007; Liu *et al.* 2010). Likewise, in membrane bioreactors (MBR) half-saturation index determination can be affected by mixing limitations. For instance, biological phosphorus removal has been demonstrated in fully aerobic MBR systems (Rosenberger *et al.* 2002; Verrecht *et al.* 2010); this phenomenon is consistent with mixing limitations causing some fractions of the reactor to become anaerobic and as such to stimulate biological phosphorus removal. It is important to note that high liquid density and viscosity stemming from high solids concentrations in MBR systems can augment the effects of mixing limitations. Another recent study has shown that the observed process rates for organic matter removal changed in a moving bed biofilm reactor by an order of magnitude depending on the mixing energy imposed on the system (Nogueira *et al.* 2015). Furthermore, in the same study, the

observed process rates for ammonium consumption were shown to also change significantly despite the fact that AOB were located deep in the biofilm (where they could be thought of as not being affected by mixing conditions) (Nogueira *et al.* 2015). These observations could impact the calculated values of the half-saturation indices, especially when dealing with low substrate concentrations.

Beyond mixing deficiencies in full-scale and pilot systems, advection limitations have also been detected in more simple and downsized systems such as respirometers. Mixing limitations are not a common assumption in respirometric systems, but the consequences could be significant. For instance, Stenstrom & Song (1991) have reported different nitrification rates at different mixing speeds in respirometric assays. This observation was also made by Chu *et al.* (2003), who reported decreasing half-saturation indices for dissolved oxygen at increasing mixing energies. This could be due to initial mixing limitations in the respirometer, enhanced oxygen dissolution in water, as well as to floc breakup due to excessive mixing. Additionally, Esquivel-Rios *et al.* (2014) reported very high half-saturation indices for different substrates as compared to values reported in the literature when using micro respirometers. This was linked by the authors to insufficient mixing in the respirometric vessels.

The main objective of the present study is to evaluate the importance of mixing limitations as a source of inconsistencies between half-saturation indices in similar or even identical systems when operated at different conditions or different scales. As a proof-of-principle, an exemplary simple system consisting of the second vessel of a hybrid respirometer (Vanrolleghem & Spanjers 1998) was chosen for this study, but the conclusions are more generic and hold for bioreactors at different scales in general. This system has been modeled using both (1) an integrated computational fluid dynamics (CFD)-biokinetic model and (2) a very simple tanks-in-series, completely stirred tank reactor (TIS-CSTR)-biokinetic model. Given the simplicity of the system and the maturity of CFD nowadays, the hybrid CFD-biokinetic model, as a state-of-the-art modelling approach, is assumed to be the best feasible representation of the real physical system (describing advective effects in detail), while the TIS-CSTR-biokinetic model represents the applied model (strongly simplifying the advective process). Different mixing conditions have been imposed on the CFD-biokinetic model and the associated process performance has been obtained; this can be considered to reflect the actual performance of a real respirometer. Then, this process performance has been compared to that obtained

by the TIS-CSTR-biokinetic model; this would represent the results obtained when using an applied simplified model. Subsequently, the half-saturation indices have been adjusted in the TIS-CSTR-biokinetic model in order to represent effluent quality for each mixing condition in the real system, mimicking one of the current process model calibration practices. The results for the calibrated half-saturation indices and their relative deviation from the real values have been presented and discussed. The importance of mixing conditions in half-saturation index determination has been further illustrated by calculating the resulting index as a function of sensor placement in the respirometer. Finally, the potential consequences of not taking into account mixing conditions in half-saturation indices in process operation and system design have been discussed.

MATERIALS AND METHODS

Description of the exemplary respirometric system

The system investigated for the effects of mixing conditions on half-saturation indices is the second vessel of a hypothetical hybrid respirometer. This system has been chosen to have a cylindrical shape, with a volume of 2 L (12 cm diameter, 20 cm height, 1 cm inlet and outlet diameter) (Figure 1(a)); these dimensions are representative of actual hybrid respirometers. An agitation device was not included as part of the hypothetical system given that the mixing associated with the incoming flow is commonly assumed

to be sufficient for complete mixing. This system has been chosen for its simplicity, which allows it to be modeled fairly easily using a CFD-biokinetic approach in a representative manner of actual process conditions. As stated in the Introduction, the governing assumption is that the CFD-biokinetic model represents the physical system in sufficient detail, while the TIS-CSTR-biokinetic model represents the applied model.

The influent composition has been chosen to mimic a typical mixed liquor coming from the first vessel of a hybrid respirometer. As such, it contains readily biodegradable organic carbon and soluble nitrogen species (in concentrations typical of wastewater treatment plants) as substrates for heterotrophic and autotrophic biomass, respectively, as well as a high concentration of dissolved oxygen due to the fact that aeration is performed in the first vessel. The detailed influent composition is shown in Table 1 and was collected from the wastewater treatment plant of Eindhoven (The Netherlands) (Amerlinck 2015), except for the concentration of soluble ammonium, which was set to be present in abundance (to achieve oxygen limiting conditions).

Description of the CFD-biokinetic model

First, the geometry of the respirometer was created in the design module of the commercial tool ANSYS (version R14.5, USA) taking into account all the details which can influence reactor hydrodynamics (such as accurate location, size and shape of inlet and outlet). Next, meshing using a

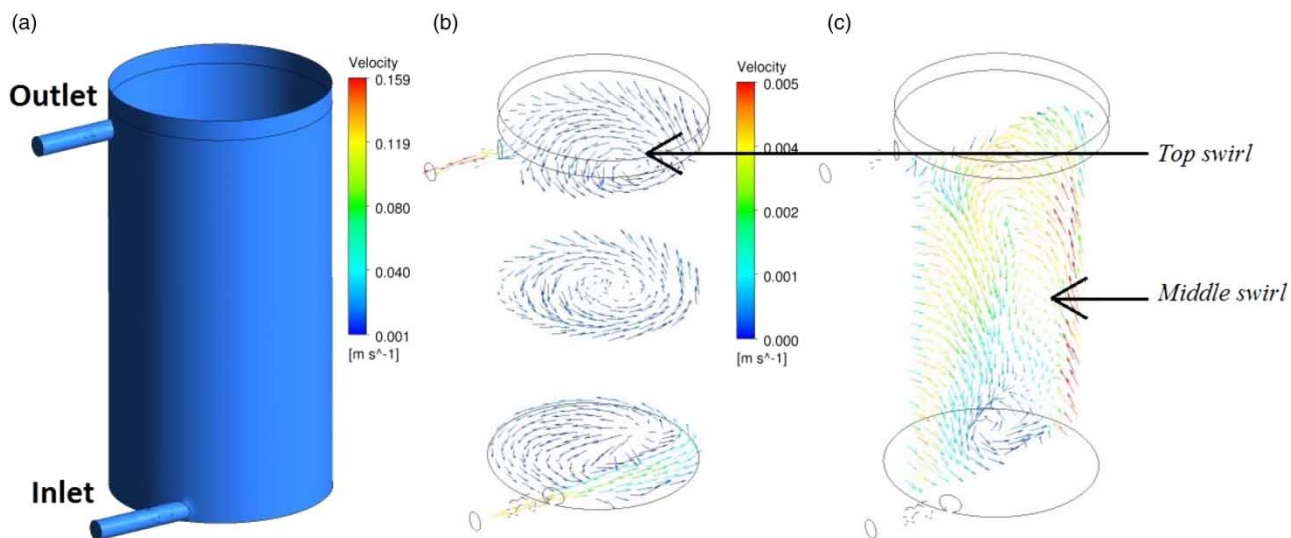


Figure 1 | Geometry of the respirometer (a) and velocity vector plots in the base case in horizontal cross-sections (b) and a vertical cross-section (c) of the system (note the difference in scales used).

Table 1 | Wastewater composition used in both the CFD-biokinetic and TIS-CSTR-biokinetic models

Component	Concentration (mg COD/L)
Readily biodegradable carbon	41.00
Heterotrophic biomass	500.00
Autotrophic biomass	98.91
Dissolved oxygen	9.00
Soluble nitrites and nitrates (expressed as N)	15.21
Soluble ammonium (expressed as N)	40.00
Soluble alkalinity	30.00

COD: chemical oxygen demand.

commercial mesh generator tool (ICEM CFD, ANSYS, Pennsylvania, USA) was performed. The mesh was kept structured (all hexahedral cells) and very fine (67,000 hexahedral cells, i.e. $3 \times 10^{-8} \text{ m}^3$ average cell size) in order to achieve a mesh-independent solution. Subsequently, steady-state CFD simulations were performed in FLUENT (v14.5) (ANSYS, Pennsylvania, USA) by solving the Navier–Stokes equations using a finite volume method for a single phase. Turbulence was modeled with a Reynolds averaged Navier–Stokes model and the realizable k - ϵ model (Ishii 1975). The equations pertaining to these models can be found in Versteeg & Malalasekera (2007). In addition, the effect of gravity over the flow patterns was incorporated in the solution of the Navier–Stokes equations, which is important for realistic hydrodynamic modelling of the system. The sludge density was calculated applying a correlation with the suspended solids concentration (De Clercq 2003; Rehman *et al.* 2017). At the inlet, the velocity calculated from the volumetric flowrate was used as a boundary condition (Table 2, Base case). However, the outlet velocity was not known and thus a boundary condition of ‘outflow’ was used, so that the CFD model can calculate it itself using the continuity (mass balance) of the system. The details on the inlet flowrates, velocities and the associated hydraulic

retention times (HRTs) for the different mixing conditions scenarios in this study can be found in Table 2.

Rehman *et al.* (2017) integrated a biokinetic model with a CFD model; this same framework has been used in the present study. Specifically, the kinetic equations included in the CFD model are those describing carbon and nitrogen removal through aerobic and anoxic reactions in the ASM1 model (Henze *et al.* 2000). The used kinetic parameter values (including the half-saturation indices) are the default values of the ASM1 model. Hence, in this study it is premised that the respirometer sludge is characterized by the half-saturation indices from the ASM1 model.

Description of the TIS-CSTR-biokinetic model

The TIS-CSTR-biokinetic model has been set up in WEST (www.mikepoweredbydhi.com, Denmark). Virtual tracer tests were performed with this model and compared with a virtual CFD tracer test to determine the number of tanks required for the TIS-CSTR-biokinetic model. From these, it was found that a single tank configuration would best represent the physical system; this would moreover be a reasonable approach from a practical perspective, given the scale and simplicity of the system. The biokinetic model chosen within the WEST platform has been ASM1, as for the CFD-biokinetic model case. The influent flowrates and composition used in the TIS-CSTR-biokinetic model were chosen equal to those used in the CFD-biokinetic model (Table 2 and Table 1, respectively), and the simulations were carried out in steady-state conditions.

Investigation of the effect of mixing conditions on half-saturation indices determination

A step-wise procedure has been followed to investigate the effects of mixing conditions on the determination of half-saturation indices. First, the mixing limitations in the real system have been studied by using the CFD model (without the integrated biokinetic model) for the base case mixing conditions; mixing patterns and the existence of dead zones have been described. Then, the effect of different mixing conditions on process performance in the real system has been studied by calculating the distribution of substrate concentrations and substrate consumption rates with the CFD-biokinetic model. Subsequently, the deviations in process performance between the modeled and physical systems at different mixing conditions have been investigated by comparing the effluent quality at different flowrates. In a further step, the effects of mixing limitations on half-saturation index

Table 2 | Simulated scenarios in terms of flowrates and HRTs

Simulation scenario	Flowrate (L/h)	Velocity (m/s)	HRT (min)
Base case (X)	16	0.056	7.5
2X	32	0.113	3.75
0.75X	12	0.042	10
0.5X	8	0.028	15
0.25X	4	0.014	30

calibration have been studied; for each flowrate used in the CFD-biokinetic model, the half-saturation index for oxygen in autotrophic organisms ($K_{O,A}$) has been estimated in order to fit the physical system behavior, thus mimicking one of the methods followed in modelling practices. It is important to note that the $K_{O,A}$ value was the sole half-saturation index modified during calibration since oxygen was present in concentrations close to its half-saturation index in many of the scenarios; this was not the case for the rest of substrates/electron acceptors which were abundantly available (in the saturated zone of the Monod curve). The importance of mixing conditions in half-saturation index determination has been further illustrated by calculating the different indices obtained when locating a sensor in different parts of the respirometer.

RESULTS AND DISCUSSION

Mixing limitations in the physical system

First, mixing limitations in the physical system have been investigated by simulating it for the base case conditions using the CFD model without including the biokinetic equations. Figure 1 shows the velocity vector plots for the base case in both horizontal cross-sections and a vertical cross-section. As can be seen, the flow follows a very distinctive pattern, moving in a circular motion along the

horizontal sections (Figure 1(b)). Furthermore, the flow proceeds from the inlet to the outlet and then part of it swirls back to the bottom of the tank where it swirls again to finally exit the system (Figure 1(c)). The consequence is the existence of two areas in the tank where flow slows down and thus the resulting mixing is deficient (center of the previously described swirls); as can be observed, the major dead zone is located in the center of the respirometer base (middle swirl). A minor dead zone can be found towards the top of the reactor (top swirl); both these dead zones have been pointed out in Figure 1(b) and 1(c). These mixing limitations could result in a heterogeneous distribution of substrate concentration and HRTs thus yielding a different effluent quality as compared to a fully mixed system. It is noteworthy that this conclusion is intimately related to the reactor design (e.g. location of inlets and outlets). The latter is usually not accounted for in detail when studying mixing limitations by means of CFD studies. Hence, some sort of mixing limitations – that could be avoided with minor design adaptations – are likely to be present in most currently used reactors, which underlines the relevance of the present work.

Effect of mixing conditions on process performance in the physical system

Figure 2 shows the steady-state distribution of dissolved oxygen, ammonium and nitrate concentrations in the base

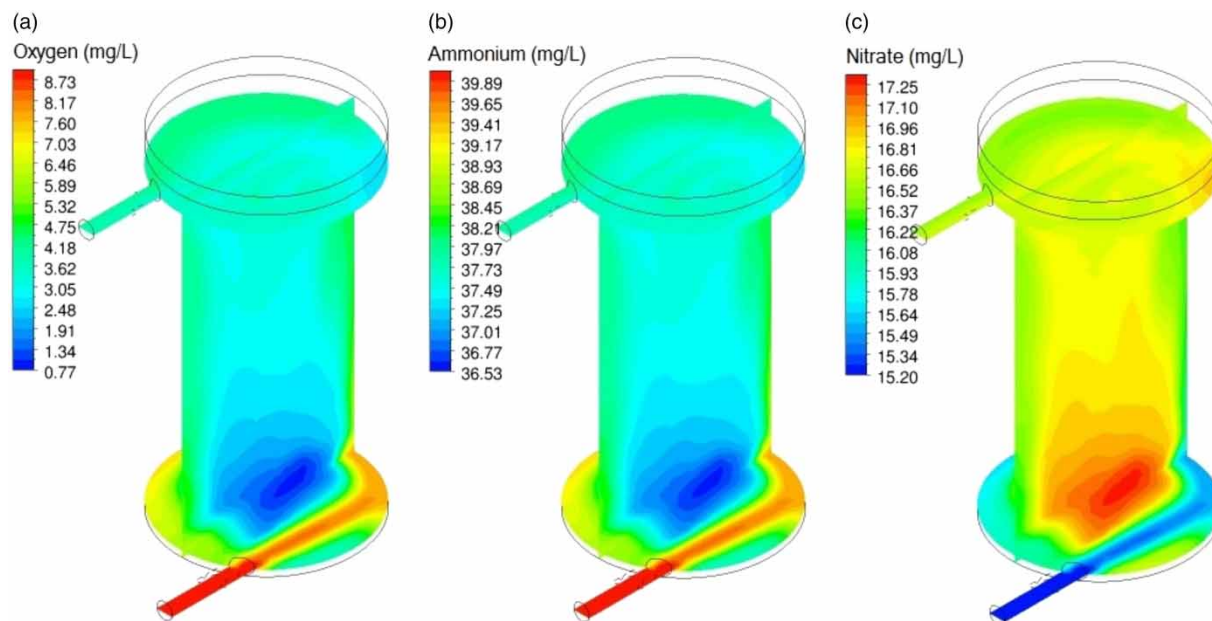


Figure 2 | Spatial distribution of dissolved oxygen (a), ammonium (b) and nitrate (c) in the base case.

case when using the CFD-biokinetic model. As can be seen, the distributions of oxygen and ammonium follow a similar pattern that is coherent with the velocity vector plots shown in Figure 1(b) and 1(c). Substrate concentrations are lower in the area where the major dead zone is, indicating that the higher retention times promote high conversion efficiencies, but also advective mass transfer limitations. It is important to note that ammonium concentrations are well above the reported ammonium half-saturation index of 1.0 mg N/L for autotrophic organisms at 20 °C (Rittmann & McCarty 2001) throughout the reactor. This means that there is no substrate limitation and, thus, half-saturation indices are not playing a significant role in the description of the ammonium oxidation kinetics. The reported oxygen half-saturation index for autotrophic organisms at 20 °C is 0.4 mg O₂/L (Rittmann & McCarty 2001). Given that the low range of oxygen concentrations is close to the half-saturation index (0.7 mg O₂/L, Figure 2), this index will probably play a significant role in the description of process kinetics (i.e. the model is sensitive to its value). From a practical perspective, this means that it will be modified through a typical calibration process in order to fit model results with observed process performance.

Nitrate follows a trend that is also coherent with the flow patterns shown in Figure 1(b) and 1(c). However, nitrate is different to ammonium and oxygen in the sense that it is

both formed and consumed in the reactor, instead of solely being consumed (as is the case for the two former components). As can be deduced, the concentration of nitrates is maximal where ammonium and oxygen are minimal (major dead zone), which is consistent with an efficient aerobic transformation of ammonium to nitrate in this area (related to the higher HRT for the substrates in it); this process dominates the nitrate balance over the anoxic denitrification process. This can be verified by looking at Figure A1(a), Appendix (available with the online version of this paper), where the simulation results excluding oxygen in the wastewater composition (thus only allowing denitrification) indeed show denitrifying activity. On the other hand, excluding the carbon source leads to a process where only aerobic nitrification is promoted (Figure A1(b), Appendix), and results show a similar but more pronounced nitrate distribution as in Figure 2 (nitrate concentrations are higher in the second dead zone near the top of the reactor).

Figure 3 shows the distribution of organic carbon in the respirometer (a), together with the distribution of the substrate consumption rate in the system (b). As can be observed, the substrate consumption rates are lowest in the major dead zone due to the lower oxygen concentrations in this area (26% lower as compared to the maximum consumption rate achieved). This is consistent with the previous observations carried out for oxygen, ammonium and nitrate.

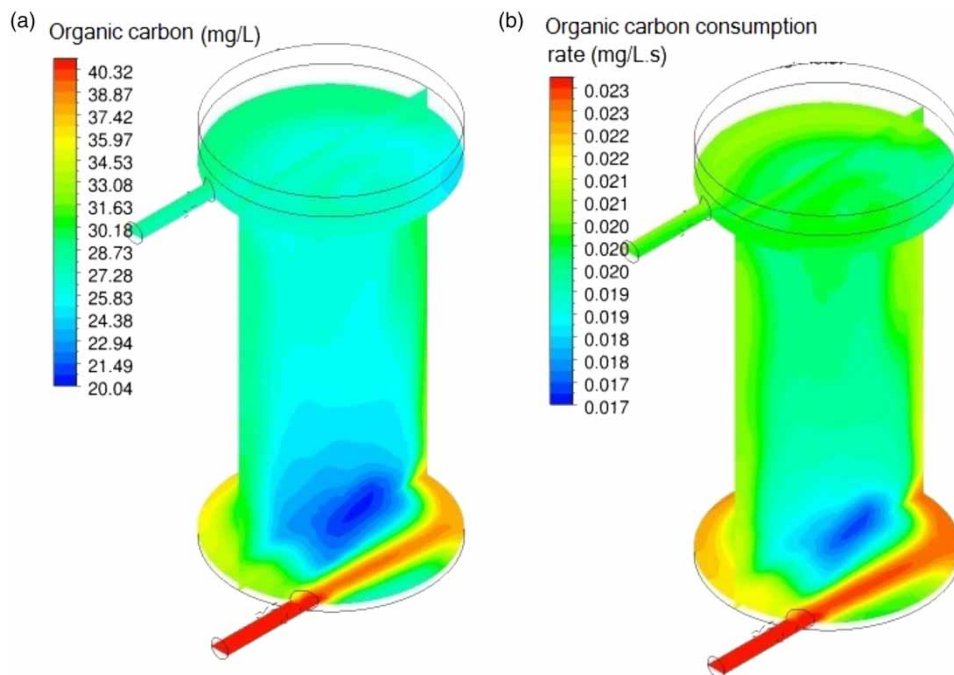


Figure 3 | Spatial distribution of organic carbon concentrations (a) and organic carbon consumption rates (b).

Figure 4 shows the distribution of organic carbon consumption rates throughout the respirometer under different mixing conditions. Higher HRTs cause lower steady-state consumption rates due to the lower substrate concentrations that prevail under these conditions. For all mixing conditions, the substrate consumption rates are lower in the two reactor dead zones, similarly as was observed for the base case (Table 3, showing the decrease in organic carbon consumption rate as compared to the base case). It is important to note that at high flowrates (low HRTs), the differences in substrate consumption rates throughout the tank are significantly smaller than in the low flowrate cases (high HRTs) (Figure 5 and Table 3). This is due to the fact that high HRT values have simultaneously better conversion efficiencies (due to the fact that there is more time available to degrade the substrates) and worse mixing conditions. From a practical perspective, this entails that the substrate concentration heterogeneity is higher in the high HRT cases, potentially causing significant departures between predicted and observed process performance.

Figure 6(a) shows the concentration of organic carbon for the different mixing scenarios considered. As can be seen, the organic carbon concentration is slightly lower in the dead zones for all cases; this was also observed for the base case. As can be seen, organic carbon concentrations in the majority of the reactor space vary between 20 and 30 mg/L throughout all mixing conditions. Given that the half-saturation index reported for aerobic and anoxic heterotrophs is 20 mg O₂/L (Rittmann & McCarty 2001), this

entails that this half-saturation index will not affect the description of organic matter consumption rates between the different mixing scenarios. Thus, it will not be modified through a common calibration exercise (as a sensitivity analysis will report it to be insensitive). Figure 6(b) shows the distribution of oxygen concentrations in the different mixing scenarios. For all mixing conditions, the oxygen concentrations are lower in the two reactor dead zones, as was observed for the base case; this is coherent with the patterns observed in Figure 4. The existence of a wider range of concentrations at higher HRTs can also be observed for the oxygen concentrations in general. However, it is important to note that the heterogeneity in concentrations throughout the tank decreases at increasing HRTs given that most of the oxygen is consumed under these conditions. Ammonium follows a very similar trend as the one seen for oxygen concentrations (Figure 6(c)), whereas nitrate does not (Figure 6(d)). As HRT values increase, there is a maximum nitrate concentration achieved around the two dead zones (found in the 0.75X case), and nitrate concentrations drop for the highest HRT cases. This complexity is due to the fact that nitrate is again, besides being transported and converted by denitrification, being formed by nitrification. Moving from the highest HRT case towards lower HRTs increases nitrification rates, since ammonium concentrations as well as oxygen concentration are higher. This in turn leads to higher nitrate values in the (still nitrification dominated) nitrate balance. However, further decreasing HRT reduces the dead zones that are present, allowing nitrate build-up. The consequence is that nitrate

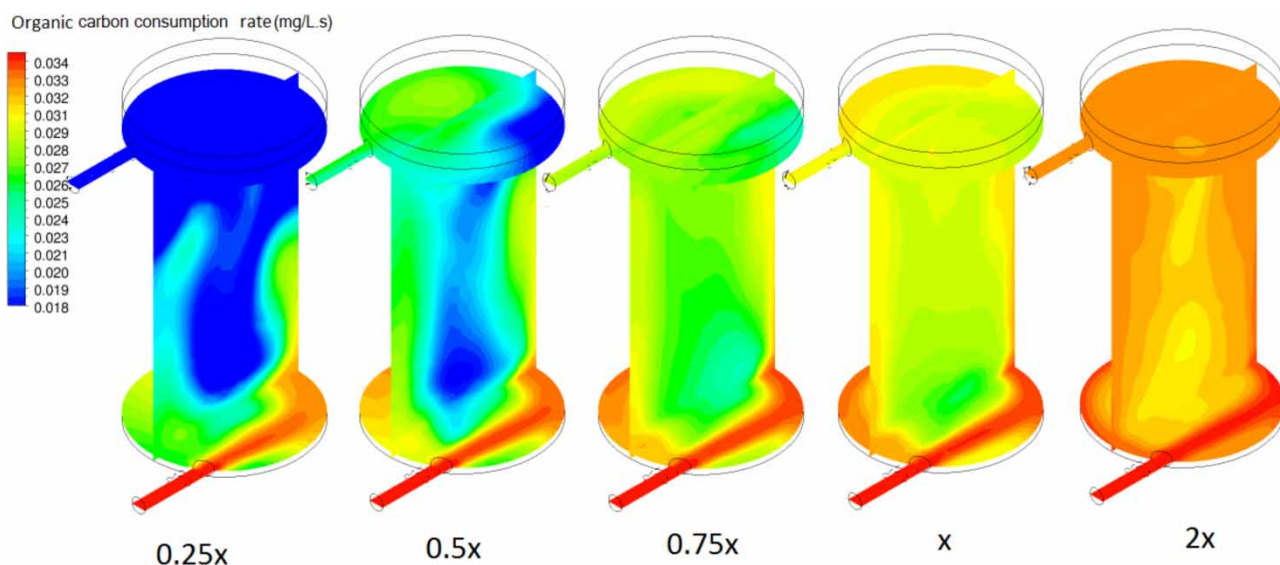
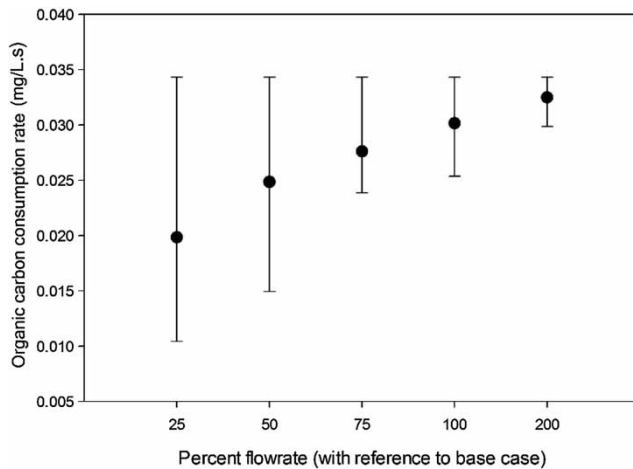


Figure 4 | Spatial distribution of organic carbon consumption rates for different mixing scenarios in the system.

Table 3 | Decrease in organic carbon consumption rates at different mixing conditions

Mixing condition	Decrease in organic carbon consumption rate (%)
0.25X	34.25
0.5X	17.57
0.75X	8.45
X	0
2X	-7.73

**Figure 5** | Range of maximum to minimum organic carbon consumption rates for the different mixing scenarios (the point shows the mean value).

concentrations drop in both the tank and effluent as the formed nitrate is being washed out.

Deviation in process performance between the physical and modeled systems at different mixing conditions

Figure 7 shows the difference between the effluent quality parameters (organic carbon, dissolved oxygen, ammonium and nitrate concentrations) for the CFD-biokinetic and TIS-CSTR-biokinetic models. As a general trend, the differences between models become more evident as HRT values become higher. This is consistent with the previous observation that there are wider concentration ranges with decreasing flowrates. This can be attributed to the combination of worse mixing conditions (i.e. worse advective transport) at lower flowrates together with more time to degrade substrates through biological conversions at higher HRTs. As can be observed, the difference between predicted organic matter concentrations diminishes fast with decreasing HRT values (Figure 7(a)). This is in contrast to ammonium and nitrate, which only become equal at the lowest HRT level. This is due to the fact that oxygen only

becomes limiting for heterotrophic aerobic reactions in the highest HRT case, affecting process rates in several areas of the tank and thus rendering effluent levels significantly dissimilar to the TIS-CSTR-biokinetic model results (Figure 7(b)). Aerobic autotrophic reactions, on the other hand, become oxygen limited at concentrations higher than the heterotrophic ones, given that nitrifying organisms are generally more intolerant to low oxygen concentrations as compared to heterotrophic organisms (i.e., they present higher half-saturation indices for oxygen). This causes a larger difference between predicted ammonium and nitrate concentrations between the CFD-biokinetic model (physical system behavior) and the TIS-CSTR-biokinetic model (Figure 7(c)). Recall that all concentrations are well above or approximately similar to their respective half-saturation indices, except for the oxygen values reached in the highest HRT case. It is also important to note that the TIS-CSTR-biokinetic model is not able to capture the change of ammonium oxidation and nitrate formation and reduction very accurately for the different mixing conditions (and particularly so for the nitrate case). As can be observed in Figure 7(d), there exists a maximum nitrate concentration at intermediate HRT levels that is not at all captured by the TIS-CSTR-biokinetic model, due to the fact that it assumes perfect mixing.

Study of the effect of mixing conditions on half-saturation indices calibration

Based on expert knowledge (mentioned in the previous sections), the $K_{O,A}$ index for autotrophic bacteria was the most sensitive of the calibration parameters given the fact that oxygen was close to its value in several of the simulated scenarios and it varies significantly throughout the different mixing scenarios. Therefore, this system provides an opportunity to illustrate the effects of mixing conditions on the calculation of half-saturation indices when following commonly implemented methods for model calibration. Figure A2, Appendix (available with the online version of this paper), shows the results for the calibrated $K_{O,A}$ for autotrophs for the TIS-CSTR-biokinetic model so the effluent predictions fit those of the CFD-biokinetic model; this is equivalent to closing the gap between both predictions in Figure 7. The calibrated $K_{O,A}$ values will be sensitive to the effluent concentrations when oxygen concentrations are in the order of $K_{O,A}$ values. As seen in Figure 7, the oxygen concentration for the 200% case is almost 3 mg/L, much higher than the default $K_{O,A}$ values (i.e. 0.4 mg O₂/L). Therefore, calibrated $K_{O,A}$ values are not sensitive to the

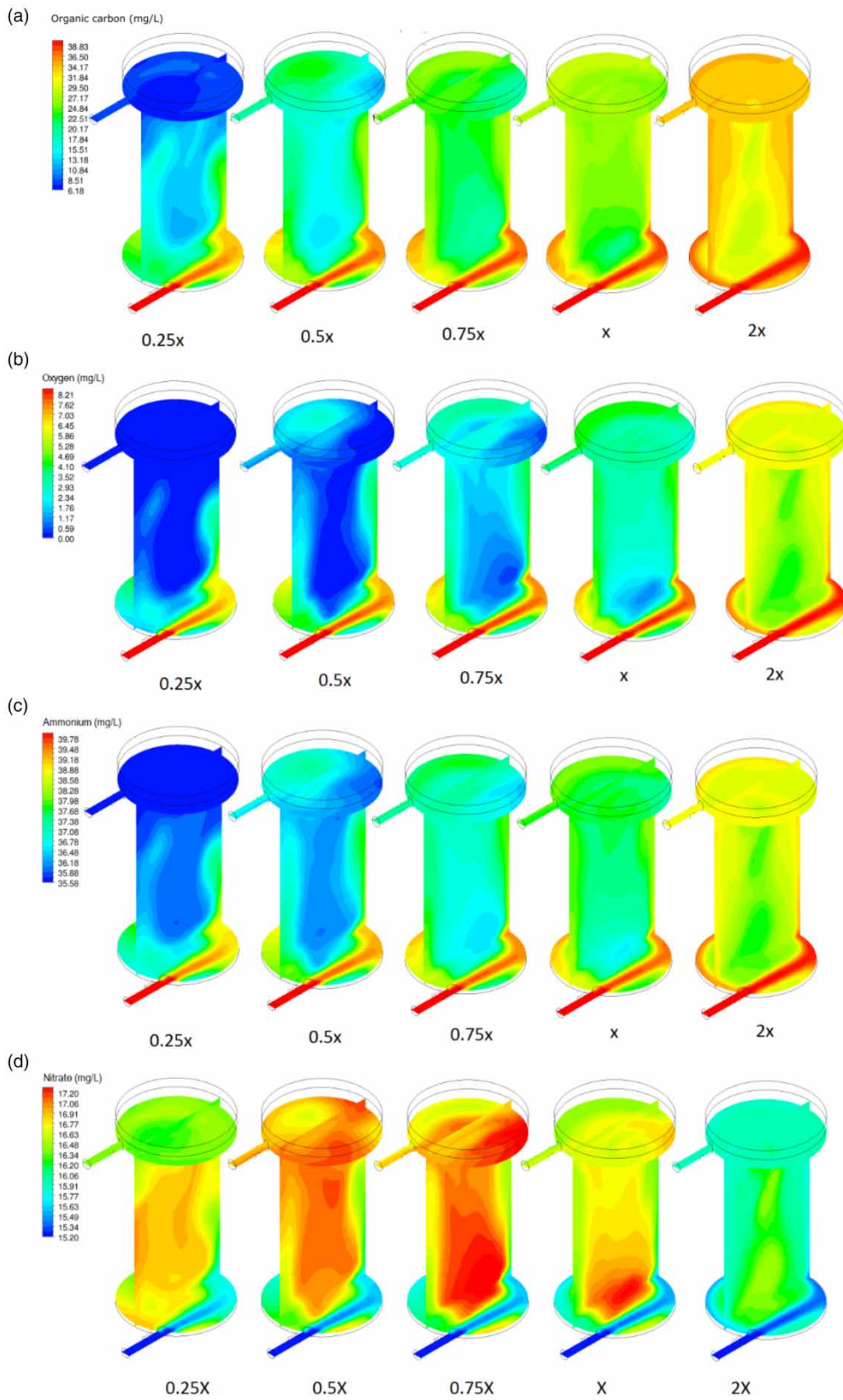


Figure 6 | Distribution of (a) organic carbon, (b) oxygen, (c) ammonium and (d) nitrate concentrations for the different mixing scenarios.

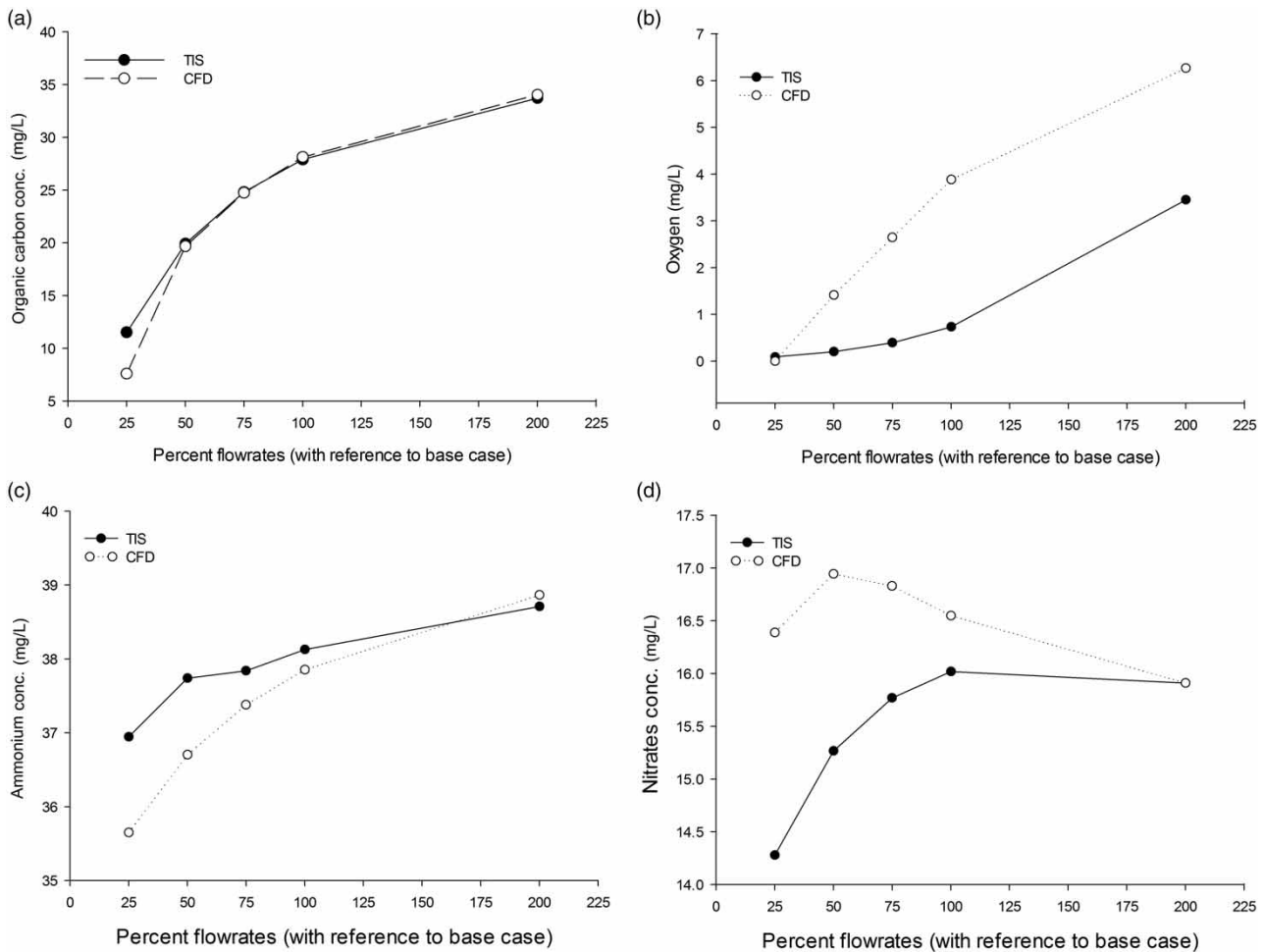


Figure 7 | Difference between CFD-biokinetic and TIS-CSTR-biokinetic predicted concentrations for organic matter (a), oxygen (b), ammonium (c) and nitrates (d).

effluent concentration in this case. As can be seen in Figure A2, Appendix, the calibrated $K_{O,A}$ values are only shown for the 25–100% cases. In these cases, the half-saturation index would be calibrated to a value that is below the real one (results from 25 to 100%) and would change an order of magnitude with the change in mixing conditions. Commonly, respirometric systems are considered to present no mixing limitations and thus constitute an ideal system to measure the diffusional and biological contributions to half-saturation indices. Under the assumption of no mixing limitations, researchers neglect or postulate different explanations for the need for calibration of the $K_{O,A}$ index. An explanation that could be provided is that there has been a selection of organisms with improved substrate transport characteristics (Arnaldos *et al.* 2015) in the case where the calibrated half-saturation index values fall well below the default. Alternatively, another biology-related explanation that could be put forward is that either alternative enzymatic

pathways have been activated in the organisms, or the expression of transport enzymes has taken place (Arnaldos *et al.* 2015). Furthermore, a commonly provided diffusional-related explanation is the fact that high mixing energy has caused floc breakage in the full-scale system, bringing about lower half-saturation indices than expected. All of these explanations, that are based on basic biology and diffusional characteristics of the system, would theoretically match the obtained results and yet are not reflecting what is actually happening in the process. In this study, the respirometer presents mixing limitations that bring about an additional contribution (either by increasing or decreasing its value) to the half-saturation index when it is calculated as a result of the calibration of a TIS-CSTR-biokinetic model. As a result of incorrect $K_{O,A}$ calculation, suboptimal and even counter-productive process operational and design decisions could be taken. Additionally, opportunities for improved reactor design by modifying the advective

characteristics of the full-scale process will be lost. In fact, in the present study, the respirometric analysis could indicate that advection limitations could be advantageous – in some occasions – for improved substrate removal by creating areas of relatively high retention times that still preserve adequate substrate and electron acceptor concentrations (i.e. complete mix might not be the best operational mode). It is important to note that this conclusion would have to be verified, carrying out a similar study as the one presented here, on a full-scale system.

In the case that the calibrated half-saturation index values fall above the real one, commonly provided explanations would refer to the diffusional conditions of the system, with the formation of flocs of large sizes and/or bio-film occurrence. This explanation also matches the results obtained from the respirometry, but would not reflect the actual behavior of the full-scale process. As an example of the potential consequences of drawing this conclusion from the results obtained, a plausible operational decision would be to increase the mixing of the full-scale system in order to reduce the size of the flocs and improve process performance. This would increase energy consumption with no actual performance improvement (or at least, no guaranteed performance improvement with the information existent).

Consequences of sensor placement on half-saturation indices calibration

In order to further illustrate the importance of mixing conditions, the effect of sensor placement in the respirometer on the calculated half-saturation indices has been quantified. A previous study using a combined CFD-biokinetic modeling framework has shown that bulk mixing can impact the performance of the control systems in full-scale plants, given that the sensors will provide inputs that are not representative of the entire treatment basin (Rehman *et al.* 2015). In this previous study, it was clearly demonstrated that a dissolved oxygen controller with a fixed setpoint performs differently as a function of the location of the sensor. In the present study, it has been shown that these heterogeneities can also happen in downsized and simple bioreactors such as respirometers, commonly assumed to exhibit ideal completely mixed behavior. This implies that sensor placement has the potential to also significantly affect the calibration of half-saturation indices through measurements that are not representative of a significant fraction of the reactor volume. This is very significant, given that an incorrect calculation of half-saturation indices can again lead to

inappropriate design and/or operational decisions in processes where substrate concentrations are low or where effluent requirements are very stringent.

Figure A3, Appendix (available with the online version of this paper), shows the distribution of calibrated $K_{O,A}$ values for autotrophic organisms for the base case if the ammonium sensor used was placed in specific locations (A: in the outlet of the reactor; B: middle of the reactor near the surface; C: near the wall and near the surface and D: near the wall at half depth of the reactor). As it can be observed, these vary significantly, from 0.02 mg O₂/L in location B to 0.24 mg O₂/L in location D. This clearly confirms the hypothesis that sensor placement plays an important role in half-saturation index calibration and that mixing heterogeneities can affect the calculation of half-saturation indices not only in pilot and full-scale systems but also in simpler systems such as respirometers.

Implications at the process design and operational levels

This study provides important information that should be taken into account from an operational perspective. When trying to use half-saturation indices information for process evaluation or optimization, mixing conditions and sensor placement should be carefully considered. A possible approach to accomplish this would be to calculate half-saturation indices with well-characterized and standardized respirometers. This would allow isolation of the biological and diffusional contributions from the advective one in a certain calibration exercise. Afterwards, fine tuning of the calculated indices could be carried out with data taken from the full-scale system at different locations. In this case, the mixing characteristics of the full-scale plant should be well understood in order to select the appropriate sampling locations. This could be carried out through CFD studies that would lead to well performing compartmental models and/or integrated CFD-biokinetic models that can be used to appropriately characterize the performance of the system.

From a design perspective, the added benefit of performing detailed CFD studies leading to robust CFD-biokinetic or compartmental models is that systems can be designed purposefully to create mixing limitations in certain sections. This could enable simultaneous oxic/anoxic/aerobic reactions to take place in different sections of the bioreactor. It could also be useful to create conditions to outcompete certain organisms (e.g. AOB vs. nitrite-oxidizing bacteria in mainstream deammonification). Careful hydraulic process design that takes into account typical influent flow

variations would ensure that this happens in a directed manner and in a way that operational and capital expenditures are minimized. As has been shown in the present study, varying influent flowrates can affect mixing patterns and subsequently impact process performance; this could be accounted for in system design through advanced hydraulic analysis. However, bringing this into practice requires further studies to get a thorough understanding of the impact of reactor design on process performance. This study has laid out the basics for this.

CONCLUSIONS

The following conclusions can be drawn from the present study.

- The present study has demonstrated the importance of mixing conditions as a source of inconsistencies between calculated half-saturation indices values in an exemplary system consisting of the second vessel of a hybrid respirometer. These inconsistencies have been found to take place despite the simplicity and small scale of the system. But these findings will also occur at larger scales, where these effects can be expected to be even larger.
- The results have shown that the half-saturation index calculated from respirometric information might not reflect the actual half-saturation index of the full-scale plant. A possible solution to this issue is to calculate half-saturation indices from standardized and well-characterized respirometers (CFD can be a useful tool to accomplish this). Afterwards, some fine tuning could be carried out on a case-to-case basis from full-scale plant data.
- Sensor placement has been shown to significantly impact the estimation of half-saturation indices in the respirometric system considered. This conclusion can be extended to full-scale systems, where half-saturation indices could be very different depending on the measurement/sampling location chosen. This entails that mixing limitations could significantly affect decisions at the process monitoring, optimization and control level. In current practice, sensor location is usually ignored when optimizing plant performance. Only dedicated models that are sufficiently describing spatial variations will be able to soundly support such decisions.
- When designing a wastewater treatment plant, the estimation/choice of a half-saturation index has to be considered carefully and in combination with either a CFD or compartmental model of the system which

allows proper understanding of mixing conditions. This use of these models (beyond their current use as troubleshooting tools) could create opportunities at the design level by promoting certain biological reactions in different parts of the tank in a directed manner.

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