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Impact of cell cluster size on apparent half-saturation coefficients for oxygen in nitrifying sludge and biofilms

Cristian Picioreanu*, Julio Pérez [1], Mark C.M. van Loosdrecht

Department of Biotechnology, Faculty of Applied Sciences, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

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ABSTRACT

A three-dimensional (3-D) diffusion-reaction model was used to assess the effects of nitrifiers growing in cell clusters on the apparent oxygen half-saturation coefficients in activated sludge flocs. The model allows conciliation of seemingly contradictory reports by several research groups. Although intrinsic half-saturation coefficients (i.e., not affected by diffusion) show a better affinity for oxygen for ammonia oxidizing (AOB) than for nitrite oxidizing bacteria (NOB) ($K_{O,AOB} < K_{O,NOB}$), measurements in flocs often produced reversed apparent values ($K_{O,AOB,app} > K_{O,NOB,app}$), which can now be explained by the 3-D model with AOB and NOB microcolonies. This effect cannot be described with a conventional 1-D homogeneous model because the reversion of the AOB/NOB apparent K_O is caused by the high biomass density and resulting concentration gradients inside the microcolonies. Two main factors explain the reversion of the half-saturation coefficients: the difference in oxygen yields (for NOB lower than for AOB) and the difference in colony size (NOB colonies are smaller than those of AOB). The strongest increase in the apparent half-saturation coefficients is linked to the colony size, rather than to the floc size. For high-density microbial aggregates (i.e., granular sludge), the need for a stratified population (AOB outer shell, NOB inner layers) was revealed in order to outcompete NOB. This study stresses the need for a more detailed description of the biomass distribution in activated sludge, granular sludge and biofilm reactors when elucidating the mechanisms for NOB repression.

1. Introduction

In activated sludge systems the measurements of half-saturation coefficients (K_s) and affinity for substrate (maximum specific growth rate per half-saturation coefficient, μ_m/K_s) are impacted by the floc size (Beccari et al., 1992; Chu et al., 2003; Manser et al., 2005a; Pérez et al., 2005). Activated sludge models (Henze et al., 2000) do not explicitly consider diffusion of substrate in the flocs, and therefore the half-saturation coefficients used are denominated “apparent” ($K_{S,app}$). Experiments (Beccari et al., 1992; Manser et al., 2005a) and theoretical evaluations (Pérez et al., 2005) show a strong increase of the $K_{S,app}$ value with increasing average floc size. Often, the presence of microbial aggregates (e.g., flocs) hampers the determination of the intrinsic (i.e., not affected by mass transfer limitations) half-saturation coefficients, K_S (for instance, Manser et al., 2005a). For models including explicitly diffusion of substrates (e.g. biofilm models) intrinsic half-saturation coefficients K_S should be used instead of the apparent coefficients (Pérez et al., 2005).

Nitrifiers grow in microcolonies (Daims et al., 2001; Mobarry et al., 1996; Wagner et al., 1995). These structures are composed of very dense cell clusters (Okabe et al., 2004; Wagner et al., 1995) surrounded by an EPS layer and often with near spherical shape (Vejmelkova et al., 2012; Wagner et al., 1995). Distribution and size of microcolonies in the floc could have an impact on the performance of the process, as pointed out in several studies (Harper et al., 2009; Kindaichi et al., 2004). For nitrifiers immobilized in gel beads, Wijffels et al. (1995) considered oxygen diffusion limitation over microcolonies in a numerical model for biomass development. However, for the mathematical description of activated or granular sludge reactors, the presence of these cell clusters is often neglected. For activated sludge, a $K_{S,app}$ value is used to avoid explicit description of the diffusion of substrate in the floc as in general activated sludge models (ASM, Henze et al., 2000). In

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* Corresponding author.
E-mail addresses: C.Picioreanu@tudelft.nl (C. Picioreanu), julio.perez@uab.es (J. Pérez), M.C.M.vanLoosdrecht@tudelft.nl (M.C.M. van Loosdrecht).

[1] Present address: Department of Chemical Engineering, School of Engineering, Universitat Autònoma de Barcelona, Barcelona 08193, Spain.

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more complex mathematical modelling approaches, when diffusion is explicitly described, very often the system is simplified to the variation of cell concentration in one dimension (Wanner and Gujer, 1986). For instance, assuming spherical flocs and calculating variations only along the radius. Two- or three-dimensional models were seldom considered for the description of flocculent sludge (Martins et al., 2004; Ofiţeru et al., 2014).

Research in advanced nitrogen removal systems in wastewater treatment plants focusses on establishing a steady nitritation process to accommodate anaerobic ammonium oxidation (anammox) in the main water line (Jetten et al., 1997; Kartal et al., 2010; Siegrist et al., 2008). Repression of nitrite-oxidizing bacteria (NOB) is essential to succeed in the application of partial nitritation/anammox (PN/A) processes at low temperatures (De Clippeleir et al., 2013; Hao et al., 2002a, 2002b; Winkler et al., 2011). The competition for oxygen between ammonia-oxidizing bacteria (AOB) and NOB has been identified as the key mechanism to achieve stable nitritation in granular sludge reactors at moderate (20°C, Jemaat et al., 2013) and low temperatures (12.5 and 10 °C; Isanta et al., 2015; Reino et al., 2016). It is rather well accepted that AOB have a higher affinity for oxygen than NOB, i.e. $(\mu_m/K_O)_{\mathrm{AOB}} > (\mu_m/K_O)_{\mathrm{NOB}}$ (Jubany et al., 2008; Wiesmann, 1994). This may be a competitive advantage responsible for the repression of the nitrite oxidation in biofilm reactors that was also identified through modelling and experiments (Brockmann and Morgenroth, 2010; Isanta et al., 2015; Jemaat et al., 2013; Pérez et al., 2009; Wang et al., 2009).

Several experimental reports apparently contradict the general observation that AOB possess a better oxygen affinity than NOB (Manser et al., 2005a; Regmi et al., 2014; Sliekers et al., 2005). In a membrane bioreactor (MBR) in which the floc size was kept minimal (80 μm) thus avoiding diffusion limitations, it was found that $K_{O,AOB} > K_{O,NOB}$ (i.e., a better oxygen affinity for NOB) (Manser et al., 2005a). In addition, in the development of strategies for the integration of anammox for sewage treatment, Regmi et al. (2014) reported a large difference between the apparent half-saturation coefficients for oxygen of AOB and NOB ($K_{O,AOB} = 1.2$, $K_{O,NOB} = 0.2$ mg O_2/L). Often, explanations for the contradicting observations include the presence of different types of AOB and/or NOB than the typical strains or methodological problems in the experimental assessment of the affinity coefficients, among others. Up to date, no consensus has been achieved to provide detailed explanations that could describe those observations reported in the literature.

We hypothesize that the variations in oxygen affinity reported in literature are largely due to neglecting the importance of diffusion gradients in the three-dimensional structure of flocs and granules. To evaluate this hypothesis, a three-dimensional diffusion-reaction model was developed and used to explain how cell cluster (microcolony) size may impact the observed half-saturation values, and how neglecting the floc organization in microcolonies could influence conclusions derived from macroscopic observations. This may reconcile the findings of Manser et al. (2005a) (and other authors) with the previously established understanding. Moreover, the gained insight will help in developing better strategies for nitrogen removal with anammox in the mainstream of wastewater treatment plants.

2. Materials and methods

2.1. Model description

A three-dimensional (3-D) numerical model was constructed with the aim of calculating the apparent half-saturation coefficient values for oxygen ($K_{O,AOB,3d}$ and $K_{O,NOB,3d}$) when both ammonia-oxidizing (AOB) and nitrite-oxidizing (NOB) cells are clustered in dense spherical colonies embedded in flocs or granules. Furthermore, the apparent half-saturation coefficients found with the 3-D model were compared with the apparent coefficients obtained when the nitrifiers are uniformly distributed in the microbial aggregate ($K_{O,AOB,1d}$ and $K_{O,NOB,1d}$, 1-D model) and with the intrinsic coefficients ($K_{O,AOB}$ and $K_{O,NOB}$).

In particular, we chose to describe the experimental measurements presented by (Manser et al., 2005a, 2005b) because, when determining the apparent half-saturation coefficients, a very detailed characterization of the flocculent sludge was provided (e.g., floc size, floc density, colony size distribution for both AOB and NOB, among others). The model was applied to three practical cases:

1. Small flocs, as those obtained in membrane bioreactors (MBR), where the effects of mass transfer resistance are claimed to be minimal (Manser et al., 2005a).
2. Large flocs, as in conventional activated sludge (CAS) systems, which are known to be affected by diffusional resistance (Manser et al., 2005a).
3. Granular sludge or biofilms, where the biomass density is much higher than in flocs and stratification of nitrifiers is sometimes observed (Matsumoto et al., 2010; Vlaeminck et al., 2010).

2.1.1. Three-dimensional model with clustered biomass (the heterogeneous model)

2.1.1.1. Model geometry. The 3-D model was constructed to represent the diffusion and reaction of dissolved oxygen as the sole growth-limiting component, in a nitrifying floc with clustered AOB and NOB. A spherical activated sludge floc was assumed, with size (diameter) d_{floc}. A number of spherical microcolonies (clusters) was placed at random locations in the floc. Each of these colonies contained either AOB or NOB densely clustered in a high biomass concentration, $C_{X,col}$. Two situations were investigated: (i) in the first simple case, all colonies of the same type had the same size, d_{AOB} or d_{NOB}, and $K_{O,AOB,3d}$ and $K_{O,NOB,3d}$ were then computed for a range of colony sizes; (ii) the second case considered a more realistic situation, where the size of colonies placed in the floc followed a log-normal distribution derived from experimental measurements, $d_{AOB,LN}$ and $d_{NOB,LN}$.

The position of each colony in the spherical floc affects the microbial activity within the colony because: (i) proximity to the bulk liquid determines overall mass transfer limitations, i.e., a deeper position in the floc results in less activity; (ii) vicinity between colonies causes a gradient of oxygen impacted by the consumption in each colony, i.e., cell clusters close to each other interact strongly. Therefore, to obtain statistically meaningful results, for each colony size case at least 10 replicate simulations were performed with randomly chosen colony positions.

2.1.1.2. Flocs with uniform colony sizes. In case of uniform sizes d_{AOB} and d_{NOB}, a given number of AOB colonies in the floc, n_{AOB}, was first assumed. The colony sizes were then calculated so that two main conditions were satisfied: (i) the concentration of nitrifiers in the floc was a fraction f_{nit} from the total biomass concentration measured by (Manser et al., 2005a), thus $C_{AOB,floc} + C_{NOB,floc} = f_{nit} \cdot C_{X,floc}$; (ii) the ratio between the concentrations of the two nitrifiers in the floc, f_Y, was set by the ratio of microbial growth yields on N substrates, $C_{AOB,floc}/C_{NOB,floc} = Y_{AOB}/Y_{NOB} = f_Y$. Additionally, the size of NOB colonies had to be a given fraction of that of AOB colonies (following experimentally determined mean colony sizes), which sets the number of NOB colonies to $n_{NOB} = (n_{AOB}/f_Y)\,(d_{AOB}/d_{NOB})^3$ for a given ratio d_{AOB}/d_{NOB}. With these assumptions, the colony sizes resulted as: