SRT increases with biofilm thickness in MBBR systems

S. Jane Fowler*, Elena Torresi, Barth F. Smets*, Benedek G. Plósz*'*****

*Department of Environmental Engineering, Technical University of Denmark, Kongens Lyngby, Denmark. jfow@env.dtu.dk, bfsm@env.dtu.dk
** Veolia Water Technologies, Lund, Sweden. elena.torresi@anoxkaldnes.com
*** Department of Chemical Engineering, University of Bath, Claverton Down, Bath BA2 7AY, UK. b.g.plosz@bath.ac.uk

Abstract: SRT is a key operational parameter that can impact the functionality of biological treatment systems. Little is known about SRTs of organisms in attached growth systems, though due to higher biomass retention it is likely that these systems would exhibit higher SRTs than suspended growth systems. We calculated the SRT of individual sequence variants (SVs) (referred to herein as taxon-SRT) in nitrifying MBBR reactors in which we controlled biofilm thickness using Veolia/ ANOX ™ Z-carriers with maximum biofilm thicknesses of 50 µm, 200 µm, 300 µm, 400 µm and 500 µm. We find that average residence time of microbes in nitrifying MBBRs are long, exceeding 38 days, and that taxon-SRTs generally increased as biofilm thickness increased with lowest average Taxon-SRT in the thinnest biofilms. Differences in the patterns of taxon-SRT with biofilm thickness within the nitrifying guild point towards variations in ecological strategies of individual nitrifiers.

Keywords: wastewater treatment; MBBR; solids retention time; micropollutant; nitrification

Solids retention time (SRT) is a key operational parameter that represents the average time that solids reside in a bioreactor. SRT impacts both the diversity and composition of microbial communities due to its relationship with microbial growth rates[1]. In suspended growth systems, SRT positively correlates with the functional potential of the microbial community. For example, above a critical SRT value (4-6- days[2]), nitrification is facilitated by the retention of nitrifying bacteria. In activated sludge, SRT is controlled by sludge wastage, but in biofilm systems like moving bed biofilm reactors (MBBRs), there is no intentional biomass removal. Instead, processes including growth, attachment, detachment and dispersal limitation affect the retention of individual taxa. In granular activated sludge, variations in SRTs of different guilds have been associated with guild localization on the exterior or interior of granules[3]. Here, we calculated the SRT of individual taxa in MBBRs with different biofilm thickness. We predicted that thicker biofilms would have both longer system SRTs and harbour taxa with longer SRTs due to increased dispersal limitation from deeper layers. We examined the variations in taxon-SRTs with biofilm thickness of functional groups such as nitrifiers. We hypothesized that highly active organisms such as nitrifiers would have shorter SRTs due to higher growth rates and surface localization compared to slow growing organisms and those present in deeper layers subject to dispersal limitation (e.g. anaerobes).

Methods: Lab-scale MBBRs were fed with WWTP effluent supplemented with 50 mg/L NH_4-N. Samples from the influent, effluent and carriers of different thickness. were subject to DNA extraction, 16S rRNA gene sequencing, and qPCR of total Bacteria. SRT was calculated for each taxon (Eq. 1) detected in the carriers and effluent samples.

$$Taxon - SRT_i = \frac{A_{i,biofilm} \cdot X_{biofilm}}{A_{i,Eff} \cdot X_{eff} \cdot Q_{Eff}} \quad \text{(Eq. 1)}$$

where $A_{i,biofilm}$ is the relative abundance of SV *i* in a carrier group, $X_{biofilm}$ is the total biomass present in that carrier group. $A_{i,Eff}$ is the relative abundance of SV *i* in the effluent, X_{Eff} is the biomass present in the effluent and Q_{Eff} is the flow rate.

Table 1. The number of sequence variants (SVs) with infinite SRT values (where SVs were never detected in the effluent but were present in carriers) and with SRT=0 (where SVs were not detected in the carriers but were present in the effluent). A total of 1874 SVs, which were present in carriers and/or effluent were considered

Biofilm Thickness (um)	Taxon-SRT = ∞	Taxon-SRT = 0	Mean Taxon-SRT*
50	210	1304	38.2
200	317	1040	42.8
300	261	1126	44.2
400	398	936	44.5
500	299	1084	42.8

*includes only non-infinite and non-zero values

Results: Considerably more infinite SRT values, as well as a higher mean SRT were observed in thicker biofilms (Table 1). We looked more closely at the nitrifying guild, which we expect to be present in the aerobic top layers of biofilms. Interestingly, *Nitrobacter* spp. (2 SVs) were only detected in the thinnest biofilms, and here displayed infinite SRTs. Similarly, members of *Candidatus* Nitrotoga had highest SRTs in the thinnest biofilms (10.2 days vs. < 3 days). Conversely, the SRT of Nitrosomonadaceae and *Nitrospira* spp. increased with biofilm thickness, particularly comparing the thinnest to thicker biofilms. Up to 10 fold variation in Taxon-SRTs were observed for SVs of the same genus, which could reflect variations in growth rates and environmental adaptation of individual species (Figure 1).

Significance: Our results indicate a trend of increasing Taxon-SRTs with biofilm thickness. These observations indicate that dispersal limitation results in higher Taxon-SRT. On the other hand, some nitrifiers had either increased SRT, or were exclusively present in thinner biofilms, which emphasizes differing metabolic strategies within the NOB guild.

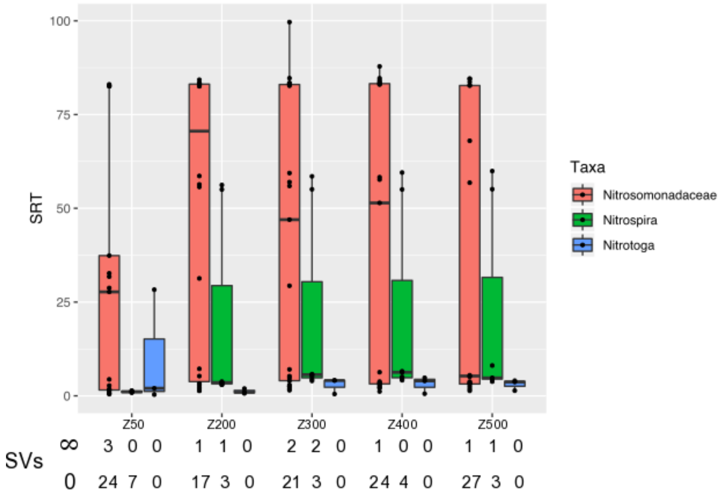

Figure 1. Distribution of SRT values for nitrifying taxa in different biofilm thicknesses. The number of SVs with SRT values of infinite or zero are indicated below the chart for each corresponding thickness and genus.

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