Zero-dimensional modelling and bacterial characterization of an aerobic granular sludge reactor

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Abstract

A laboratory aerobic granular sludge (AGS) sequencing batch reactor (SBR) removing soluble organic substrate, nitrogen and phosphorous was fed with artificial wastewater and modelled using a zero-dimensional approach. Model development was supported by bacterial characterization using 16S rRNA gene amplicon high-throughput sequencing. The mathematical model was based on the activated sludge model nr. 2d (ASM2d) and extended with both two-step nitrification as implemented in the wastewater treatment plant simulator Sumo19 (Dynamita SARL) and ammonia nitrogen adsorption and desorption according to Langmuir. The model developed for the AGS was thus based on a model formulated for conventional activated sludge. The anaerobic filling and aerobic reaction phases were dynamically simulated. The mean simulated soluble chemical oxygen demand (11.0 mgCOD/l) was offset to the measured data (37.2 mgCOD/l). This was attributed to the formation of soluble microbial products, which were not considered in the model. The measured ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, ortho-phosphate phosphorous, and dissolved oxygen concentrations were very well reproduced by the model (all Pearson's r values $\geq$ 0.9479). However, anoxic (denitrification) processes by ordinary heterotrophic organism during the aeration phase had to be introduced, which underpinned the importance of anoxic zones in the granules under oxic bulk conditions.

Keywords
Aerobic granular sludge, biofilm, diffusion, half-saturation coefficient, modelling, Monod

1. Introduction

Aerobic granular sludge (AGS) has emerged as an advanced wastewater treatment technology and as a suppressing alternative to the conventional activated sludge (CAS) process [1]. This is because AGS has many advantages compared to CAS. In AGS the purifying biomass grows in compact cell aggregates that settle very fast and that feature stratification in aerobic, anoxic and anaerobic zones of the granules. The properties of AGS lead to a small footprint of the wastewater treatment plants (WWTPs) and small capital and operational expenses [2]. AGS technology as realized in sequencing batch reactors (SBRs) has the advantage that all processes can take place in one reactor and that there is no need for separate settlers and for recycle of sludge and wastewater as in CAS with secondary settlers.
AGS was first obtained at laboratory scale in a sequencing batch reactor (SBR) in 1997 [3]. The SBR was operated in cycles (fill, react, settle and draw) characterized by a very short settling time ($\leq$ 1 min). This very short settling time resulted in the growth and selection of very fast settling granules in the reactor, i.e. slower settling aggregates were washed out whilst the granules were retained. Since then AGS is considered as a promising technology for wastewater treatment and as a major research topic. The research presented here develops on zero-dimensional (0D) mathematical modelling of AGS, i.e. not taking the structure of the granules into account.
Modelling can be a useful tool to describe and compile knowledge about AGS in a mathematical form. Ultimately, models can be used by practitioners for process design and optimization as demonstrated for CAS treatment by the state of the art activated sludge models (ASMs) from the ASM model family [4]. Note that AGS is seen as a biofilm system [5] and that biofilm models have first been used to mathematically describe the AGS system.
The first mathematical model of AGS grown in an SBR was proposed by Beun et al. [6]. This model was a one-dimensional (1D) model. It was implemented in AQUASIM, a simulator for aquatic systems [7]. The model used an extension of the biofilm model of Wanner & Gujer [8] as implemented in AQUASIM. However, the biofilm compartment in AQUASIM does not allow variable volumes and the model layout required the use of advective and diffusive links in order to implement the SBR. Following this concept, early AGS modelling was determined by the capacities of the

simulator AQUASIM, i.e. diffusive and/or advective links were needed in order to implement the variable volume of the SBR. This AGS modelling concept, i.e. 1D with advective and diffusive links, has been adopted in further research, and simulations have shown predictive power (see for example [9]).
More complex (2D and 3D) models have also been formulated for AGS [10-11]. As 1D models, these more complex models simulate AGS structure and use *intrinsic* kinetic parameters; diffusion is modelled explicitly using Fick's laws. Thus, in AGS modelling dimensional (1D, 2D, and 3D) biofilm models have been mainly used, because AGS is considered to be a biofilm system (see [12] for a thorough review). In this research we elaborate on using the 0D modelling approach with *extant* (apparent and measureable) kinetic parameters for modelling the AGS system.
Only one 0D AGS modelling study using ASM3 [4] is reported in early literature [13]. The 0D concept was demonstrated in principle. However, the 0D approach was not further developed in the following years, and dimensional models have pertained in AGS research. It was only recently that another 0D AGS model has been compared to a 1D model in a simulation study [14] and it was found that the 0D model approximated the 1D model. Additionally it was demonstrated in this study that this simple 0D (double Monod) model could dynamically predict real effluent ammonia nitrogen (NH4-N) concentrations of a full scale AGS plant.
Hence, although considerable research has been dedicated to AGS modelling, there is still a need to develop models of the AGS process that adequately describe relevant processes and that can be used in engineering practice. This study aims at further testing the 0D modelling approach for AGS in view of developing an AGS model for engineering practice. - The 0D approach is encouraged by the previous development of a 0D biofilm model for dynamic simulation of moving bed bioreactor (MBBR) systems [15] presented more recently elsewhere [16]. In order to do so, real data was collected from a laboratory scale AGS SBR; the data was used to formulate the proposed 0D mathematical model. Simulation results of unseen extend and quality (according to our knowledge) are presented, evaluated on real data, analysed and discussed. This modelling study was complemented by microbiological characterization of the developed granules, which was set into context of the mathematical modelling work.

2. Materials and methods

2.1 Reactor operation and analysis

The laboratory scale AGS reactor was based on the study of Beun et al. [6]: The reactor consisted of a transparent acrylic glass tube (inner diameter = 6.2 cm, height = 120 cm, working volume = 3 dm^3). The reactor was inoculated with activated sludge from a dairy wastewater treatment plant that (biologically) removed COD, N, and P. The reactor was operated in cycles using 60 min anaerobic fill (unmixed), 120 min react (aerated), 5 min settle, and 10 min decant. The reactor was filled to a level of 100 cm and decanted from half the filling height, i.e. 50 cm (volume exchange ratio = 50 %). Aeration was provided by an aquarium air pump (hygger) and an aeration stone. Wastewater was pumped in and out the reactor using two peristaltic pumps (Thermo SCIENTIFIC, Masterflex P/S, Easy Load II). The aeration and peristaltic pumps were controlled by an in house made control system using a Raspberry Pi and LABVIEW. The reactor was operated at room temperature (23 ± 0.5°C).
Artifical wastewater was prepared in batches using commercially available chemicals (Fisher Scientific) with the following concentrations: Sodium acetate (342 mgCOD/l), ammonium chloride (17.5 mgN/l), mono- and di-potassium phosphate (3.3 mgP/l in total), magnesium sulfate heptahydrate (83 mg/l), and potassium chloride (33 mg/l). The COD:N:P ratio was thus 100:5:1 in the artificial wastewater. A volume of 1.5 ml of trace element solution (adapted from [17]) was added to a batch of 83 l of articifial wastewater prepared with tap water.
Standard wastewater components (COD, ammonium-N, nitrite-N, nitrate-N, and orthophosphate-P) were analysed using cuvette tests (Hach). Wastewater samples were filtered through a 0.7 µm pore filter (WTW) before analysis. The dissolved oxygen (DO) concentration was measured during the aerobic phase using a benchtop meter and an on-line probe (WTW, inoLAB). A detailed analysis (sampling interval of 10 min during one aeration phase) of wastewater characteristics was performed on operational day 367.

2.2 Bacterial Characterization

Granules were collected from the AGS reactor in order to evaluate their bacterial structure on operational day 368. For that purpose, a volume of approximately 250 µL of AGS containing granules was sampled during the aeration phase and directly frozen in liquid nitrogen. The frozen aliquots were stored at -80 °C prior to analysis. Before DNA extraction, the sample was thawed, centrifuged for 15 min at 14 000 g, and supernatent was removed. DNA from the pellet (granules) was extracted using the PowerSoil DNA Isolation kit (MoBio) according to the manufacturer's protocol. The bacterial 16S rRNA gene amplicon libraries were prepared as previously described (see [18] and references in there). Briefly, the modified universal primer pair S-D-Bact-0909-a-S-18 and S-*-Univ-*-1392-a-A-15 and Nextera XT Index Kit V2

(Illumina) were used along with Q5 Hot Start High-Fidelity 2x Master Mix (New England Biolabs) to perform two-step polymerase-chain reaction (PCR). In the first PCR, selective amplification of the 484 bp long fragments of bacterial 16S rRNA gene V6-V8 region was performed. In the second PCR, Illumina-compatible adapters and barcodes were attached to the previously amplified fragments. Purified libraries were sequenced along with PhiX control (Illumina) using Miseq Reagent Kit V3-600 on in-house Illumina MiSeq Platform. The sequencing results analysis (quality trimming, chimera check, singletons removal and assignment of the obtained sequences to operational taxonomic units (OTUs) at 97% similarity level) was done using usearch v11.0.667_i86linux64 software. The taxonomic affiliation of the resulting bacterial OTUs was performed using Mothur and SILVA database v.138. Most dominant OTUs were also taxonomically affiliated using the MiDAS database in order to get indication about their putative activity [19] and their closest cultivated sequences were determined using NCBI's BLAST and the refseq_rna database. The bacterial nucleotide sequences were deposited in the GenBank database under the accession numbers MZ410808 to MZ411347.

2.3 Modelling

The mathematical model of the AGS reactor was implemented in the wastewater treatment plant simulator Sumo19 (Dynamita SARL). The SBR object available in Sumo was used. This object/module is normally used to model SBRs with CAS. This SBR module has variable volume. The SBR was set-up as completely mixed during the fillind and aeration phase. The mathematical model of the biochemical conversions behind this object was Sumo2, an activated sludge model based on ASM2d [4] and extended with two-step nitrification (see Sumo19 model sheet if available or contact Dynamita SARL for further information). The influent of the AGS reactor model was defined according to the composition of the artificial wastewater. The simulation was run over one filling and react phase; the settling and decant phases were not modelled. The initial amounts of active biomass, i.e. ordinary heterotrophic organisms (OHOs), phosphorous accumulating organisms (PAOs), ammonia oxidizing organisms (AOOs), and nitrite oxydizing organisms (NOOs) were estimated. Glycogen accumulating organisms (GAOs) were not considered. The author is aware that the estimates of active biomass are not absolute estimates since microbial activity is a product of amount of active biomass and specific growth rate, and depends of various environmental parameters. The respective specific growth rates were used with typical values used in ASM2d and Sumo2 ([4] and Sumo2 default values). Initial storage products were also estimated and some half-saturation coefficients and other parameters were calibrated (see 3.4 and Appendix I).
Simulation results were evaluated by correlation with the measured data. Pearson's r values were calculated using the relevant dynamic data ranges (i.e. omitting concentrations that had become and stayed zero in the series).

3. Results and discussion

3.1 Reactor performance

The removal rates of chemical oxygen demand (COD), ammonium nitrogen (NH4-N), and orthophosphate (PO-P) were excellent (Tab. 1). The soluble substrate (COD) was completely consumed before the beginning of the aeration phase (60 min) and it was assumed that the remaining COD was inert, because it's amount did not further decrease during the aeration phase (60 – 180 min). This recalcitrant COD was attributed to the formation of soluble microbial products (SMP) as reported in the same range for AGS reactors in the literature [20]. Some nitrate nitrogen (NO3-N) was found in the influent, which originated from the tap water. Also, a net production of NO3-N took place during the aeration phase due to nitrification; therefore the associated removal appeared with a negative value.
The data set presented in table 1 was used for model calibration.

Table 1: Data set resulting from chemical analysis during one cycle of the AGS reactor and used for modelling.

Sample Nr.	Time	COD	NH4-N	NO2-N	NO3-N	TIN	PO4-P
	[min]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]
Influent	0	340	17.9	0.091	4.07	22.061	3.76
0	60	39.9	6.5	0	2.34	8.84	32.6
1	70	32.5	4.62	0.299	2.55	7.469	27.9
2	80	35.1	2.72	0.671	2.65	6.041	25.5
3	90	41.4	0.809	1.07	4.46	6.339	19.2
4	100	48.4	0.16	0.751	5.55	6.461	14.4
5	110	21	0	0	5.86	5.86	10.7
6	120	40.6	0	0	6.02	6.02	6.75
7	130	26.4	0	0	5.94	5.94	4
8	140	46.7	0	0	5.97	5.97	2.71
9	150	34.9	0	0	5.73	5.73	1.26
10	160	57.3	0	0	5.94	5.94	0.602
11	170	21.9	0	0	5.96	5.96	0.301
12	180	37.5	0	0	5.94	5.94	0.313
Removal	[%]	**89.0**	**100.0**	**100.0**	**-45.9**	**73.1**	**91.7**

3.2 Bacterial community structure of the granules

To characterise the bacterial structure of the granules developed in the studied AGS reactor, we sequenced the bacterial 16S rRNA gene previously amplified of a sample of granules collected after 368 days of reactor operation. The high-throughput sequencing resulted in 114,135 reads, assigned to 584 bacterial OTUs (defined at 97 % sequence similarity). The calculated rarefaction curve reached a plateau (Figure 1c), indicating that most of the bacterial diversity was described by our sequencing. Indeed, the Boneh estimate indicates that no more than 28 OTUs (out of 584, *i.e.* less than 5 %) could have been additionally described by increasing the sequencing depth.

The studied aerobic granular sludge was mostly composed of bacteria affiliated to the phyla Proteobacteria (representing 44.3 % of the total relative abundance), Bacteroidota (33.3% of the total relative abundance), Planctomycetota (8.4 % of the total relative abundance), and Chloroflexi (4.4 % of the total relative abundance) (Figure 1a). Organisms affiliated to other phyla such as Acidobacteriota, Myxococcota, Nitrospirota, Verrucomicrobiota for example represented less than 10 % of the whole bacterial community in terms of relative abundance. This observation is consistent with the current knowledge on granular sludge in which Proteobacteria and Bacteroidota are found to be the dominant phyla (see for example [21]).

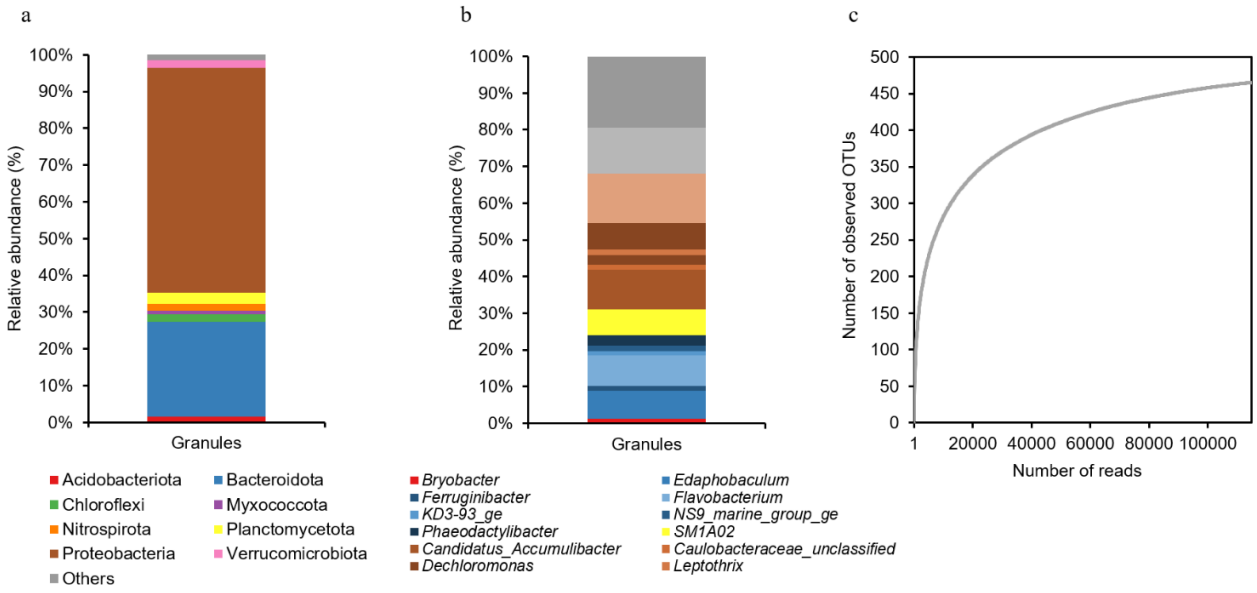

Figure 1: Bacterial structure of the granules sampled in the studied AGS. Results are presented at the phylum level (a) and at the genus level (b). Panel c shows the observed richness estimator rarefaction curve of the high-throughput amplicon sequencing of the bacterial 16S rRNA gene for the analysed AGS sample.

Table 2: Phylogenetic affiliation and closest cultivated species of the 18 OTUs having a relative abundance above 1% in the studied granules

OTUs	Relative abundance (%)	Phylogenetic affiliation[a]			Closest cultivated species[b]		
		Phyla level	Genus level	Putative function	Specie name	Accession n.	Score (% identity)
Otu1	12.38	Proteobacteria	*Zoogloea*	OHO	*Zoogloea caeni*	NR_043795.1	100.00
Otu2	10.74	Proteobacteria	*Candidatus* Accumulibacter	PAO	*Propionivibrio pelophilus*	NR_024855.1	97.42
Otu3	8.27	Bacteroidota	*Flavobacterium*	OHO	*Flavobacterium keumense*	NR_157621.1	98.92
Otu10	7.29	Proteobacteria	*Thauera*	OHO	*Thauera aminoaromatica*	NR_027211.1	100.00
Otu5	4.96	Bacteroidota	*midas_g_729*		*Taibaiella yonginensis*	NR_145567.1	88.75
Otu4	4.94	Bacteroidota	*Taibaiella*		*Mucibacter soli*	NR_165710.1	94.68
Otu9	3.85	Chloroflexi	*Candidatus* Villigracillis		*Bellilinea caldifistulae*	NR_041354.1	92.51
Otu12	3.64	Planctomycetota	*SM1A02*		*Phycisphaera mikurensis NBRC 102666*	NR_074491.2	86.97
Otu14	3.12	Planctomycetota	*SM1A02*		*Thermogutta hypogea*	NR_134825.1	88.42
Otu7	3.00	Bacteroidota	*Phaeodactylibacter*	OHO	*Haliscomenobacter hydrossis*	NR_074420.1	95.46
Otu11	2.75	Proteobacteria	*Dechloromonas*	OHO	*Dechloromonas hortensis*	NR_042819.1	99.14
Otu8	2.18	Bacteroidota	*Ferruginibacter*		*Mucibacter soli*	NR_165710.1	95.11
Otu25	1.50	Proteobacteria	*Ideonella*	OHO	*Aquincola amnicola*	NR_159088.1	98.05
Otu26	1.32	Proteobacteria	*Caulobacter*		*Brevundimonas aveniformis*	NR_043770.1	99.78
Otu23	1.16	Bacteroidota	*Ferruginibacte*r	OHO	*Ferruginibacter lapsinanis*	NR_044589.1	97.01
Otu290	1.09	Proteobacteria	*Zoogloea*	OHO	*Zoogloea ramigera*	NR_113749.1	98.93
Otu30	1.06	Bacteroidota	*midas_g_3492*		*Tenacibaculum caenipelagi*	NR_125675.1	86.72
Otu29	1.04	Bacteroidota	*midas_g_3846*		*Sphingobacterium spiritivorum*	NR_113707.1	88.63
Total	74.32						

[a] Phylogenetic affiliation was performed using the MiDAS database. [b] Closest cultivated sequences were determined using NCBI's BLAST and the refseq_rna database. OHO: ordinary heteroptrophic organism; PAO: phosphorous accumulating biomass.

At the genus level (Figure 1b), *Zoogloea* was the dominant genus, representing 13.48 % of the whole bacterial community, followed by *Candidatus* Accumulibacter (10.74 %), *Flavobacterium* (8.37), *Edaphobaculum* (7.64 %), *Thauera* (7.29 %) and *SM1A02* (6.94%) for example. It is interresting to highlight the high percentage of uncultured (*i.e.* uncharacterised) organisms at this taxonomic level (12.52 %). The presence of members of the genus *Zoogloea* which form cell aggregates embedded in gelatunous matrices, called zoogloeal matrices [22], and members of genus *Flavobacterium* which support the granules formation by the production of extracellular polymeric substances [23] are typical and logical for the studied environment (AGS).

At the OTU level, the bacterial community structure was dominated by only 18 OTUs having each a relative abundance above 1 % (Table 2). These 18 OTUs accounted for roughly 74 % of the total relative bacterial community abundance. Two OTUs out of the 18 had a relative abundance above 10 %. Otu1 was the most dominant one in the granules, representing around 12.38 % of the total bacterial community. This OTU was taxonomically affiliated to *Zoogloea caeni* sp. nov. (100 percent identity of the 16s rRNA gene sequence), a floc-forming bacterium isolated from activated sludge, and responsible for a quick reduction of nitrate to nitrite [24]. The second most abundant OTU, Otu2 representing 10.74 % of the bacterial community of the studied granules, was affiliated to *candidatus* Accumulibacter. This bacterial group is known to be able to accumulate large amounts of intracellular polyphosphate [25]. No representative organism with a percentage identity of 100 % could be identified for this specific OTU. According to the MiDAS database used to identify putative functions of the main OTUs present in the studied granules, most of them are ordinary heterotophic organisms which mediates the biological processes of COD removal and denitrification [26] (Table 2).

These results were taken into account as much as possible for the model development described below, bearing in mind that more than 12 % of the identified OTUs could not be classified at the genus level and that the organisms for which a putative function could be associated, are representing only 48 % in terms of relative abundance of the whole bacterial community of the studied granules. These made the microbiological data set rather incomplete and indicative only.

3.3 Model Development

In a preliminary modelling attempt Sumo2 (see 2.3) was used as implemented in the software Sumo19 for conventional activated sludge modelling. Two significant observations and interpretations were made:

1. The ammonia nitrogen concentration (NH4-N) at the beginning of aeration phase (6.5 mgN/l) was smaller than expected from the dilution effect only (17.90 mgN/l * 0.5 = 8.95 mgN/l; volume exchange rate = 50 %). This was explained by adsorption of ammonium nitrogen to the granules as proposed in the literature [27].
2. The nitrate nitrogen concentration (NO3-N) at the end of the aeration phase was much smaller than predicted by the model (data not shown). This was explained by the presence of anoxic zones in the granules during the aeration phase. (The simulation results could not be improved by adapting kinetic parameters sensibly in the model.)

Adsorption (Eq. 1) and desorption (Eq. 2) of ammonia nitrogen were therefore introduced according to Langmuir [28]. It was thus assumed that the ammonia nitrogen adsorption rate (r_{ads}) is proportional to the product of the adsorption rate constant (k_{ads}), the ammonia nitrogen concentration in the bulk (S_{NHx}), the concentration of adsorbed ammonia nitrogen at saturation ($S_{NHx,sat}$), and the relative amount of available adsorption sites, given by 1 minus the concentration of adsorbed ammonia nitrogen ($S_{NHx,ads}$) devided by the concentration of adsorbed ammonia nitrogen at saturation ($S_{NHx,sat}$). The desorption rate (r_{des}) was assumed to be proportional to the product of the desorption rate constant (k_{des}), the concentration of adsorbed ammonia nitrogen at saturation ($S_{NHx,sat}$), and the relative coverage.

$$r_{ads} = k_{ads} \cdot S_{NHx} \cdot S_{NHx,sat} \cdot \left(1 - \frac{S_{NHx,ads}}{S_{NHx,sat}}\right) \qquad Eq.1$$

$$r_{des} = k_{des} \cdot S_{NHx,sat} \cdot \left(\frac{S_{NHx,ads}}{S_{NHx,sat}}\right) \qquad Eq.2$$

Two new processes, two new parameters and one new state variable were thus added to Sumo2. The stoichiometric factors for the conversions between S_{NHx} and $S_{NHx,ads}$ were set to 1 and -1 respectively. The concentration of adsorbed ammonia nitrogen at saturation ($S_{NHx,sat}$) was assumed to be a constant, because the amount of biomass in the reactor, and hence the number of adsorption sites did not change significantly during a phase.

The model matrix was further changed by emphasizing anoxic processes of ordinary heterotrophic organisms (OHOs) during the aeration phase. Note that denitrification by phosphorous accumulating organisms (PAOs) was "switched off" by setting the reduction factor for anoxic growth of these organisms to 0, this was in the sense of the original ASM2 [4] and supported by numerous simulations (data not shown).
In order to do so, inhibition functions related to the bulk oxygen concentration for anoxic growth of OHOs were removed. As a result, anoxic growth could take place under aerobic bulk conditions. An inhibition function related to the bulk oxygen concentration for oxic growth of OHOs was introduced. As a result, this aerobic growth of OHOs did not take place under aerobic bulk conditions, and the organic substrate remained available for denitrification.

Glycogen accumulating organisms (GAOs) were not considered in the model and their concentration was set to 0, because this organism group was not evidenced by the sequencing analysis (Table 2) and because all the organic substrate was taken up by PAOs. (GAOs will more likely have to be considered in AGS reactors fed with real wastewater.)

3.4 Calibration and simulation Results

During calibration, the initial amounts of active biomass, i.e. ordinary heterotrophic organisms (OHOs), phosphorous accumulating organisms (PAOs), ammonia oxidizing organisms (AOOs), and nitrite oxydizing organisms (NOOs) were estimated using the results presented in Table 1 and the charts presented below (Fig. 3-6). An overview of these and other estimated parameters can be found in Appendix I.

The (soluble) chemical oxygen demand (***COD***) fluctuated during the aerobic reaction phase of the AGS reactor (Fig. 2); no continuous decline of this wastewater component could be observed. The mean value was found to be 37.2 mgCOD/l with a standard deviation of 10.4 mgCOD/l. This indicated that the soluble COD during the aerobic reaction phase was inert (as mentioned above) and originated from soluble microbial products (SMP). This inert COD did not originate from EDTA of the trace solution, that was added to the artificial wastewater only, and which had a final concentration of 1.8 mg/l (i.e. 1.9 mgCOD/l), in the influent. However, formation of SMP was not modelled. This result meant that the soluble substrate provided as acetate was almost completely stored as polyhydroxyalkanoate (PHA) by the phosphorous accumulating organisms (PAOs) during the anaerobic feeding of the reactor. Some influent soluble substrate might also have been consumed by denitrifying biomass during anaerobic feeding, since nitrate nitrogen (NO3-N) available from the influent (4.07 mgN/l), which was prepared with tap water, was slightly reduced after the anaerobic reaction phase (Fig. 4).

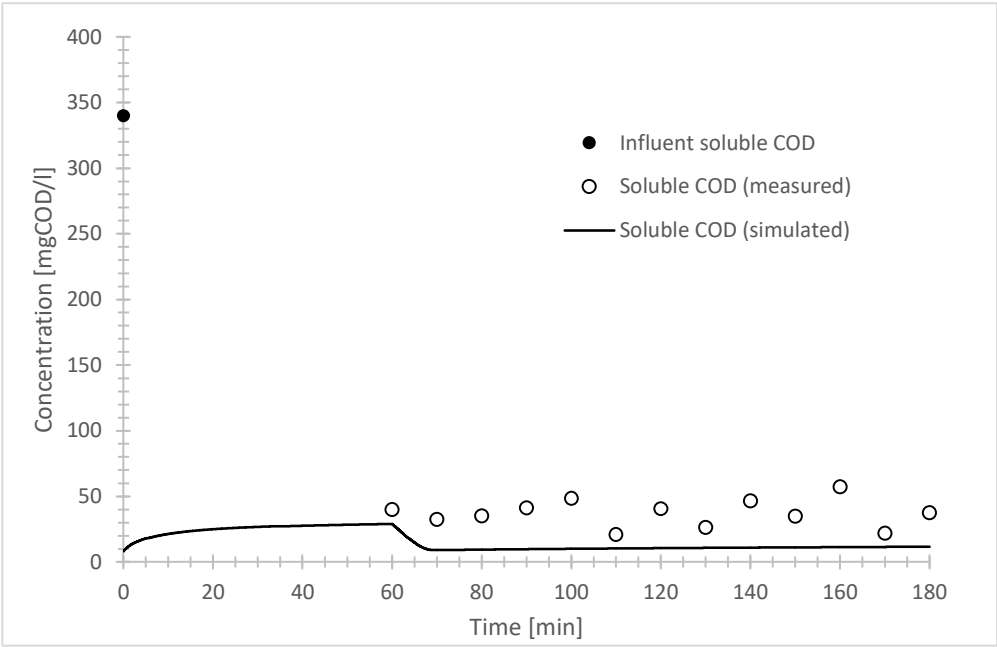

Figure 2: Measured and simulated soluble COD concentrations in the influent and the AGS reactor.

The ammonium nitrogen concentration in the bulk (***NH4-N*** called ***SNHx*** in the model) dropped from 17.9 mgN/l in the influent to 6.5 mgN/l at the beginning of the aerobic reaction phase, i.e. after 60 min anaerobic feeding (measured data in Fig. 3). As mentioned before, this was explained by dilution of the influent (volume exhange rate 50 %) and adsorption of ammonium nitrogen by the granules as proposed in the literature (Bassin *et al*. 2011). It was thus suggested, that 2.4 mgN/l were adsorbed by the granules. This value seems to be plausible according to the literature (Bassin *et al*. 2011). Indeed, the model predicted an adsorbed ammonia nitrogen concentration (SNHx,ads) of 2.1 mgN/l at the beginning of the aeration phase (Fig. 3). It is further suggested, that the "missing" 0.3 mgN/l were taken up as nutrient by the biomass (OHOs and PAOs). The initial amount of AOOs was estimated to be 80 mgCOD/l and the respective half-saturation coefficient K_{NH4} was set to 0.35 mgN/l. The default value for this parameter in ASMs is 1.00 mgN/l (Henze *et al*. 2000). This indicated that diffusion of ammonium nitrogen into the cell aggregate was less important for the aerobic granules than for typical conventional activated sludge, since diffusional mass transfer limitations manifest by increased half-saturation coefficients in activated sludge models [29-30].

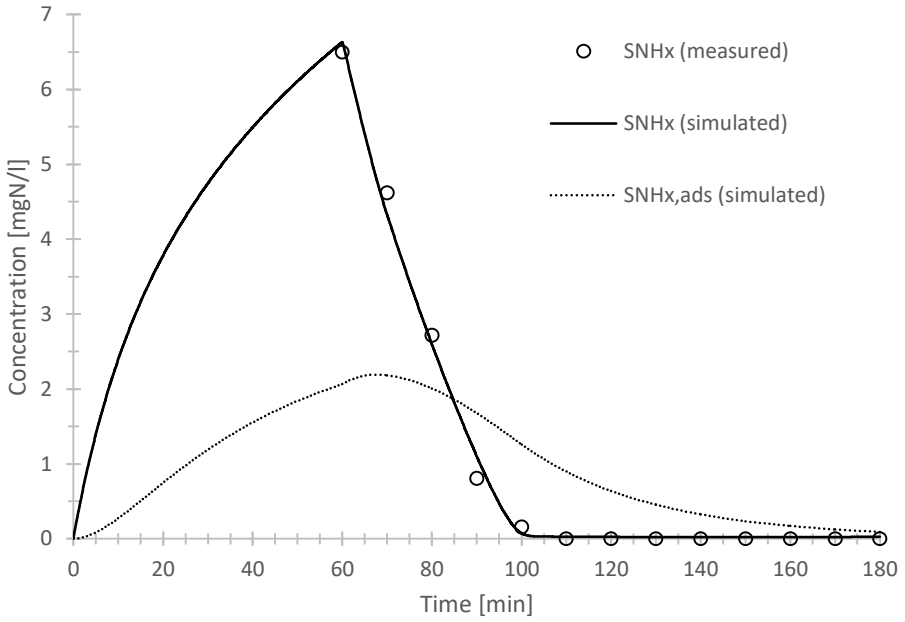

Figure 3: Measured and simulated ammonia nitrogen concentration in the bulk (SNHx), and (simulated) adsorbed ammonia nitrogen concentration (SNHx,ads) in the AGS reactor (Pearson's r = 0.9971).

The measured nitrite nitrogen concentration (***NO2-N*** called ***SNO2*** in the model) during the aerobic reaction phase of the AGS reactor showed a peak (Fig. 4). The initial amount of NOOs was set to 50 mgCOD/l. The respective half-saturation coefficient was not changed. This peak was also well reproduced by the model. The nitrate nitrogen concentration (***NO3-N*** called ***SNO3*** in the model) at the beginning of the aerobic reaction phase (2.34 mgN/l) was well reproduced by the simulation (Fig. 4), but the subsequent increase of the measured data delayed relative to the simulated data. The authors have no explanation for this at the moment. The agreement of the simulation with the final measured nitrate nitrogen concentration was good. Overall it can be said that NO2-N and NO3-N were well reproduced by the model and quantitative agreement was found. Note that denitrification by PAOs was not considered by the model. Nitrite and nitrate depletion was thus due to anoxic growth of heterotrophic organisms only. This deserves further investigation..

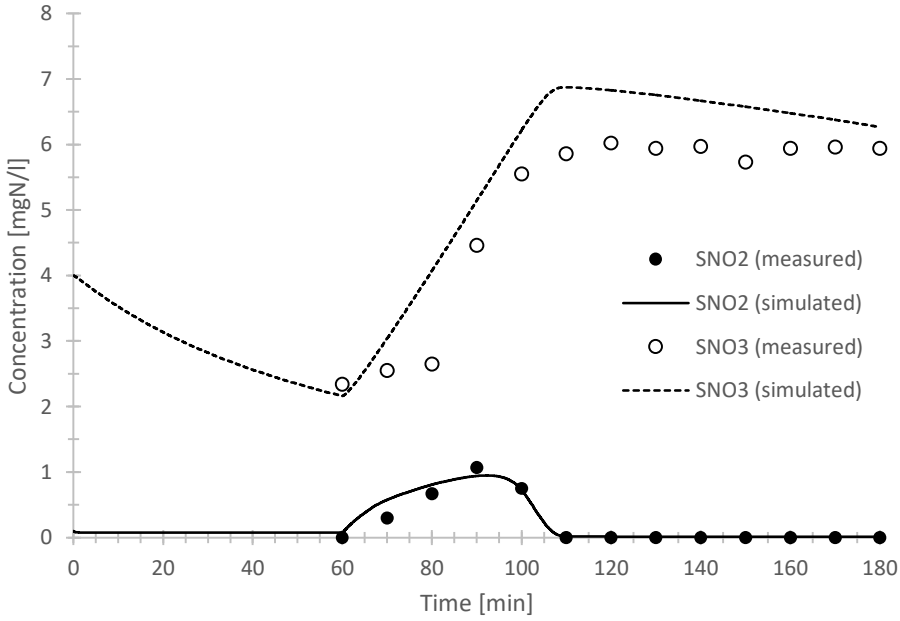

Figure 4: Measured and simulated NO2-N (Pearson's r = 0.9479) and NO3-N (Pearson's r = 0.9716) in the AGS reactor.

The total othophosphate (***PO4-P*** called ***SPO4*** in the model) release during the anaerobic feeding phase was well simulated by the model (Fig. 5). However, PO4-P was not measured during anaerobic feeding, but the final PO4-P concentration after feeding (32.6 mgP/l) was closely reproduced by the model. The uptake of PO4-P during the aerobic reaction phase was well simulated by the model (Fig. 5). The initial amount of PAOs was set to 2500 mgCOD/l, the initial amount of PHA was 200 mgCOD/l and the initial amount of polyphosphate (PP) was 250 mgP/l. The half saturation coefficient for P-uptake (K_{PS}) was set to 8.0 mgP/l. The typical value for K_{PS} is 0.2 mgP/l (Henze *et al*. 2000) or 0.5 mgP/l (in Sumo2). This indicated that diffusion of PO4-P into the cell aggregate was much more important for the aerobic granules than for conventional activated sludge, since diffusional mass transfer limitations manifest by increased half-saturation coefficients in activated sludge models [29-30].

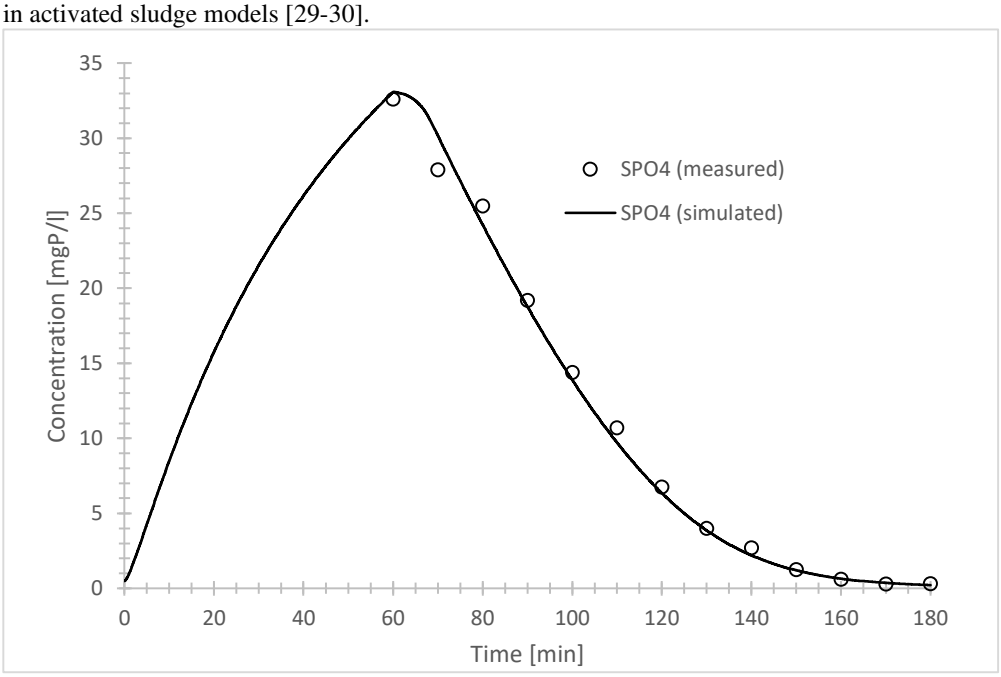

Figure 5: Measured and simulated PO4-P in the AGS reactor (Pearson's r = 0.9976).

The dissolved oxygen concentration (***DO*** called ***SO2*** in the model) featured several bends which were very well reproduced by the model (Fig. 6). The air flow was estimated in the model to be 1.8 m^3/d. The resulting oxygen mass transfer coefficient (K_La) was 827 1/d.

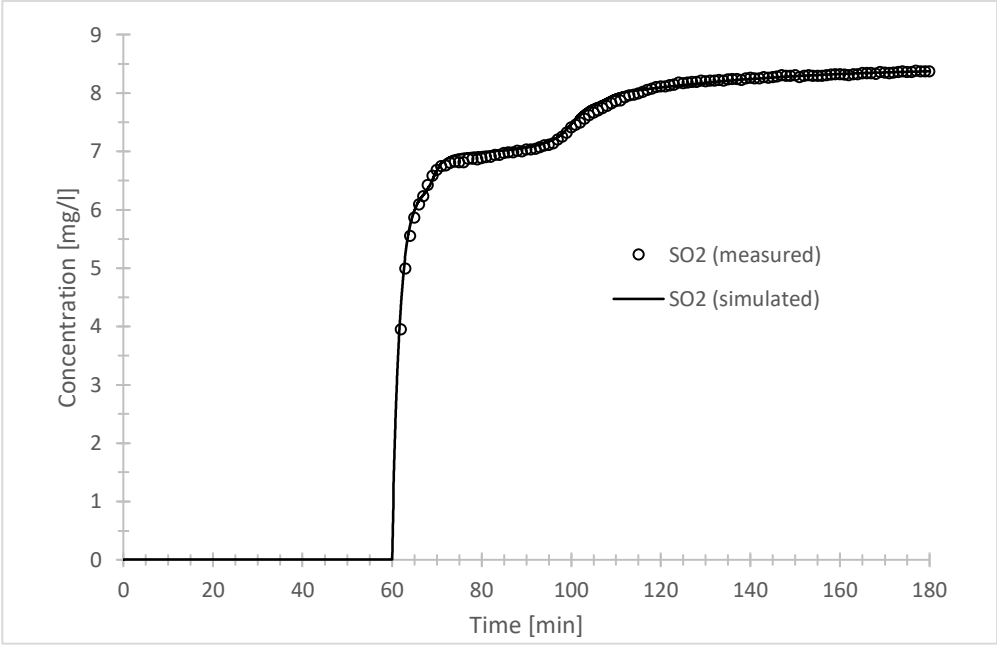

Figure 6: Measured and simulated DO in the AGS reactor (Pearson's r = 0.9978).

OHOs and PAOs were found to be the dominant species in the model, which corroborated the microbiological characterization of the granules. However, the relative abundance (expressed genetically) did not correspond to the relative amounts (expressed in biomass) found in the model. The authors think that this is because both measures cannot be directly correlated, and there are to the authors knowledge no correlation factors available in the literature. Further kinetic activity can be affected by various factors (e.g. temperature and pH) which might boost or impede activity of specific organism groups differently, thereby making a direct link of both measures difficult.

4. Conclusions

The zero-dimensional (0D) modelling approach was taken to model an AGS system fed with artificial wastewater and removing (soluble) COD, N, and P. The simulation results were very good for all reactants, i.e. COD, NH4-N, NO2-N, NO3-N, PO4-P, and DO. Anoxic (denitrification) processes during the aeration phase were introduced. This underpinned the presence and importance of relatively large anoxic zones in the granules during oxic bulk conditions.
This modelling approach deserves further testing with reactors fed with real wastewater, also on pilot and full scale, in order to confirm the applicability in engineering practice. The results are very promising and the 0D modelling approach offers an alternative to more complex dimensional (biofilm) models that emphasize cell aggregate structure. The authors believe that the research presented here will contribute to filling the gap that has developed over the past decades between dimensional biofilm research and engineering practice in the wastewater treatment modelling community.

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Appendix I

Initial conditions, kinetic parameters, common switches, stochiometric yields, and ammonia nitrogen adsorption parameters used in the model.

Initial conditions (state variables)			
Active biomass	**Unit**	**Value**	**Default**
Ordinary Heterotrophic organisms (OHOs)	[mgCOD/l]	500	-
Phosphorus accumulating organisms (PAOs)	[mgCOD/l]	2500	-
Glycogen accumulating organisms (GAOs)	[mgCOD/l]	0	-
Ammonia oxydizing organisms (AOOs)	[mgCOD/l]	80	-
Nitrite oxydizing organisms (NOOs)	[mgCOD/l]	50	-
Storage products	**Unit**	**Value**	**Default**
Stored polyhydroxyalkanoates (PHA)	[mgCOD/l]	200	-
Stored polyphosphate (PP)	[mgP/l]	250	-
Kinetic parameters			
Ordinary Heterotrophic organisms (OHOs)	**Unit**	**Value**	**Default**
Reduction factor for anoxic growth of OHOs	-	0.10	0.6 [1]
Phosphorus accumulating organisms (PAOs)	**Unit**	**Value**	**Default**
Reduction factor for anoxic growth of PAOs	-	0.00	0.66 [1]
Half-saturation of PO4 for PAOs	[mgP/l]	8.00	0.50 [1]
Ammonia oxydizing organisms (AOOs)	**Unit**	**Value**	**Default**
Half-saturation of NHx for AOOs	[mgN/l]	0.35	0.70 [1]
Common switches	**Unit**	**Value**	**Default**
Half-saturation of NHx as nutrient for biomass	[mgN/l]	0.000	0.005 [1]
Stochiometric yiels	**Unit**	**Value**	**Default**
Ratio of P released per VFA stored	[mgP/mgVFA]	0.25	0.65 [1]
Ammonia nitrogen adsorption	**Unit**	**Value**	**Default[2]**
Adsorption rate constant for total ammonia N	[mgN/l.d]	6.00	-
Desorption rate constant for total adsorbed ammonia N	[1/d]	50.00	-
Maximum concentration of afsorbed ammonia N	[mgN/l]	6.00	-

[1] Default value in Sumo19

[2] Not defined in Sumo19