Comparison of aerobic granulation in SBR and continuous-flow plants

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Abstract

Up to now, aerobic granulation of activated sludge is only realized in SBRs, where the discontinuous feed and sedimentation allow the formation of dense granules with excellent settling properties. However, aerobic granulation in continuous-flow plants (CFP) is gaining more and more interest in order to exploit the advantages of these excellent sludge properties to construct compact and efficient WWTP. Within the scope of this project, a SBR and CFP were operated in parallel to investigate the aerobic granulation of activated sludge and to compare the biomass in terms of their structure and settling behavior. CFP operation included two experimental phases with different reactor designs. The use of synthetic wastewater during phase I led to a biomass with a SVI of 42 ml g^{-1}, whereby the SVI declined only to 85 ml g^{-1} in the second phase and the use of municipal sewage. After the start-up period, microscopic images of the biomass from CFP comprised small compact granules with a high flocculent fraction. Particle size distribution for phase II confirm, that 72% of the particles had a size over 200 µm. A strong correlation was observed between the appearance of NO_x-N in the first reactor and the SVI. The results illustrate, that the anaerobic conditions during feeding are essential to keep stable granules.

1. Introduction

Aerobic granular sludge (AGS) is an innovative technology for the biological wastewater treatment worldwide. AGS is characterised as compact and dense biomass which settles much faster than flocculent sludge. Up to now, AGS is only realised in sequencing batch reactors (SBR). These systems offer an easy handling and the adjustment of individual phases within one single reactor. Hereby, the cycle operation consists of an anaerobic feeding, aeration and sedimentation phases. In recent years, a lot of research has been undertaken to understand the requirements for the aerobic granulation, whereby initially SBR systems were considered. Especially, the anaerobic feed was found to improve the stability of the granules by maintaining substrate-storing organism with slow growth rates (de Kreuk and van Loosdrecht, 2004). These organisms are promoted by a batch-wise feeding strategy, which ensures feast and famine conditions and subsequently oppress the growth of filaments. Beside a well-described plug-flow feed into the settled biomass at the bottom of the reactor and a subsequently high F/M ratio, some studies relate to mixed anaerobic conditions during the feed. Rocktäschel et al. (2013) found that this steep substrate gradient between bulk phase and biomass is not absolutely necessary to form stable granules. Compact and dense granules with excellent settling properties were hereby achieved with a fast influent pumping and a subsequently anaerobic mixing phase, although the granules were overall smaller compared to the non-mixed feeding strategy. The results of this study indicate that aerobic granulation is also possible under mixed anaerobic conditions (Rocktäschel et al., 2013), which is important for the continuous-flow operation. Beside the anaerobic feed, an increased hydraulic pressure can be set to washout fine sludge flocs and to promote aerobic granulation. The discharge of flocculent biomass during the start-up of an AGS lab scale reactor is often desired in order to enrich a biomass which settles much faster and owns already a dense and compact structure. Hereby, the minimum settling velocity, defined as $v_{s,min}$, influences the extent of sludge particles that is discharged during the decant phase. The parameter $v_{s,min}$ is calculated as quotient of the sedimentation height L and the settling time t [Unit: $m\ h^{-1}$] and can be either enhanced by increasing the settling height inside the reactor or by reducing the settling time. In order to achieve a rapid granulation, $v_{s,min}$ should be higher than the settling velocity of the flocculent sludge.

First full scale plants based on aerobic granular sludge were constructed as Nereda process. Pronk et al. (2015b) summarize some first operating data from an aerobic granular sludge plant treating domestic wastewater. The authors report a sludge and volumetric loading rate of 0.1 $kgCOD\ kgTSS^{-1}\ d^{-1}$ and 1.5 $kgCOD\ m^{-3}\ d^{-1}$. Hydraulic retention time was 17 h. The dry and rain weather cycle comprised 390 and 180 min with an anaerobic plug-flow feed of 60 to 90 min. Hereby, the plug-flow feed under non-mixed conditions creates high substrate concentrations which promotes the formation of large granules due to an increased diffusion. The desired biomass concentration of 8 $gTSS\ L^{-1}$ was reached after 9 months (Pronk et al., 2015b).

Beside this suitable handling of SBR systems for an aerobic granulation, in which an anaerobic plug-flow is easy to realise and $v_{s,min}$ can be controlled over the sedimentation time, most of the large scale WWTP are designed as continuous-flow plants (CFP). It is essential to have well-mixed conditions in these systems in order to avoid sludge deposits on the bottom of the tanks. Moreover, the clarification takes place continuously in a separate tank, which makes the sludge separation difficult to control. However, the implementation of AGS in existing CFP would offer the opportunity to retrofit existing plants and to increase the hydraulic treatment capacity without the need to build additional tank volume. The successful transfer of AGS in a CFP operation would allow constructing compact WWTP with smaller sedimentation tanks. Moreover, there are publications reporting a reduced energy demand for the activated sludge tank (Giesen and Thompson, 2013; Niermans et al., 2009). With regard to the several advantages of the AGS process, a current research question is, under which conditions is it possible to realise an aerobic granulation in a continuous-flow operation.

Until now, there are only few publications reporting about AGS in continuous-flow systems. For example, Liu et al. (2012) used a continuous-flow setup with a SBAR (sequencing batch airlift reactor), a settling tank and a tank with a dynamic membrane, which was used to ensure good effluent quality. The sludge separation was realised over a sieve with a mesh size of 600 µm. Sieving methods are accepted to separate granules from floccelent sludge since the second granular sludge workshop (de Kreuk et al., 2007). The plant was inoculated with granules from a SBR and fed with synthetic wastewater. The retained biomass had a loose structure with particle sizes in a range of 0.1 to 1.0 mm and settling velocities between 15 and 25 m h^{-1}. The authors report, that the granules had an overall smaller diameter, because under continuous feeding conditions, the diffusion of nutrients is limited by the lower substrate gradient. Li et al. (2015) investigated the aerobic granulation in a reverse-flow baffled reactor (RFBR), where the feed of raw wastewater (30% municipal and 70% industrial) was periodically switched between the two ends of the reactor via control valves. During the first half of the cycle (2 h), the flow direction was from right to left through the reactor and was switched from left to right in the second phase. A main advantage of this operation is, that the need of sludge pumping is minimised. The seed sludge was from an anaerobic-aerobic tank of a WWTP with a sludge volume index (SVI) of 66 ml g^{-1}. The operation resulted in periodic feast and famine conditions, which allowed the formation of granules after 21 days and finally a SVI of 33 ml g^{-1}. However, the granules from the RFBR had a small mean diameter of 130 µm and the raw wastewater obtained a high amount of inorganic compounds (metal ions and salts), which probably affected the settling behavior. Another study from Li et al. (2016) relates to the operation of a CFP, where the setup included an anaerobic reactor with 6 L and an aerobic reactor with 9 L. The sludge separation was realised in a secondary clarifier constructed as a tube with 0.125 L. HRT was 6 h and compromised an anaerobic time of 2.4 h and an aerobic time of 3.6 h. The granules were distinguished by particle sizes of 600 µm and a loose structure with a SVI of about 20 ml g^{-1}. However, the plant was inoculated with AGS from a SBR and synthetic wastewater was used as feed, which is known to favor for regular granules. More references of aerobic granulation in CFPs are summarized below in Table 1.

The few studies to aerobic granulation in CFPs illustrate, that there is still research demand in order to establish aerobic granulation in continuous-flow systems with the use of municipal wastewater. Especially, the implementation of the anaerobic feed and the sludge separation are important for the large scale application. In this study, two experimental setups for SBR and CFP were operated to investigate the aerobic granulation and settling properties of the activated sludge. The main objective of the SBR was to investigate the influence of the anaerobic feeding and settling time on the granules structure. AGS cultivated in the SBR served as reference to compare the sludge properties of the biomass cultivated in the CFP. Hereby, the known conditions required for the aerobic granulation in SBRs were set to individual compartments of the CFP. For the experiments municipal wastewater was used to set a representative feed composition. The focus of this study was to test operational conditions under which AGS could be established in a CFP. Furthermore, the study was used to earn general operational experiences for the handling of AGS.

Table 1. Literature overview to continuous flow-operation with aerobic granular sludge

Author	**Setup (reactor type, media)**	**Results**	**Information**
Liu et al. (2012)	SBAR, settling tank, DMBR (dynamic membrane) tank and a sludge selection tank (sieve with 600 µm mesh size) AGS as inoculum, synthetic wastewater	0.1- 1.0 mm, 15- 25 m h^{-1} No data for SVI	Continuous feeding conditions and limited diffusion lead to overall smaller granules.
Chen et al. (2013)	Two CFP with a completely stirred reactor (18 L), clarifier: 4.5 L, DO: 4.2 mg/L, synthetic wastewater, no anaerobic conditions R1: seed sludge with filaments R2: seed sludge without filaments	Seed sludge from a WWTP SVI between 50 and 90 ml g^{-1} Size in a range of 0.18 to 1.25 mm	Granules could form with a sufficient number of filaments and high shear force. High H/D ratio of the reactor and short settling times were not essential for the formation of AGS.
Li et al. (2015)	Reverse Flow Baffled Reactor (RFBR), feed switched periodically between the two reactors endings, sewage from WWTP with some metal compounds, seed sludge SVI: 66 ml g^{-1}	AGS with a mean diameter of 130 µm and SVI of 33 ml g^{-1}, high inorganic compounds (VSS/TSS of 0.55)	Higher EPS content, PN/PS ratio in the EPS was about 10:1, precipitation inside the granules, Gamma proteobacteria and Nitrospira sp. dominate in RFBR.
Li et al. (2016)	Anaerobic zone: 6 L, Aerobic zone: 9 L Settling tank: 0.125 L, HRT of 6 h with 2.4 h anaerobic and 3.6 h aerobic HRT	AGS as inoculum, synthetic wastewater, 900 µm diameter SVI of 20 ml g^{-1}	Granules with large size were more influenced by the inoculation than those with smaller sizes.
Corsino et al. (2016)	Reactor of 7.5 L was divided into five compartements with risers/downcomers, high loaded and low loaded zones to ensure feast and famine conditions, ultrafiltration membrane	Granules with loose structure, improved structure with an intermittent feed.	Feast/famine conditions and the hydraulic selection pressure are essential to washout flocculent sludge.
Zou et al. (2018)	Two-zone sedimentation tank, with 26.8 L, real and low-strength wastewater, micropowder with metal ions, seed sludge from a WWTP	Mean diameter of 105 µm, SVI 26 ml g^{-1}	Micropowder served as nuclei for the microbial attachment.

2. Material and Methods

2.1 Experimental setup

2.1.1 SBR

Aerobic granules were cultivated in a lab scale SBR with a reactor volume of 8 L. H/D ratio of the reactor was 5.7. The experiments lasted 200 days, whereby the reactor was fed with wastewater from a municipal WWTP. Cycle time was 3 h including an anoxic-anaerobic plug-flow feed of 60 min. Settling times were set in a range of 5 to 1 min to vary the selection pressure during the startup. Stirrers were not used throughout the experiments. Exchange ratios were between 30 and 40% and thus within a common range for SBR plants (DWA, 2010). HRT was on average 8.9 h. During the experiments, an alternate aeration strategy was applied with aeration intervals of 5 min. DO was not controlled during the aeration and reached concentrations up to 5 mg L^{-1}. Figure 3 shows a typical DO profile as well as N and P concentrations during the feeding phase and aeration. Since there was NO_x-N left over from the earlier cycle, the first minutes during the feeding included denitrification. However, anaerobic conditions were reached about 20 to 30 min after the start of the feed.

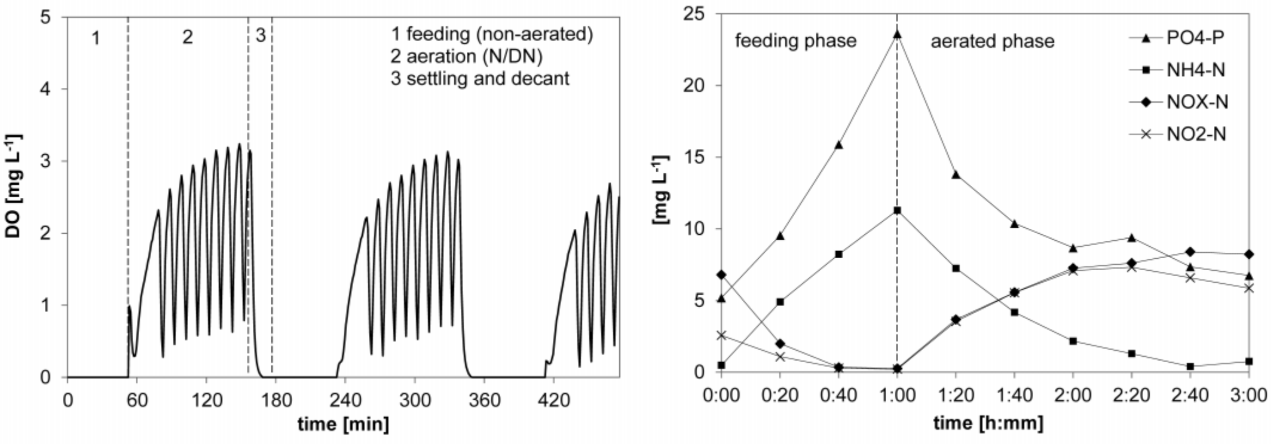

Figure 1. DO profile of the SBR with phases (left), N and P during feeding and the aerated phase (right)

2.1.2 CFP

The CFP setup included a first reactor (selector), which was operated in order to ensure anaerobic conditions similar to the anaerobic feed of the SBR. The reactor volume and layout of this anaerobic tank was changed between the two phases. The relevant operational data of the two experimental phases for the CFP are summarized in Table 2. Figure 2 shows a schematic chart of the CFP.

Table 2. Settings and approaches during the experimental phase I and II

Phase	**Medium**	**Approach**	$\mathbf{V_{anaerob}}$	$\mathbf{V_{BB}}$	$\mathbf{q_A}$	**OLR**
			[L]	[L]	[m h^{-1}]	[gCOD $(L\ d)^{-1}$]
Phase I	synthetic	complete mixed anaerobic recator	9.7	39.3	0.23	0.19
Phase II	sewage	plug-flow and mixed anaerobic reactor	2.4/6.4	39.3	0.23	0.36

Phase I lasted 65 days, whereby the CFP was inoculated with activated sludge from a municipal WWTP and the feed was synthetic media containing $C_{12}H_{22}O_{11}$, $C_6H_8O_7$, CH_4N_2O and K_2HPO_4. An additional trace element solution was dosed in regular periods to avoid limitations. The setup comprised a completely mixed anaerobic reactor with 9.7 L and a mixed aerobic-anoxic reactor with 39.3 L. Thus, the ratio between anaerobic and total volume was 20%. The return sludge ratio was 2.2 until day 39 and was reduced on day 46 to 1.3. The mean surface and sludge loading of the clarifier was 0.23 m h^{-1} and 51.3 L m^{-2} h^{-1} respectively.

Phase II comprised a period of 120 days. Hereby municipal sewage from a nearby WWTP was feed to the plant. The anaerobic part was divided in two sections, a tube reactor with 2.4 L and a completely mixed reactor with 6.4 L. The aerobic-anoxic reactor was continuously stirred. The tube reactor was operated in a horizontal plug-flow to achieve high substrate concentrations as usually realized in SBRs. Twice a week the walls inside the tube were cleaned to avoid a biofilm growth. The ratio between anaerobic to the total volume was 18% and thus similar to the anaerobic volume in phase I. HRT was 3.0 h in the anaerobic part. The return sludge ratio was set to 1.6. The mean sludge volume loading was 49.1 L m^{-2} h^{-1} with a mean surface loading of 0.23 m h^{-1}.

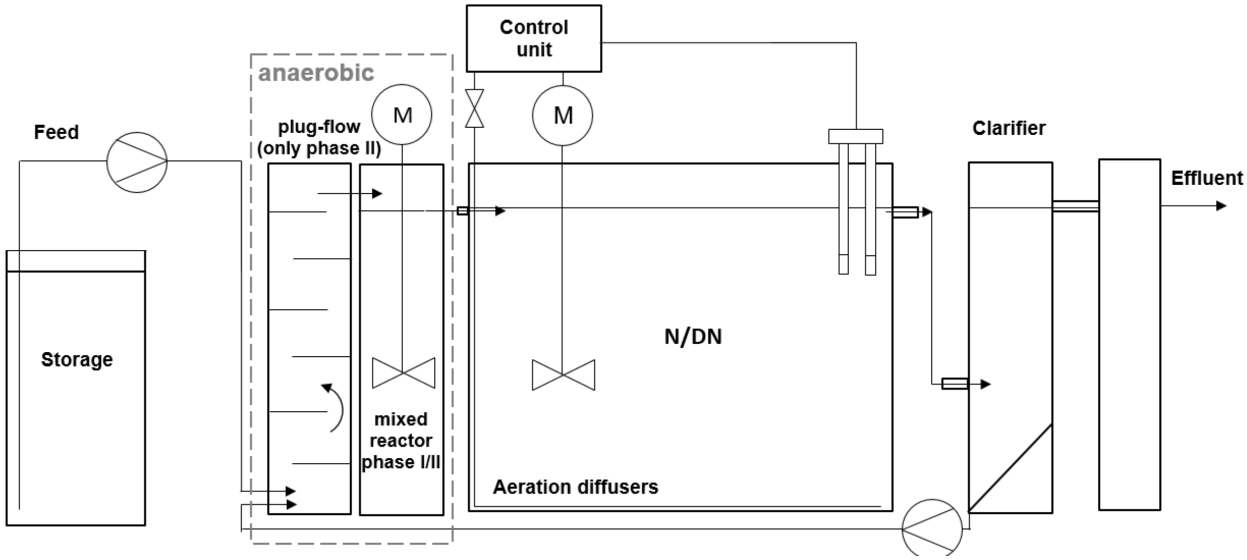

Figure 2. Schematic chart of the setup of the continuous-flow plant

The aerated reactor was equipped with a combined pH/redox sensor (Endress und Hauser, Memosens, Model CPS16D) and a DO sensor (Endress und Hauser, Oxymax COS61D). Figure 3 shows typical DO profils for the CFP during phases I and II. The aeration was set between 2 and 3 mg L^{-1} for a period of 40 (30) min. In the subsequent non-aerated phase of 20 (30) min, the DO decreased to zero which allowed denitrification. Moreover, a siemens logo control tool was used to set the sequential arrangement of pumps, valves and aeration modes. Suspended solids were accumulated in a downstream collecting tank and analysed once a week to calculate the biomass washout and sludge age.

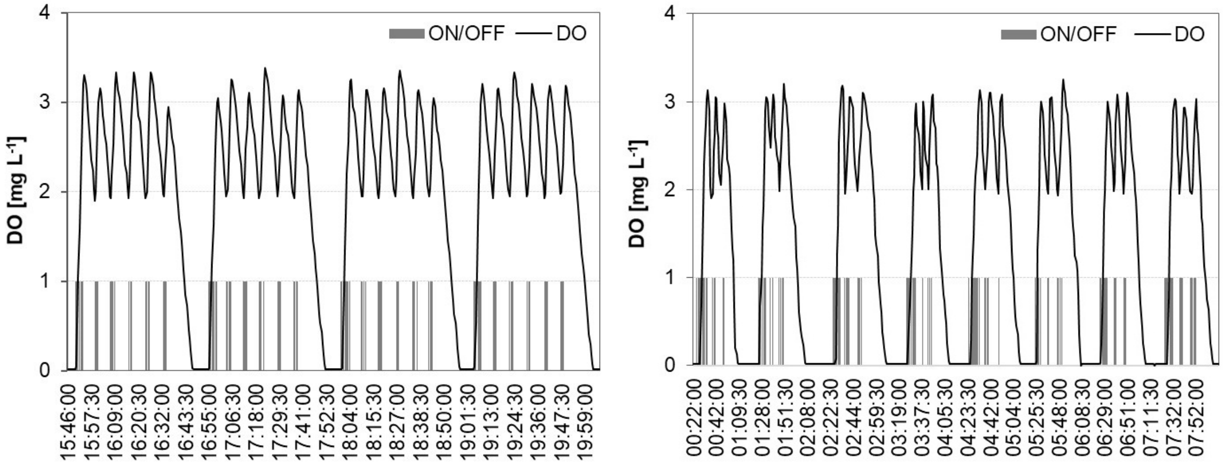

Figure 3. Typical DO profils in the aerated tank for the CFP during phase I and II (lines show DO, columns show aeration mode ON/OFF)

2.2 Sampling

Each charge of wastewater used for the CFP and SBR was sampled and analysed for COD (chemical oxygen demand), TN (total nitrogen), TP (total phosphorous), NH_4-N and PO_4-P. Effluent samples were taken twice a week from both plants and analysed for COD, NH_4-N and PO_4-P. Additional samples were taken from the effluent of the anaerobic reactor of the CFP. MLSS (mixed liquor suspended solids) were sampled during aerated conditions twice a week. SVI as well as SV_5/SV_{30} and SV_{10}/SV_{30} ratio and pH value were measured daily. Table 3 shows the mean analytical data of the wastewater fed to the CFP and SBR. Mircoscopic images were prepared weekly by a Leica microscope to track changes in the sludge structure. Particle size distributions were measured by a Malvern Mastersizer 2000 to compare the sizes of the AGS from the SBR and CFP with seed sludge from a WWTP. This technology allows to identify the granulation grade and a percental amount of particles in a defined size range.

Table 3. Number of samples, mean composition of the wastewater for the plants and OLR

Plant	**Nr.**	**COD**	**TN**	**TP**	**OLR**	**OLR**
	[-]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[gCOD L^{-1} d^{-1}]	[gCOD $gTSS^{-1}$ d^{-1}]
CFP (Phase I)	23	457	46.3	18.6	0.5	0.19
CFP (Phase II)	38	560	59.1	11.8	0.7	0.36
SBR	65	388	44.7	9.4	1.0	0.36

3. Results

3.1 SBR

Aerobic granulation in the SBR was achieved within 28 days, whereby first small granules were detected via microscopic images. Figure 4 shows the measured SVI and $v_{s,min}$. During the startup, the settling time was decreased to 3 min and thus $v_{s,min}$ was changed to 3 m h^{-1}. With these settings, SVI decreased from 120 to 60 ml g^{-1} within 42 days. The further increase of $v_{s,min}$ to 4 m h^{-1} lead to a even lower SVI of 40 ml g^{-1}. The settling time was afterwards reduced to 1 min, which resulted in $v_{s,min}$ of 8.5 m h^{-1} and consequently in a strong washout of biomass. SVI was not affected by this increased washout and was stable at 40 ml g^{-1} but climbed afterwards to 64 ml g^{-1} (day 126). The higher SVI was probably caused by an increased sludge loading (0.5 g COD $gTSS^{-1}$ d^{-1}) due to the intensive biomass washout. Settling time was set back to 2 and 3 min and $v_{s,min}$ was again between 3.0 and 4.7 m h^{-1}. However, with these settings, it was not possible to return to this low SVI of 40 ml g^{-1}. SVI was mainly between 50 and 60 ml g^{-1} till the end of the experiments. SV_{10}/SV_{30} ratio of the AGS was 1.0 to 1.1; while the ratio of the inoculated activated sludge was about 1.5 to 1.8. Settling velocity of individual granules were determined with 23 m h^{-1}, whereas for activated sludge a range of 7 to 10 m h^{-1} is reported (Qin et al., 2004).

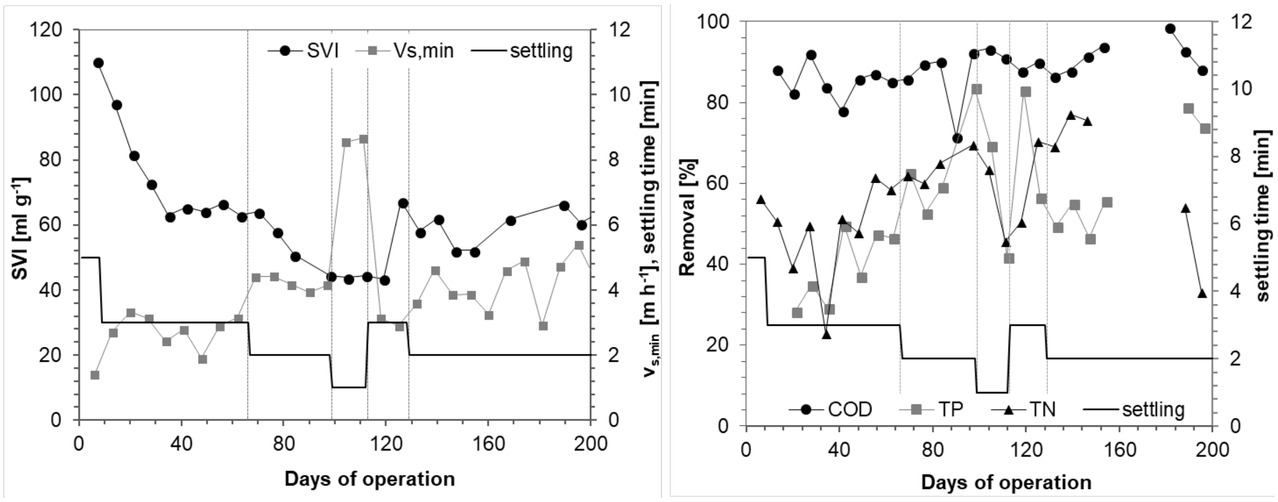

Figure 4. SVI, settling time and $v_{s,min}$ of the SBR (left) and COD, TN and TP removal of the SBR (right)

Additionally, the particle size distributions of the flocculent seed sludge and the AGS from the SBR were measured and compared. The analysed granules were removed from the SBR on day 186 and had a SVI of 63 ml g^{-1} and a SV_{10}/SV_{30} ratio of 1.14. The particle size distribution indicates that approx. 50% of the particles of the flocculent activated sludge were smaller than 235 µm, while the AGS obtained 50% of the particles larger than 548 µm and only 10% being smaller than 143 µm. The measurement illustrates the change of the particle sizes within the granulation from a flocculent to a granular structure. Beside the high amount of large granules, there was a significant proportion of flocculent biomass present in the AGS. This observation is in line with earlier reports from Pronk et al. (2015b) and Wagner et al. (2015). There is usually a flocculent proportion present in a granular sludge suspension especially when real wastewater is used as feed. Reasons for the flocs are suspended particular matter from the sewage, separated particles from the granules and biomass growth on the supplied polymeric substrate (Pronk et al., 2015b). Overall, there was a good removal performance observed with the SBR operation. Figure 4 shows the COD, TN and TP removal calculated per week. COD and NH_4-N removal were on average 88.3% and 82.6% respectively. Nitrification rate was between 3.3 and 3.7 mgNH_4-N $gTSS^{-1}$ h^{-1}. The maximum respiration rate at the beginning of the aerated phase was between 24 and 36 mgO_2 h^{-1}. A continuous improvement of the TN removal was observed from day 100 on. TN removal depends beside the nitrogen load, from the DO concentration and the diameter of the granules. Thus, the oxygen diffusion determines the distribution of aerobic and anoxic zones inside the granules. The larger particle sizes are responsible for the increased anoxic zones within the granules and explain the increased TN removal.

Moreover, the results of this study show a strong correlation between TN and TP removal. TP removal reached up to 83.6%. The extent of biological phosphate removal depends on several factors, such as sludge age, temperature, wastewater composition and content of phosphate accumulating organisms (PAO). Bassin et al. (2012) investigated the TP removal at different temperatures and found a higher removal of 90% with 20 °C compared to an operation with 30 °C (TP removal of 70%). Similar observations were reported by Winkler et al. (2011), whereby the lower TP removal at higher temperatures was explained with growth advantages for GAOs (glycogen accumulating organisms). Since PAOs and GAOs compete for the same substrate and GAOs are not able to remove phosphate, the growth of GAOs is not desired. Since PAOs are more present in granules of the lower sludge bed, a suitable method to increase the growth of PAOs was found in a selective removal of granules from the top of the sludge bed (Winkler et al., 2011). This strategy resulted in a complete TP removal. However, in the present study, waste sludge was removed under mixed conditions during the aerated phase to remove also older and larger granules. Probably, this sludge wasting strategy caused a lower TP removal compared to results from literature. The average pH value in the reactor was 7.56.

3.2 CFP

The following section describes the experiments of the CFP during the two experimental phases. In both phases the plant was inoculated with activated sludge from a municipal WWTP. The main focus was to describe changes in the sludge structure and settling behavior. Figure 5 shows the course of the SVI, SV_5/SV_{30} and SV_{10}/SV_{30} ratios of the CFP for phase I and II.

The SVI of the seed sludge at the beginning of phase I was about 130 ml g^{-1} and decreased to 66 ml g^{-1} within 11 days. Over the same period, the SV_{10}/SV_{30} ratio dropped from 1.8 to 1.5. NO_x-N and DO measured in the effluent of the first tank (selector) was 0 mg L^{-1} till day 44. Suspended solids (SS) in the effluent were between 32 and 200 mg L^{-1} up to the day 18. As a result of the better settling properties, the washout of SS decreased, thus the effluent concentrations fluctuated between 8 and 25 mg L^{-1} until the end of phase I. By day 32, the SVI remained between 60 and 80 ml g^{-1} with a SV_{10}/SV_{30} ratio of approximately 1.4. A further decrease of the SVI to 42 ml g^{-1} was recorded until day 46 and was probably caused by a massive growth of Arcella (microscopic images below). In the further operation, the SVI increased again to 70 ml g^{-1} caused by the growth of filaments. Anaerobic conditions were not longer ensured from day 45 on, thus there is probable reason for the increased SVI. MLSS concentration during phase I ranged between 1.8 and 4.0 g L^{-1}.

During the startup of phase II, there was an intensive washout of flocculent biomass with SS in the effluent in a range of 35 to 227 mg L^{-1}. While the SV_5/SV_{30} ratio was approximately 2.5 at the beginning of the experiments, the ratio declined to 1.5 till day 100. A similar trend was observed for the SV_{10}/SV_{30} ratio with a steady decrease from 1.6 to 1.2. MLSS was between 1.2 and 3.2 g L^{-1}. At the beginning of the experiments, the SVI was approx. 140 ml g^{-1} and declined to 90 and 80 ml g^{-1} between the days 44 and 74. During phase II (till day 74), NO_x-N and DO was 0 mg L^{-1} in the effluent of the first tank and confirm anaerobic conditions in the first reactor. Between the days 74 and 79, a sudden increase of the SVI from 85 to 102 ml g^{-1} was recorded, while simultaneously NO_x-N concentrations up to 6.4 mg L^{-1} appeared in the effluent of the anaerobic reactor. A similar correlation was seen between days 109 and 114, where NO_x-N concentrations of 7.6 and 9.0 mg L^{-1} led to an increase of the SVI to 100 ml g^{-1}. Form these findings, it seems that the presence of NO$_x$-N and subsequently anoxic instead of anaerobic conditions in the first reactor (selector) are responsible for higher SVI. A tight control of the nitrogen removal is necessary to ensure anaerobic conditions in the selector and to allows a stable granules formation.

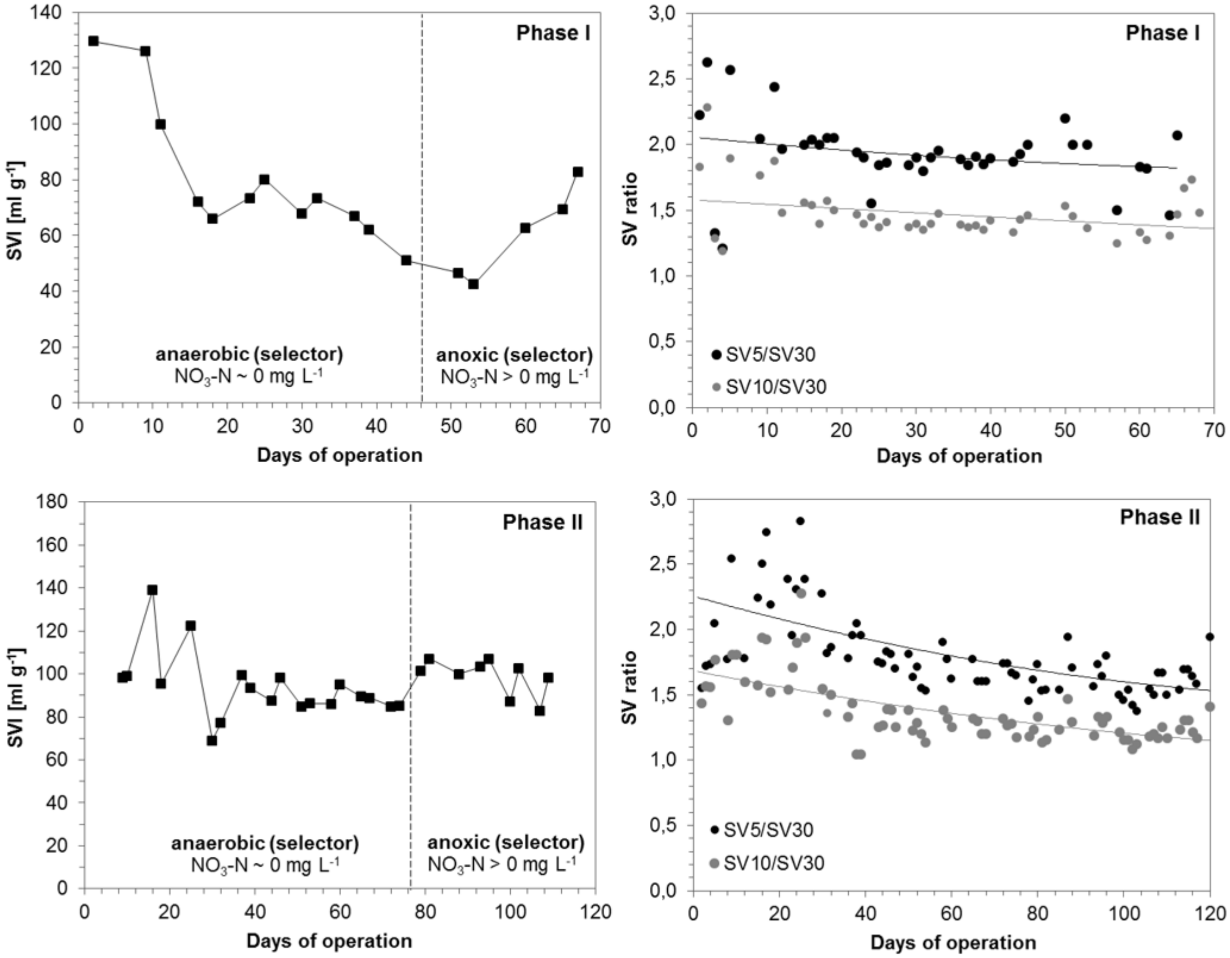

Figure 5. SVI, SV$_5$/SV$_{30}$ and SV$_{10}$/SV$_{30}$ ratio for the CFP during phase I and II

Figure 6 shows microscopic images of the sludge during phase I and II. Hereby, significant larger and more compact structures of the biomass were observed during the startup. An increased growth of sessile ciliates, especially vorticella-like organism, as it is often postulated for AGS in GSBR, did not appear during phase I using synthetic wastewater. Unfortunately, there was a massive growth of arcella from day 40. The occurrence of arcella is characteristic for good oxygen supply and high SRT. It can be assumed, that the round shape of these organisms with sizes up to 200 µm probably caused an increased settling velocity. Thus, a further decrease of the SVI to 42 ml g^{-1} could be linked to the appearance of these organisms. From day 55 onwards, an increase in filamentous organisms was observed, leading to a rising SVI. The operation was stopped at this point. During phase II, an increased occurrence of zoogloea was observed, which is often postulated for AGS (Adav et al., 2009; Li et al., 2008; Sheng et al., 2010). The presence of fixed ciliates was less pronounced compared to the granules from the SBR. Nevertheless, isolated colonies of vorticella and also rotaria were observed on the surface of the granules.

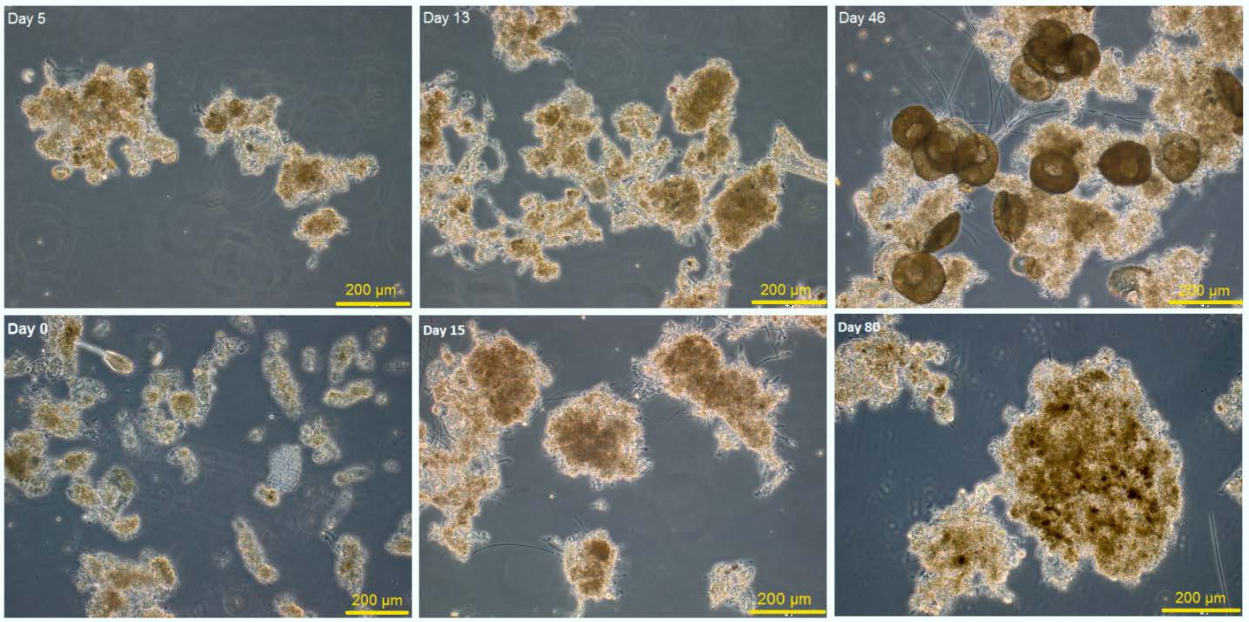

Figure 6. Microscopic images of granules (first line: CFP – phase I , second line: CFP – phase II)

Microscopic images show that in both CFP phases there were large fractions of flocculent biomass in a coexistence with granules, which appeared similar to the AGS of the SBR. To describe the ratio between flocculent and AGS, particle size distributions were prepared for the sludge from the CFP (phase II), from the SBR as well as from the flocculent seed sludge. Figure 7 shows the distribution for the analysed sludge samples. Overall, larger structures were found for the AGS from the SBR. Here, about 50% of the particles had a size of more than 450 µm and only 10% were smaller than 146 µm. For the AGS from the CFP, approx. 50% of the particles had a size above 300 µm with only 10% being smaller than 110 µm. The measurement illustrates a significant change of the seed sludge to AGS, whereby for the flocculent activated sludge, approximately 90% of the particles were smaller than 400 µm. According to the definition of AGS, a size of more than 200 µm is characteristic for aerobic granules. Thus, the measured particle sizes illustrate, that about 72% of the biomass reached this defined size.

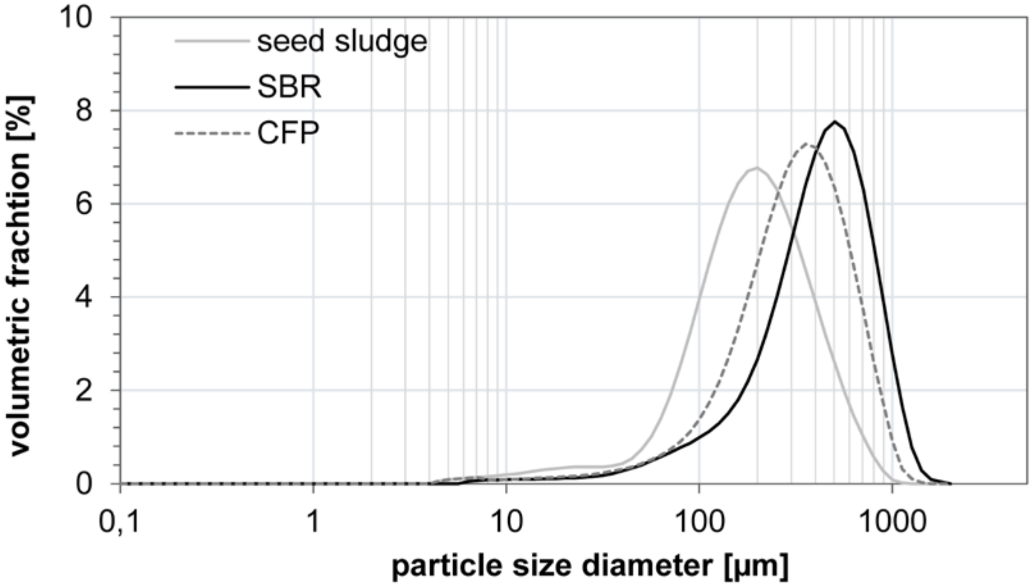

Figure 7. Particle size distribution of sludge probes (left: inoculum, middle: CFP, right: SBR)

With the settings of phase I and II it was possible to form small and dense granules. Overall smaller granules sizes were also reported by Li et al. (2015) and Chen et al. (2013) operating continuous-flow and can be explained by a reduced nutrient diffusion due to a lower substrate gradient. The results of both experimental phases point out the importance to ensure anaerobic conditions to achieve excellent settling properties of the biomass. The SVI of phase I was significantly lower compared to phase II, whereby the use of synthetic wastewater and thus a soluble carbon source can be assumed as the reason for this. With phase II, it was only possible to reduce the SVI to 85 ml g^{-1}, this was far above the results of the SBR operation. Similar findings were made for the sludge volume ratios. Overall, the SV_5/SV_{30} and SV_{10}/SV_{30} ratios in the CFP were higher compared to the AGS cultivated in the reference SBR, where SV_{10}/SV_{30} ratios between 1.05 and 1.1 were measured.

3.2.2 Removal performance

The anaerobic reactor is essential to provide growth conditions for substrate-storing organisms, which are necessary to form stable granules. During anaerobic conditions, the supplied COD is taken up by these organisms and stored as internal products. Some studies report that COD should be extensivley stored in order to avoid an irregular growth during the aerated periods (Pronk et al., 2015a). Moreover, the breakthrough of substrate into the aerobic reactor can lead to the development of filaments (van den Akker et al., 2015). During phase I, the average COD effluent concentration of the anaerobic reactors was 47 mg L^{-1} and was related to a mean COD reduction of 76%. TP concentrations in the effluent were mostly increased (up to 24 mg L^{-1}) compared to the influent concentrations, which indicates that there were PAO present in the sludge and responsible for a phosphate release. However, on individual days it was not possible to ensure complete anaerobic conditions due to inefficient TN removal in the aerobic-anoxic reactor, subsequently there was no phosphate release in the anaerobic reactor.

Figure 8 shows the NH_4-N, TN and TP removal during the phases I and II. During phase I, COD was almost completely eliminated with an average removal of 92.1%. Similar results were observed with phase II, while the COD removal was between 87.1 and 97.8% and reached a mean COD removal of 95.3%. The average NH_4-N removal was 99.6% (phase I) and 99.1% (phase II). With a few exceptions, the TN removal reached about 80%. TP removal during phase I declined within the startup. This observation can be explained by the use of synthetic media as feed. Similar effects were reported in earlier studies from Wang et al. (2010), where the dosage of glucose lead to an advanced GAO metabolism with a worsened TP removal.

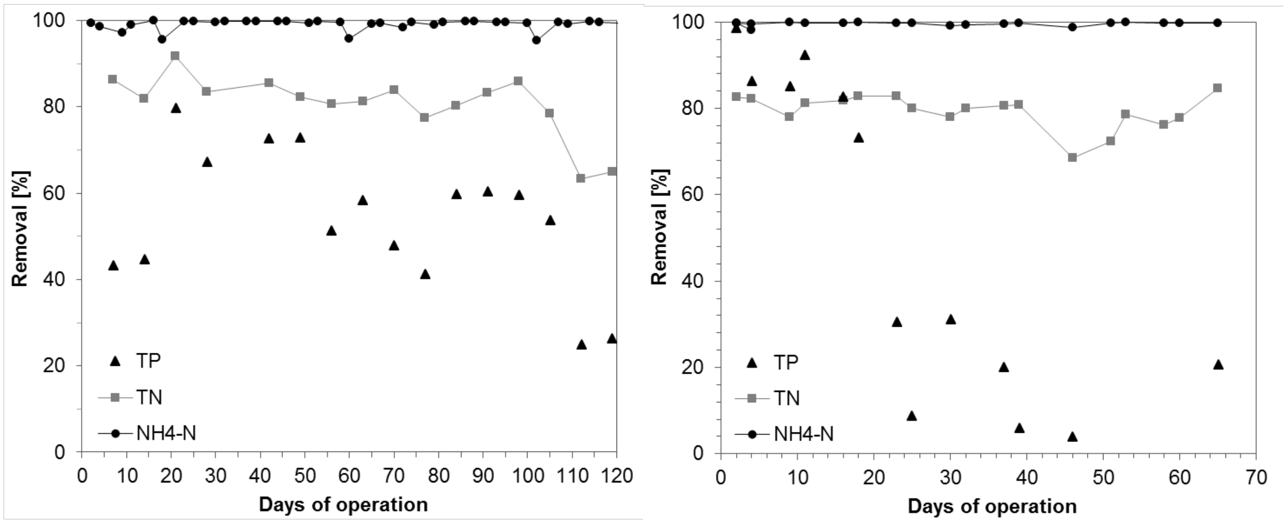

Figure 8. NH_4-N, TN and TP removal during phase I (left) and phase II (right)

4. Disscusion

The importance of the anaerobic feeding phase for aerobic granulation was already explained in some earlier studies by de Kreuk and van Loosdrecht (2004) as well as by de Kreuk and van Loosdrecht (2006). This known requirement for the aerobic granulation was picked up for the present study and applied for the SBR and CFP. Due to the presence of NO_x-N in the beginning of the feed (SBR), the feeding started under anoxic conditions and was followed by an anaerobic feed as soon as NO_x-N was denitrified in the sludge bed. However, the operation with an anoxic-anaerobic feeding and the use of municipal wastewater allowed the formation of dense and compact granules with excellent settling properties. SVI of the AGS in the SBR was in a range of 50 to 60 ml g^{-1} and thus in line with studies, which also relate to the treatment of municipal sewage (de Kreuk et al., 2010; Wagner et al., 2015). The plug-flow feeding strategy is suitable to ensure anaerobic conditions, even if low NO_x-N concentrations are present in liquid phase above the sludge bed. However, the occurrence of high NO_x-N concentrations can contract the anaerobic feeding and should be considered carfully. In this case, a declining phosphate removal performance can be accepted. One further advantage of the plug-flow feed is that there result high substrate gradients, which promote the formation of larger granules. The substrate gradient is hereby one driving parameter for the substrate diffusion inside the granules and influences the granules size.

For the startup of the CFP, activated sludge from a municipal WWTP was inoculated to the plant. The first phase of the CFP is not considered in detail due to the massive growth arcella and a potential impact on the SVI. Furthermore, phase I lasted only 65 d and was not long enough under stable operation. Beside anaerobic conditions for aerobic granulation, the substrate gradient should be considered for the design and setup of continuous-flow reactors too. In this study, the tube reactor of the anaerobic part of the CFP (phase II) was one possibility to build up increased substrate gradients compared to mixed conditions. Low substrate gradients on the surface of the biomass can limit the diffusion into the granules and thus the overall granules size. In the course of the first weeks, the sludge structure and settling performance of the inoculated seed sludges (CFP II) changed clearly to larger particles with a compact structure (particle size distribution, microscopic images). According to the granular sludge definition, the measured particle size distribution indicates the shift of the flocculent seed sludge towards larger granules, while only 28% of the particles had a size less than 200 µm. SVI was successfully reduced to 85 ml g^{-1} during phase II (CFP). However, the settling properties were not as good as like the AGS from the SBR. SV_5/SV_{30} and SV_{10}/SV_{30} ratio were reduced only to about 1.5 and 1.2 and were clear higher than in the SBR. Since the settling velocity relates to the size of a settling particle (Stokes Law), the overall smaller granules can be named as one reason for the different settling properties. Moreover, a negative effect on the SVI was observed when no anaerobic conditions were realized in the first reactor (selector), which confirms the fact that anaerobic conditions are necessary to form stable granules with excellent settling performance.

Furthermore, the higher SVI and SV ratios were probably a result of an overall larger fraction of flocs, which was additionally caused by a lower selection pressure in the clarifier. The sludge volume feed q_{SV} is a parameter to characterize the hydraulic load to the clarifier. For the experiments, q_{SV} was only about 51.3 L m^{-2} h^{-1} and the sufarce loading q_A was calculated with 0.23 m h^{-1}. It can be assumed, that the lower q_A leads to a higher retention of floccuelent biomass. From the results it is clear, that there is a further research demand, how the sludge separation can be optimised and adjusted to the granulation process. A possible approach would be the use of hydrocyclones, however this could not be tested in the presented study. Another reason for smaller granules and higher SVI can be the shear forces caused by the use of stirrers in the anaerobic as well as in the aerobic reactor. For example, Rocktäschel et al. (2013) reported smaller granules in a SBR, in which the feed was followed by an anaerobic mixing compared to a reactor with plug-flow feed without stirrers. Nor Anuar et al. (2007) examined in a study the effect of mechanical mixers on the settleability of AGS and found an insignificant effect of the shear stress, however there was a slightly decreased settling velocity with an increased stirrer speed. The results demonstrate, that the turbulence and shear stress caused by mixing systems can have an influence on the settling properties of AGS, also when the effect of these advices on the sludge structure is not clearly described at the moment. Similarly, a further negative effect can be seen in the return sludge pump (peristaltic pump), which was used to recycle the biomass from the secondary clarifier to the first reactor. Since there is no need for stirrers or pumps in full scale SBR like in Nereda plants, the effects on the structure is up to now less investigated. However, in the CFP it is essential to have mixed conditions in the tanks, so that the use of technical devices such as pumps and stirrers for AGS should be given careful consideration. Further research work is needed to optimize the continuous-flow systems in order to achieve similar settling properties compared to the granules from GSBR.

5. Conclusion

With the experimental setup and operation of the CFP, it was possible to achieve changes of flocculent activated sludge structure towards a compact and dense biomass. With the use of municipal sewage and an anaerobic plug-flow, the SVI of a flocculent activated sludge declined up to 85 ml g^{-1}, while for 72% of the particles a size of 200 µm was realised. The use of a synthetic wastewater and completely mixed conditions lead to a SVI of 42 ml g^{-1}, which was probably caused by the soluble carbon source and a growth of Arcella. A direct link was seen between NO_x-N in the effluent of the first reactor and an increase of the SVI. The results indicate the importance of anaerobic conditions for good settling properties. Particular attention should be paid to the implementation of a continuous sludge separation and the use of technical devices such as pumps and stirrers. Moreover, high substrate gradients should be considered in order to enrich large granules. There is a further research demand for the application of AGS in CFP.

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