Doctoral Thesis

Wastewater treatment with aerobic granular sludge in continuous-flow reactors and effects on downstream processes

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Abstract

The activated sludge process is the most widely applied technology for the treatment of municipal and industrial wastewater worldwide. Conventional WWTPs usually comprise separate activated sludge tanks for the biological treatment processes as well as clarifiers for the separation of the activated sludge from the purified water. The size of the clarifiers depends mainly on the settling properties of the activated sludge, whereby the settling rate tends to decrease with higher biomass concentrations and necessitates large post-clarification tanks with increased footprint. The concentration of the biomass in the activated sludge tank is therefore limited by declining settling properties. Generally, lower sinking rates are related to the growth of filamentous organisms, which are usually responsible for sludge wash-out and lower effluent quality. Filamentous bacteria grow preferably in low or high loaded WWTPs as well as under carbon or nutrient limitations. In regions with demographic increase, additional quantities of wastewater can favor the development of bulking sludge that imperils the compliance of the effluent quality. In that case, capacity expansions with the upgrade of the biological stage and the construction of additional tank volume are necessary. Alternatively, the treatment capacity could be increased by raising the number of the involved organisms to avoid the construction of new plant components. For instance, the use of growth carriers or membranes are possibilities to enhance the biomass concentration and plant capacity. However, these processes are usually expensive and mostly applied for industrial wastewater treatment.

In order to counteract the limited settling properties, the activated sludge process has been modified in recent years with the target to enhance the biological treatment capacity by increasing the microbial density. An innovative approach for the design of compact treatment plants was found with the formation of aerobic granular sludge, a biomass that offers a more compact structure compared to suspended activated sludge. Consequently, aerobic granules settle significantly faster than conventional sludge flocs. Beside improved settling properties, the compact and dense sludge structure enables zones with different oxygen and substrate gradients and thus the ability for simultaneous nitrogen and carbon removal. Moreover, the granulation allows the enrichment of phosphate accumulating organisms and subsequently enhanced biological phosphate removal. In consequence, a reduced demand for precipitation agents oppresses the operational treatment costs. Moreover, aerobic granules offer an increased content of extracellular polymeric substances which was found to improve the resistance against load fluctuations and toxic shock loads.

Previous publications mainly report about the operation of aerobic granular sludge in sequencing batch reactors (SBR), in which the biological processes and the sludge separation appear time-seperated in one single reactor. In this process, the adjustment of cycle times and settling intervals are easy to handle. Since only 15% of the WWTPs in Austria with a size above 50 p.e. are designed as SBR, there is still an interest in making the granular sludge process accessible also for continuous-flow plants. Lack of experiences in the application of the aerobic granular sludge process in continuous-flow illustrates the demand for further research. The establishment of aerobic granules in continuous-flow was investigated within the framework of the project "KontiGran - Aerobic granular sludge process in continuous-flow plants" supported by the Federal Ministry of Sustainability and Tourism. As part of the project, lab-scale experiments were performed to examine the cleaning performance of the biomass in a continuous-flow

setup consisting of an anaerobic and aerobic reactor volume as well as a post-clarifier. The setup obtained an anaerobic reactor, which was divided into a plug-flow and a fully mixed reactor. Additional experiments were carried out in lab-scale SBRs in order to receive experiences in the aerobic granulation process as well as in the operation and treatment performance. Further investigations belonged to the effects on the down-stream processes, especially on the sludge treatment and dewatering as well as on the nitrous oxides emissions during the biological nutrient removal.

With the operation of the continuous-flow setup, it was possible to improve the settling properties of the biomass to an overall lower sludge volume index of approx. 85 ml g^{-1}. The particle size distribution as well as the microscopic images revealed a more compact structure of the sludge and overall larger sizes. Nevertheless, the sludge offered lower settling properties compared to the granules from the SBR, which was confirmed by an increased SV_{10}/SV_{30} ratio. Aerobic granulation in continuous-flow was limited due to insufficient substrate gradients and the use of stirrers. In contrast to SBR systems, where stirrers are not entirely necessary, continuous-flow plants always require mixing systems to avoid sludge deposits. The use of stirrers and the presence of nitrate in the return sludge makes it difficult to realize anaerobic plug-flow conditions, which were found to be the most important requirement for the aerobic granulation. Moreover, stirrers reduce the substrate gradients among the bulk liquid and the biomass which causes lower penetration depths into the granules and smaller sizes (diffusion limitations).

N_2O measurements in the exhaust air of the SBR operated with aerobic granular sludge revealed emission factors in a range of 0.54 to 4.8% (gN_2O/gNH_4-$N_{ox.}$). A clear correlation was found between the N_2O emissions and the nitrification, since N_2O was measured in the exhaust air as long as NH_4-N was present in the bulk liquid. Higher emissions were observed with increased organic loading rates and elevated nitrite levels. Moreover, the investigations comprised anaerobic tests under mesophilic temperatures and an hydraulic retention time of 25 d. The outcomes of the anaerobic biodegradation revealed a specific methane production of 263 to 285 $mlCH_4$ $gVSS^{-1}$. VSS reduction was calculated with 51 and 59%. The experiments disclosed an overall lower dewaterability of the digested aerobic granules. However, further studies with large-scale dewatering aggregates are required to verify the lower dewatering behavior.

In addition, operational data of a full-scale SBR with excellent settling properties were evaluated as part of this work. The activated sludge of this plant comprised a high amount of granules and a sludge volume index in a range of 30 to 50 ml g^{-1}, which is significantly lower than for conventional activated sludge plants. The plant operation included a feeding phase under non-aerated conditions, which ensured the formation of anoxic-anaerobic periods at the beginning of the cycle. Anaerobic feeding conditions were identified as essential for the aerobic granulation in earlier studies. Furthermore, the increased proportion of industrial wastewater with a high amount of readily available carbon as well as short stirring intervals are recognized as favorable conditions for the formation of granules in this SBR plant.

Kurzfassung

Das Belebtschlammverfahren ist das weltweit am häufigsten eingesetzte Verfahren zur Behandlung kommunaler sowie industrieller Abwässer. Der Abtrennung und Rückführung des belebten Schlammes kommen bei diesem Verfahren eine besondere Bedeutung zu. Herkömmliche Belebungsanlagen benötigen üblicherweise große Nachklärbecken mit einem entsprechend hohen Flächenbedarf. Die Größe der Nachklärung hängt vor allem von den Absetzeigenschaften des Belebtschlammes ab, wobei es bei höheren Biomassekonzentrationen zu einem eingeschränktem Absetzen der Schlammpartikel kommen kann. Die Konzentration der Biomasse in der biologischen Stufe ist daher durch die Absetzeigenschaften des Schlammes begrenzt. Schlechte Absetzeigenschaften, die oftmals durch das Wachstum fadenförmiger Organismen verursacht werden, sind häufig für einen Schlammabtrieb und eine verschlechterte Ablaufqualität verantwortlich. Fadenförmige Bakterien vermehren sich bevorzugt in schwach- als auch in hochbelasteten Anlagen. In Regionen mit Bevölkerungszuwachs können zusätzliche Abwassermengen die langfristige Sicherstellung der vorgeschriebenen Ablaufqualität gefährden. In diesem Fall werden Kapazitätserweiterungen mit dem Ausbau der biologischen Stufe und der Schaffung zusätzlicher Beckenvolumen erforderlich. Eine Möglichkeit zur Steigerung der Behandlungskapazität besteht in der Erhöhung der Anzahl der an der Abwasserreinigung beteiligten Organismen, wodurch der Neubau von Anlagenkomponenten umgangen werden kann. Eine Erhöhung der Biomassekonzentration zur Steigerung der Reinigungsleistung ist beispielsweise durch den Einsatz von Aufwuchsträgern möglich. Diese Verfahren sind jedoch meist kostenintensiv und werden hauptsächlich für die industrielle Abwasserreinigung angewendet.

Um den begrenzten Absetzeigenschaften des Belebtschlammes entgegenzuwirken und die Kapazität der biologischen Abwasserreinigung zu erhöhen, kam es in den vergangenen Jahren zur Modifikation des Belebtschlammverfahrens mit der gezielten Anreicherung von Mikroorganismen über die Bildung kompakter Biofilmaggregate. Dieser sogenannte aerob granulierte Schlamm zeichnet sich durch eine kompakte und dichte Schlammstruktur und hohe Sedimentationsgeschwindigkeiten aus. Durch die Kompaktheit der Granula entstehen Zonen mit unterschiedlichen Sauerstoff- und Substratgradienten, welche die simultane Stickstoff- und Kohlenstoffentfernung ermöglichen. Die vermehrte Anreicherung von Phosphat akkumulierenden Organismen ermöglicht zudem die erhöhte biologische Phosphatentfernung und die Reduktion des Fällmittelbedarfs sowie der Betriebskosten. In einigen Studien wurden erhöhte Gehalte an extrazellulären polymeren Substanzen in aerob granulierten Schlamm nachgewiesen, welche eine hohe Resistenz der Biomasse gegenüber Schwankungen in der Zulauffracht und auftretende Stoßbelastungen ermöglichen.

Bisherige Publikationen berichten hauptsächlich von der Umsetzung des Verfahrens mit aerob granuliertem Schlamm in SBR-Anlagen, in denen die Einstellung der Zykluszeiten und der Absetzintervalle relativ leicht handhabbar sind. Da in Österreich lediglich 15% der Kläranlagen größer 50 EW als SBR konzipiert sind, besteht ein Interesse das Verfahren mit aerob granuliertem Schlamm auch in kontinuierlich durchflossene Anlagen umzusetzen. Mangelnde Erfahrungen zur Anwendung des granuliertem Belebtschlammverfahrens in kontinuierlich durchflossenen Anlagen verdeutlichen die Notwendigkeit zur weiteren Forschung und großtechnischen Umsetzung. Im Rahmen des Forschungsprojektes KontiGran (Granulares Belebtschlammverfahren in kontinuierlich durchflossenen Anlagen) gefördert von Ministerium für Nachhaltigkeit

und Tourismus (BMNT) wurde die Etablierung des aerob granuliertem Belebtschlammverfahrens in kontinuierlich durchflossenen Belebungsanlagen untersucht. Im Rahmen des Projektes wurde in Laborversuchen die Reinigungsleistung des aerob granulierten Belebtschlammverfahrens in einem kontinuierlich durchflossenen System bestehend aus einem anaeroben und aeroben Becken in Kombination mit einer Nachklärung untersucht. Neben einem vollständig durchmischten anaeroben Reaktor, wurde ein geteilt anaerobes Volumen genutzt, wobei ein Schlauchreaktor (plug-flow) und ein vollständig durchmischter Reaktor kombiniert wurden. Begleitend wurden Versuche im SBR durchgeführt, um Erfahrungen zum Betrieb und der Reinigungsleistung der Biomasse zu erarbeiten. Darüber hinaus wurden Untersuchungen zur Beantwortung weiterer Fragestellungen bezüglich potentieller Auswirkungen auf den Kläranlagenbetrieb durchgeführt.

Mit dem Betrieb der kontinuierlich durchflossenen Anlage konnte eine Verbesserung der Absetzeigenschaften mit einer Senkung des Schlammindex auf ca. 85 ml g^{-1} realisiert werden. Die Messung der Partikelgrößenverteilung und die mikroskopischen Aufnahmen zeigten eine deutlich kompaktere Struktur des Schlammes. Dennoch ergaben sich im Vergleich zur SBR Betriebsweise insgesamt schlechtere Absetzeigenschaften des granulierten Schlammes, was in einem höheren Schlammindex sowie SV_{10}/SV_{30}-Verhältnis ersichtlich wurde. Im Gegensatz zum SBR ist in kontinuierlich durchflossenen Anlagen der Einsatz von Rührern erforderlich und die Realisierung von plug-flow Bedingungen schwer realisierbar. Die Granulierung war aufgrund niedriger Substratgradienten und dem Einsatz von Rührern limitiert. Die Ausbildung großer Granula ist lediglich beim Vorliegen hoher Substratkonzentrationen und einer hohen Eindringtiefe (Diffusion) in die Granula möglich.

Messungen von N_2O in der Abluft der Versuchsreaktoren mit aerob granuliertem Schlamm ergaben Emissionsfaktoren zwischen 0,54 und 4,8% (gN_2O/gNH_4-$N_{ox.}$). Ein deutlicher Zusammenhang konnte zwischen den N_2O-Emissionen und der Aktivität der Nitrifikanten gesehen werden. Höhere Emissionen wurden mit steigender Schlammbelastung sowie hohen Nitrit-Konzentrationen festgestellt. Die anaerobe Abbaubarkeit des aerob granulierten Schlammes wurde in anaeroben Faulreaktoren untersucht. Das Entwässerungsverhalten des Schlammes wurde mit Hilfe einer Zentrifuge untersucht. Die anaeroben Versuche ergaben eine spezifische Methanproduktion von 263 bis 285 $mlCH_4$ $goTS^{-1}$. Der oTS-Abbau errechnete sich mit 51 und 59%. Die Laboruntersuchungen zeigten ein schlechteres Entwässerungsverhalten des aerob granulierten Schlammes, wobei weitere Untersuchungen unter Einbezug großtechnischer Entwässerungsaggregate erforderlich sind.

Im Rahmen der Arbeit wurden zudem die Betriebsdaten einer großtechnischen SBR-Anlage mit ausgesprochen guten Absetzeigenschaften und einem hohen Anteil an Granulas ausgewertet. Der Schlammindex lag ganzjährig in einem Bereich zwischen 30 bis 50 ml g^{-1} und somit deutlich niedriger als für suspendierten Belebtschlamm. Der Betrieb der Anlagen mit der Beschickung unter nicht belüfteten Bedingungen ermöglicht die Ausbildung anoxisch-anaerober Phasen, welche eine wesentliche Voraussetzung für die Granulierung bilden. Im Weiteren werden ein hoher industrieller Abwasseranteil mit leicht verfügbaren Kohlenstoffverbindungen sowie kurze Rührintervalle als günstige Faktoren für die Ausbildung von Granulas in dieser SBR-Anlage angesehen.

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Chapter 1

Introduction

1.1 Background

Aerobic granules were first observed by Mishima and Nakamura (1991) in the operation of upflow airlift reactors and described as compact and dense aggregates owning fast settling velocities. Although anaerobic granular sludge has been known since the early 1980s for the anaerobic wastewater treatment, especially for the operation of UASB reactors, the discovery of aerobic granules has attracted the interest of several research groups to exploit the very good settling properties for biological wastewater treatment. In the following years, intensive research was undertaken to understand the mechanisms for the granules formation and to describe the relevant reactor settings and their effects on the biomass structure and treatment performance.

Up to now, aerobic granulation relates to the operation of sequencing batch reactors (SBR). This process is based on a cycle operation, which is characterized by the periodic change between phases with high substrate availability (feast phase) and starvation periods (famine phase). In initial lab-scale experiments, aerobic granular sludge (AGS) was formed using an aerobic feeding phase and high dissolved oxygen (DO) concentrations (Beun et al. 1999, Morgenroth et al. 1997). However, lowering the DO after the start-up led to the disintegration and loss of granules (Mosquera-Corral et al. 2005). Only with the implementation of an anaerobic feeding phase, it was possible to form stable aerobic granules and long-term operation (de Kreuk and van Loosdrecht 2004). The anaerobic feeding phase was therefore identified as the most important requirement for the AGS operation. As an outcome of the research with laboratory and pilot-scale reactors (Ede, the Netherlands), a first commercial AGS process was developed under the tradename Nereda® in cooperation between the University of Delft and Royal Haskoning DHV. A first municipal sewage treatment plant (Epe, Netherlands) was commissioned in 2011 based on this AGS technology. Currently, more than 40 Nereda® plants are in operation or in design worldwide. Despite a growing number of full-scale plants operated with AGS, the large-scale application so far only relates to the operation of SBR systems. Since the number of plants operated in continuous-flow prevails the SBR, current studies focus on the development of aerobic granules in continuous-flow reactors. Beside an intensive scientific knowledge of the AGS formation and operation, there are still research gaps on the impact of the compact sludge structure on downstream processes including sludge stabilization and dewatering.

1.2 Research Questions

In the beginning of the reasearch on aerobic granular sludge, various publications reported detailed about the operational settings and influencing parameter under the use of synthetic media. The practical work of the thesis is arranged to a time, in which only a few publications related to the municipal wastewater treatment with AGS. The currently proven operational requirements and the scientific progress in the handling of AGS are summed up in the introduction part of this thesis. The questions in this present work were based on further effects of the application of AGS on municipal wastewater treatment plants. The following section briefly describes the specific research questions addressed in this thesis.

1.2.1 Aerobic granulation in continuous-flow

Despite numerous publications on aerobic granulation and purification with AGS in SBR systems, only a few studies relate to the continuous-flow operation. A successful establishment of AGS in continuous-flow operation is not documented yet and represents a desirable milestone since only a few wastewater treatment plants are designed as SBR. The main issue of the present work was to investigate under which conditions an aerobic granulation can be achieved in a continuous-flow system and to what extent it is possible to improve the settling properties.

1.2.2 Sludge Stabilization

The use of AGS on municipal sewage treatment plants is still largely new. Numerous questions arise regarding the large-scale application of the AGS process and the economic effects on the plant operation. Only a few studies focus on the effects of aerobic granular sludge on downstream sludge treatment. Due to the structural differences compared to flocculent activated sludge, possible effects on the anaerobic biodegradability must be considered carefully. Energetic and economic aspects concerning energy recovery from biogas production, costs of sewage sludge dewatering and disposal form the background for considering potential impacts on the treatment process. For these reasons, the anaerobic biodegradation and dewatering behavior of AGS were investigated and evaluated in this thesis.

1.2.3 Nitrous oxide emissions

Nitrous oxide (N_2O) is a greenhouse gas and approximately 300-times more potent than CO_2 in its effectiveness (IPCC 2007). For this reason, the N_2O emissions are a topic of current discussions on climate protection. Beside attempts in reducing N_2O emissions from agriculture and industry, more and more focus is paid to reducing emissions from wastewater treatment processes. So far, only a few publications report about N_2O emissions from the AGS process, which illustrates the need for further detailed measurements. The aim of the N_2O measurements was to evaluate the extent of N_2O that is emitted during the wastewater treatment with AGS compared to conventional activated sludge, and which triggers contribute the N_2O formation. N_2O emissions were tested and evaluated under different organic loading rates and aeration strategies.

1.2.4 Constructive requirements for aerobic granulation

Repeatedly, there are some WWTP, where the activated sludge offers extremely good settling properties. Such a WWTP (SBR) is located nearby Vienna and reveals over the years a sludge volume index (SVI) in a range of 30 to 50 ml g^{-1}. The work examined the reasons for these excellent settling properties by evaluating the process data from the plant for a period of one year.

1.3 Structure of the thesis

This thesis is a cumulative research work on aerobic granular sludge technology for wastewater treatment and comprises four peer-reviewed articles published in scientific journals.

1) The first chapter briefly describes the current state of the scientific knowledge including the requirements for an aerobic granulation, the structural and microbial differences compared to suspended activated sludge as well as the operational strategies. The chapter shortly describes the effect of different settings on the cleaning performance and settling properties.

2) The second chapter "Comparison of aerobic granulation in SBR and continuous-flow plants" deals with aerobic granulation in a continuous-flow setup. The settling properties and the removal of the cultivated sludge were compared with AGS from a lab-scale SBR. The article was published in the Journal of Environmental Management, 2019, Volume 231, 953-961.

3) The implementation of AGS technologies into full-scale could affect downstream processes like sludge stabilization and dewaterability. The questions of possible impacts on the methane yield and dewatering behavior were considered in the article "Anaerobic biodegradation and dewaterability of aerobic granular sludge". The article was published in the Journal of Chemical Technology and Biotechnology, 2019, Volume 94(9), 2908-2916.

4) N_2O emissions from the biological wastewater treatment with AGS were investigated under different aeration strategies and organic loading rates. The results are presented in the article "Nitrous oxide emissions from aerobic granular sludge", which was published in the Journal of Water Science and Technology, 2019, 80(7), 1304-1314.

5) An extensive data evaluation was undertaken for a large-scale SBR, where the activated sludge offers excellent settling properties. The paper with the title "Accidental aerobic granules – Data evaluation of a full-scale SBR plant" discusses different reasons for the formation of aerobic granules on this wastewater treatment plant. The article was published in the Journal Desalination and Water treatment, 2019, Volume 164, 11-17.

6) The last chapter gives a short summary of the presented work and an overall conclusion to the prospective wastewater treatment with aerobic granular sludge.

Chapter 2

State of the Art regarding aerobic granulation

2.1 Definition and Benefits

The most apparent difference between aerobic granular sludge (AGS) and activated suspended sludge is the structure and size of the biomass. Aerobic granules are characterized by particle sizes above 0.2 mm and higher settling velocities compared to conventional sludge. In two workshops (Munich 2004/Delft 2006), further definitions for AGS were formulated to distinguish the biomass from conventional activated sludge. According to these definitions, AGS consists of suspended biofilm units (aggregates of microbial origin), which do not coagulate under reduced shear stress and settle as separate units (de Kreuk et al. 2007a). It is recognized that aerobic granulation appears without the use of carrier materials, only sieving is an accepted method to separate aerobic granules from sludge flocs since the last AGS workshop.

Several advantages of AGS arise for the wastewater treatment due to its compact and dense structure. The large sizes of the granules enable higher settling velocities compared to activated sludge (Bathe et al. 2005), which are mostly reported within a range of 20 to 40 m h^{-1} (Table 2.1). In contrast, suspended activated sludge sinks with settling velocities of 7 to 10 m h^{-1} (Ni et. al, 2009; Su and Yu, 2005). Since the settling of the suspended sludge is limited to higher total suspended solid (TSS) concentrations, the operation of conventional activated sludge tanks is recommended to 5 gTSS L^{-1}. The faster sedimentation of aerobic granules ensures an excellent settling behavior also at higher TSS concentrations. Thus, AGS operation usually relates to TSS of 8 g L^{-1} (Giesen et al. 2013), which illustrates the increased treatment capacity compared to conventional activated sludge plants. Overall, the AGS operation allows the design of compact SBR systems due to shorter settling times and shorter SBR cycles (Pronk et al. 2015b). De Kreuk (2006) calculated that the footprint of AGS systems is only 20% compared to conventional processes due to an increased volumetric load. Bengtsson et al. (2019) reported saving in footprint for AGS systems in a range of 39–50%. Similiarly, Pronk et al. (2017) summarized a potential in saving footprint for AGS plants with 25–75% mainly due to increased biomass concentrations and the lack of clarifiers. However, it should be mentioned that SBR systems generally require a lower footprint compared to conventional activated sludge plants, which is in the range of 20 to 30% and related to a lack of clarifiers (Hobus et al. 2020).

Beside a lower footprint, the granular sludge process is characterized by a lower energy demand due to the lack of stirrers and return sludge pumps. Some authors reported an overall lower energy consumption for the biological stage, whereby the energy savings are named within a range of 53 to 60% (Giesen 2013, Pronk et al. 2017). Bengtsson et al. (2019) compared different wastewater treatment processes and calculated that the energy demand for AGS is approx. 23% lower than for conventional treatment processes including enhanced biological phosphorus removal. Further full-scale studies are needed to confirm these findings. The SBR operation for AGS focuses on the enrichment of phosphate accumulating organisms, thus another main advantage of the process is the enhanced biological phosphate removal (EBPR) with a reduced demand for precipitant agents. Furthermore, it is reported that an elevated amount of extracellular polymeric substances (EPS) that is produced by the bacteria provides greater resistance against toxic effects (Fang et al. 2002) making the AGS operation attractive for the treatment of industrial wastewater .

Although the wastewater treatment with AGS offers numerous advantages for the large-scale application, there is still a need for research. Sarma et al. (2017) summarized existing knowledge gaps for AGS, which mainly belong to biodegradation, bioaccumulation, biosorption, and mass transfer behaviors of emerging contaminants within the granules. Bengtsson et al. (2018) allude that further research should focus on a better understanding of nitrous oxide emissions from AGS as well as on sludge handling processes with respect to thickening and digestion. Moreover, there is still an interest in the transfer of AGS in continuous-flow operation in order to design full-scale plants with a smaller footprint.

2.2 Settling behavior

The settling behavior of the activated sludge is an essential parameter for the design of secondary clarifiers (DWA 2016). The settling process depends on the size, density, and shape of the biomass, whereby these properties are mainly related to the reactor operation and wastewater quality. A common value to control the settling behavior of the activated sludge is the sludge volume index (SVI), which indicates the volume of one gram biomass after a settling time of 30 min. The SVI for activated sludge is usually around 100 ml g^{-1} and can strongly depend on industrial influences, the concept and operating mode of the WWTP as well as the sludge retention time (DWA 2016). AGS is characterized by a much lower SVI, which is reported within a range of 45 to 59 ml g^{-1} (de Kreuk and van Loosdrecht 2006, Pronk et al. 2015b, Wagner et al. 2015). Although, values below 50 ml g^{-1} are often reported, there is up to now no SVI value defined for AGS. A further parameter to evaluate the settling properties of AGS is the ratio between the sludge volume after 10 to 30 min of settling. SV_{10}/SV_{30} ratios of approx. 1.0 indicate that the biomass reaches a comparable sludge volume already after 10 min instead of 30 min of settling. Small differences in the settling times and SV_{10}/SV_{30} ratios near to 1.0 were achieved in previous studies especially with the use of synthetic wastewater. However, the treatment of municipal wastewater with AGS always causes a fraction of flocculent sludge within the granules resulting in SV_{10}/SV_{30} ratios slightly above 1.0 (Pronk et al. 2015b).

The settling velocity is mainly determined by the size and density of the granules and can be described by the Law of Stokes. Both parameter, the increased density and the larger size contribute to a higher settling velocity. Granule sizes vary widely and are mostly reported within a range of 0.2 to 3 mm (Bengsston et al. 2018). In the past, various authors reported an increased density of AGS. For example, Su and Yu (2005) found a rising biomass density from 1.006 g cm^{-3} to 1.020 g cm^{-3} approx. 6 weeks after starting a granular sludge reactor. The increased density correlated with rising settling velocities from 8.9 m h^{-1} to 33.2 m h^{-1}. It was found that granules own different densities, which causes stratification over the settled sludge bed. Winkler (2012a) determined a density of 1.018 g L^{-1} for bottom granules of a settled sludge bed, while the granules from the top of the sludge bed had a density of 1.004 g L^{-1}. The higher density of AGS results not only from the compact structure, but also from mineral precipitation inside the biomass (Angela et al. 2011, Huang et al. 2015, Ren et al. 2008, Winkler et al. 2013). Angela et al. (2011) as well as Winkler et al. (2013) found apatite inside the core of the granules. Precipitations inside the granules were explained by increased pH values and ortho-phosphate levels, which originate from a phosphate release during anaerobic conditions (de Kreuk 2006; Maurer et al. 1999). Apatite formation was elevated when the pH value was in a range of 7.5 to 8.8, while minor apatite levels were detected under controlled pH values of 7.0 to 7.3 (Lochmatter et al. 2013). Table 2.1 summarizes literature references on settling properties including settling velocities (v_{sink}), SVI and diameters of AGS cultivated under synthetic, municipal and industrial wastewater.

Table 2.1: Literature review on SVI, v_{sink} and diameter for AGS cultivated with different media

Media	Reference	SVI [ml g^{-1}]	v_{sink} [m h^{-1}]	ø [mm]
synthetic	Arrojo et al. (2004)	60.0	20.0	0.25-4.0
	Zheng et al. (2005)	23.0	18-31	0.5-1.2
	Rosman et al. (2014)	20.0	61.0	2.0
	Long et al. (2014)	67.0	43.8	1.6
industrial	Rosman et al. (2014)	20.1	61.0	2.0
	Su and Yu (2005)	30.8	36.6	1.22
	Muda et al. (2012)	61.0	42.0	0.85-1.0
	Wang et al. (2007a)	32.0	91.0	2.0-7.0
	Cassidy and Belia (2005)	22.0	51.0	1.7
municipal	Li et al. (2014)	47.1	42.0	0.5
	Ni et al. (2009)	20-40	18-40	0.2-0.8
	De Kreuk et al. (2006)	38.0	n.n.	1.1
	De Kreuk et al. (2010)	59.0	n.n.	1.6
	Liu et al. (2010)	30.0	n.n.	0.8
	Pronk et al. (2015b)	45.0	n.n.	> 1.0 (60%)
	Derlon et al. (2016)	65.0	n.n.	0.25-0.63

Beside the size of the granules, the shape and surface delimitation are further essential parameters for the settling behavior. Ideal granules obtain a compact and round shape with a regular surface and without filamentous growth. However, the structure of the granules is affected by different parameters such as shear stress and wastewater quality. Some researchers found that high shear stress favors the formation of more compact and dense granules (Tay et al. 2001). The shear stress was increased by setting higher aeration rates or mixing intensities. From an economic point of view, increasing the shear stress by higher aeration rates is not suitable for full-scale design.

An increased growth of protozoa on the granules surface was often observed within the course of different studies. With the beginning of the aerobic granulation, the fraction of sludge flocs decreases and thus the possibility to incorporate suspended solids from the influent into the sludge structure. Subsequently, there appears an increased suspended solid concentration, which favors the growth of ciliates. Several researchers reported a meantime massive growth of vorticella-like organisms as well as rotifers. Thwaites et al. (2018) compared the abundance of higher organisms in AGS and flocculent sludge and observed that AGS was more colonized by fixed ciliates and rotifers than the sludge flocs. Li et al. (2013) investigated the appearance of vorticella-like organisms and rotifers in an AGS reactor operated with municipal wastewater and reported an alternating growth of vorticella, whereby a successive decrease of these organisms was linked to a simultaneous growth of rotifers. Moussa et al. (2005) reported that rotifers own a longer growth rate of 6 to 45 days and therefore prefer enhanced sludge retention times (SRT) to establish inside the granules. After this time, rotifers are able to reduce the substrate availability for vorticella and therefore limit their abundance leading to an alternating enrichment of vorticella-like organisms and rotifers in the granules.

Up to now, the influence of fixed ciliates on the settling velocity of AGS is not completely clarified. Pronk et al. (2015b) stated that protozoa, especially ciliates, slightly hinder the settling process but do not disturb the plant operation. Jahn et al. (2017) investigated the settling rates of AGS with different sizes and surface delimitations. Granules without growth of vorticella-like organisms and rotifers had a much higher sedimentation rate than those with surface growth. While the settling velocity of aerobic granules with a diameter of 1.3 mm and a regular surface resulted in a settling velocity of 23 m h^{-1}, the settling velocity for the granules with surface growth and the same size was reduced to approx. 17 m h^{-1}. Although ciliates could slightly affect the settling velocity, they are important organisms to retain suspended solids from the influent and to reduce the effluent turbidity.

2.3 Spatial arrangement of bacterial groups

Aerobic granules are characterized by a compact and dense structure with the formation of zones with different oxygen and substrate gradients similar to biofilms. The distribution of aerobic, anoxic and anaerobic zones within the biomass allows the enrichment of different bacterial groups. Figure 2.1 illustrates the AGS structure compared to suspended activated sludge, as well as the arrangement of relevant microorganisms. While the aerobic surface area is predominantly populated with nitrifiers and heterotrophic organisms, the deeper anoxic-anaerobic zones are dominated by phosphate and glycogen accumulating organisms (Winkler 2012). Beside substrate and oxygen gradients, there is a spatial distribution of the nitrogen fractions over the granules. Nitrate is formed by nitrifiers in the aerobic surface areas and diffuses into the interior of the granules, where it is denitrified. As a result, nitrifying as well as denitrifying processes take place at the same time resulting in simultaneous nitrogen removal which is characteristic for biofilms and AGS.

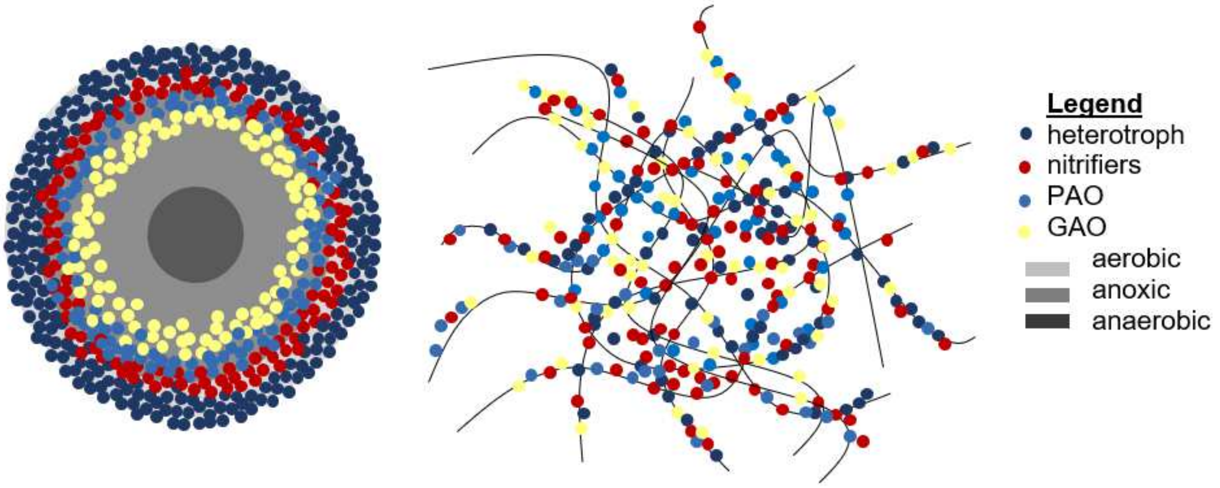


Figure 2.1: Structural differences of AGS (left) and suspended activated sludge (right) (adopted from Winkler 2012)

2.4 Formation of aerobic granular sludge

Most of the lab-scale studies used activated sludge as inoculum to cultivate aerobic granules. The quality of the used inoculum was found to affect the granulation process significantly (Sheng et al. 2010). Especially the macroscopic characteristics, the settleability, the surface properties, and the microbial activity of the seeding sludge were identified to influence the granule formation (Liu and Tay 2004). Startup times were reported within a broad range due to different reactor configurations and settings (Gao et al. 2011, Pijuan et al. 2011). Long et al. (2014) inoculated aerobic granules to a pilot-scale SBR and observed a fast granulation within 18 days, whereby the seeded granules acted as nucleis and carriers for new particles. Overall, the use of crushed granules as inoculum was found to shorten the startup time considerably (Pijuan et al. 2011). Pronk et al. (2015b) provided experiences of the startup of a full-scale Nereda® plant and reported that the design TSS was reached after 9 months of operation. Longer startup times for full-scale plants are attributed to the need to achieve the effluent quality.

Different hypotheses were presented in earlier publications explaining the formation of aerobic granules. Liu and Tay (2002) explained the granulation process by four formation steps. The first step includes the cell-to-cell contact, which is contributed by hydrodynamic, diffusion, gravitational and thermodynamic forces. The stabilization of the attached cells is supported by physical, chemical and biochemical interactions. Especially van der Waals and thermodynamic forces as well as surface tension are named in this hypothesis as relevant forces for the granulation. Moreover, it is known that the operation with a periodic feed ensures feast and famine conditions. It was found that the periods without substrate (famine phase) promote the enrichment of hydrophobic bacteria, which increase the microbial adhesion (Kjelleberg and Hermansson 1984, Liu and Tay 2002). The further development of cellular aggregates is supported by the production of EPS, which ensures interactions between the cells and the growth of large and compact aggregates.

In contrast to publications describing the granule formation, some studies relate to the granules disintegration, for example Verawaty et al. (2013), who described the break of AGS. For granules above a critical size, the diffusion into the inner zones is limited which leads to an under-supply of organisms in deeper zones. Dead cells and EPS consumption reduces the granule stability and causes week structures and the break of the biomass. However, the fragments of the break can serve again as new cores for granules.

2.5 Metabolic processes in aerobic granular sludge

2.5.1 Substrate-storing organisms

The enrichment of substrate-storing organisms was found to be the most crucial requirement for the stable long-term operation of aerobic granular sludge (de Kreuk and van Loosdrecht 2004, Liu et al. 2004). Substrate-storing organisms require an alternating aerobic and anaerobic operation strategy, which is usually ensured by the implementation of an anaerobic feeding phase into the SBR cycle. The most relevant substrate-storing organisms for the wastewater treatment are phosphate and glycogen accumulating organisms. These organisms are characterized by slow growth rates (Liu et al. 2004), which were found to contribute to the formation of compact and dense sludge aggregates. The following section explains the metabolic processes and required conditions of these organisms in detail.

2.5.2 Phosphate accumulating organisms (PAO)

Phosphorus is consumed by humans through food products and is afterward almost completely excreted to the sewer systems. The daily specific population-load to the wastewater is about 2 gP. In the inflow of the WWTP, about 80% of the total phosphorus is present as orthophosphate (Schönberger 1990) and 20% as inorganic and organically bound polyphosphate. The removal of particulate phosphorus via mechanical pre-treatment is usually about 15% (Schönberger 1990). During the biological treatment, a part of the phosphate is consumed by bacteria to build up new cell substance, thus approx. 1% of the removed BSB_5 is incorporated as phosphorus. In total, approx. 30% of the incoming phosphorus is removed from the wastewater without a targeted removal strategy. To meet the legal effluent quality, the dosage of iron or aluminum salts for chemical precipitation is the most widely applied removal strategy for phosphorus.

In addition to mechanical and chemical removal processes, there is also the possibility for an enhanced biological phosphorus removal (EBPR). A broad group of bacteria is able to store more phosphate than they need for their growth. The main representative of these phosphate accumulating bacteria (PAO) is Candidatus Accumulibacter phosphatis. The overall growth rate of PAO is expected with 1 d^{-1} (Morgenroth 1998). The design of anaerobic tanks for EBPR requires a minimum contact time of 0.75 h during dry weather conditions and a return sludge ratio of 1 (DWA 2016). The activated sludge usually contains a P content in the range of 1 to 2%, whereas the P content of sludge with EBPR can rise up to 5% (ATV 1994). The internal stored phosphate is removed with the excess sludge.

The mechanism of the biological phosphate uptake is summarized in Figure 2.2. One main requirement for an increased phosphate uptake is the periodic change between anaerobic and aerobic conditions. A further requirement for the growth of PAO is the presence of low molecular organic carbons during anaerobic conditions (Morgenroth 1998). Fermentative organisms convert high molecular organic compounds of the incoming raw wastewater into acetate and provide thus the substrate for PAO. Under anaerobic conditions, PAO absorb acetate and convert them into cell internal storage products. These storage materials are high in energy-rich poly-ß-hydroxybutyrates (PHB), which belong to the group of polyhydroxyalkanoates (PHA). PHB is hereby the most present form of PHA. In a further step, glycogen is converted via glycolysis to produce ATP and NADH (van Loosdrecht et al. 1997; Mino et al. 1998). NADH is used for acetate conversion to PHA. In the cells, acetate is activated to acetyl-CoA with a simultaneous ATP hydrolysis and ADP production. A part of the ATP, which is required for the assimilation of acetate, is provided via the cleavage of polyphosphates and leads to the resolution of ortho-phosphate into the bulk liquid (Wentzel et al. 1985). As soon as the glycogen is consumed, the acetate uptake and the phosphate resolution stop simultaneously.

Under aerobic conditions, oxygen is available as electron acceptor. The phosphate, which is released in advance, is absorbed again by PAO and the poly-phosphate storage within the cell is expanded. The PHA energy storage is used to re-create glycogen reserves, while PHA is used as an energy source to restore glycogen reserves, to generate ATP and to build up new cell substance. Problems in the subsequent anaerobic phase can occur if the glycogen reserve is not fully recharged. In this context, insufficient reduction potential (NADPH) is available for glycolysis of glycogen. With a balanced energy ratio of the cells, more phosphate is absorbed under aerobic conditions than it was released under anaerobic conditions.

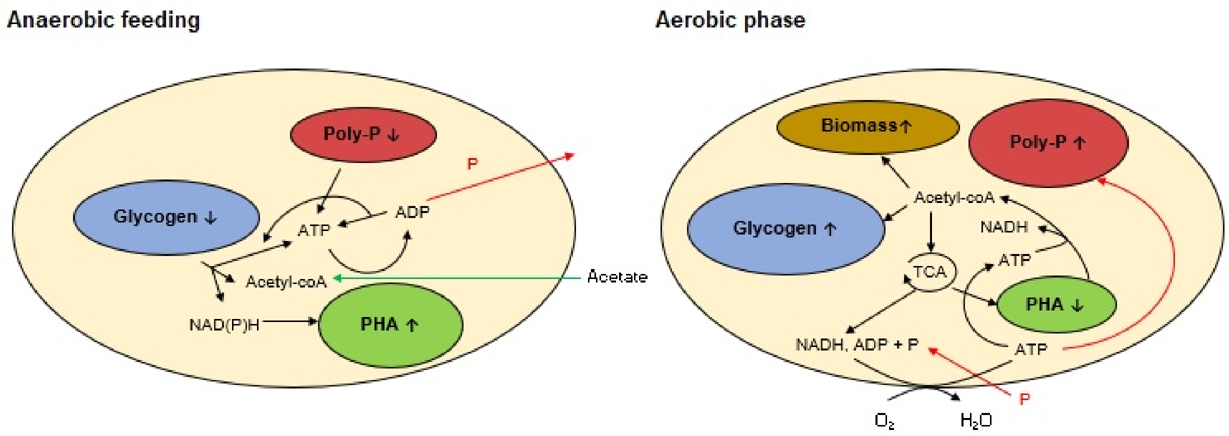


Figure 2.2: Anaerobic conversion of acetate and glycogen to PHA and the aerobic metabolism of phosphate accumulating bacteria (adopted from Winkler 2012)

In municipal sewage treatment plants with EBPR, the biological P removal is usually supported by the dosage of chemical precipitants to ensure the effluent quality. Chemical and biological P removal processes can hereby influence each other. Chemical-physical processes for the elimination of phosphorus from wastewater are described by DWA (2011b). For full-scale WWTP, different parameters can be calculated to determine the extent of the biological phosphate removal (DWA 2011a). One evaluation criteria is the K_p value, which indicates the relationship between the amount of precipitants and the P load in the inflow to the WWTP. For plants with EBPR, the K_p value is usually in a range of 11 mol metal/kg P (DWA 2011a).

Further parameters to evaluate the biological P removal are listed below with the $f_{P,Bio\text{-}P}$ and the $ß_{prec}$ value. The $f_{P,Bio\text{-}P}$ value relates to the organic inflow load and is usually between 1.0 and 1.5% for WWTP with EBPR, while the $ß_{prec}$ value amounts to 1 (DWA 2011a).

$$K_P = \frac{FM_d}{C_{P,Z} \cdot Q_d} \qquad f_{P,Bio\text{-}P} = \frac{X_{P,bio\text{-}P}}{C_{BSB,ZB}} \qquad ß_{prec} = \frac{31 \cdot FM_d}{X_{P,prec} \cdot Q_d}$$

2.5.3 Glycogen accumulating organisms (GAO)

A further group of organisms that stores organic carbon under anaerobic conditions is the group of glycogen accumulating organisms (GAO). These bacteria are of special interest for the EBPR since they compete with PAO against the same substrate and do not perform an enhanced P uptake. Figure 2.3 shows the metabolisms of the GAO under anaerobic and aerobic conditions. Under anaerobic conditions, GAO use internal stored glycogen to gain energy and to take up acetate from the bulk liquid for PHA synthesis. During oxygen supply, GAO start to build up new cell substance using the internal stored PHA. Simultaneously, the glycogen storage is replenished. Bassin et al. (2012a) and Winkler et al. (2011) observed a growth advantage of GAO against PAO at increased temperatures. Since GAO are usually responsible for a declining biological P removal, different studies investigated strategies to restrict their growth. One method to improve the biological P removal was found in the targeted removal of GAO-rich sludge, which is usually located in the upper part of the sludge bed. This strategy is only possible since the granules with PAO and GAO own different densities, which leads to a stratification over the settled sludge bed as it was explained beforehand in Chapter 2.2.

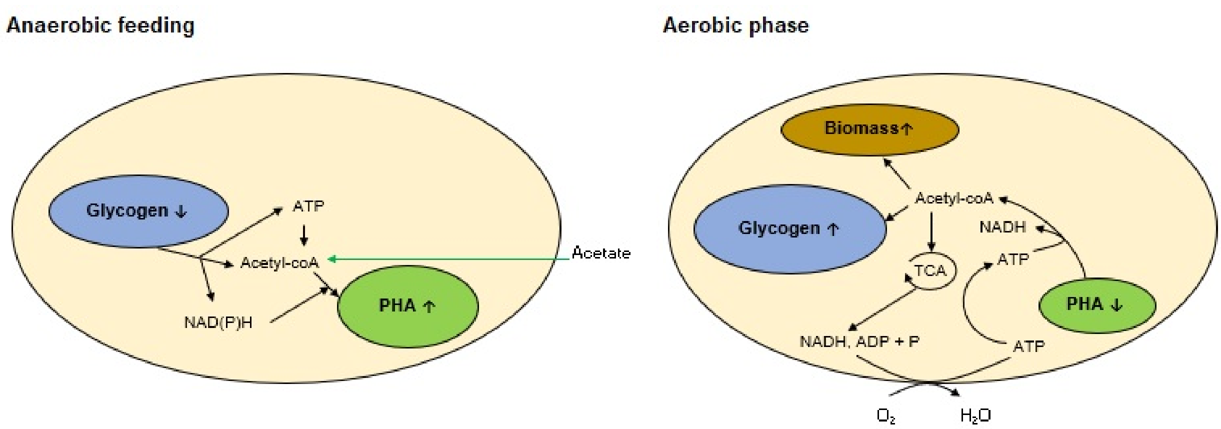


Figure 2.3: Anaerobic conversion of acetate and glycogen to PHA and the aerobic metabolism of glycogen accumulating bacteria (adopted from Winkler 2012)

2.5.4 Denitrifying PAO and GAO

PAO and GAO are able to use nitrate and nitrite as electron acceptors and therefore enable denitrification. In this case, the literature speaks of denitrifying PAO or GAO (dPAO, dGAO) (Bassin et al. 2012a, Hu et al. 2002). Some studies proved that two main groups of PAO can be found in activated sludge plants. PAO of group I can use nitrate and nitrite as electron acceptors, while PAO of Group II only use nitrite (Flowers et al. 2009). The proportion of PAO denitrifying nitrate or nitrite simultaneous to the phosphate uptake depends on the distribution of aerobic and anoxic zones within the granules as well as on the availability of nitrite and nitrate. Denitrifying GAO also take over denitrification, mainly reducing nitrate and only a small proportion of nitrite. Thus, there is a symbiosis between PAO and GAO with GAO providing nitrite for PAO (Group I). Winkler et al. (2011) reported that strictly aerobic PAO, which use only oxygen, can be found in the outer area of aerobic granules, while optional aerobic PAO, also grow inside the granules and use oxygen, nitrate as well as nitrite.

2.5.5 Simultaneous nitrification and denitrification

AGS is known for simultaneous nitrogen removal (SND) with nitrifying and denitrifying processes taking place in parallel. The extent of the SND can be determined by the content of the denitrified nitrate to the amount of nitrified ammonium (Derlon et al. 2016). The amount of SND depends on the reactor operation, especially the aeration and the presence of anoxic zones, the organic loading as well as on the nature of the incoming wastewater (C/N ratio). Generally, the aeration strategy and the oxygen diffusion decisively influence the distribution of aerobic and anoxic zones as well as the size of the granules. Results of studies investigating different aeration strategies are summed up in Chapter 2.7.3. Simulation results from de Kreuk et al. (2007b) reveal that with organic loading rates above 1.9 kgCOD m^{-3} d^{-1}, the amount of ammonium oxidizing bacteria (AOB) is not enough to nitrify all the incoming ammonium. The simulation results and the experiments from de Kreuk et al. (2007b) indicate that the optimal diameter for the nutrient removal is in a range between 1.2 and 1.4 mm.

2.5.6 Extracellular polymeric substances (EPS)

AGS obtains a higher amount of extracellular polymeric substances (EPS) compared to suspended activated sludge (Adav et al. 2008a, Adav et al. 2008b, Wang et al. 2006). Increased EPS contents are also known for biofilms, where the gel-like EPS matrix acts as agglomeration agents. EPS is produced by bacteria themselves and consists mainly of proteins, polysaccharide, and nucleic acids. A detailed description of the EPS function and composition can be found in Section 4.2.

2.5.7 Toxic resilience of AGS

Some studies investigated the resilience of AGS against toxic impacts and their suitability to degrade chemical substances. Chen et al. (2019) investigated the treatment of petroleum wastewater containing alkanes, olefins, arenes, heterocyclic N-compounds with AGS. Petroleum compounds are usually toxic for bacteria. However, the COD removal efficiency of AGS declined only from 95% to 90% by the treatment of petroleum wastewater. Adav et al. (2007) reported that aerobic granules are able to degrade wastewater with pyridine concentrations of 200 to 2,500 mg L^{-1}. Further studies belong to the treatment of phenol-containing wastewater with AGS (Jiang et al. 2004, Tay et al. 2005). The role of EPS in the adsorption of toxic chemicals is supported by a high surface area as it was also mentioned in earlier studies by Adav et al. (2008a). This effect is probably related to the high binding capacity of the EPS. Moreover, Wang et al. (2019) investigated the removal of antibiotics with AGS, namely kanamycin, tetracycline, ciprofloxacin, ampicillin, and erythromycin. The total removal efficiency was 88.4%, whereby 62.5% of antibiotics were degraded and 33.2% adsorbed to the surface of the granules. Nevertheless, there are up to now no comparable reference tests with suspended activated sludge, which would confirm an increased aptitude of AGS for antibiotic removal.

2.6 Factors influencing granulation

2.6.1 Nature of the wastewater

The nature of the incoming wastewater is subject to regional circumstances and sometimes dominated by a high industrial proportion or a high amount of foreign sewer infiltration. Moreover, in coastal areas, the wastewater is often characterized by an increased salinity. Although an excellent aerobic granulation was also achieved with high-saline wastewater, the biological phosphate removal efficiency was clearly limited under saline conditions (van den Akker et al. 2015, Bassin et al. 2011a) and probably induced by salt inhibition (Welles et al. 2014).

Further studies focused on the effects of the wastewater composition on the AGS formation and the sludge structure. Numerous lab-scale studies with AGS included the use of synthetic wastewater containing a high amount of readily available carbon. These substances can be generally used well by the organisms and lead to large granules and high settling velocities. In contrast, experiments with wastewater containing particulate substances resulted in a more irregular biomass growth and lower settling rates. The reason for the irregular structure was found in an incomplete hydrolysis of the complex wastewater ingredients during the anaerobic feeding phase (de Kreuk et al. 2010). The merely adsorbed particulate matter causes substrate gradients over the granules, which promote an irregular aerobic growth and the formation of finger-type structures. Finger-type structures can decrease the settling behavior significantly and their breaking off under shear stress can subsequently increase the suspended solids concentration in the effluent. A strategy to overcome the issues of a delayed hydrolysis of particulate matter was found in an extended anaerobic feeding phase (Wagner et al. 2015). Pronk et al. (2015a) investigated the role of different substrates and their behavior during the granules formation. Again, the complete uptake of biodegradable substrates during feeding and the formation of storage polymers was named as relevant to achieve stable granulation. Similar findings were made by Layer et al. (2019), who investigated the granulation process under the use of four types of wastewater containing different amounts of diffusible and non-diffusible organic substrates. In this study, the increased content of diffusible substances was found to shorten the startup time considerably. Figure 2.4 shows different structures of aerobic granules cultivated under municipal wastewater as well as granules with finger-type structures.

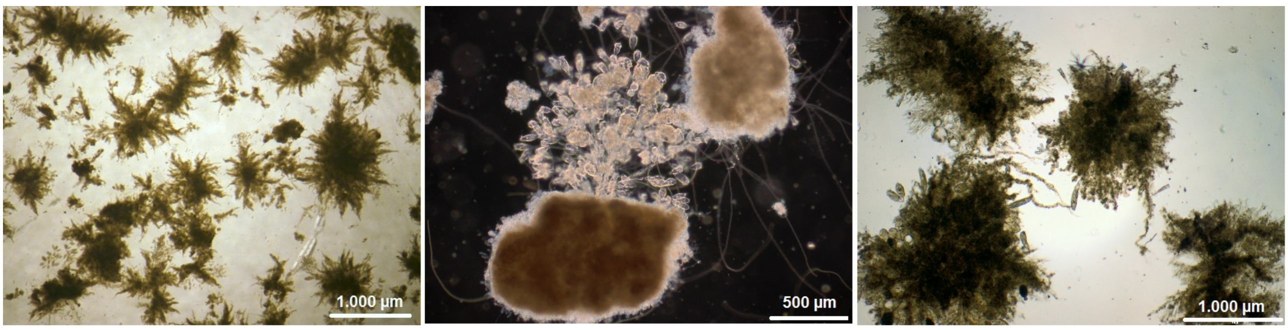


Figure 2.4: AGS with finger-type structure (left), AGS with a regular shape and surface growth (middle), AGS with an irregular shape and rotifers (right)

The organic loading rate (OLR) is a further essential parameter for the formation of stable granules and was thus intensively studied in their importance for AGS operation. Moy et al. (2002) used aerobic granules for the treatment of highly contaminated wastewater, whereby the gradual increase of the OLR from 6.0 to 15.0 kgCOD m^{-3} d^{-1} showed no impairment on the granule formation. However, Kim et al. (2008) and Li et al. (2008) found that the OLR affects the morphology and structure of the granules as well as the startup time for the granulation. Increasing the load resulted in a faster formation of large but less dense granules. The highest OLR showed the lowest biodiversity, while the lowest loaded reactor had a high variety of bacteria. An optimal OLR is moreover relevant to prevent the growth of filamentous organisms, which tend to develop under high loads. Strategies to suppress filamentous growth and bulking sludge in AGS systems are summed up by Liu et al. (2006a).

2.6.2 Temperature

Aerobic granulation was observed for a temperature range of 7 to 50°C (Gonzalez-Martinez et al. 2017, Ab Halim et al. 2015, Ab Halim et al. 2016). The temperature affects the microbial community mainly (PAO/GAO distribution, Section 2.6) and gets especially important during winter months, since temperatures below 10°C reduce the growth rates of the nitrifiers and in turn decline the nitrification capacity. De Kreuk et al. (2005b) studied the influence of lower temperatures on the cleaning performance of AGS. During the granule formation, temperatures of 8°C led to an irregular growth and increased discharge of sludge flocs. In contrast, after a complete granulation, lowering the temperature from 20 to 8°C did not give any appreciable differences in nitrification performance. Although Gonzalez-Martinez et al. (2017) reported a successful startup of a granular sludge reactor at temperatures of 7°C using a cold-adapted biomass as inoculum, the startup for AGS reactors is generally recommended during the summer months.

2.7 Operational strategies

2.7.1 Feeding strategy

The anaerobic feeding is the most important setting to achieve aerobic granulation and is performed under mixed or non-mixed conditions. Rocktäschel et al. (2013) compared the aerobic granulation with an anaerobic plug-flow feed and a fast-influent pumping, which was followed by stirred conditions. The authors reported that under anaerobic mixing the biomass is longer in contact with the substrate, which allows a better COD uptake. With the anaerobic mixing, a comparable and good granulation was achieved, even if the biomass was significantly smaller than in the reference test without stirring. Better nitrogen removal has also been reported under mixed conditions, which was explained by an increased specific surface area of the granules and thus a higher conversion rate. However, with the mixing strategy, the biomass can get in contact with nitrate, which is probably leftover from the previous cleaning cycle. In that case, mixing can reduce the anaerobic phase considerably. The full-scale Nereda® process is related to an anaerobic feeding strategy under non-mixed conditions, whereby the wastewater is supplied from the bottom of the reactor with an upflow velocity of 3.0 to 3.3 m h^{-1} (Pronk et al. 2015b). The benefit of an anaerobic plug-flow is that the settled sludge bed is exposed to high substrate concentrations, which ensure high diffusion depths inside the granules. High diffusion forces build the requirement for the formation of larger granules sizes. Further studies reported about the AGS operation with an intermittent feeding strategy (McSwain et al. 2004, Wang et al. 2012). Although the step feed ensured enhanced TN removal, this feeding strategy is so far not applied for full-scale AGS plants.

The construction of the inflow pipes is one of the most important elements in the reactor design for AGS. Rocktäschel et al. (2013) found that the operation of a reactor with a low H/D ratio causes a heterogeneous flow pattern in the sludge bed with a shorter contact between the supplied substrate and settled biomass. As a result, an incomplete COD uptake appears so that the easily available COD is still present during the aerated phase causing aerobic growth and irregular forms. To avoid the formation of flow channels through the settled sludge bed, full-scale Nereda® plants are equipped with a perforated inlet system, which allows an equal distribution of the incoming wastewater.

2.7.2 Organic loading rate (OLR)

Up to now, only little information on the OLR of large-scale AGS plants is available. Li et al. (2014) named an OLR of 0.56 kgCOD m^{-3} d^{-1} for a full-scale SBR operated with a TSS of approx. 4 g L^{-1}. Pronk et al. (2015b) reported an OLR of 0.1 kgCOD $kgTSS^{-1}$ d^{-1} for a full-scale AGS plant, which is comparable to a hydraulic load of 1.5 m^3 m^{-3} d^{-1}. As mentioned beforehand, the OLR was found to affect the strength of the granules considerably. However, the reported values for full-scale plant operation do not differ from conventional loading rates applied for activated sludge plants.

2.7.3 Hydraulic retention time (HRT)

The hydraulic retention time (HRT) is the average time the supplied wastewater remains in a WWTP. For SBR systems, the HRT correlates with the cycle length and the exchange ratio and is usually between 12 and 24 h (DWA 2009). In practice, cycle lengths within a range of 6 to 8 h are well proven for full-scale SBR operation under dry weather conditions (DWA 2009). The cycle duration determines the number of cycles per day and thus the number of decant phases. The periodic washout of flocculent biomass was identified to favor the granulation process in lab-scale. However, too short HRT can cause unstable granulation due to a strong washout of biomass. Rosman et al. (2014) reported an optimal HRT of 6 h for the aerobic granulation. Liu et al. (2016) investigated HRT in a SBR with 4, 6 and 8 h and found the highest biomass increase with a HRT of 4 h. The HRT of a full-scale SBR operated with AGS was reported with 17 h (Pronk et al. 2015b).

2.7.4 Aeration strategy

The aeration strategy combines different settings including the intensity, the upflow air velocity as well as the time-controlled onset of the aerated phases. The effect of the aeration intensity and the upflow air velocity on the aerobic granulation were studied intensively in early research projects (Adav et al. 2007; Liu et al. 2006b; Lochmatter et al. 2013). Tay et al. (2001) found more compact and regular granules under increased superficial upflow air velocities and explained this observation by an increased polysaccharide production. A positive effect of the shear force was also reported by McSwain et al. (2008) investigating the granule formation under different upflow gas velocities. The authors observed a disintegration of the granules with upflow velocities below 1 cm s^{-1}. However, a further experiment with similar upflow velocities showed that the dissolved oxygen (DO) concentration is more relevant for the granule formation than the shear forces (McSwain et al. 2008).

Mosquera-Corral et al. (2005) investigated the aerobic granulation under different oxygen levels, thereby lowering the DO led to a massive biomass washout. Until this time, an operation with reduced DO was not possible since there was no anaerobic feeding phase implemented in the SBR cycle. Stable operation of AGS under lower DO levels was achieved for the first time with an anaerobic feeding phase. As mentioned beforehand, this anaerobic feeding strategy is now widely applied for the AGS operation. Since AGS is suitable for SND, further studies focused on the effect of different aeration strategies on the extent of SND. Lochmatter et al. (2013) compared a constant aeration with an intermittent aeration and found an improved TN removal for the intermittent aeration strategy. Chen et al. (2011) also observed a higher nitrogen removal after switching from a simultaneous to an alternating aeration strategy. Phases without aeration increase the anoxic zones inside the granules and thus the SND capacity. Pronk et al. (2015b) reported for a full-scale Nereda® plant DO levels in the range of 1.8 to 2.5 mg L^{-1} during the aerated phases. As soon as the NH_4-N is depleted in these systems, the aeration is reduced to ensure denitrification. This aeration strategy allows an average TN removal above 85% (Pronk et al. 2015b).

2.7.5 Effluent discharge

Previous studies are based on the operation with a constant or variable reactor volume. The operation with a variable reactor volume is characterized by the discharge of the purified wastewater after a controlled settling time, which causes a declining water level. This operation mode allows to washout slow settable substances that do not settle within the given settling time. The minimum settling velocity ($v_{s,min}$) determines the extent of the sludge particles that are washed out during the decant phase. Increasing $v_{s,min}$ leads to an elevated washout of fine biomass, which is desired in order to enrich AGS in the system faster. In order to achieve rapid granulation, $v_{s,min}$ must be higher than the sinking velocity of the flocculent sludge. Wang et al. (2006) reported that a $v_{s,min}$ smaller than 4 m h^{-1} is not enough for aerobic granulation and promotes the growth of flocculent biomass. Although the operation with a varying water level allows a fast granulation, the realization of this decant strategy is very challenging for full-scale plants since it would require a very high pumping rate. Another way to remove the purified water out of the SBR was found in a simultaneous feeding and decant phase, whereby the purified water is replaced by the incoming raw wastewater. This operation mode is applied for full-scale Nereda® plants and requires a constant control of the effluent quality with online analyzers, which are situated 0.5 mbelow the discharge point.

Wagner et al. (2015) investigated the aerobic granulation with wastewater containing particulate material under $v_{s,min}$ of 4.5 and 9.0 m h^{-1}. In both cases, the suspended solids in the effluent were high with 55 to 490 mg L^{-1} and no increase of the TSS in the reactor was possible. The high suspended solids in the effluent of AGS reactors were identified as a potential problem for full-scale implementation (Arrojo et al. 2004). Thus, first AGS plants were equipped with a post-treatment, usually a filter unit, to retain suspended solids and to ensure excellent effluent quality. Van Dijk et al. (2018) investigated the reasons for the increased suspended solids in the effluent of AGS plants and found that rising sludge particles were caused by degasification of nitrogen gas. Another reason for the washout of particles is the presence of not settable substances such as fats and foam. A strategy to decrease the sludge washout caused by degasification was found by stripping nitrogen gas before the settling phase. Moreover, the implementation of a vertical scum baffle before the effluent weir was reported as further action to keep particles away from the outlet (van Dijk et al. 2018). With these arrangements it was possible to reduce the suspended solids in the effluent of a full-scale Nereda® plant to 7.8 mg L^{-1}.

2.7.6 Waste activated sludge extraction

Bassin et al. (2012b) as well as Winkler et al. (2011) investigated the effect of the sludge discharge on the PAO-GAO distribution and on the EBPR efficiency. The sludge was removed under mixed conditions, as well as from the top and the bottom of the settled sludge bed. The background for applying this strategy was that a stratification across the settled sludge bed appears, whereby PAO were mainly found on the bottom of the sludge bed. GAO-rich granules were situated on the top of the sludge bed. With this strategy, the amount of GAO in a full-scale AGS plant was below 5% indicating that the biomass was dominated by PAO. Benstöm et al. (2019) summarized different patents for the waste sludge removal of AGS systems. One patent of the Company DHV relates to the removal of flocculent sludge from the upper sludge bed over a fixed removal height (WO 2007/089141 A1). Another patent from Veolia includes the operation of a floating decant facility, which removes the purified wastewater and afterwards the flocs from the settled sludge bed (WO 2012/175489 A1). One operation strategy according to a patent by Company Degremont includes the anaerobic feeding in a pulsating sludge bed and the removal of sludge with low settling properties by a fixed removal setpoint (WO 2009/050347 A2). Further patents relate to the operation of microsieves to select for dense and large granules (H2Oland) (de Blois et al. 2018) or to the separation of compact granules from sludge flocs by hydrocyclones (S::Select technology, EssDe GmbH). The primary application of the S::Select process was the separation of bulking sludge in order to improve the sludge settleability. Up to now, 10 facilities are equipped with the S::Select technology (Sepúlveda-Mardones et al. 2019).

2.8 Resource recovery from AGS

Some earlier studies focused on the recovery of biomaterial from activated sludge, whereby special attention was paid to the metabolism of substrate-storing organisms, which are known to synthesize PHA. PHA owns similar properties to polypropylene and is therefore of interest for renewable resource production (Fang et al. 2009). With this background, some research is based on the PHA accumulation and extraction from AGS. Different yields for PHA out of aerobic granules can be found in literature, which vary from 0.1 to 0.68 g PHA $gVSS^{-1}$ (Qin et al. 2005; Fang et al. 2009; Gobi and Vadivelu 2014, 2015). Further researchers reported about alginate-like exopolysaccharides (ALE) in AGS. ALE is a polysaccharide-based fraction of the EPS (Lin et al. 2010, Meng et al. 2019) and usually extracted from brown algae. ALE are preferably used as thickeners in the food and paper industry and for textile printing due to their gel-like structure (McHugh 1987). Lin et al. (2010) as well as Meng et al. (2019) reported yields in a range of 0.03 to 0.16 gALE $gVSS^{-1}$, whereby the results strongly depend on the applied extraction method. Lin et al. (2015) investigated the possible use of polysaccharide-based biomaterial from AGS and found the formation of a film on hydrophilic surfaces, indicating the probable suitability as surface coating material.

Chapter 3

Comparison of aerobic granulation in SBR and continuous-flow plants

Jahn, L., Svardal, K., Krampe, J. (2019) Comparison of aerobic granulation in SBR and continuous-flow plants. Journal of Environmental Management 231, 953–961, doi: 10.1016/j.jenvman.2018.10.101.

3.1 Abstract

Up to now, aerobic granulation of activated sludge is only realised 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.

3.2 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 most applied cycle operation for aerobic granulation consists of an anaerobic feeding, aeration and sedimentation phase. In recent years, a lot of research has been undertaken to understand the requirements for the aerobic granulation. 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 sludge bed 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) summarized some first operating data from an aerobic granular sludge plant treating domestic wastewater. The authors reported 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 60–90 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 with aerobic granules (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 flocculent sludge since the second granular sludge workshop (de Kreuk et al., 2007a). 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 0.1–1.0 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. Further references of aerobic granulation in CFPs are summarized below in Table 3.1.

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 3.1: 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^{-1}, 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 compartments 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.

3.3 Material and Methods

3.3.1 Experimental setup

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 (see Fig. 3.1). 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 2009). 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}. Fig. 3.1 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.

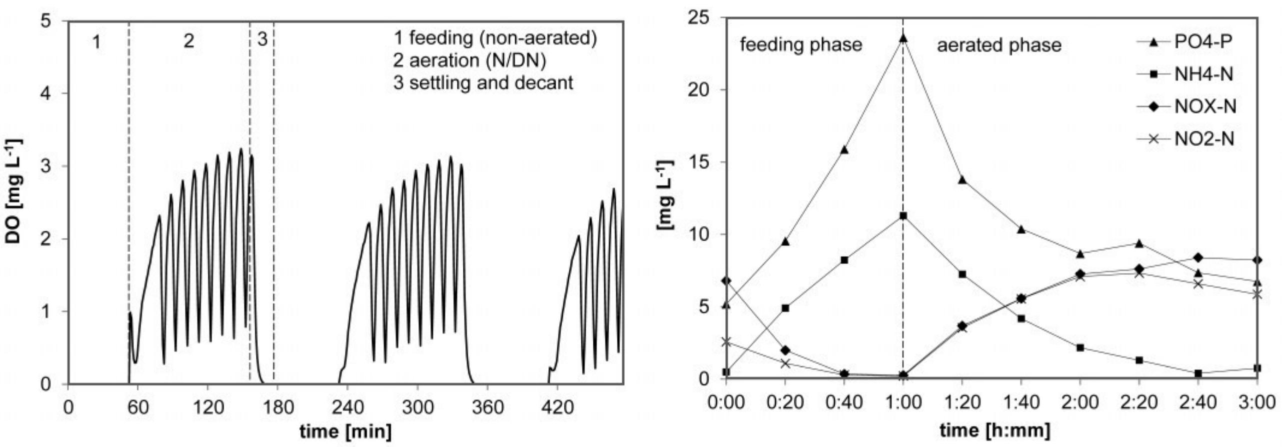


Figure 3.1: DO profile of the SBR with phases (left), N and P during feeding and the aerated phase (right)

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 3.2. Fig. 3.2 shows a schematic chart of the CFP.

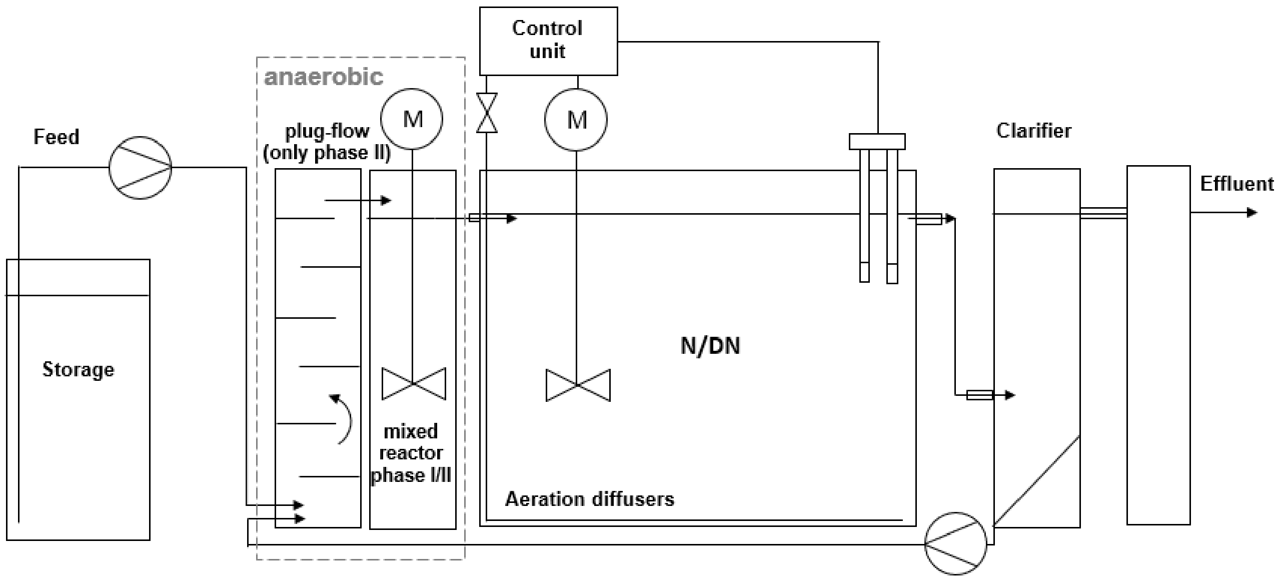


Figure 3.2: Schematic chart of the setup of the continuous-flow plant

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 realised 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}.

Table 3.2: Settings and approaches during the experimental phase I and II

Phase	Medium	Approach	$V_{anaerob}$ [L]	V_{BB} [L]	q_A [m h^{-1}]	OLR [gCOD L^{-1} d^{-1}]
Phase I	synthetic	complete mixed anaerobic reactor	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

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). Fig. 3.3 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 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.

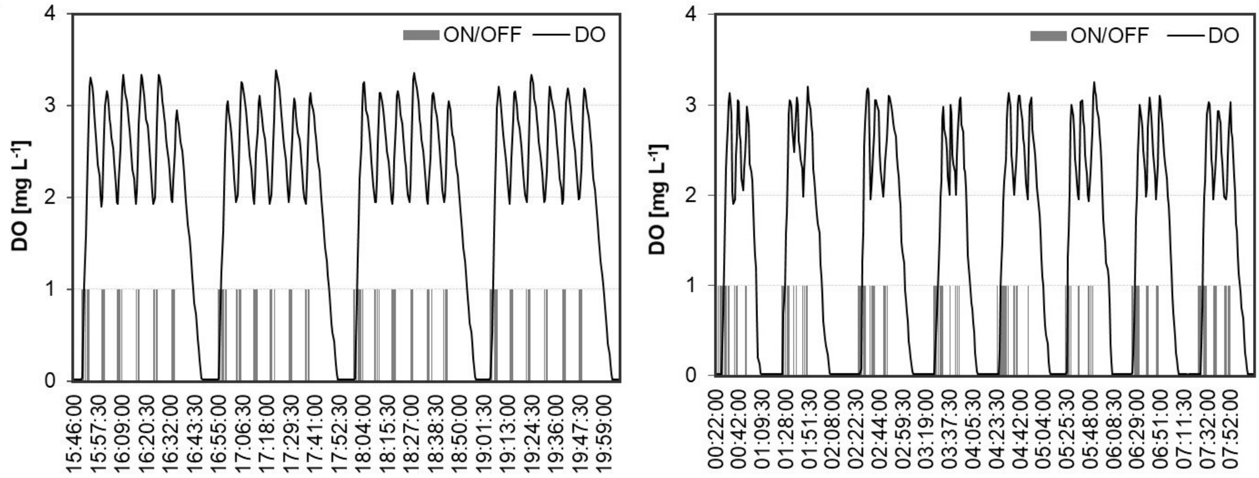


Figure 3.3: Typical DO profils in the aerated tank for the CFP during phase I and II (lines show DO, columns show aeration mode ON/OFF).

3.3.2 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 3.3 shows the mean analytical data of the wastewater fed to the CFP and SBR. Microscopic 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.

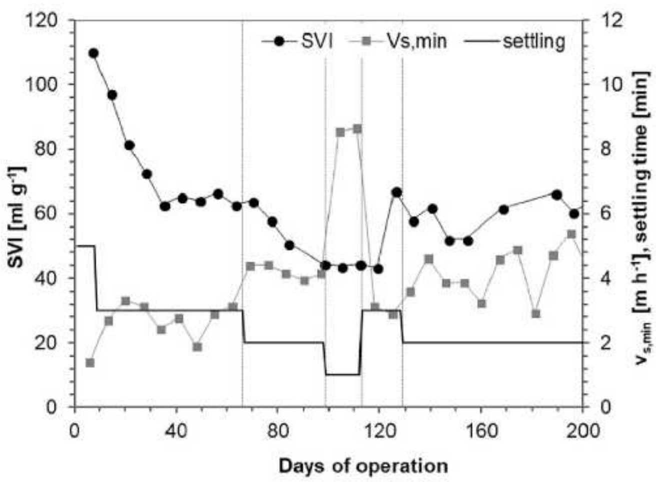
Table 3.3: Number of samples, mean composition of the wastewater for the plants and OLR.

Plant	Nr. [-]	COD [mg L^{-1}]	TN [mg L^{-1}]	TP [mg L^{-1}]	OLR [gCOD L^{-1} d^{-1}]	OLR [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

3.4 Results

3.4.1 SBR

Aerobic granulation in the SBR was achieved within 28 days, whereby first small granules were detected via microscopic images. Fig. 3.4 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 an 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 gCOD 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 1.0–1.1; while the ratio of the inoculated activated sludge was about 1.5–1.8. Settling velocity of individual granules were determined with 23 m h^{-1}, whereas for activated sludge a range of 7–10 m h^{-1} is reported (Qin et al., 2004).



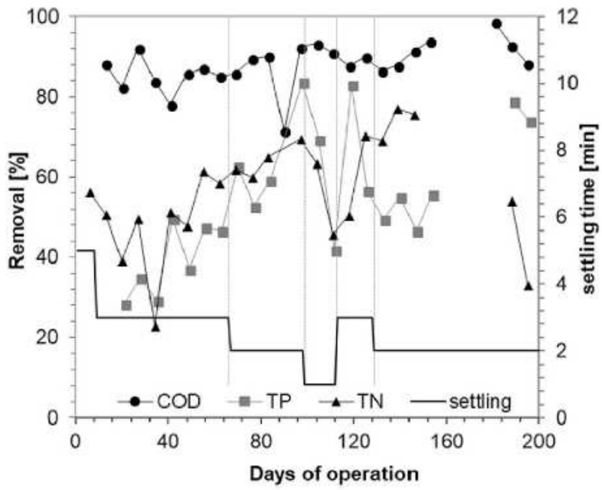


Figure 3.4: SVI, settling time and $v_{s,min}$ of the SBR (left) and COD, TN and TP removal of the SBR (right)

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. The average pH value in the reactor was 7.56. Fig. 3.4 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} L^{-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. (2012a) 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.

3.4.2 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. Fig. 3.5 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 mg L^{-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 related to 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 35–227 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 allow a stable granules formation.

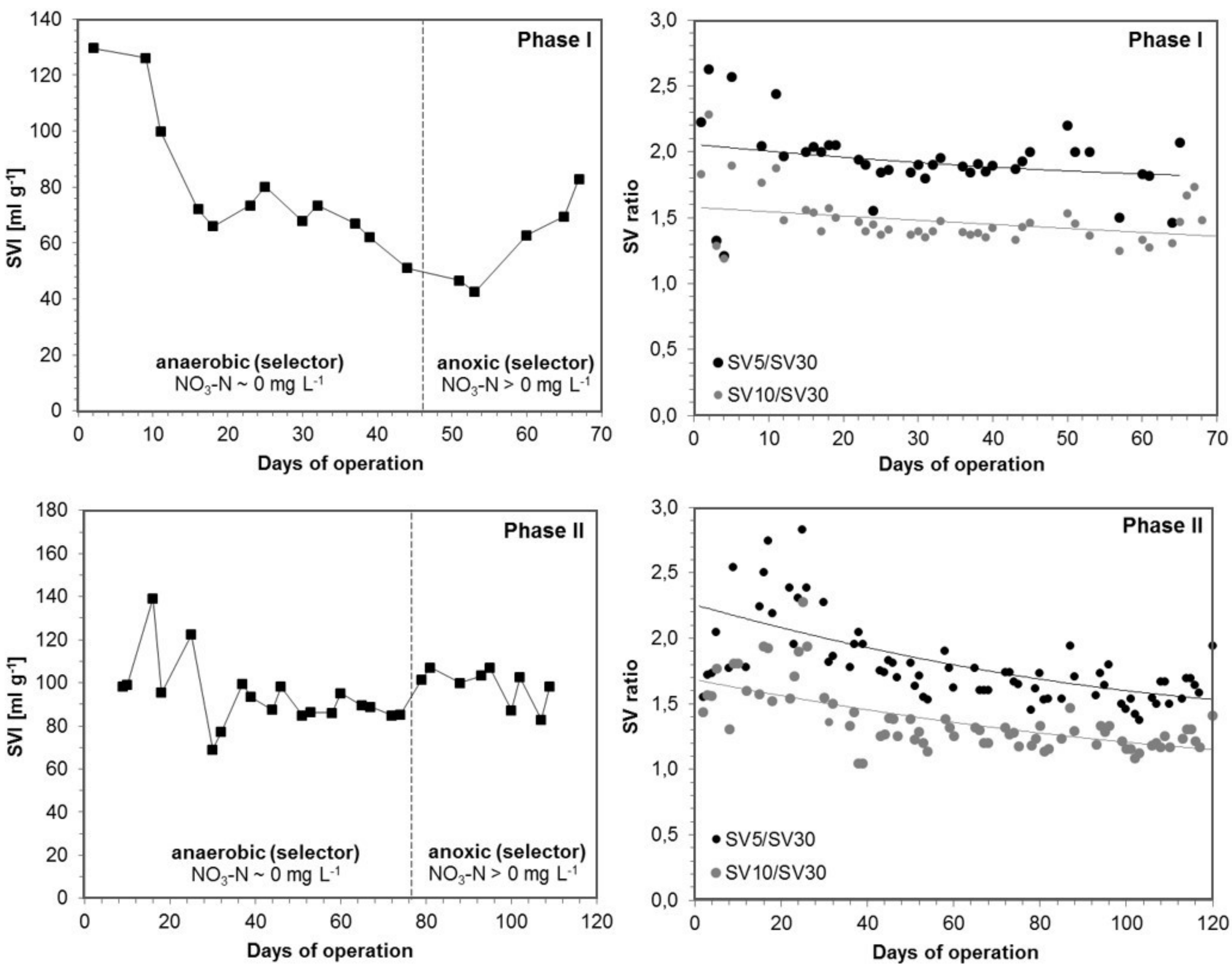


Figure 3.5: SVI, SV_{10}/SV_5 and SV $_{10}/SV_{30}$ ratio for the CFP during phase I and II

Fig. 3.6 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.

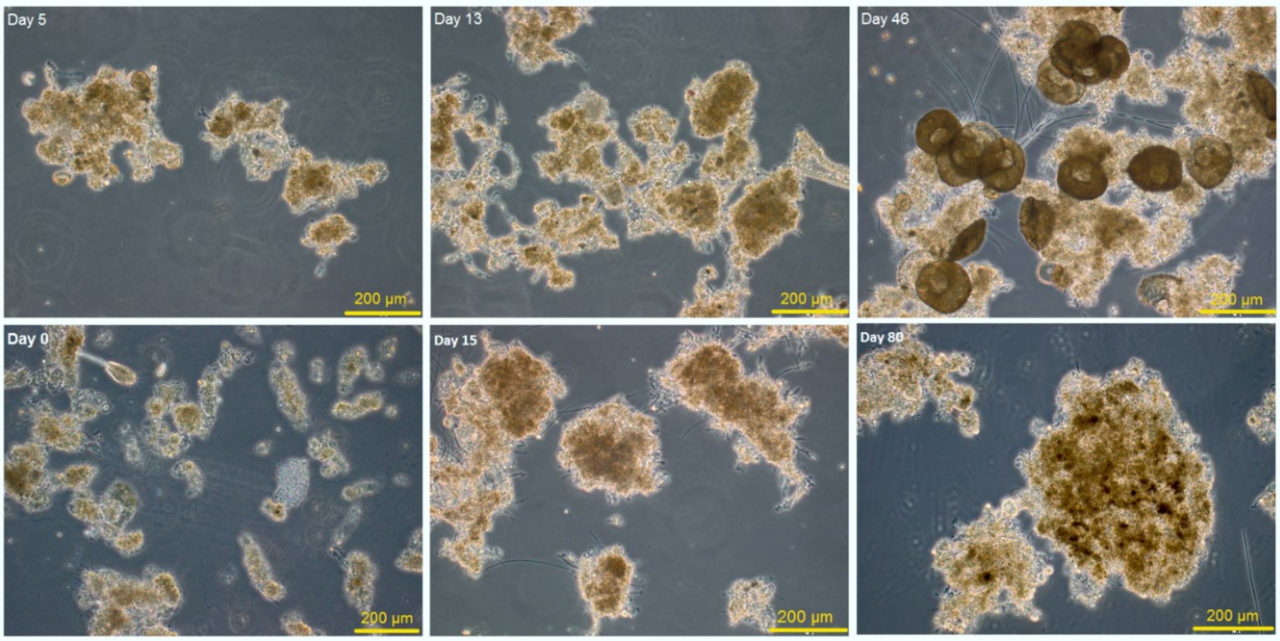


Figure 3.6: Microscopic images of granules (first line: CFP – phase I, second line: CFP – phase II)

Microscopic images show that in both CFP phases there were large amounts 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. Fig. 3.7 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.

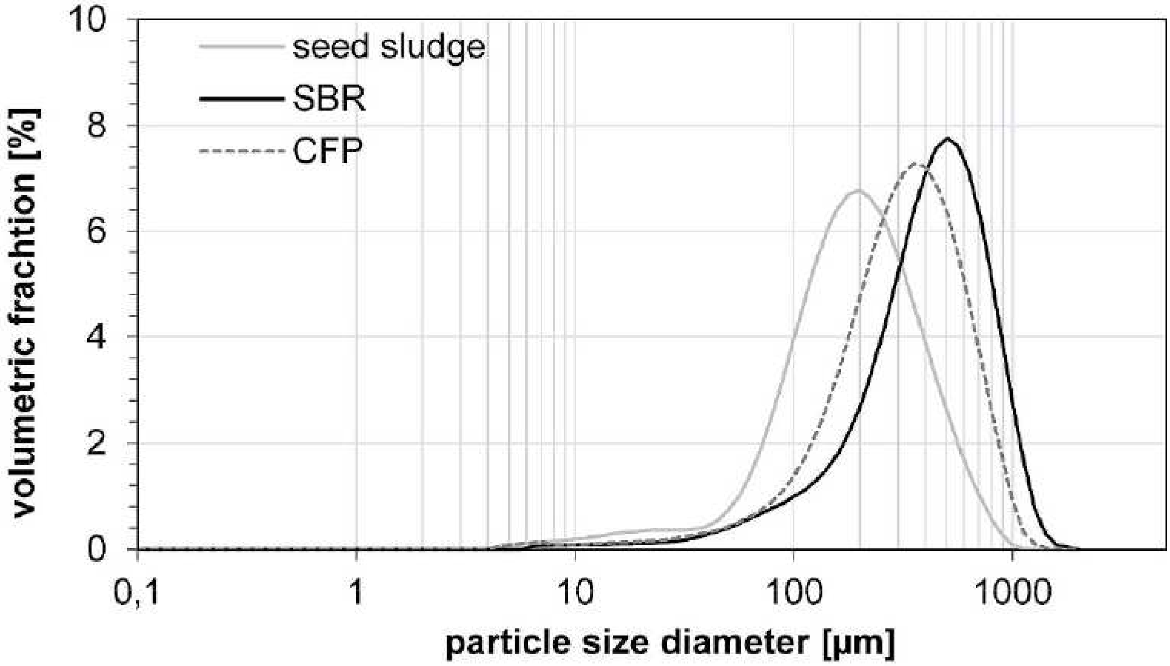


Figure 3.7: Particle size distribution of sludge probes (left: inoculum, middle: CFP, right: SBR)

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, which 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 an easy available 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.

3.5 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 extensively 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.

Fig. 3.8 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.

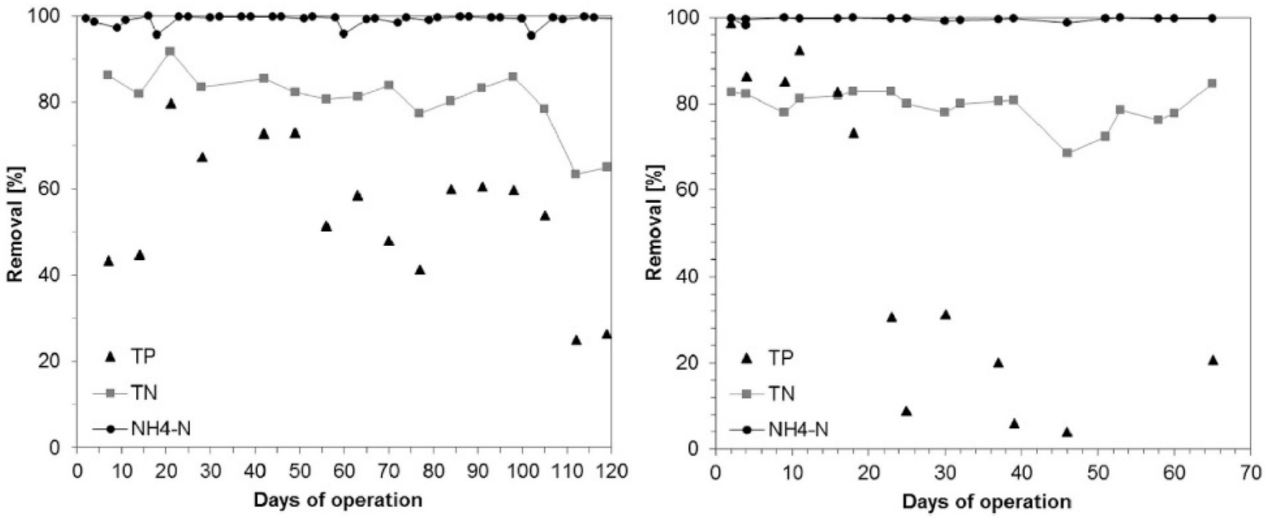


Figure 3.8: NH_4-N, TN and TP removal during phase I (left) and phase II (right)

3.6 Discussion

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 50–60 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 the liquid phase above the sludge bed. However, the occurrence of high NO_x-N concentrations can contract the anaerobic feeding and should be considered carefully. 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} ratios 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 realised 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 surface 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 flocculent 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 and 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.

3.7 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.

3.8 Acknowledgements

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Chapter 4

Anaerobic biodegradation and dewaterability of aerobic granular sludge

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4.1 Abstract

BACKGROUND: Although a growing number of full-scale wastewater treatment plants have already been constructed and operated with aerobic granular sludge (AGS), only limited information is available about further post-treatment, in particular about sludge stabilization and dewaterability. The aim of the present study was to investigate the biodegradation and methane yield of AGS by the use of anaerobic laboratory-scale reactors operated under mesophilic conditions and hydraulic retention times of 25 and 40 days.

RESULTS: The methane yield of AGS was ca 260 ml $gVSS^{-1}$ (volatile suspended solids) and thus slightly increased compared to that of suspended activated sludge (SAS; 240 ml $gVSS^{-1}$). A clear difference between the methane yield was found for separated pure granules (500 μm), which was ca 50% higher compared to that for SAS. VSS removal of AGS during anaerobic degradation was ca 52%. Dewaterability of AGS after anaerobic digestion was slightly lower compared to SAS. Extracellular polymeric substance (EPS) extraction and fluorescence analysis showed tryptophan contents which were almost twice as high compared to the EPS extracted from SAS.

CONCLUSIONS: Overall, the anaerobic digestion of AGS was found to be a suitable stabilization strategy with the benefit of recovering energy in the form of methane. Further tests are needed to validate the decreased dewatering behaviour with full-scale applications. The presented approach for tryptophan measurement allows the transfer of qualitative results from a fluorescence analysis into quantitative values and could be further adapted for identifying relevant EPS constituents.

4.2 Introduction

Aerobic granular sludge (AGS) is an upcoming wastewater treatment technology, which is increasingly applied in full-scale wastewater treatment plants (WWTPs). The compact and dense sludge structure offers excellent settling properties and the possibility for enhancing the activated sludge concentration and thus the biological treatment capacity. Aerobic granules are characterized by the formation of zones with different substrate and dissolved oxygen levels, which allow biological phosphate and simultaneous nitrogen removal. A rising number of full-scale sequencing batch reactors (SBRs) are operated using the commercial Nereda process, which is reported to be more cost-efficient compared to the conventional wastewater treatment technology with an overall lower energy consumption (Giesen and Thompson 2013). Although numerous studies have focused on the process stability and treatment performance of AGS cultivated under laboratory-scale as well as under full-scale conditions (de Kreuk et al. 2005b, Li et al. 2008, Bassin et al. 2012b, Lochmatter et al. 2013, Thwaites et al. 2017), information about the impacts on advanced sludge stabilization is limited.

During biological wastewater treatment, biomass growth leads to a continuous surplus sludge generation. For suspended activated sludge (SAS), the surplus sludge production depends on the sludge retention time (SRT) and is normally between 0.5 to 1.0 gTSS $gBSB_5^{-1}$. Muda et al. (2011) reported a biomass yield for aerobic granules in the range of 0.2 to 0.4 gTSS $gCOD^{-1}$, which is lower compared to that for suspended sludge. Ni et al. (2008) explained the lower sludge production of AGS by the fact that heterotrophs grow partially on internal stored polyhydroxybutyrate which leads to an overall lower grow rate. One further reason for the lower surplus sludge generation is an overall longer SRT for the granules. However, the generated surplus sludge has to be removed from the biological system constantly. Almost all larger WWTPs apply an aerobic (external or simultaneous) or anaerobic sludge stabilization, which primarily serves to decrease the organic compounds of the biomass. Sludge stabilization with an enhanced degradation of organic compounds improves the dewaterability of the sewage sludge and finally reduces the polymer demand and disposal costs (Kopp 2001). Most large WWTPs apply an anaerobic stabilization process using digesters, where the organic sludge compounds are converted into carbon dioxide (CO_2) and energy-rich methane (CH_4). The benefits of this anaerobic stabilization strategy compared to aerobic stabilization are an overall reduced energy demand and a smaller required aerobic tank volume. A subsequent water separation step by centrifuges, screw presses or chamber filter presses is necessary to reduce the volume of the digested sludge in order to ensure low disposal costs.

For economic reasons, the biodegradation and dewaterability of AGS should be considered in detail, especially since the structure and composition of the aerobic granules are quite different compared to SAS. Moreover, characterization of AGS has found increased content of extracellular polymeric substance (EPS)(Adav and Lee 2008b, Li et al. 2014, McSwain et al. 2005). EPS is a gel-like hydrated secretion which is produced by microorganisms themselves (Wingender et al. 1999) and consists mainly of proteins, polysaccharides, glycoproteins, nucleic acids, and phospholipids (Nielsen and Jahn 1999). The function of the EPS to protect the biomass against external environmental influences and to enhance the stability of microbial aggregates can adversely affect the degradability and dewaterability. Wang et al. (2005) and Wang et al. (2007b) investigated the biodegradability of EPS extracted from AGS and found that the EPS from the outer layer was not biodegradable in contrast to EPS found in the inner layer. The biodegradability is thereby linked to the composition of the EPS. Especially, the presence of multivalent ions and crosslinks was found to reduce the biodegradability. Leenen (1996) showed that Ba-Ca-EPS is not biodegradable, whereas Sawabe et al. (1995) reported a simple degradability of EPS with Na^{+} compounds.

Further studies focused on the correlations between the EPS content and the dewaterability of activated sludge. For example, Jin et al. (2004) quantified the filterability of activated sludge samples by a capillary suction time test and compared the results with the amount of extracted EPS. Those authors found that a high amount of total extracted EPS was associated with low capillary suction time, indicating a better filterability. Similar results were published by Mikkelsen et al. (2002) while the EPS improved the floc stability and filterability, the cake solid content decreased with higher EPS content. Moreover, Kopp et al. (1998) reported that the EPS content affects the polymer demand during dewatering. Skinner et al. (2015) investigated the dewaterability of digested sludge samples from different WWTPs and identified a strong correlation between the volatile suspended solids (VSS) and the final cake solid content. Cetin et al. (2004) found higher cake solid concentrations with lower EPS protein. Regarding that fact, EPS proteins seem to be an important parameter for the dewatering behaviour and water holding capacity.

Although a number of studies have investigated the dewaterability of digested SAS, limited comparative information exists for aerobic granules. Lehmann and Kasper (2017) reported a lower total suspended solids (TSS) concentration after thickening an AGS compared to a conventional sludge using the same polymer dosage. Here again, the EPS proteins were named as a potential reason due to their increased water binding capacity. In contrast to these results, the polymer demand to thicken the AGS of another WWTP operating the Nereda process was only half that for a conventional activated sludge (Lehmann and Kasper 2017). These contradictory results confirm that more investigations are needed to predict the dewaterability of AGS. Some further studies reported the methane production and biodegradation of AGS. For example, Val del Rio et al. (2011) investigated the anaerobic digestibility of AGS samples, which were cultivated using pig manure and synthetic wastewater. Those authors found a specific methane production for the granules grown on synthetic wastewater with 243 ml $gVSS^{-1}$ and 170 ml $gCOD^{-1}$ as well as a VSS reduction of 49%. Thermal pre-treatment was found to slightly enhance the biodegradation. Hogendoorn (2013) investigated the VSS removal of AGS sludge from a Nereda plant and reported a VSS degradation of ca 42.5% with hydraulic retention times of 12 and 20 days. Palmeiro-Sánchez et al. (2013) compared the anaerobic biodegradation of AGS and flocculent sludge under saline conditions and found a higher degradation of AGS with 32% compared to the flocculent sludge with 27%. The overall reduced biodegradation of that study was explained by an increased salt concentration in the treated wastewater.

Since only a few publications are so far available concerning the anaerobic stabilization of AGS, the purpose of the study reported here was to characterize the biodegradation and dewatering behaviour of AGS compared to SAS. Laboratory-scale tests were applied to investigate VSS and COD (chemical oxygen demand) removal as well as the specific methane production. Moreover, the digested sludge samples were centrifuged and compared in terms of their water separation. The outcomes of the study were used to quantify the AGS post-treatment in terms of anaerobic biodegradation and dewatering efficiency compared to conventional SAS. EPS was extracted and subsequently analysed via fluorescence spectroscopy to quantify the tryptophan content. The decision to investigate the tryptophan content was driven by the fact that tryptophan is a typical EPS protein[1], which can be easily detected using fluorescence spectroscopy.

4.3 Material and Methods

4.3.1 SBRs (AGS)

Laboratory-scale SBRs with a reactor volume of 6–8 L were operated over a period of 36 months to investigate various operational strategies. Cycle times were in the range 3–6 h with an anaerobic plug-flow feed of 60–90 min. Settling times were varied between 1 and 10 min. The biomass was mainly fed with sewage from a municipal WWTP. However, there were some tests with synthetic wastewater containing $C_{12}H_{22}O_{11}$, $C_6H_8O_7$, CH_4N_2O and K_2HPO_4 in a similar composition to that of municipal sewage. After a start-up period of ca 28 days, the granules were removed from the SBR to control the SRT and afterwards used for anaerobic tests. Further information about the SBR operation can be found in Jahn et al. (2019).

4.3.2 Anaerobic digestion

Five identical flasks with a volume of 525 mL were operated to characterize the anaerobic digestion of AGS under semi-continuous feeding conditions. The feed was carried out on 5 of 7 days, where 25 mL of input sludge was added per day. Due to the low feeding quantity, the digested sludge was removed only once a week (125 mL) and analysed for the parameters COD, TSS and VSS. Hydraulic retention time in the digesters was on average 25 days. Since the degradation also depends on the reactor operation, the temperature was always adjusted to 37°C using a thermostatic bath. The produced biogas was collected in gas-tight cylinders with gas capture reset once a week. Methane content in the biogas was measured with a gas analyser (GFM series). COD balances were calculated to control the analysed parameters and the measured methane gas.

Table 4.1 summarizes the mean composition of the input sludges and the experimental settings of the anaerobic reactors. During the reference run, the anaerobic reactor was fed with thickened SAS from a municipal WWTP. During runs I to IV, the anaerobic reactors received thickened AGS from the laboratory-scale SBR. The dosed granules during the first and second runs were cultivated with the same municipal sewage as the reference activated sludge. However, the third run was carried out with granules, which grew on synthetic wastewater. Since the biodegradation correlates with the SRT, the anaerobic tests were performed for granules operated under different SRTs. AGS during the fourth run came from a reactor operated with a SRT above 40 days, while the granules for runs I to III were cultivated under a SRT of 25 days.

All feed sludges (SAS and AGS) were thickened via centrifuge to ca 4.0 to 5.0% TSS before feeding. During the experiments the OLR of the anaerobic reactors were in the range 0.7-0.9 gCOD L^{-1} d^{-1}. AGS samples offered an overall increased VSS/TSS ratio, which can be explained by a lack of precipitation agents added to the reactor. Biological phosphate removal was the highest during runs I and II. During these tests, slightly lower VSS/TSS ratios were observed compared to runs III and IV. The increased inorganic compounds were probably related

[1] amino acid

to mineralic precipitations inside the granules. Earlier studies assume that increased phosphorus removal can cause apatite precipitation inside the granules, especially under anaerobic conditions and phosphate release (de Kreuk et al. 2005a). In contrast, a reduced biological phosphate removal and a lower VSS/TSS ratio were found during run III and the use of a synthetic medium.

Table 4.1: Composition of the feed sludge and settings of the anaerobic reactors [1) gCOD L^{-1} d^{-1}]

Run	Feed (origin)	VSS/TSS	COD/VSS	OLR[1)]	WW (SBR)	SRT (feed)
Ref	AS (WWTP)	0.73	1.48	0.93	Municipal	15 d
I	AGS (SBR)	0.78	1.44	0.68	Municipal	25 d
II	AGS (SBR)	0.83	1.48	0.96	Municipal	25 d
III	AGS (SBR)	0.90	1.36	0.89	Synthetic	25 d
IV	AGS (SBR)	0.90	1.37	0.76	Municipal	$>$ 40 d

In the frame of the tests, the particle size distributions of the feed sludges were measured with a Malvern Matersizer 2000 (Table 4.2). Aerobic granules normally coexist within a mixture of sludge flocs; thus the applied measurement analyses all particle sizes including flocs and granules. The smallest particle sizes were found for the SAS with 50% being smaller than 235 μm. During the second run, ca 50% of the particles reached a size above 550 μm. The largest particles were found for the AGS investigated during the third run, for which about 90% of the particles were larger than 453 μm. This observation can probably be attributed to the synthetic feed and a more compact and regular growth of the granules. The smallest diameters of the aerobic granules were observed during the fourth run, for which 50% of the particles were smaller than 366 μm. The particles size distribution provides an indication of the degree of granulation of the sludge. Pronk et al. (2015b) reported for a full-scale WWTP with AGS that ca 80% of the granules were larger than 0.2 mm. Our measurements confirm similar granulation grades, since the fraction of flocs ($<$200 μm) was only between 4.3 and 16.5%. The highest granulation grade of 95.7% was found for the granules cultivated under synthetic wastewater.

Table 4.2: Particle size distribution and volumetric equivalence diameter[1)] of AGS and SAS

Run	Input Sludge	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	diameter[1)] (μm)	Granulation grade (%)
Ref.	SAS (WWTP)	83	235	571	287	-
I	AGS (SBR)	143	548	1,255	630	83.5
III	AGS (SBR)	453	854	1,451	899	95.7
IV	AGS (SBR)	166	366	693	400	84.7

4.3.3 Anaerobic batch tests

Anaerobic batch tests were performed to differentiate the methane yield of the AGS as well as from the separated sludge fractions (large granules and sludge flocs). This method is characterised by a one-time feed, while the gas production is subsequently measured over a period of 21 days. Glass flasks with a volume of 525 mL were placed in a temperature-controlled (37°C) water bath. Gas was collected in graduated tubes. Overall, there were two measurements with AGS as a mixture of sludge flocs and granules as well as two tests with the sieved fractions. For this purpose, AGS from a SBR was separated by a sieve with a mesh size of 500 μm and then divided into flocculent and granulated fractions. For the dosage of the input sludge, a recommended ratio of maximum 50% to the VSS load of the seed sludge was taken into account (VDI 4630, 2016). Table 4.3 presents the mean composition of the samples investigated with the batch tests as well as the SV_{10}/SV_{30} ratio before thickening.

Table 4.3: Composition of the feed sludge and settings of the anaerobic reactors

Run	Feed (origin)	VSS/TSS	COD/VSS	SV_{10}/SV_{30}
I	sludge flocs ($<$ 500 μm)	0.87	1.11	1.39
II	granules($>$ 500 μm)	0.86	1.24	1.17
III	AGS (mixed)	0.86	1.27	1.10
IV	AGS (mixed)	0.86	1.32	1.13

4.3.4 Dewaterability tests

The quantification of the full-scale dewatering results with laboratory-scale tests is very challenging, since several parameters, like the polymer dosage and the operation of the dewatering aggregate, affect the results significantly. Moreover, there are many dewatering aggregates used at WWTPs, like screw press, decanter or chamber filter press, which achieve different TSS in the dewatered sludge. Since the quantification of TSS is challenging for the different aggregates, the study focused on given qualitative information on the dewaterability of AGS compared to conventional SAS. The applied method used in this study was based on von der Emde et al. (1982), whereby those author centrifuged primary and surplus sludge under a constant centrifugation time and z values between 20 and 2,000 g. The procedure allows calculating approximately the solid content of the dewatered sludge under full-scale conditions. To comply with the method described of von der Emde et al. (1982), a laboratory centrifuge (Sigma 3K30) was used to investigate the sludge cake solids of the digested probes. A sample volume of 30 mL was centrifuged for 10 min at 20,000 g (14,000 rpm), whereby a higher z value was chosen to get an almost complete separation of the loosely bound water. After centrifugation, the centrate was removed from the centrifuge tube and analysed for NH_4-N and PO_4-P. TSS and VSS content in the remaining residue was determined by drying the probe at 105°C and 505°C.

4.3.5 EPS extraction

Different kinds of EPS are described in literature as soluble EPS, loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) (Guo et al. 2016). EPS outside cells can be subdivided into bound EPS (sheaths, capsules, polymers, gels) and soluble EPS (soluble macromolecules, colloids, slimes) (Nielsen and Jahn 1999, Laspidou and Rittmann 2002). The structure of the bound EPS can be illustrated as a two-layer model (Nielsen and Jahn 1999). The inner layer consists of TB-EPS, which is in contact with the cell surface. The outer area comprises a loosely bound dispersible EPS layer without clear delimitation. The proportion of LB-EPS in microbial aggregates is reported to be smaller than that of TB-EPS (Sheng et al. 2006, Li and Yang 2007). Different methods are applied for the extraction of the different EPS types. A detailed list of such extraction methods can be found in More et al. (2014).

For this study, a method published by Adav et al. (2008b) and Liu and Fang (2002) was used to extract the bound EPS from SAS and AGS samples. Sludge samples were washed three times with distilled water before extraction. A triple determination was prepared for each probe with a sample volume of 10 mL. For a sufficient breakup of the granule structure, the samples were pre-treated in an ultrasonic bath for 30 min. Subsequently, 0.06 mL of formamide was dosed to stabilize the cell walls and to avoid cell lysis and contamination through inner cell content. During a reaction time of 1 h, the samples were stored at 4°C. After the reaction time, 4 mL of NaOH (1 N) was dosed to increase the pH and to subsequently dissociate acidic groups in EPS (Adav and Lee 2008a). The samples were stored again for 3 h at 4°C. Afterwards, the samples were centrifuged for 10 min at 10,000 g. The supernatant was separated by a 0.2 μm filter, whereby the filtrate contained the dissolved components of the bound EPS. Beside the extraction of the bound EPS, the soluble EPS was separated from the biomass by filtration through a 1 μm glass microfibre filter.

Further EPS analysis is based on three-dimensional fluorescence spectroscopy. The recording of the fluorescence spectrum makes it possible to obtain qualitative information on the distribution of different substance groups. This technique was also used in earlier studies to determine characteristic EPS compounds (Adav and Lee 2008a). Especially aromatic proteins can be detected through fluorescence analysis. Zeng et al. (2016) reported by means of measured fluorescence intensities that tryptophan-like substances are the major group of fluorescence substances in EPS. Since tryptophan is a characteristic protein of EPS, the general idea was that higher tryptophan concentrations correlate with increased EPS proteins. Therefore, the extracted EPS was analysed using an Aqualog fluorescence absorption spectrometer (Horiba), which simultaneously records the absorption and fluorescence spectra as excitation (Ex) emission (Em) matrix (EEM). Figure 4.1 shows the assignment of areas in an EEM for detectable substance groups. Tryptophan-like substances are visible at 280/350–360 nm (Ex/Em), while tyrosine-like substances appear in a region of 270–280/305–310 nm (Ex/Em) (Fox et al. 2017). The investigation of this study focused on the tryptophan content in the extracted bound EPS of AGS and SAS samples. For the reason that the EEM gives only qualitative values in the form of intensity peaks, the samples were spiked with known tryptophan concentrations and afterwards measured using the fluorescence spectrometer. This approach allows the transfer of qualitative results into quantitative values.

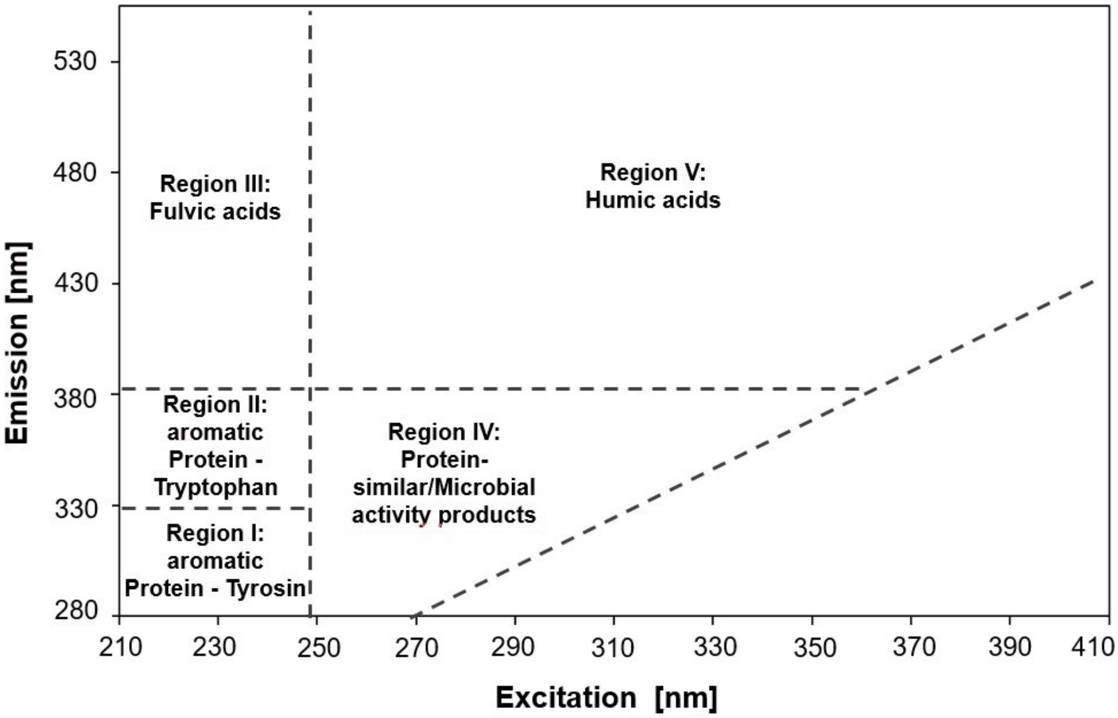

Figure 4.1: Assignment of regions of different substance groups in EEM (Chen et al. 2003, Ishii and Boyer 2012)

4.4 Results and Discussion

4.4.1 EPS Protein

This section summarizes the results of the EPS examination. For the reason that the EEM gives only qualitative values in the form of peaks, the samples were enhanced with known tryptophan concentrations to determine the increase in the peak intensity. In a first step, tryptophan was spiked to deionat in different concentrations. The resulting fluorescence peaks in the region of tryptophan (Ex/Em: 273/347 nm) were correlated to the added tryptophan concentrations, which resulted in a linear correlation as shown in Fig. 4.2 (left, black dots). This validation indicates that it is possible to recalculate from peak intensity to substance concentration by having the same background matrix.

The tryptophan concentration in the extracted EPS was analysed for all probes with the described spiking method. Figure 4.3 shows two examples of fluorescence peaks, which resulted from added tryptophan concentration (+0.5, +1.0, +1.5, +2.0 mg L^{-1}). The examples relate to the extracted EPS from a SAS (left) and AGS (right). The peaks in the EEM appeared in the characteristic area of tryptophan with 273/347 nm (Ex/Em). A clear link was found between the measured EEM peaks and the increased tryptophan concentrations, while the double determination confirms a coefficient of determination of ca 0.99. Since bacteria produce the EPS, the concentrations were related to the VSS content to obtain comparable results. The calculated tryptophan concentrations for these samples were 1.2 mg $gVSS^{-1}$ (Fig. 4.3, left: SAS) and 2.5 mg $gVSS^{-1}$ (Fig. 2, right: AGS).

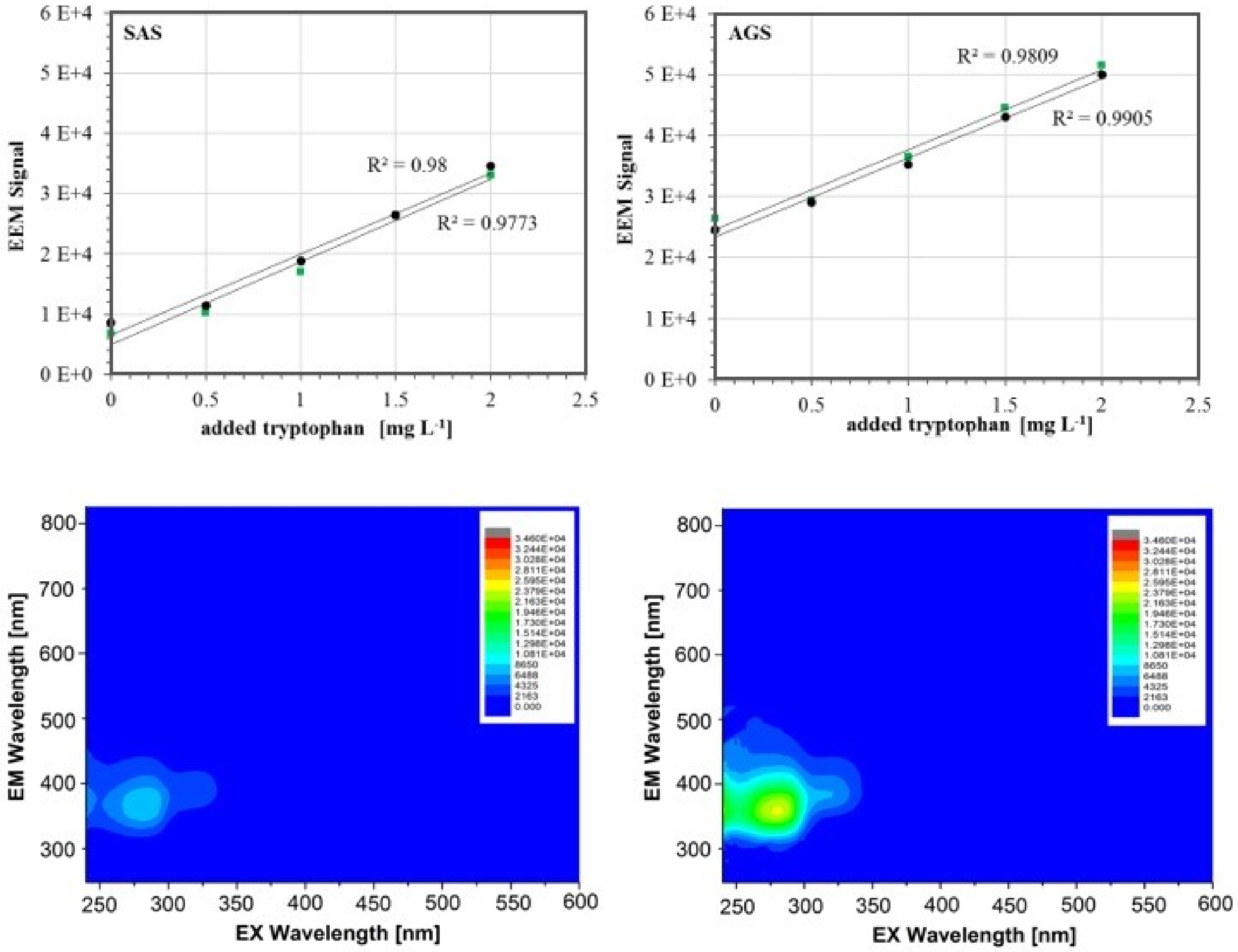

Figure 4.2: EEM Signal (273/347 nm) with added tryptophan concentrations for the extracted EPS from an SAS and AGS (top) and EEM of the untreated samples (bottom)

Figure 4.3 shows the analysed tryptophan concentrations of the soluble and bound EPS. AGS samples were taken from a laboratory-scale SBR operated for aerobic granulation; the SAS samples were collected from a pilot plant operated in continuous flow with sludge flocs. The left-hand panel of Fig. 4.3 shows the tryptophan concentrations of the soluble EPS of samples were collected over a two-month period. The results show that the tryptophan concentrations were always higher in the soluble EPS extracted from the granular sludge probes compared to the EPS of the SAS. In a further step, the tryptophan concentrations were related again to the organic solid contents. The results for the EPS from the AGS samples were found in a range between 30 and 75 μgTry gVSS^{-1} (mean 55 μgTry gVSS^{-1}). This result was approximately three times higher than for the EPS extracted from the SAS reaching on average 17 μgTry gVSS^{-1}.

Figure 4.3 (right) summarizes the results for the bound EPS extracted from AGS and suspended sludge. The results confirm a significantly increased tryptophan concentration in the AGS compared to SAS (data points 1, 5). Although all measurements result in a linear correlation, the fluorescent peaks were overall lower than for the validation with deionized water. This observation is probably due to a different background matrix (salts, ions) which is known to slightly affect the fluorescence signal.

The tryptophan concentration of the soluble EPS was ca 10% of that measured for the bound EPS. Guo et al. (2016) investigated the fraction of soluble EPS, LB-EPS and TB-EPS and found a ratio of 0.1:0.3:1. This ratio would explain the overall lower tryptophan concentration in the soluble EPS. All measurements reveal tryptophan concentrations in the extracted bound EPS from the SAS in a range of 1.2 to 1.9 mg $gVSS^{-1}$, while the tryptophan concentration in the bound EPS from the AGS ranged between 2.5 to 4.6 mg $gVSS^{-1}$. The total protein contents of the extracted EPS were measured by a modified TKN analysis according to DIN EN ISO 11732. Proteins in the EPS extracted from AGS ranged between 211 and 338 mg $gVSS^{-1}$, whereas proteins in suspended sludge EPS were ca 150 mg $gVSS^{-1}$. The amount of tryptophan was 1.2±0.12% of the measured protein content. The reported amount of proteins in EPS varies widely; while Dai et al. (2013) reported 3.5 mg g^{-1} in a municipal raw sludge, Zeng et al. (2016) measured proteins in a range between 35 and 68 mg $gVSS^{-1}$ and Adav et al. (2008b) found concentrations even up to 540 mgPN $gVSS^{-1}$ in AGS. Our outcomes are thus in a similar range compared to the literature values.

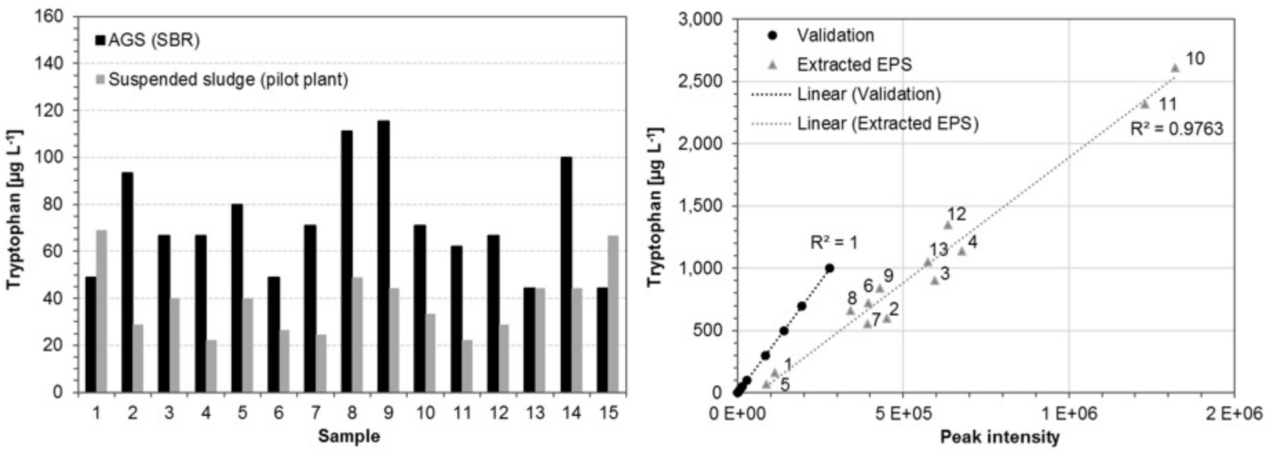

Figure 4.3: Tryptophan of soluble EPS (left), tryptophan of extracted bound EPS, validation (right)

4.4.2 Anaerobic digestion

Table 4.4 summarizes the VSS and COD removal as well as the specific methane production of the anaerobic tests with AGS and SAS. COD balances were satisfactory for all reactors with variances below 10%. The pH values of the reactors were within an optimal range of 6.7 to 7.5. For the reference test with SAS, the VSS and COD removal was calculated at 46 and 47%, which are slightly above the reported literature values of 30–40% (DWA 2014, Rosenwinkel et al. 2015). Biogas yield for waste activated sludge depends on the sludge age and is typically between 250 and 350 ml $gVSS^{-1}$ (Mennerich et al. 2001). AGS used for the first and second run was fed with the same municipal wastewater as the reference sludge, which allows a direct comparison of the results. In the digested sludge, the granules were no longer visible, which confirms that the compact structure was completely degraded. Methane production of the AGS was about 20 ml $gVSS^{-1}$ higher than for the reference run and thus in a range comparable to that for the suspended sludge samples. A slightly increased COD and VSS removal of ca 51% (runs II and III) was calculated for the AGS, which is in line with findings of Palmeiro-Sánchez et al. (2013), who also reported a slightly increased biodegradation of AGS. Since the formation of aerobic granules is associated with the implementation of an anaerobic feeding phase at the beginning of the SBR cycle, glycogen- and phosphate-accumulating organisms are enriched inside the biomass. The metabolism of these organisms is coupled to an increased formation of internal stored substrates (polyhydroxyalkanoates) and volatile fatty acids formation. Val del Rio et al. (2011) state that the degradation of polyhydroxyalkanoates and volatile fatty acids can contribute to the biodegradability and methane production. A further reason for the higher specific gas production of AGS can be seen in the increased EPS. The applied extraction method and fluorescence measurements revealed clear increased tryptophan and protein concentrations in the EPS of the AGS. An increased protein content in the EPS of AGS was also reported by Zhang et al. (2007). EPS in microbial aggregates has numerous sites for the adsorption of metals as well as organic substances such as aromatics, aliphatics and carbohydrates (Flemming and Leis 2002). Due to the high number of carboxyl and hydroxyl groups, the EPS has a very high binding capacity (Flemming and Leis 2002). A high absorption rate of EPS was also reported by Wei et al. (2015). It can be assumed that the higher specific methane production was caused by an increased uptake of easily degradable organic substances into the EPS.

A clear increased VSS and COD removal of about 60% was achieved within the third run, where the granules were cultivated using synthetic wastewater. The increased specific methane production is probably attributed to the sewage composition with an overall increased easily available carbon source. In contrast, a clear decreased removal was found for the fourth run. Here, VSS and COD removal reached only 35 and 37%. Moreover, the methane production was decreased to about 170 ml $gVSS^{-1}$. This observation can be explained by a longer SRT in the SBR. Mennerich et al. (2001) investigated the biogas production of activated sludge samples from WWTPs with different SRTs and showed a clearly declining gas production with longer SRT. Since the SRT of the reference sludge was only 15 days, it can be assumed that a higher SRT would result in a slightly lower methane yield and a greater difference between the methane potential calculated for SAS and AGS. According to a regression calculated by Mennerich et al. (2001) a lowering of methane yield by ca 10% would result from changing the SRT in the biological stage from 15 to 25 days.

Table 4.4: COD and VSS removal and specific methane yield during runs I to IV

Parameter	Run SRT Evaluation time	Ref 15 d 36 d	Run I 25 d 28 d	Run II 25 d 36 d	Run III 25 d 14 d	Run IV > 40 d 25 d
VSS removal	[%]	45.9±2.5	51.1±4.2	52.6±4.5	59.9±4.4	37.2±2.4
COD removal	[%]	47.5±2.5	50.9±4.2	52.4±4.5	59.6±4.4	35.2±2.4
Methane yield	[ml $gVSS_{IN}^{-1}$]	245±13	263±21	271±23	285±21	168±12
Methane yield	[ml $gCOD_{IN}^{-1}$]	166±9	178±15	183±16	209±15	123±8

4.4.3 Anaerobic batch tests

Figure 4.4 shows the cumulative curves of the gas production for the batch tests recorded for a period of 18 to 21 days. Since AGS always contains a fraction of flocs and granules, two batch tests were performed for AGS as complete mixture of flocs and granules. The samples were removed from a SBR operated under similar conditions (SRT of ca 25 days, same feed). The gas development appeared similar for both tests with a specific methane production of 250 and

260 ml gVSS^{-1}. These results are in line with the findings of the continuous anaerobic tests. In a further test, the granulated granulated sludge was separated into a flocculent fraction (R1) and granules with sizes above 500 μm (R2). The specific methane production of the pure granules was 294 ml gVSS^{-1} and thus clearly increased compared to the batch test with the flocculent fraction (206 ml gVSS^{-1}). Although there was no EPS extracted from these samples, the separated granules were covered by a shiny gel-like matrix which was characteristic of EPS.

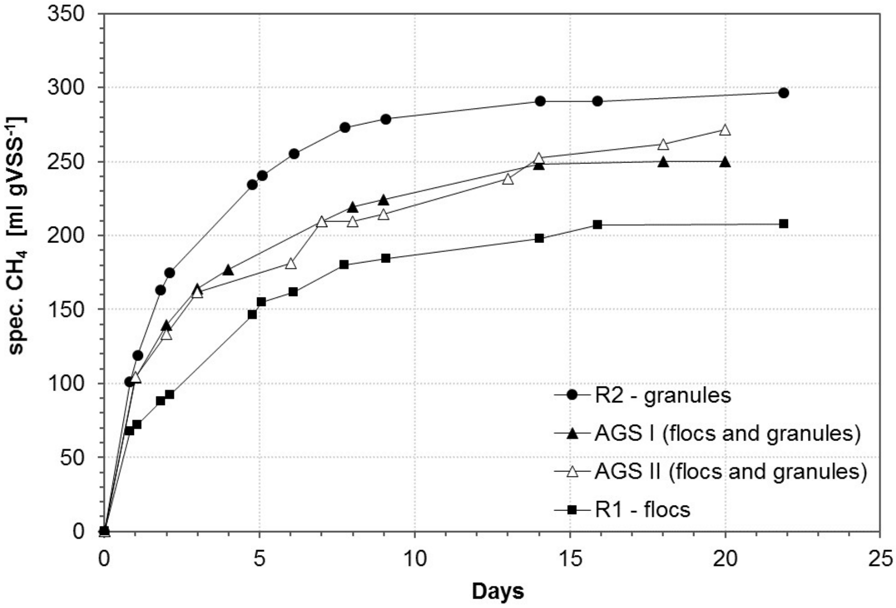

Figure 4.4: Sum curve of gas production for batch tests

4.4.4 Dewaterability

Figure 4.5 shows the results of the dewatering tests as TSS in the digested granules after centrifugation. A clear decreased dewatering behaviour was found during the start-up of phase IV with TSS decreasing from 16 to 9% within 35 days (Fig. 4.5, right). In the same time, a clear increased VSS fraction was observed for the digested sludge samples. Similar results of the dewatering behaviour were found for the digested reference sludge and the granules from the first run. The biomass from both runs was fed with the same municipal wastewater, which allowed a direct comparison of the dewatering results. The digested and dewatered SAS comprised a TSS of 9.6%, while the TSS for the AGS was only about 6.6%. During these tests, the organic fraction of the digested granules was significantly increased with 69.1% compared to the digested reference sludge (61.1%).

From the dewatering results, it can be concluded that the increased organic fraction caused a lower water separation. This is line with the results of Thomé-Kozmiensky (1998), who reported a lower dewaterability of sewage sludge with a higher ignition loss. Organic compounds have a higher water binding capacity, which hinders an advanced water separation. Moreover, the lower dewatering behaviour was probably a result of the increased PO_4-P concentrations in the AGS. Hougthon et al. (2002) reported that increased phosphate concentrations can negatively affect the dewatering results. Increased concentration of orthophosphate (PO_4-P) enhances the water binding capacity and finally the demand for polymer agents (Ortwein 2016). A steady increase from 13 to 186 mgPO_4-P L^{-1} in the centrate was found during the startup of run IV.

Furthermore, the centrate of the digested AGS of the first run was on average 396 mgPO_4-P L^{-1} and 1,645 mgNH_4-N L^{-1}, while the concentrations in the centrate of the flocculent sludge were significantly lower with 143 mgPO_4-P L^{-1} and 1,400 mgNH_4-N L^{-1}. Biological phosphate removal during the treatment process with AGS is responsible for increased phosphate uptake under aerobic conditions, while the stored phosphate is released during anaerobic conditions and the degradation of organic compounds. Additionally, the increased EPS can be named as a further potential reason for a decreased dewaterability. However, the EPS left after the anaerobic treatment was not analysed in this study. Further tests are recommended for quantifying the degradation of EPS under anaerobic conditions.

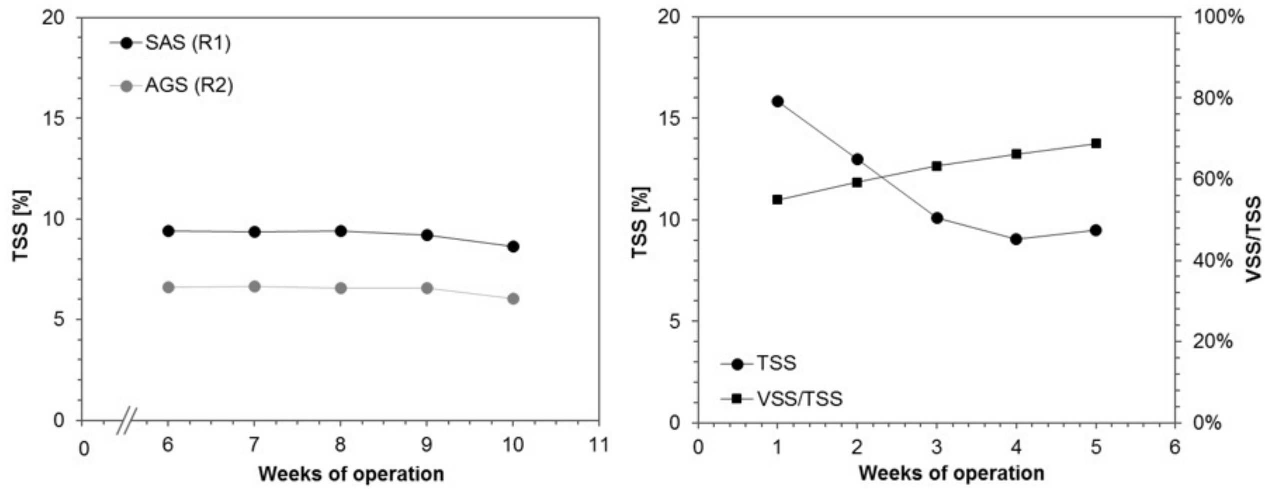

Figure 4.5: TSS in the residue (left: run I, after startup, right: during the startup of run IV)

The outcomes indicate that there are various parameters having an impact on the dewaterability of digested AGS. However, two prospects can be named in order to improve the dewaterability of digested sludge samples. For example, it can convenient to apply an increased hydraulic retention time in the digester in order to decrease the organic solids and EPS through further degradation. However, an extended hydraulic retention time for the digester operation can only be applied when there is enough digester volume available. Another possibility for improving the dewatering behaviour could be selective PO_4-P removal. Kopp (2017) and Bergmans et al. (2014) observed an improved sludge dewaterability after a targeted struvite formation. The conclusion from these studies was that a better mechanical dewatering can be expected with less dissolved PO_4-P in the sewage sludge. Further tests for the quantification of the dewaterability are recommended at this point with respect to the EPS and PO_4-P content of AGS.

4.5 Conclusion

Anaerobic degradation and methane production of AGS and SAS were investigated in this study using laboratory-scale reactors. The specific methane production of AGS cultivated with municipal wastewater was found to be ca 260 ml $gVSS^{-1}$ and was thus slightly increased compared to that of conventional activated sludge. However, it could be confirmed that SRT above 40 days led to a clearly decreased gas yield (-35%). A higher specific methane production was found for granules separated from a mixed granular sludge, whereby the higher methane yield was probably linked to an increased EPS content. Proteins and tryptophan were clearly increased in the extracted EPS of the granules. The use of fluorescence to analyse EPS allowed the transfer of qualitative results into quantitative data. This approach can be further adapted to investigate special substance groups in the extracted EPS. The dewatering test showed a slightly decreased dewatering behaviour of the digested granules, which was probably caused by an increased organic fraction due to an absence of chemical precipitation and an increased amount of PO_4-P. The dewatering results require further validation with full-scale aggregates under real operational conditions.

4.6 Acknowledgements

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Chapter 5

Nitrous oxide emissions from aerobic granular sludge

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5.1 Abstract

The emissions of climate-relevant nitrous oxides from wastewater treatment with aerobic granular sludge (AGS) are of special interest due to considerable structural as well as microbiological differences compared to flocculent sludge. Due to the compact and large structures, AGS is characterized by the formation of zones with different dissolved oxygen (DO) and substrate gradients, which allows simultaneous nitrification and denitrification (SND). N_2O emissions from AGS were investigated using lab-scale SBR fed with municipal wastewater. Special attention was paid to the effects of different organic loading rates (OLR) and aeration strategies. Emission factors (EF) were in a range of 0.54 to 4.8% (gN_2O/gNH_4-$N_{ox.}$) under constant aerobic conditions during the aerated phase and different OLR. Higher OLR and SND were found to increase the N_2O emissions. A comparative measurement of two similarly operated SBR with AGS showed that the reactor operated under constant aerobic conditions (DO of 2 mg L^{-1}) emitted more N_2O than the SBR with an alternating aeration strategy. TN removal was significantly higher with the alternating aeration since non-aerated periods lead to increased anoxic zones inside the granules. The constant aerobic operation was found to promote the accumulation of NO_2-N, which could explain the differences in the N_2O levels.

5.2 Introduction

Today's focus in wastewater treatment plant (WWTP) operation moves towards sustainable treatment and low greenhouse gas emissions. In this regard, special attention is paid to the N_2O formation during biological wastewater treatment. Relevant pathways for the N_2O formation in wastewater treatment are described by Wunderlin et al. (2012) as well as by Kampschreur et al. (2009). The most relevant mechanism for the N_2O production was reported with the autotrophic nitrification pathway. During nitrification, ammonium is oxidised to hydroxylamine and in a further step to nitrite. Hereby it is assumed that an incomplete hydroxylamine oxidation is responsible for the N_2O release. The rate of the incomplete hydroxylamine oxidase reaction increases when both NH_4-N and NO_2-N concentrations are elevated. Another pathway for the formation of N_2O is the so-called nitrifier denitrification. In the absence of oxygen, ammonium oxidising bacteria (AOB) can no longer transfer part of the electrons from nitrite to the oxygen and therefore not only produce NO_2- as end product, but also N_2O, NO and N_2. Furthermore, N_2O is a by-product of the denitrification process and produced during the conversion of nitrate to nitrogen. High N/COD ratios (COD: chemical oxygen demand) can promote the N_2O formation due to an incomplete denitrification. Moreover, since the N_2O reductase is sensitive to oxygen, micro-aerobic conditions (low DO) during denitrification can promote the N_2O formation too. The following equations describe the formation pathways through nitrification, nitrifier denitrification and heterotrophic denitrification. Further less relevant formation pathways for N_2O are found in literature as chemo-denitrification and dissimilatory nitrate-ammonification (Chalk and Smith 1983, Hu et al. 2011, Tallec et al. 2008).

Nitrification

$NH_3 + 2H^+ + 2e^- + O_2 \longrightarrow NH_2OH + H_2O$

$NH_2OH \longrightarrow HNO \longrightarrow NO \longrightarrow NO_2^-$

Nitrifier denitrification

$2NH_3 + 2O_2 \longrightarrow 2HNO_2 + 4[H]$

$NH_3 + O_2 + 2[H] \longrightarrow NH_2OH + H_2O$

$NH_2OH + HNO_2 \longrightarrow N_2O + 2H_2O$

Heterotrophic denitrification

$2NO_3^- \longrightarrow 2NO_2^- \longrightarrow 2NO \longrightarrow N_2O \longrightarrow N_2$

Although a high number of publications focus on the emissions of climate-relevant N_2O from conventional activated sludge systems, only a few studies relate to the emissions from AGS. Table 5.2 summarises N_2O emission factors (EF) of different AGS studies as well as measurements from conventional full scale WWTP with suspended activated sludge. The EF of these studies appear in a broad range, which is probably attributed to the different operational strategies. Quan et al. (2012) found increased EF under lower aeration rates and limited carbon availability. The authors claimed that elevated N_2O emissions might be due to structural reasons and an increased SND for aerobic granules with larger diameter. Lochmatter et al. (2013) investigated the nitrogen removal and N_2O emissions of AGS under different aeration strategies and COD loads (constant nitrogen load). The highest EF of 9.0% was measured under the lowest COD load and under alternating high/low DO. Lower N_2O emissions of 2.1 and 1.0% occurred at higher COD loads (C/N ratios). Van den Akker (2015) determined N_2O emissions of an AGS system operated with high saline municipal wastewater in a range of 2.3 to 6.8%. The lowest N_2O emissions were measured when nitrite and ammonium were consumed and the DO in the bulk liquid was above 1.0 mg L^{-1} (van den Akker et al. 2015). Similar findings were made by Peng et al. (2018), whereby decreased DO levels promoted SND and subsequently increased N_2O emissions. However, low DO levels are required to achieve maximum SND, indicating that there has to be a compromise between nitrogen removal and N_2O emissions.

Parravicini et al. (2015) as well as Foley et al. (2010) investigated the N_2O emissions of full-scale conventional WWTP and found EF up to approx. 1.5%. Table 5.2 demonstrates that the EF of AGS systems are overall higher compared with full-scale measurements on conventional WWTP. It can be supposed that results from lab-scale cannot be directly compared with full-scale measurements, which is probably due to different reactor geometry and aeration rates. Since AGS is dominated by larger sizes (>200 μm), the compact structure promotes the formation of zones with different DO and substrate levels. In contrast to suspended activated sludge, where the bulk liquid concentrations of the substrate and DO are similar in all zones, AGS is characterised by gradients, which lead to the formation of intermediates in different layers.

Table 5.1: Literature review on N_2O emissions of AGS and conventional WWTP with suspended activated sludge

Reference	EF [%]	Referring to	Scale
Aerobic granular sludge			
Lochmatter et al. (2013)	1.0-9.0	TN_{IN}	lab-scale
van den Akker et al. (2015)	2.3-6.8	TN_{IN}	pilot-scale
Quan et al. (2012)	2.2-8.2	TN_{IN}	lab-scale
Gao et al. (2016)	2.72 ± 0.52	NH_4-$N_{ox.}$	lab-scale
Zhang et al. (2015)	7.0-21.9	$TN_{rem.}$	lab-scale
Suspended activated sludge			
Parravicini et al. (2015)	0.002-1.52	TN_{IN}	full-scale
Foley et al. (2010)	0.006-0.253	$TN_{rem.}$	full-scale

In regard to the structural differences of AGS compared with conventional sludge flocs and the formation of zones with different DO, several N_2O formation pathways can coexist within the biomass (Lochmatter et al. 2013) and illustrate that the emissions of climate-relevant N_2O out of AGS deserve further research. The complexity of the different formation pathways for N_2O during biological wastewater treatment indicates the difficulty to quantify the impact of operational settings, especially the aeration strategy. Further research is needed in order to understand the different triggers for N_2O formation, which allows setting arrangements for declining emissions from biological wastewater treatment. This study focused on N_2O emissions in correlation to different aeration strategies and OLR of AGS operated with municipal wastewater.

5.3 Methods

5.3.1 SBR setup

AGS was cultivated in two lab-scale SBR with a volume of 6 to 8 L. After 261 days (data points 1 to 6), the reactors were restarted and operated again for 240 days (data points 7 and 8). The first experimental phase includes measurements under different OLR, while during the second period different aeration strategies were investigated. The operation comprised an anaerobic plug-flow feed of 60 to 90 min. Cycles lasted 4 to 6 h with settling times between 1 and 10 min. The exchange ratios were 30 to 40%. Temperatures and pH values were monitored daily as well as sludge volume measurements. SBR were fed with sewage from a municipal WWTP. Table 1 shows the relevant concentrations of the wastewater during the measurements. COD was mostly between 230 and 530 mg L^{-1} with TN concentrations between 28 and 50 mg L^{-1}.

N/COD ratios were in a range of 0.08 to 0.18. The samples for the sequencing were collected on day 501 (second period).

The different settling properties were attributed to a slightly diverse reactor operation. The applied settling time affected the minimum settling velocities ($v_{s,min}$), which were in a range of 0.7 to 3.2 m h^{-1}. The minimal settling velocity is described as quotient of the drawn water level and the settling time and determines the slow settable substances that are discharged with the effluent. Samples 4 and 5 had a high SV_{10}/SV_{30} ratio compared with the other measurements, which were attributed to a low $v_{s,min}$ of approx. 0.7 m h^{-1} and a reduced washout of flocculent sludge. Although the biomass was occasionally dominated by flocculent structures, there were large granules with sizes above 200 μm present in the SBR during all measurements.

Table 5.2: Wastewater composition, cycle operation and settling properties during different measurements

Data point		1	2	3	4	5	6	7	8
SBR		2	2	2	1	1	1	1	2
Day		80	101	110	198	205	260	501	501
COD	[mg L^{-1}]	448	283	309	244	470	530	228	228
TN	[mg L^{-1}]	45.6	50.2	39.3	28.0	48.0	42.8	34.7	34.7
TP	[mg L^{-1}]	9.2	10.6	11.4	15.3	17.7	11.2	5.8	5.8
Cycle time	[min]	240	240	240	240	240	240	360	360
Anaerobic	[min]	60	60	60	90	90	90	90	90
Settling	[min]	2	3	3	10	10	2	10	10
decant	[min]	5	5	5	5	5	5	5	5
SVI	[ml g^{-1}]	69.2	78.1	89.6	64.3	75.9	64.1	47.8	70.3
SV_{10}/SV_{30}	[-]	1.10	1.07	1.26	1.35	1.84	1.66	1.17	1.17
$v_{s,min}$	[m h^{-1}]	3.21	2.89	2.28	0.69	0.77	2.82	0.84	0.84

During the off-gas sampling, bulk liquid samples were taken in regular intervals for analysing NH_4-N, NO_2-N, NO_3-N according to DIN 38406. The formation of anoxic zones within the biomass depends on the granules sizes and the DO level in the bulk liquid. Smaller sizes limit the formation of anoxic zones and thus the ability for SND. COD and mixed liquor suspended solids were measured according to DIN 38409. Based on the distribution of the nitrogen fractions, the simultaneous nitrogen removal was calculated according to the following equation (Zhang et al. 2015).

$$SND = \frac{NH_4^{+}{}_{oxidized} - NO_x^{-}{}_{accumulated}}{NH_4^{+}{}_{oxidized}} \text{ x } 100\%$$

Microbial community was investigated by Illumina MiSeq sequencing Nextera XT library, 341F – 802R primers. It should be noted that *Nitrobacter* may well be present, however *Nitrospira* is not always detected by Illumina MiSeq sequencing.

5.3.2 Gasprobes

The SBR were completely covered during the aerated phase, while sampling the exhaust air. Gas chromatography-mass spectrometry (GC-MS) was used for the N_2O analysis of the gas samples. Exhaust air samples from the SBR were injected into 20 ml sealed and evacuated headspace gas bottles by means of a gas-tight glass syringe. The sample amount was 10 ml. For the GC-MS, a Porapak column (ID 0.32 mm and length about 30 m) was used. The headspace gas cylinders filled with the exhaust air samples were analysed immediately or stored in a thermostat at 20°C until analysis to avoid temperature fluctuations. Gas samples with N_2O contents between 0.5 and 40 ppmv were used for the validation of the GC-MS measurement for N_2O according to DIN 32645. Moreover, the validation parameters including detection and determination limit as well as the standard deviation were determined. N_2O samples were analysed to check the zero point. According to DIN 32645, a detection limit (DL1) of 0.5 ppmv, a determination limit (DL2) of 1.0 ppmv and the standard deviation of 0.56 ppmv are calculated. These values are even lower (DL1 = 0.25 ppmv. DL2 = 0.50 ppmv) when the usual "signal to noise" ratio (S/N) in chromatography is considered (Kromidas 2011).

For the evaluations, the measured ppmv in the exhaust air was converted to the concentration of N_2O as gram per liter air. To compare the results, the gas volume was adjusted to standard temperature. During the sampling, the aeration intervals were recorded to calculate the N_2O load emitted from the reactor per cycle. For this calculation, the required aeration rate was checked in preparation of the measurements. EF were related to the oxidised NH_4-N according to the following equation.

$$\mathrm{EF} = \frac{\sum(\mathrm{Q_{air}} \cdot \mathrm{c_{N2O,air}})}{\mathrm{Q_{Feed}} \cdot \mathrm{NH_4{}^+{}_{oxidized}}} \quad [\frac{\mathrm{gN_2O}}{\mathrm{gNH_4\text{-}N}}]$$

5.3.3 Respiration tests

Oxygen utilisation rates for carbon and nitrogen (OUC, OUN) were determined by respiration tests. OUC was determined without the use of ATU (allylthiourea, nitrifier inhibitor) since the sludge was afterwards returned to the reactor. OUC was measured at the end of the cycle to guarantee that the nitrogen respiration was zero. In contrast, the highest oxygen utilisation rate was measured at the beginning of the aerated phase, when nitrogen was in a range of 5 to 10 mg L^{-1}. OUN was calculated with the following equations considering the measured temperatures. Oxygen utilisation rates were needed to create COD and nitrogen mass balances.

$$\mathrm{OUC_{t20}} = \mathrm{OUC_t} \cdot 1.072^{(20\text{-}t)}$$

$$\mathrm{OU_{t20,max}} = \mathrm{OU_t} \cdot 1.103(^{(20\text{-}t)}$$

$$\mathrm{OUN_{t20}} = \mathrm{OU_{t20,max}} - \mathrm{OUC_{t20}}$$

5.4 Results and Discussion

5.4.1 Upstart and Operation

The reactors were inoculated with a mixture of AGS from an earlier phase and suspended activated sludge from a municipal WWTP. Minimal settling velocity was 6 m h^{-1} during the first 50 days. Sludge volume index (SVI) of both SBR was 94 ml g^{-1} in the beginning of the tests. SVI of SBR 1 decreased to 67 ml g^{-1} within 23 days and fluctuated between 60 to 80 ml g^{-1} till day 64. Due to a technical failure, a great amount of sludge of SBR 1 was lost at day 74, thus, the results of the following five weeks were excluded from the evaluation. Till the end of the first phase (day 261), the SVI of SBR 1 ranged again between 61 to 79 ml g^{-1}. Similar results during the startup were observed for SBR 2. SVI declined to 60 ml g^{-1} within 18 days. The average sludge loading during the first phase was 0.34 gCOD $gTSS^{-1}$ d^{-1}. Both SBR were aerated to a DO in the range of 1.8 to 2.2 mg L^{-1} without anoxic conditions in the bulk liquid. The operation of SBR 2 included a stirrer during non-aerated phases, which probably caused increased shear stress and SVI in a range of 64 to 97 ml g $^{-1}$. From day 119 on, the SVI of SBR 2 climbed up to 300 ml g^{-1}, which was attributed to the growth of filaments. SBR 2 was no longer sampled for N_2O under these unstable conditions.

During the second startup with activated sludge from a municipal WWTP, $v_{s,min}$ of both reactors was increased to approx. 4 m h^{-1} by reducing the settling time from 6 to 3 min. Stirrers were not used during these experiments. SVI declined to 42 (SBR 1) and 48 ml g^{-1} (SBR 2) within 35 days. For both reactors, SVI was between 40 to 60 ml g^{-1} till day 91. Since a high amount of suspended solids was present in the effluent, the settling time was enlarged and $v_{s,min}$ decreased to approx. 1.4 m h^{-1}. In between the tests, there were elevated NO_x-N concentrations measured in both reactors and mean-time high SVI up to 100 ml g^{-1}. However, compact granules with sizes above 200 μm were observed by regular microscopy. A recirculation of purified water through the settled sludge bed was applied to improve the TN removal. With this strategy, it was possible to reduce the NO_x-N concentration to approx. 5 to 10 mg L^{-1}, which ensured again anoxic-anaerobic feeding conditions and declining SVI. N_2O sampling was performed at the end of the phase (day 501), when SVI and NO_x-N concentration were in a representative range. The average OLR during the second phase was 0.25 gCOD $gTSS^{-1}$ d^{-1}.

5.4.2 Mass balance

Figure 5.1 shows the calculated COD and nitrogen mass balances exemplary for data point 1. The calculations consider the input and output loads to the SBR during the measured cycle as well as the carbon and nitrogen respiration rates. The balances were calculated for the cycles with N_2O sampling according to the following equations.

COD balance

$$OUC = COD_{IN} + COD_{XA} - COD_{OUT} - \Delta COD_{SP} \text{ [mg]}$$

$$OUC = COD_{IN} + 0.3\,(N_{TN,IN} - N_{NH4\text{-}N,OUT}) - COD_{OUT} - Y_{BM} \cdot (COD_{IN} - COD_{OUT}) \text{ [mg]}$$

Nitrogen balance

$$N_D = N_{TN,IN} + N_{NOX\text{-}N,IN} - N_{NH4\text{-}N,OUT} - N_{NOX\text{-}N,OUT} - \Delta N_{NOX\text{-}N} - N_{N2O}\ [mg]$$

The sludge production (SP) was determined with a biomass yield (Y_{BM}) of 0.25 gTSS gCOD^{-1} (0.3 gCOD gCOD^{-1}). Since there was no sludge removed during the cycle, the biomass growth is presented as ΔCOD_{SP}. COD_{XA} is the COD load produced through the autotrophic growth of the nitrifiers due to biomass cell synthesis of CO_2 and assumed as 30% of the oxidised nitrogen load. Oxygen utilisation for carbon removal (OUC) was calculated with the measured carbon respiration (OU_{CO2}) as well as with the oxygen gained through denitrification (OUD). The oxygen utilisation (OU_{CO2}) was related to the entire aerobic phase and the reactor volume. The deviation was referred to the input COD and amounted to 6.3%. Other data were checked according to the procedure and the deviations were found up to 12%.

The nitrogen mass balances were calculated with the in- and output nitrogen loads and the NO_x-N left over from the previous cycles. Oxygen utilisation rate for nitrogen (OUN) during the aerated cycle was measured with 29.3 mgO_2, which is close to the theoretical OUN calculated based on the oxidised ammonium loads. The difference in the OUN was found with 1.8% and up to 10% for all measurements.

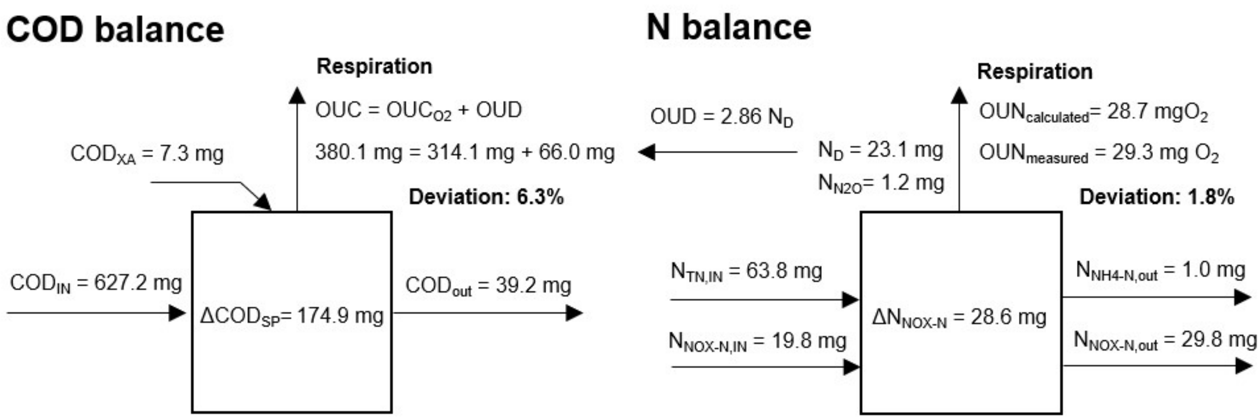


Figure 5.1: COD (left) and nitrogen mass balance (right) for data point 1

5.4.3 Effect of Organic loading rate

Since SND is a promising ability of aerobic granules, the first tests focused on the operation under fully aerobic conditions during the aerated phase without anoxic periods in the bulk liquid. DO was throughout adjusted to 2 mg L^{-1} with aeration rates of 2 L min^{-1}. Under these conditions, the effect of different OLR on the N_2O emissions from AGS was investigated including seven measurements (data points 1 to 7). OLR ranged between 0.13 and 0.42 gCOD gTSS^{-1} d^{-1}. Temperatures were in a range between 21.2 and 24.0°C. NH_4-N removal reached 66.0 and 99.4%, while TN removal was calculated with 41.6 to 78.5%.

Figure 5.2 shows the nutrients and N_2O levels for the data points 3 and 5 during the aerated phase. Generally, it was found, that the measured NH_4-N concentrations were approx. 25% lower than the theoretical NH_4-N concentration that should be measured after feeding.

We assume that this fact was due to NH_4-N adsorption on the granules as it was reported by Bassin et al. (2011b). The authors observed an increased adsorption capacity of AGS compared to activated sludge with an NH_4-N adsorption in a range of 23 to 36% at 20°C. During all measurements, the highest N_2O levels in the off-gas were measured at the beginning of the aerated phase with the highest substrate availability. The N_2O peaks appeared within the first 5 min with N_2O levels mostly between 10 to 35 ppm. This is in line with findings from van den Akker et al. (2015), who observed N_2O peaks in the exhaust air during the changeover from the anaerobic feed to the aerated phase, which was probably associated with an increased oxygen uptake and lower DO levels during the first few minutes of aeration. N_2O emissions steadily decreased afterwards, whereby the emissions were almost zero after all NH_4-N was oxidised (Figure 5.2, right). Since there was no full NH_4-N removal achieved during run 5, N_2O was also present at the end of the aeration with off-gas concentrations of approx. 5 ppm (Figure 5.2, left).

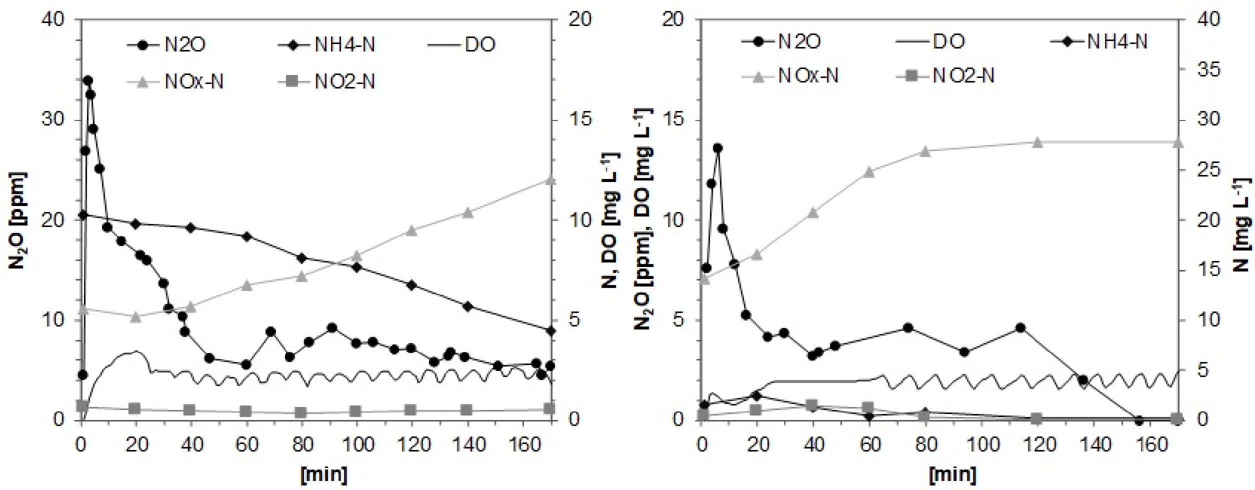


Figure 5.2: N_2O, NH_4-N, NO_2-N, NO_x-N and DO during the aerated cycle for data points 3 (left) and 5 (right)

Figure 5.3 (left) shows the EF under different OLR, which were calculated in a range of 0.54 to 4.8% (N_2O/NH_4-$N_{ox.}$). During the tests, nitrite was identified as a relevant trigger for increased N_2O emissions and an indicator for elevated AOB activity. Clearly increased N_2O emissions appeared for data points 6 and 7 (triangle), whereby enhanced nitrite levels of 3.3 and 4.8 mg L^{-1} were detected within the aerated phase. During run 1 to 5 (dots), the nitrite concentrations were much lower at 0.7 and 1.8 mg L^{-1}. Overall, the results indicate that higher OLR lead to increased EF, which is in line with an earlier study from Guo et al. (2017). The authors investigated the N_2O emissions from a SBR operated with AGS under different food/microorganism (F/M) ratios and found EF between 2.77 and 3.79%. In the study of Guo et al. (2017), the increased F/M ratios led to decreased DO levels (approx. 1 mg L^{-1}). In this case, the OLR was increased and the DO decreased, which promotes the N_2O formation of autotrophic nitrification and nitrifier denitrification. Especially during the beginning of the aeration, when the oxygen uptake is the highest, nitrifier denitrification pathway could contribute to increased N_2O emissions. It has to be mentioned that municipal WWTP are normally operated with OLR between 0.05 and 0.15 gCOD $gTSS^{-1}$ d^{-1}. In this loading range, the N_2O emissions of our presented data are below 1%, which fits to EF measured at full-scale municipal WWTP (Parravicini et al. 2015).

A further correlation of the present study was found between the EF and the amount of SND (Figure 5.3, right). SND was detected especially in the beginning of the aeration (within the first 20 min) and was probably linked to a high oxygen uptake and increased anoxic zones inside the granules. SND decreased afterwards, since oxygen penetrated into deeper zones. In that case, the granules size plays a key role in the formation of anoxic zones. Furthermore, the DO and substrate diffusion into the granules are limited by larger diameter. The measurements were performed over a period of a half year, whereby differences in the granulation grade probably affected the SND capacity. Low emissions were found for data points 4 and 5, where the low SND capacity was attributed to an increased flocculent proportion. Gao et al. (2016) found increased EF from AGS operated under SND and claimed that the AOB and heterotrophic denitrifiers are responsible for the N_2O emissions. The authors suggested that when the external COD is limited, the internally stored substrate is used as electron donor for incomplete denitrification, which leads to an increased N_2O formation due to heterotrophic denitrification. Thus, the elevated N_2O emissions of AGS can be attributed to a limited COD diffusion into the granules with a larger diameter. Stewart (2003) summarized different diffusion coefficients for various substrates. Hereby, the nitrogen and oxygen diffusion coefficient for temperatures of 25°C were reported in a similar range with $18.8 \cdot 10^{-6}$ and $20 \cdot 10^{-6}$ cm^2 s^{-1}. Lower diffusion coefficients were reported for carbon sources like glucose and acetic acids within a range of $6.7 \cdot 10^{-6}$ cm^2 s^{-1} to $12.1 \cdot 10^{-6}$ cm^2 s^{-1}. A limited carbon diffusion into the granules could promote the N_2O formation via heterotrophic denitrification in combination with SND. Findings from Lochmatter et al. (2013) verify our assumption of a limited carbon availability, since the authors found lower N_2O emissions with higher COD loads under constant NH_4-N inflow concentrations. A further trigger for the N_2O formation over the heterotrophic denitrification is the presence of oxygen during denitrification. Since SND relates to oxygen gradients in the biomass, micro-aerobic conditions can affect the N_2O reductase and therefore increase N_2O emissions (Lemaire et al. 2006).

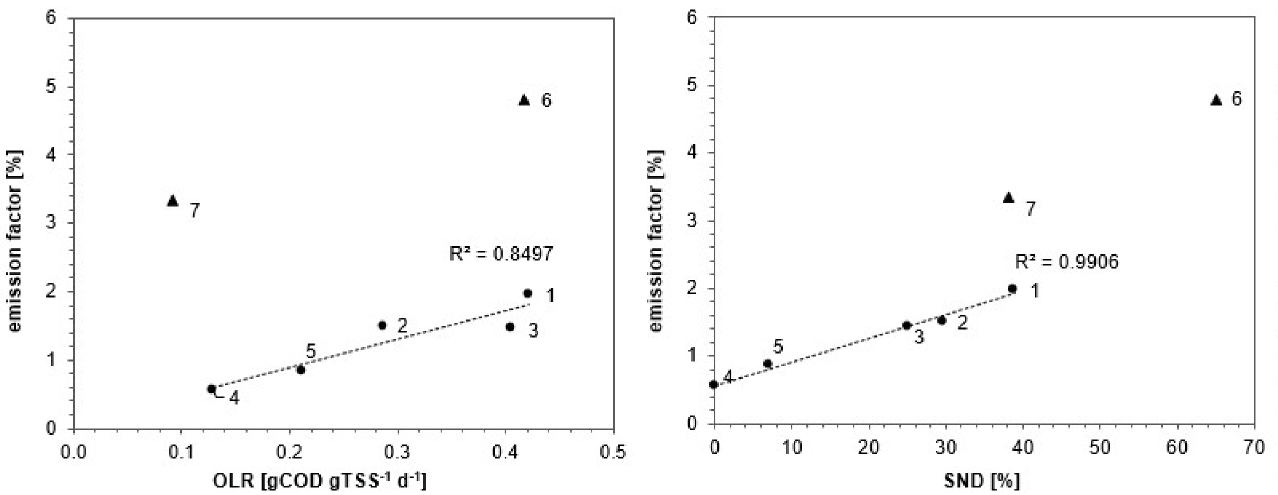


Figure 5.3: OLR to EF (left) and SND to EF (right) for the tests with a fully aerobic conditions during aeration [dots: NO_2-N $<$ 1.8 mg L^{-1}, triangle: NO_2-N $>$ 3.0 mg L^{-1}]

In summary, we suggest that the OLR affects the N_2O formation in different ways. Firstly, increased loads lead to enhanced AOB activity and the N_2O formation from autotrophic nitrification especially at the beginning of the aeration. SBR operation includes a period feed, thus, high NH_4-N levels appear in the beginning of the aeration and decline afterwards by nitrification. Moreover, the higher OLR probably leads to an increased oxygen uptake through the outer zones, which causes enlarged anoxic zones inside the granules. This would explain the increased SND activity and the N_2O formation from heterotrophic denitrification, since there is especially internal carbon used for denitrification.

Moreover, AGS systems are operated with an anaerobic feeding strategy to enrich substrate-storing organisms, that store most of the incoming COD during the feed. Denitrifying phosphate and glycogen accumulating organisms (PAO and GAO) play an important role in partial denitrification. Earlier studies state that denitrification by GAO and PAO can cause increased N_2O emission (Kampschreur et al. 2009, Lemaire et al. 2006, Zeng et al. 2003a, Zeng et al. 2003b). The metabolism of these organisms is associated to the growth on storage compounds, whereby the PHB consumption is the rate-limiting step. Schalk-Otte et al. (2000) observed elevated N_2O emissions when PHB was the substrate to grow during COD limitation. Phosphate uptake in the presented study ranged from 0 to 0.96 gP $gTSS^{-1}$, which indicates that PAO activity was different during the measurements. However, it was found that higher EF appeared when the phosphate uptake was increased. The impact of enhanced biological phosphate removal of AGS on N_2O emissions should be addressed in further research.

5.4.4 Effect of aeration strategy

Over a period of 240 days after the restart, the two SBR were operated in parallel to investigate the effect of different aeration strategies on the nitrogen removal. The simultaneous sampling for both SBR was conducted to identify which aeration strategy is more decisive for the N_2O formation. The aeration control setting for SBR 1 ensured continuous aerobic conditions during the aerated phase with DO levels between 1.8 and 2.2 mg L^{-1}. For SBR 2, an alternating aeration was applied with a 5 min aeration interval to a DO level of 2 mg L^{-1} that was followed by a 5 min aeration pause. N_2O emissions of the two SBR were measured under similar operating conditions. Both SBR were fed with the same municipal wastewater and an OLR of 0.1 gCOD $gTSS^{-1}$ d^{-1}. N/COD ratio was 0.15 and thus in an optimal range. N/COD ratios above 0.15 indicate COD limitations. The aeration rates were set to 2 L min^{-1} for both reactors. TSS in the SBR were similar with 3.0 and 3.3 g L^{-1} respectively.

Figure 5.4 shows the NH_4-N and TN removal of both SBR over the second experimental phase. At some points, there was no full NH_4-N removal achieved for SBR 1. This was mainly due to increased loading rates and a meantime growth of *Arcella* (days 385 to 430), which was observed by regular microscopy. Unfortunately, it was not possible to identify the reasons for the development of *Arcella* in SBR 1. Increased OLR of 0.6 gCOD $gTSS^{-1}$ d^{-1} appeared for SBR 1 during the days 311 and 318 and caused a decline in NH_4-N removal. TN removal for SBR 1 was mostly between 50% and 60%. With the applied aeration strategy for SBR 2, a complete NH_4-N removal was recorded over the entire period. TN removal increased until the day 501 to approx. 90%, which can be attributed due to larger granule sizes growing with time and enlarged anoxic zones for denitrification.

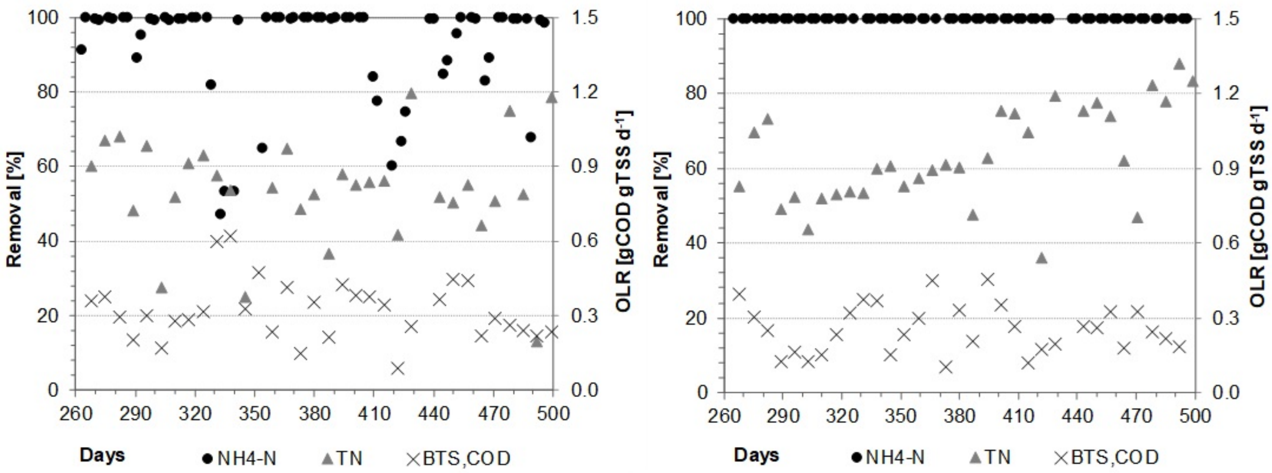
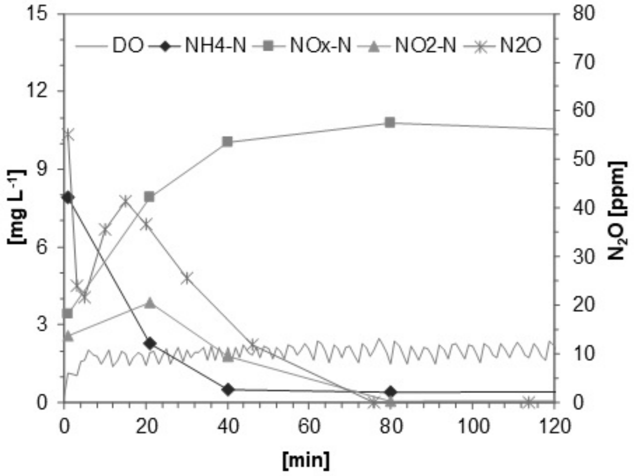


Figure 5.4: OLR, NH_4-N and TN removal for SBR 1 (left) and SBR 2 (right)

Figure 5.5 shows the course of N_2O emissions in the exhaust air and the nutrients over the aerated cycle. N_2O sampling occurred on day 501 simultaneously for both reactors. NH_4-N concentrations were similar during the start of the aeration with 7.5 mg L^{-1} (SBR 1) and 7.9 mg L^{-1} (SBR 2). The highest emissions were measured at the beginning of the sampling. With the onset of aeration, the off-gas concentrations increased to 55 ppm N_2O (SBR 1) and 70 ppm N_2O (SBR 2). Up to this time, the NH_4-N uptake rate of SBR 1 was almost twice that of SBR 2. Within the first 20 min, the NH_4-N concentrations were reduced to 2.5 and 5.4 mg L^{-1} resulting in ammonia-oxidising rates of 5.1 (SBR 1) and 2.8 mg NH_4-N $gTSS^{-1}$ h^{-1} (SBR 2). N_2O levels decreased with declining NH_4-N concentrations. However, for SBR 1 the N_2O emissions increased again after approx. 20 min of aeration, which was probably linked to a rising NO_2-N level (3.88 mg L^{-1}). In contrast, NO_2-N was nearly stable for SBR 2 with 1.2 to 1.8 mg L^{-1}. EF for SBR 1 and SBR 2 were calculated at 3.32 and 1.62% (N_2O/NH_4-$N_{ox.}$). Besides an increased AOB activity, the different EF can be attributed to the different NO_2-N concentrations within the aerated phase. Zeng et al. (2003a) assumed that nitrous oxide reductase is inhibited by nitrite concentrations above 1 mg L^{-1}. This reported NO_2-N level was clearly exceeded in both reactors. However, the periods without aeration (SBR 2) probably allowed denitrification of NO_2-N and subsequently lower N_2O emissions compared with SBR 1.

The results illustrate that the main source of N_2O was related to autotrophic nitrification since the emissions clearly correlated to the ammonium oxidation. N_2O was no longer detectable when ammonium was depleted. Total NH_4-N removal was found for SBR 1 and 2 with 99.0 and 99.6%. TN removal reached 69.2% for SBR 1 with the constant aerobic aeration, while SBR 2 achieved a TN removal of 77.3% with an alternating aeration mode. The results are in line with Lochmatter et al. (2013), where a higher TN removal was found with an alternating aeration mode. In this study, the constant DO also amounted to higher NO_2-N emissions compared to the alternating aeration strategy. Zhang et al. (2015) observed higher N_2O emissions in a fully aerobic SND compared with the aerobic-anoxic SND by inhibition tests and reported an increased nitrifier denitrification as the main source for N_2O. However, with our applied method we cannot prove the presence of nitrifier denitrification, even though it was mentioned as a relevant N_2O formation pathway in biofilms (Schreiber et al. 2009). Since it is known, that nitrifier denitrification is mainly related to oxygen-limited conditions, we assume that N_2O formation from nitrifier denitrification could appear temporarily within the beginning of the aeration due to an increased oxygen uptake in the outer zone of the granules.



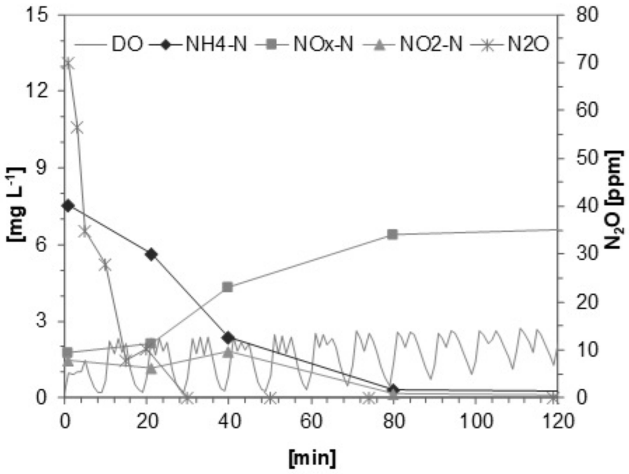


Figure 5.5: NH_4-N, NO_x-N, NO_2-N and DO in the liquid phase and N_2O in the exhaust air of the SBR 1 and 2

Figure 5.6 shows the microbial community of the investigated AGS (taxonomy, phylum, class) of the two SBR. The taxonomic distributions of the communities were quite similar. An increased abundance of the phylum *Parcubateria* was found for SBR 2. Since these bacteria were mostly observed under anoxic conditions (Castelle et al. 2017), we assume that the increased proportion of *Parcubateria* in SBR 2 was related to increased anoxic conditions due to non-aerated periods. Since N_2O is an intermediate of the metabolism of involved nitrifying and denitrifying bacteria, the microbial composition of AOB and NOB effects the N_2O formation mainly. Winkler et al. (2015) investigated the correlation between the involved microorganisms and the nitrite accumulation by modeling and experimental tests and revealed that *Nitrobacter* was the dominant NOB in aerobic granules and not *Nitrospira*. The authors claimed that in AGS systems NOB grow uncoupled from AOB, which can lead to an additional nitrite formation due to partial denitrification by *Nitrobacter*. Although, the microbial community of the present study was also characterized by an elevated NOB/AOB ratio, *Nitrobacter* was not detectable; instead *Nitrospira* was found to be the dominant NOB in both SBR, which is also in line with findings from earlier AGS studies (Lochmatter et al. 2014; Thwaites et al. 2017). NOB/AOB ratios determined by gene sequencing were 1.2 (SBR 1) and 1.7 (SBR 2), respectively, and thus increased compared to a reported NOB/AOB ratio of approx. 0.2 (± 0.1) for a conventional treatment plant (Winkler et al. 2012). Although an increased growth of NOB is coupled to the accumulation of nitrite, elevated NOB/AOB ratios were also found in correlation with increased temperatures and decreasing DO, which illustrates that further parameters affect the presence of nitrifying and denitrifying bacteria.

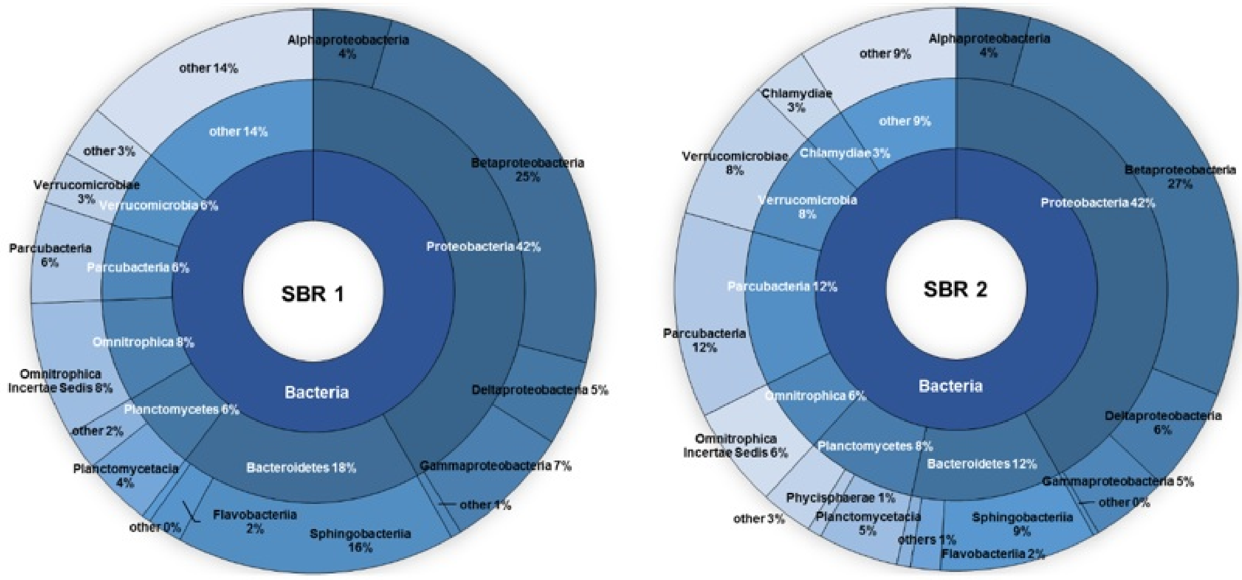


Figure 5.6: Microbial community of the AGS (taxonomy, phylum, class) by Illumina MiSeq sequencing

5.5 Conclusion

Due to the structural properties of AGS, the bacterial community and the arrangement of individual microorganisms are quite different compared to conventional activated sludge. Several triggers for the N_2O formation in AGS systems were identified in the framework of this study. The evaluated data reveal a correlation between the OLR and the SND on the N_2O emissions and were identified as relevant triggers for the N_2O formation. Increased loads promote the N_2O formation via autotrophic nitrification and probably due to nitrifier denitrification, since at the beginning of the aerated phase, higher loads lead to higher DO uptake through the outer layers. Furthermore, the increased OLR promote SND and the N_2O formation via heterotrophic denitrification, which is probably a reason for endogenous denitrification and COD limitation. EF under fully aerobic conditions during the aerated phase were calculated in a range of 0.54 to 4.8% at OLR between 0.13 and 0.42 gCOD $gTSS^{-1}$ d^{-1}. This aeration strategy was found to promote the nitrite accumulation and increased N_2O emissions. It has to be taken into account that nitrite is a sign of high AOB activity and can have a direct impact on the N_2O formation. The alternating aeration strategy was found to be more efficient for the TN removal and lower N_2O emissions. Against the background of desired low N_2O emissions, the alternating aeration strategy with anoxic-aerobic conditions should be favored for the operation of AGS systems.

5.6 Aknowledgements

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Chapter 6

Accidental aerobic granules - Data evaluation of a full-scale SBR plant

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6.1 Abstract

This paper describes and evaluates a large-scale SBR with a design capacity of 35,000 p.e. where the activated sludge exhibits excellent settling properties. The sludge volume index (SVI) of all four SBR is mostly below 50 ml g^{-1} and shows annual fluctuations; the lowest values of 30 ml g^{-1} are measured during summer. The focus of this study was to identify reasons for this excellent settling behavior. Microscopic images of the sludge showed a compact and dense structure with small granules. Particle size distribution indicates that about 74.4% of the particles had a size above 200 μm, which is a characteristic size of aerobic granules. Approx. 50% of the sludge particles were larger than 320 μm. SV_{10}/SV_{30} ratio was calculated with 1.21. Based on the existing knowledge of aerobic granular sludge it can be assumed that the long filling during denitrification leads to anaerobic conditions and promotes the formation of aerobic granules. Legal requirements for the effluent quality were met the entire year. The average COD and TN removal amounted to 94.2 and 83.3%.

6.2 Introduction

In recent years, a lot of research has been done in the field of aerobic granular sludge (AGS), a biomass that is characterized by a compact and dense structure with excellent settling properties. A common tool to control the thickening and settling behavior of the activated sludge is the sludge volume index (SVI). AGS is characterized by a lower SVI ($<$ 60 ml g^{-1}) compared to flocculent sludge (80–100 ml g^{-1}). Another criterion to differentiate between aerobic granules and flocculent sludge is the use of sludge volume ratios (SV_5/SV_{30} and SV_{10}/SV_{30}). For AGS, the sludge volume after 5 or 10 min of settling is usually already close to the final sludge volume achieved after 30 min. Since the biomass settles significantly faster than flocculent activated sludge, numerous operational advantages are possible. Shorter settling and thus shorter cycle times for SBR plants in combination with a higher biomass concentration make room for higher hydraulic and organic loads. SBR processes with AGS can be applied in order to increase the capacity of existing plants especially when space is limited. Moreover, Giesen and Thompson (2013) as well as by Pronk et al. (2017) reported a more energy-efficient operation of AGS plants.

SBR systems are especially suitable for aerobic granulation due to the periodic feeding and the formation of feast and famine conditions. Overall, the anaerobic feed was claimed as the most relevant parameter for the formation of AGS in SBR. De Kreuk and van Loosdrecht (2004) found out that the anaerobic feeding promotes the enrichment of slow growing organisms, which increase the sludge stability significantly. However, many other parameters, like sludge separation, aeration intensity and organic loading rate (OLR) also affect the sludge structure. In particular, the washout of flocculent biomass due to short settling times is mentioned in many studies to contribute to a fast granulation (Qin et al. 2004).

Most studies on AGS relate to laboratory work, thus full-scale plant experiences are limited. Pronk et al. (2015b) published an extensive study of a full-scale AGS plant (WWTP Garmerwolde) with a treatment capacity of 28,600 m^3 d^{-1}. With the Nereda process, the SVI was stable in a range of 35 to 45 ml g^{-1} with a TSS above 8 g L^{-1}. The percentage of granules exceeded 80%. The dry weather operation included a 60 min plug-flow feed with an OLR of 0.1 kgCOD $kgTSS^{-1}$ d^{-1}. Li et al. (2014) investigated a full-scale SBR treating approx. 50,000 m^3 d^{-1}. Hereby, the SVI averaged on 47.1 ml g^{-1} with a mean diameter of 0.5 mm. The SBR plant was operated with a 40 min filling time and an OLR of 0.56 kgCOD m^3 d^{-1}. The authors report that aerobic granules could not develop, if the settling time was not well controlled, even though a periodic feast-famine regime was present. Świątczak et al. (2017) investigated a full-scale WWTP after upgrading towards AGS and reported a SVI_5 and SVI_{10} of 64 and 48 ml g^{-1}.

The present paper deals with a conventional SBR plant nearby Vienna, where stable granules were observed over several years. SVI of the activated sludge was mostly between 30 and 50 ml g^{-1}. A detailed analysis of the WWTP was undertaken to identify the reasons for this extraordinary good settling behavior. Operational data of one year were considered. Additional sludge investigations were conducted and supplemented by online nutrient measurements.

6.3 Description of the plant

Figure 6.1 shows a scheme of the WWTP Wolkersdorf, which includes a buffer tank with 450 m^3 and four activated sludge tanks as SBR with a volume of 2,646 m^3 each (21m x 21m x 6m). The operation of each SBR is placed in series (e.g. SBR 1 is filled, while SBR 2 is in reaction mode). The SBR are equipped with hyperboloid stirrer, but the stirrers are only operated during feeding and between aeration with a mean daily mixing time of 4.4 h. Exchange ratios depend on the inlet flow and are normally in the range of 11–19%. The plant was designed for 35,000 p.e., while the average load amounts to approx. 15,500 p.e. (COD) with peak loads of approx. 20,000 p.e. during the wine campaign. Sludge is stabilized aerobically in the reactors and is subsequently dewatered via centrifuge. Centrate from the sludge dewatering is pumped into the buffer tank, where $FeCl_3$ is dosed for phosphate removal. The SBR are filled with wastewater from a pipeline from above under mixed conditions.

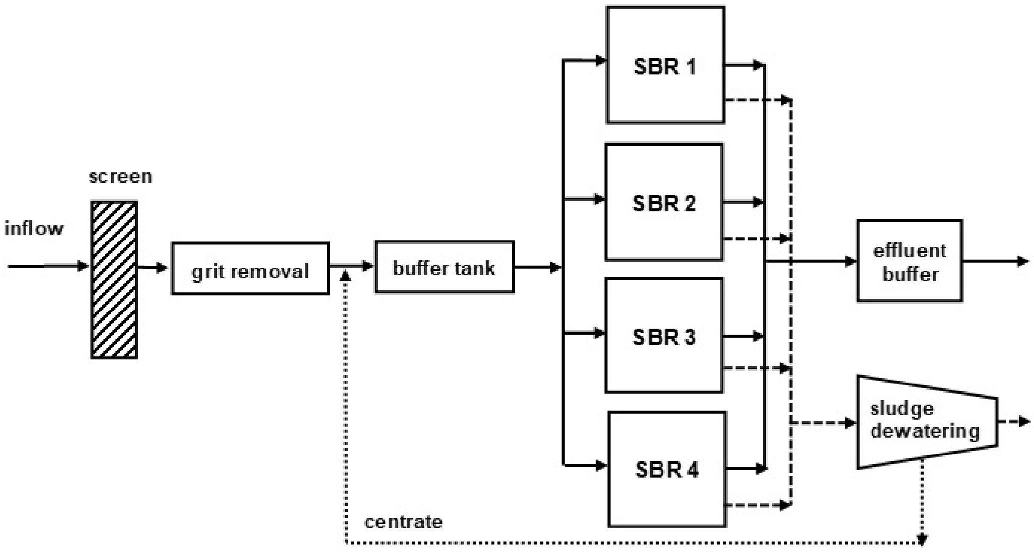

Figure 6.1: Scheme of the WWTP Wolkersdorf

Table 6.1 shows the cycle time operation during dry and wet weather conditions. The dry weather operation includes a 500 min cycle with a denitrification time of 170 min. The wet weather cycle lasts 300 min with a feeding time of 60 min. Settling time is 60 min for both operation modes with a subsequent 60 min decant period. The aeration of the SBR is controlled to a set point of 2 mgDO L^{-1} and is stopped to achieve nitrification and denitrification. Moreover, pre-denitrification is achieved during the feeding time.

Table 6.1: Cycle time operation during dry and rain weather conditions [min]

	Denitrification (Feeding)	Aeration (Feeding)	Settling	Decant
Dry weather	170 (130)	230 (0)	60	50
Wet weather	-	120(60)	60	50

6.4 Wastewater characteristic

The WWTP treats municipal sewage and industrial wastewater from food industry and viniculture. About 20% of the COD results from food industry. Average COD/BOD_5 (biological oxygen demand) ratios was 1.6, which indicates a higher biological availability of the COD. Moreover, a metalworking company is connected to the sewer system and causes slightly increased nickel and copper concentrations. Table 6.2 presents the average influent concentrations as well as the 85-percentile. The sewage is characterized by a low N/COD ratio of about 0.06 (P/COD 0.01). Settle able substances in the wastewater were on average 10.7 ml L^{-1}.

Table 6.2: Mean influent concentration, 85-percentile during 2016

Influent	COD [mg L^{-1}] (n=200)	BOD_5 [mg L $^{-1}$] (n=52)	TN [mg L^{-1}] (n=202)	TP [mg L $^{-1}$] (n=298)
Mean	770	466	43.1	7.3
85-percentile	1,034	580	55.6	9.0

6.5 Structure and settling behavior

As a first step of the investigation, the activated sludge was examined via microscopy (Fig. 6.2). The images of the biomass showed a mixture of sludge flocs and compact dense granules. Numerous granules comprised sizes in a range of 200–500 μm. These compact sludge particles were colonized by many sessile ciliates, which is often reported for aerobic granules (de Kreuk et al. 2010, Li et al. 2013). Moreover, the biomass obtained a high abundance of *Zoogloea spp.*, which was also reported by (Long et al. 2014, Sheng et al. 2010). The presence of filaments was low.

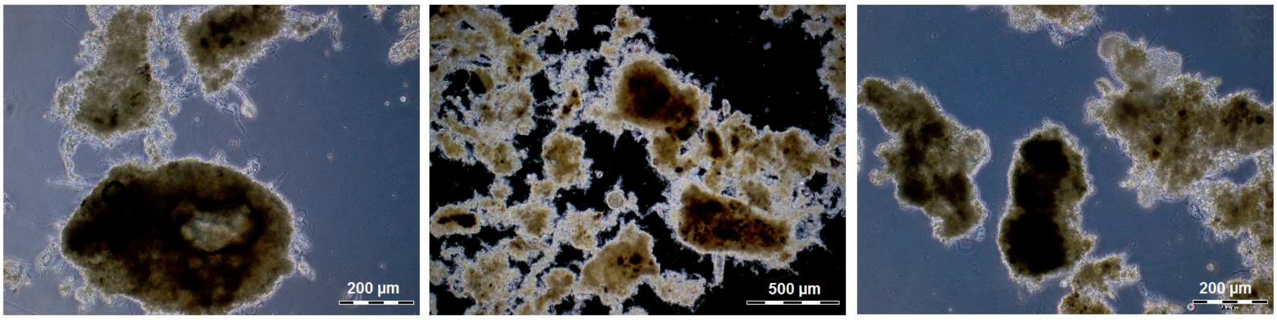

Figure 6.2: Microscopic image of the activated sludge (Leica microscope)

Additionally, a Malvern Mastersizer 2000 was used to analyze the particle size distribution of the activated sludge (Fig. 6.3). The investigated sludge sample had a SVI of 57 ml g^{-1} with a SV_{10}/SV_{30} ratio of 1.21. VSS/TSS and COD/VSS ratio were 0.65 and 1.48, respectively. The results were compared with particle size distributions of two reference samples from a conventional activated sludge plant (CAS) in continuous-flow operation and additionally with samples of a lab-scale SBR (AGS). Detailed information on the operation of the lab-scale SBR can be retrieved from Jahn et al. (2019). Figure 6.3 indicates that the particle size diameters of the full-scale SBR plant (WWTP Wolkersdorf) were between the results of the sludge removed from the CAS and the AGS from the laboratory SBR. Approx. 10% of the particles were smaller than 117 μm, while 50% had a size above 329 μm; in total, 74.4% of the analyzed sludge particles from the WWTP Wolkersdorf reached a particle size above 200 μm, which is characteristic for aerobic granular sludge. Overall, there was a higher fraction of middle ranged particles. In comparison, the curves of the CAS samples and the granules from the SBR are more flat, which is attributed to an overall wider size range.

Fig. 6.4 presents the SVI of the full-scale SBR for a period of 365 days, whereby the trends are similar for all reactors. Due to refurbishment of the aeration system, SBR 3 was not in operation for 83 days during summer. After the restart of SBR 3, the SVI increased to about 50 ml g^{-1} and was thus slightly above the values measured for SBR 1, 2 and 4. Up to May, the SVI of all SBR were mostly between 40 and 50 ml g^{-1}, whereas during June and July the SVI dropped below 30 ml g^{-1}. All reactors showed a reverse correlation between the temperature and the SVI. The lowest SVI of approx. 30 ml g^{-1} was measured at 22°C, while higher SVI above 50 ml g^{-1} were related to temperatures of 15°C. Comparable, the settling velocity of the total sludge bed (flocs and granules) was 3 m h^{-1} at 15°C and 8 m h^{-1} at 22°C. Up to now, the temperature effect on AGS is only described in a few studies. Winkler et al. (2012c) investigated the temperature effect on granules and found a two-fold difference in the settling velocity (35–63 m h^{-1}) for the same granule by increasing the temperature from 5°C to 40°C.

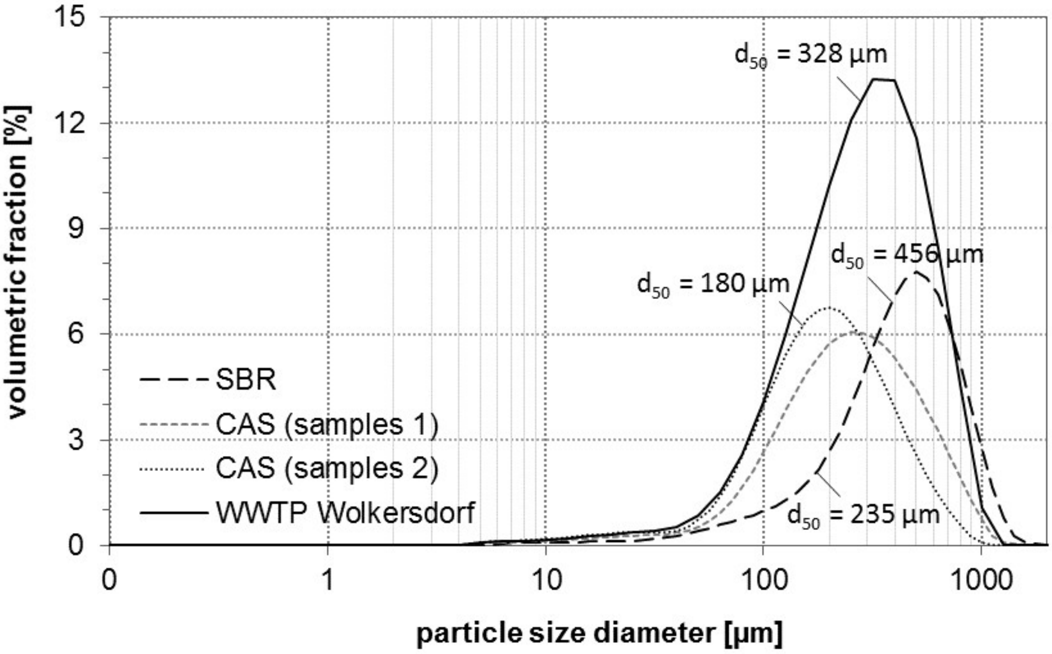

Figure 6.3: Particle size distribution and d_{50} values for samples of the WWTP Wolkersdorf, CAS samples and granules from the lab-scale SBR

The authors explained this observation by the fact that the viscosity of water decreases at higher temperatures. Although, the annual difference in temperature was only about 10°C; the viscosity effect could be a reason for the overall lower settling velocities during summer. Moreover, the hydrolysis of particulate matter depends on the temperature too. An increased hydrolysis during the feed could improve the COD uptake and thus the regular growth of the granules. Otherwise, not hydrolyzed polymeric substances can cause the formation of irregular structures with decreased settling properties and higher SVI. The trend of the settling is in line with de Kreuk et al. (2005b), where the start-up of AGS was tested under different temperatures. A more stable granulation was detected at 20°C; in contrast to the start-up at 8°C, which lead to a more irregular growth of the granules. A further reason for poorer settling properties during winter could be the increase of filamentous bacteria, especially Microthrix parvicella was found to grow under lower temperatures (Knoop and Kunst 1998).

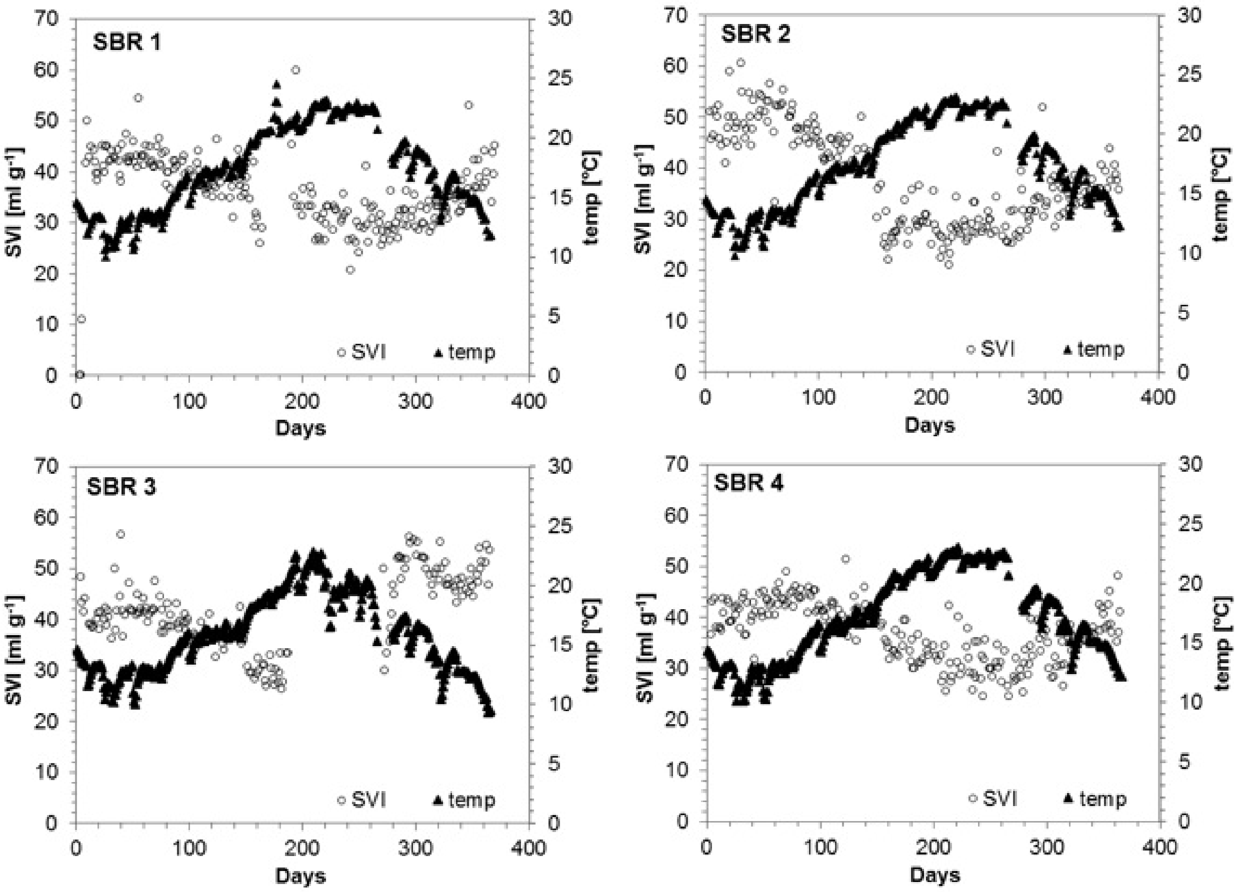

Figure 6.4: SVI and temperatures of the SBR over a period of one year, starting in January

A further correlation was identified between the SVI and the measured COD and TN influent concentrations (Fig. 6.5). Slightly increased concentrations and loads were observed by the end of the year (day 260), which correlates with increasing SVI. Average TN and COD load was 96.3 kg d^{-1} and 1,647 kg d^{-1} before day 260 and raised to 121.1 kgTN d^{-1} and 2,450 kg-COD d^{-1} afterwards. This increased load is probably attributed to the beginning of the wine campaign, which normally starts in August/September. The annual data illustrate that the higher concentrations appeared simultaneous to the declining temperatures. Thus, there could be a strengthened impact of both parameters, since changing process conditions can affect the microbial composition of the biomass, which can further affect the settling properties and SVI (Kristensen et al. 1994).

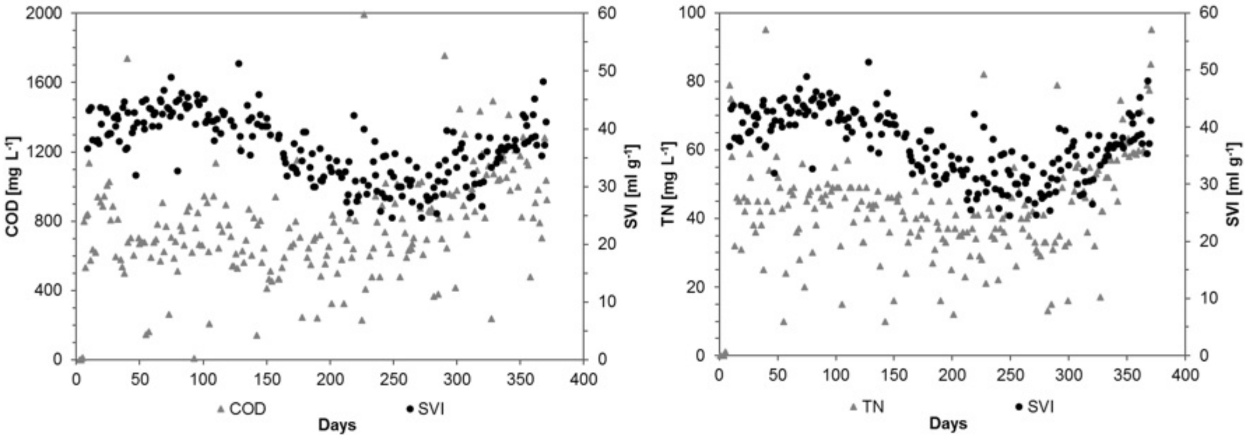

Figure 6.5: COD (left) and TN (right) influent concentration and SVI (SBR 4)

6.6 Treatment performance

Table 6.3 presents the average effluent concentrations as well as the 85-percentile of the measured data. The effluent concentrations and removal efficiencies complied with the legal requirements throughout the year. Average COD and BOD removal achieved 94.2% and 98.3%. TP removal amounted to 94.3% with an average effluent concentration of 0.4 mg L^{-1} and TN removal was calculated to be 83.3%. OLR was nearly constant over the year with an average value of 0.06 kgCOD $kgTSS^{-1}$ d^{-1}, the volumetric loading was 0.23 kgCOD m^{-3} d^{-1}. No settable substances were measured in the effluent, indicating that the biomass was completely retained in the SBR and not washed out during decant.

Table 6.3: Mean effluent concentration, 85-percentile during 2016

Effluent	COD [mg L^{-1}] (n=209)	BOD_5 [mg L^{-1}] (n=52)	TN [mg L^{-1}] (n=202)	TP [mg L^{-1}] (n=298)
Mean	25.4	7.3	6.4	0.4
85-percentile	30.5	10.0	9.0	0.5

In order to describe the processes in the SBR, the NO_3-N concentrations and the DO were monitored in SBR 3 during a period of 10 d dry weather mode. Fig. 6.6 shows the course of the measured concentrations during the cycle operation (A-E). Based on the data it can be concluded, that there were no fully anaerobic conditions during feeding (A), since NO_3-N effluent concentrations were measured up to 5.0 mg L^{-1}. Anaerobic feeding conditions were established as soon as the NO_3-N was depleted (pre-denitrification).

Low NO_3-N effluent concentration (Figure 6.6, first cycle: NO_3-N < 2 mg L^{-1}) were found to increase the anaerobic time up to 1 h. Anaerobic conditions during the feed are generally the most important parameter for an aerobic granulation (de Kreuk et al. 2005a). Thwaites et al. (2017) investigated the performance of a split anaerobic-aerobic feed to a complete anaerobic feed and found comparable results for the granulation. This study is similar to the feeding conditions of the WWTP Wolkersdorf, although this operation included first the anaerobic and then aerobic conditions. During settling and decant, NO_3-N was nearly constant since there was no further contact between the settled biomass and the purified wastewater limiting biological reactions.

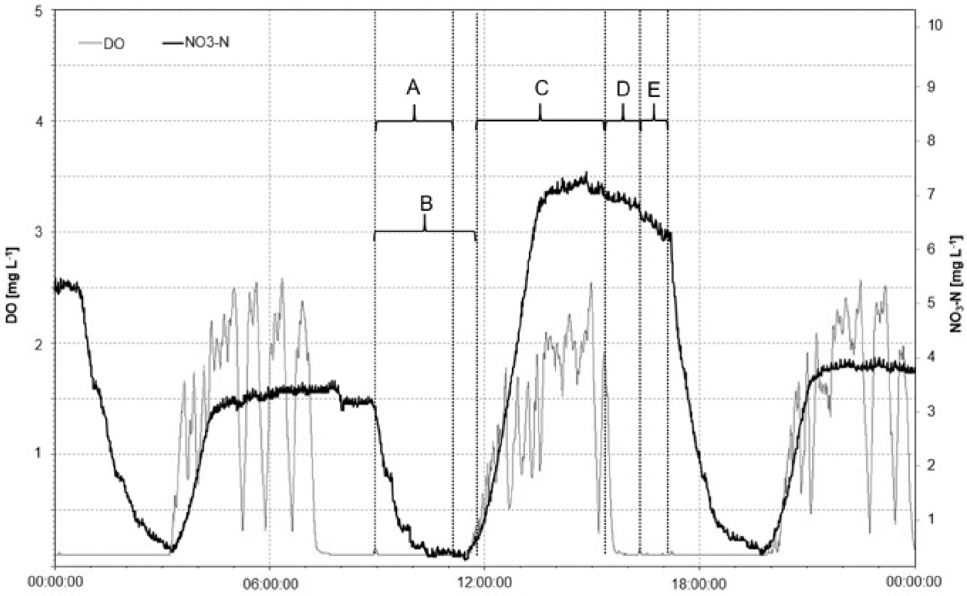

Figure 6.6: Exemplary cycle for SBR 3 (A: Feeding, B: Denitrification, C: Aeration, D: Sedimentation, E: Decant), NO_3-N and DO under dry weather operation

During the year, TSS in the SBR ranged between 3.4 and 3.7 g L^{-1} with an ash content of 67%. Excess sludge production varied between 0.38 and 0.40 gTSS $gCOD^{-1}$, which is in line with the theoretical excess sludge production calculated according to ATV-DVWK-A 131 (2016). Muda et al. (2011) reported an excess sludge production in the range of 0.24 to 0.41 gTSS $gCOD^{-1}$ for AGS, with a decreasing sludge production correlated to an increasing sludge retention time (SRT). Pronk et al. (2015b) reported a SRT of 20 to 38 d for a large-scale Nereda plant. At the WWTP Wolkersdorf, the SRT amounted to approx. 40 to 50 d, being in the range of plants operated for AGS.

The specific energy consumption of the biological stage was 21.6 kWh (p.e. a)$^{-1}$ with a total energy consumption of approx. 43 kWh (p.e. a)$^{-1}$. These values are in the common range for Austrian WWTP with aerobic stabilization and an average energy consumption of 43 kWh (p.e. a)$^{-1}$ (Haslinger et al. 2016).

6.7 Discussion

The presented data of the SVI, particle size distribution and the microscopic images confirm that the activated sludge of the WWTP Wolkersdorf have great similarities to AGS. In the following part, potential reasons for the aerobic granular sludge formation on this WWTP are discussed considering known parameters for improving the sludge structure and settling behavior.

In order to face an upcoming demographic increase of the region, the WWTP has currently some spare capacity. Although, the sewage is characterized by high COD concentration (mean 770 mg L^{-1}), the average OLR was 0.06 kgCOD $kgTSS^{-1}$ d^{-1} and thus in a range reported by Pronk et al. (2015b) for a large-scale Nereda plant. De Kreuk and van Loosdrecht (2006) state that the COD load is an important process parameter for large-scale operation, especially the availability of carbon during the feed is essential for the granulation. High concentrations of organic substances allow a deep diffusion into the core of the granules; consequently, the substrate is also available to microorganisms in the inner zones. This increased diffusion effect is strengthened by high substrate concentrations and can be seen as a driving force towards larger granules. Furthermore, the availability of the carbon source was found to effect the granules formation too. Adler et al. (2017) investigated the AGS structure and nitrogen removal under different substrates and found the largest granules for the reactor fed with volatile fatty acids. An easy available carbon source is almost completely stored during the anaerobic feed, which causes a slow grow rate of the heterotrophs with a compact granules formation. In contrast, more complex substrates and particulate COD were found to promote the growth of irregular structures (Adler et al. 2017, de Kreuk et al. 2010, Wagner et al. 2015). An irregular growth appears, when particulate substrates are not completely hydrolyzed during the anaerobic phase. Thus, the substrate is available in the aerated phase and favors a fast heterotrophic growth that can lead to irregular forms (Adler et al. 2017). The sewage to the WWTP Wolkersdorf is dominated by a large portion of industrial wastewater from food industry, which is reflected in the lower COD/BOD_5 ratio. Thus, it is assumed, that in the present study an easily available carbon source contributes to the regular formation of granules since the COD is stored during the anaerobic phase. In the case of an incomplete COD uptake, COD is available in the aerobic phase and can cause an irregular growth (van den Akker et al. 2015). A possibility to improve the anaerobic COD uptake is the enlargement of the anaerobic contact time with an increased hydrolysis of particulate organic matter (Wagner et al. 2015). A further driver for the granulation is the nutrient to carbon ratio. The average N/COD ratio of the influent to the WWTP Wolkersdorf was 0.06. This low N/COD ratio of the wastewater promotes a high nitrogen removal (denitrification), which in turn ensures enlarged anaerobic conditions during the feed.

Furthermore, it was assumed that the metalworking company and the increased heavy metals are beneficial to the sludge structure by increasing the weight of the activated sludge. Fig. 6.7 shows the heavy metal concentrations in the sewage sludge of the WWTP Wolkersdorf in comparison to average values from Germany (BMU, 2015) and Austria (Oliva et al. 2009). The data indicate that the copper, nickel and chrome concentrations were only slightly increased compared to the published average values, while other heavy metals were in the common range for municipal WWTP. Thus, an effect of the heavy metals on the sludge structure due to metal precipitation can be excluded.

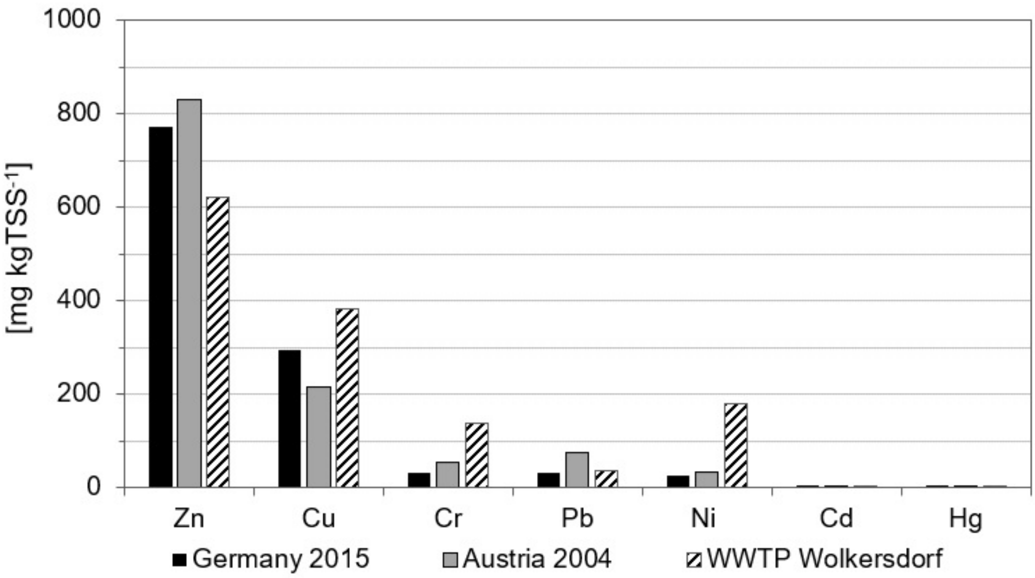

Figure 6.7: Heavy metals contents in sewage sludge compared to average values from Germany (average) and Austria (median)

Operational data of the WWTP Wolkersdorf indicate that it is possible to develop aerobic granules on a full-scale SBR plant. The operation was contradictory to the known AGS process, since there was no plug-flow feed applied. Generally, the anaerobic plug-flow feed without mixing is considered as the most favorable feeding strategy since they result in high substrate gradients in the sludge bed, which promote the growth of substrate-storing organisms.

Aerobic granulation is mostly linked to the accumulation of substrate-storing organisms, whereby the operation mode is primarily intended to promote the enrichment of phosphate-accumulating organisms (PAO). PAO are able to create polyphosphate storage and promote the aerobic granulation due to a slow growth rate. Furthermore, P-accumulating granules are characterized by an overall higher density (Winkler et al. 2011). Lin et al. (2003) investigated the effect of different P/COD ratios on the SVI and specific gravity of aerobic granules and found a higher specific gravity and lower SVI with increasing P/COD ratios. This observation was explained by the fact that a higher phosphate availability promotes the accumulation of P-storing organisms. Aerobic granules that were fed with a P/COD ratio of 0.01 in the wastewater had a SVI of approx. 30 ml g^{-1}. These results are similar to the sludge of the WWTP Wolkersdorf where the mean P/COD ratio was 0.01. Based on the operating data, the ß-value of the system was calculated. This parameter indicates the molar relationship between the precipitant consumption and the removed phosphorus. A ß-value below 1.0 usually indicates an enhanced biological phosphorus removal (EBPR). The annual average of the ß-value of the WWTP Wolkersdorf was above 1.5 and thus in a range for municipal WWTP without EBPR. In addition to PAO, aerobic granulation can also occur by the accumulation of glycogen-storing bacteria (GAO) (de Kreuk and van Loosdrecht 2004). These organisms are able to create storage polymer like PHB without an increased P-uptake. Unfortunately, there are no data available for the microbial composition of the sludge. Thus, it is recommended to differentiate the microbial composition by further investigations via DNA sequencing or FISH.

On the WWTP Wolkersdorf, the feeding is applied during denitrification by a pipeline from above, i.e. no plugflow conditions are possible. However, due to the relatively low NO_3-N concentrations in the effluent, it was proven that anaerobic conditions are achieved within the denitrification and feeding phase. Compared to previous large-scale Nereda systems, the activated sludge of the WWTP Wolkersdorf is stirred during feeding as well as between the aeration intervals. The influence of stirrers on the aerobic granular biomass has not yet been fully described yet; only a few studies investigated the effect of stirrers on the aerobic granular sludge. For example, Nor Anuar et al. (2007) found a slightly decreased settling velocity with an increased shear stress. Rocktäschel et al. (2013) reported smaller granules as a result of stirring during the anaerobic feed compared to complete plug-flow feeding conditions. However, this observation is probably more related to the lower substrate gradient caused by mixing. SBR tanks of the WWTP Wolkersdorf are equipped with hyperboloid stirrers. This type of stirrer is generally considered to be gentle to the sludge structure since the flow direction of the particles is only switched and the particles are not destroyed by the mixing plates. The overall mixing time is quite short with approx. 4–5 h per day.

Large-scale plants, which are designed according to the Nereda technology, are characterized by a simultaneous feeding and decant phase (Pronk et al. 2015b). In the case of the WWTP Wolkersdorf, the sedimentation takes place over a period of 60 min with a separate withdrawal of likewise 60 min. A reduction of the settling time is not necessary, as the capacity of the plant is currently not limited. Flocs are kept in the system, which allows the assumption that the washout of slow settable biomass is less important for the granulation than the need for anaerobic feeding conditions.

6.8 Summary

The operational data of the WWTP Wolkersdorf indicates that aerobic granular sludge with excellent settling properties (SVI 30 to 50 ml g^{-1}) developed at a full-scale SBR plant treating municipal and industrial wastewater without special design considerations for aerobic granular sludge. The granules formation on this plant can be seen as an interaction of different favorable parameters. Especially, the anaerobic feed was identified as a driving force for aerobic granules. Moreover, the sewage composition with an increased proportion of readily available carbon and a low N/COD ratio is favorable for the formation of granules on this WWTP. The results show that a high selection pressure (short settling time) is not crucial to form aerobic granules. Our conclusion of this study is that the presented SBR operation promotes the formation of granules-like aggregates, since there was a long filling time applied under non-aerated conditions. We assume that changing the SBR operation mode towards this strategy could have a positive effect on the sludge settling of existing SBR plants.

6.9 Acknowledgement

We want to thank the operators of the WWTP Wolkersdorf for providing operational data and for supporting this work by giving insight in the plant operation.

Chapter 7

Summary and Conclusion

Although the wastewater treatment with aerobic granular sludge has proven itself suitable for SBR operation, which is confirmed by a rising number of full-scale plants, the effects on subsequent treatment processes remain still unclear. The present thesis comprises three research questions for AGS operation, which were investigated by the help of laboratory experiments. The lab-scale tests were partly carried out within the framework of the research project "Granular activated sludge process in continuous-flow plants" (Grant number: GZ B401949), which was supported by the Austrian Federal Ministry of Sustainability and Tourism. In a fourth question, the reasons for the formation of aerobic granules on a full-scale SBR plant were investigated by the evaluation of operational data.

In the first objective, it was investigated under which conditions an aerobic granulation can be realized in continuous-flow plants (CFP). From prior studies it is known that stable AGS formation requires anaerobic feeding conditions to allow the enrichment of substrate-storing organisms. A continuous-flow setup was chosen, which included an anaerobic plug-flow reactor to ensure high substrate gradients. A SBR was operated with AGS in parallel to compare the biomass in terms of the particle sizes and settling properties. Togehter with the applied settings it was possible to enrich PAO, which was evident in the release of phosphate during anaerobic conditions. CFP operation resulted in a declining SVI to approx. 85 ml g^{-1}. The particle size distribution of the sludge from the CFP showed that 72% of the particle sizes were above 200 μm, which is a characteristic size of granules. SV_{10}/SV_{30} ratio was 1.2 and thus slightly above the sludge volume ratios of AGS cultivated in the SBR. Compared to the SBR operation, the granules formation in continuous-flow was less pronounced. The results indicate that the applied settings have contributed to an improved settling behavior, however, the granulation in the SBR was much more apparent with overall larger sizes and lower SVI. In contrast to the SBR operation, the use of agitators and pumps is required in continuous-flow systems to avoid sludge deposits; thus, negative effects of the technical advices on the granulation process cannot be excluded and should therefore be investigated in further studies. The separation of the flocculent biomass was not controlled, which was probably the reason for the increased amount of sludge flocs. A clear deterioration of the settling properties could be associated with the appearance of nitrate and illustrates the importance to achieve sufficient nitrogen removal and anaerobic feeding conditions.

A second research question was set to the effects on the subsequent downstream processes such as the anaerobic stabilization and dewaterability of AGS. So far, only a few studies relate to the anaerobic biodegradability of aerobic granular sludge, further investigations were performed to evaluate the methane production as well as COD and VSS degradation. The specific methane production of AGS ranged from 263 to 285 $mlCH_4$ $gVSS^{-1}$ and 178 to 209 $mlCH_4$ $gCOD^{-1}$. VSS removal was calculated with 51 and 59%, which indicates an excellent biodegradation of AGS. The experiments for assessing the dewaterability of AGS revealed an overall lower dewatering behavior for the digested AGS samples, which was confirmed by a lower TSS content in the dewatered samples after centrifugation. Reasons for the poorer water separation may be found in an increased EPS content. The measured tryptophan in the extracted EPS matrix of AGS was twice as high than for the EPS from a suspended sludge. Further reasons for the poorer dewaterability can be an increased VSS content, since there was no targeted P removal applied and no precipitants dosed to the lab-scale reactors. The increased phosphate level due to the biological phosphate removal and the phosphate release under anaerobic conditions can also be considered as reasons for a poorer dewaterability. Overall, it can be inferred from the results that AGS owns a good biodegradability, but a reduced water separation behavior after digestion, which in turn can increase the disposal costs. Due to the nature of the experimental setup, the results need to be verified with large-scale dewatering aggregates.

The third research question belonged to the emissions of N_2O from aerobic granular sludge. The measurements reveal that N_2O is formed mainly during nitrification with emission factors in a range from 0.54 to 4.8%. Literature referencens for full-scale activated sludge plants operated with comparable OLR name a similar range with emission factors up to 1.5%. Overall, higher N_2O emissions were observed with rising OLR and nitrite levels (> 3 mg L^{-1}). The operation with constant aerobic conditions resulted in a higher nitrite accumulation and N_2O formation compared to an intermittent aeration strategy. However, the intermittent aeration allowed increased TN removal compared to the constant aeration strategy . The results indicate that the same parameters (OLR, nitrite) contribute to increased N_2O emissions in AGS as in conventional activated sludge systems.

Moreover, a full-scale WWTP (SBR) was investigated, where the biomass offers excellent settling properties throughout the year with SVI ranging from 30 to 50 ml g^{-1}. Since this plant is not specially operated for aerobic granular sludge formation, the question was, which settings contribute to the excellent settling properties and the formation of aerobic granules on this WWTP. Microscopic image of the sludge showed a high amount of granules with approx. 75% of the measured particles being larger than 200 μm. Operational data of one year were evaluated. The SBR cycle comprises a long denitrification period, where the wastewater is supplied to the reactor. Online measurements reveal that this operation strategy ensures anaerobic conditions during denitrification, which is a widely applied operation strategy for aerobic granulation. Moreover, the sewage also comprises industrial wastewater with an increased amount of easy available carbon. The presented data illustrate that under suitable operation with anoxic-anaerobic feeding conditions, the formation of aerobic granules is possible at WWTP without a special design for AGS.

In this study, the wastewater treatment with aerobic granules was found to be a promising technology reaching excellent cleaning results and anaerobic biodegradation as well as comparable nitrous oxides emissions as for conventional activated sludge. The benefits of the AGS treatment process belong mainly to the structure of the biomass, which allows the design of compact WWTPs. Although it has to be mention that a lot of plants already reach low SVI due to suitable wastewater characteristics or adjusted plant design. For example, the application of anaerobic zones for suppressing filamentous growth was found to be a successful strategy for improving the settling properties of the biomass. Anaerobic or aerobic selectors are often implemented for full-scale WWTPs in Austria. The experiments clearly reveal that the AGS structure depends on the nitrogen removal, which indicates that the process requires very low nitrate concentrations to enable the growth of substrate-storing organisms. These organisms are required for stable granules formation. Although the performance under lab-scale conditions allows an easy handling of the settings, the full-scale plant operation will be challenged by realizing low nitrate levels. Nitrate removal is often limited to the nutrient ratios of the incoming wastewater or the plant design. Thus, the AGS technology is confronted with both requirements, excellent nitrogen removal and stable granules formation. Since currently, a lot of aerobic granular sludge plants are still in the construction phase, the stable long-term operation of AGS plants has to prove itself in the future.

References

Ab Halim, M.H., Anuar, A.N., Azmi, S.I., Abdul Jamal, N.S., Wahad, N.A., Ujang, Z., Schraim, A., Bob, M.M. (2015) Aerobic sludge granulation at high temperatures for domestic wastewater treatment. Bioresour Technol 185, 445-449.

Ab Halim, M.H., Anuar, A.N., Azmi, S.I., Abdul Jamal, N.S., Wahad, N.A., Ujang, Z., Bob, M.M. (2016) Influence of high temperature on the performance of aerobic granular sludge in biological treatment of wastewater. J Environ Manage 184(2), 271-280.

Adav, S.S., Lee, D.J., Ren, N.Q. (2007) Biodegradation of pyridine using aerobic granules in the presence of phenol. Water Res 41(13), 2903-2910.

Adav, S.S., Lee, D.J. (2008a) Extraction of extracellular polymeric substances from aerobic granule with compact interior structure. J Hazard Mater 154(1-3), 1120-1126.

Adav, S.S., Lee, D.J., Tay, J.H. (2008b) Extracellular polymeric substances and structural stability of aerobic granule. Water Res 42(6-7), 1644-1650.

Adav, S.S., Lee, D.J., Lai, J.Y. (2009) Functional consortium from aerobic granules under high organic loading rates. Bioresour Technol, 100, 3465-3470.

Adler, A., Reynaert, E., Layer, M., Derlon, N., Morgenroth, E., Holliger, C. (2017) Influence of wastewater compositon on microbial communities of aerobic granules and their nutriert removal performance. IWA (ed), 10th International Conference on Biofilm Reactors, Dublin.

Angela, M., Beatrice, B., Mathieu, S. (2011) Biologically induced phosphorus precipitation in aerobic granular sludge process. Water Res 45(12), 3776-3786.

Arrojo, B., Mosquera-Corral, A., Garrido, J.M., Mendez, R. (2004) Aerobic granulation with industrial wastewater in sequencing batch reactors. Water Res 38(14-15), 3389-3399.

ATV (1994) ATV-Merkblatt M 208: Biologische Phosphorentfernung bei Belebungsanlagen, Biological phosphorus removal for activated sludge plants, GFA, Hennef.

Bassin, J.P., Pronk, M., Muyzer, G., Kleerebezem, R., Dezotti, M., van Loosdrecht, M.C. (2011a) Effect of elevated salt concentrations on the aerobic granular sludge process: linking microbial activity with microbial community structure. Appl Environ Microbiol 77(22), 7942-7953.

Bassin, J.P., Pronk, M., Kraan, M., Kleerebezem, van Loosdrecht, M.C. (2011b) Ammonium adsorption in aerobic granular sludge, activated sludge and anammox granules. Water Res 45(16), 5257-5265.

Bassin, J.P., Kleerebezem, R., Dezotti, M., van Loosdrecht, M.C. (2012a) Simultaneous nitrogen and phosphate removal in aerobic granular sludge reactors operated at different temperatures. Water Res 46(12), 3805-3816.

Bassin, J.P., Winkler, M.K., Kleerebezem, R., Dezotti, M., van Loosdrecht, M.C. (2012b) Improved phosphate removal by selective sludge discharge in aerobic granular sludge reactors. Biotechnol Bioeng 109(8), 1919-1928.

Bathe, S., De Kreuk, M.K., McSwain, B.S., Schwarzenbeck, N. (2005) Aerobic granuler sludge. Water and Environmental Management Series (WEMS), London.

Bengtsson, S., de Blois, M., Wilén, B.-M., Gustavsson, D. (2018) Treatment of municipal wastewater with aerobic granular sludge. Critical Reviews in Environmental Science and Technology 48(2), 119-166.

Bengtsson, S., de Blois, M., Wilén, B.-M., Gustavsson, D. (2019) A comparison of aerobic granular sludge with conventional and compact biological treatment technologies. Environmental technol 40(21), 2769-2778.

Benstöm F., Oelscheid, S., Klasen, H., Bruszies, D., Niehof, H. Alt, K., Pinnekamp, J. (2019) Aerober Granulierter Schlamm in Sequencing-Batch-Reaktoren (AGS-SBR)Quo Vadis Deutschland? Aerobic Granulated Sludge in Sequencing Batch Reactors (AGS-SBR) Quo Vadis Germany? Korrespondenz Abwasser, Abfall 66(3), DWA, Hennef.

Bergmans, B.J., Veltman, A.M., van Loosdrecht, M.C., van Lier, J.B., Rietveld, L.C. (2014) Struvite formation for enhanced dewaterability of digested wastewater sludge. Environ Technol 35(5-8), 549-555.

Beun, J.J., Hendriks, A., van Loosdrecht, M.C.M., Morgenroth, E., Wilderer, P.A., Heijnen, J.J. (1999) Aerobic granulation in a sequencing batch reactor. Water Res 33(10), 2283–2290.

BMU (2018) Roskosch, A. and Heidecke, P. Klärschlammentsorgung in der Bundesrepublik Deutschland, Sewage sludge disposal in Germany, Fachgebiete III 2.4 – Abfalltechnik, Abfalltechniktransfer und III 2.5 – Überwachungsverfahren, Abwasserentsorgung, Umweltbundesamt, ISSN (Print) 2363-8311.

Cassidy, D.P. and Belia, E. (2005) Nitrogen and phosphorus removal from an abattoir wastewater in a SBR with aerobic granular sludge. Water Res 39(19), 4817-4823.

Castelle, C.J., Brown, C.T., Thomas, B.C., Williams, K.H., Banfield, J.F. (2017) Unusual respiratory capacity and nitrogen metabolism in a Parcubacterium (OD1) of the Candidate Phyla Radiation. Sci Rep 7, 40101.

Cetin, S. and Erdincler, A. (2004) The role of carbohydrate and protein parts of extracellular polymeric substances on the dewaterability of biological sludges. Water Sci Technol 50(9), 49-56.

Chalk, P.M., Smith, C.J. (1983) Gaseous Loss of Nitrogen from Plant–Soil Systems. J.R. Freney, J.R.S. (ed), Springer Netherlands, Dordrecht, The Hague, ISBN: 9789024728206.

Chen, W., Westerhoff, P., Leenheer, J., Booksh, K. (2003) Fluorescence Excitation - Emission Matrix Regional Integration to Quantify Spectra for Dissolved Organic Matter. Environ Sci Technol 37(24), 5701-5710.

Chen, F.Y., Liu, Y.Q., Tay, J.H., Ning, P. (2011) Operational strategies for nitrogen removal in granular sequencing batch reactor. J Hazard Mater 189(1-2), 342-348.

Chen, X., Yuan, L., Lu, W., Li, Y., Liu, P., Nie, K. (2013) Cultivation of aerobic granular sludge in a conventional, continuous flow, completely mixed activated sludge system. Frontiers of Environmental Science and Engineering 9(2), 324-333.

Chen, C., Ming, J., Yoza, B.A., Liang, J., Li, Q.X., Guo, H., Liu, Z., Deng, J., Wang, Q. (2019) Characterization of aerobic granular sludge used for the treatment of petroleum wastewater. Bioresour Technol 271, 353-359.

Corsino, S.F., Campo, R., Di Bella, G., Torregrossa, M., Viviani, G. (2016) Study of aerobic granular sludge stability in a continuous-flow membrane bioreactor. Bioresour Technol 200, 1055-1059.

Dai, X., Luo, F., Dai, L., Dong, B. (2013) Degradation of Extracellular Polymeric Substances (EPS) in Anaerobic Digestion of Dewatered Sludge. Procedia Environmental Sciences 18, 515-521.

de Blois, M., Viberg, M. (2018) Heavy sludge production in a conventional SBR in Sweden, IWA Biofilm Conference: Granular Sludge Systems, H2OLand, 18.-21.03.2018, Delft.

de Kreuk, M.K. and van Loosdrecht, M.C.M. (2004) Selection of slow growing organisms as a means for improving aerobic granular sludge stability. Water Sci Technol 49(11-12), 9-17.

de Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C. (2005a) Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. Biotechnol Bioeng 90(9), 761-769.

de Kreuk, M.K., Pronk, M., van Loosdrecht, M.C. (2005b) Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures. Water Res 39(18), 4476-4484.

de Kreuk, M.K., van Loosdrecht, M.C.M. (2006) Formation of aerobic granules with domestic sewage. J Environ Eng 132(6), 694-697.

de Kreuk (2006) Aerobic granular sludge: Scaling up a new technology. Doctoral Thesis, Technical University Delft, ISBN-13: 978-90-9020767-4.

de Kreuk, M.K., Kishida, N., van Loosdrecht, M.C.M. (2007a) Aerobic granular sludge – state of the art. Water Sci Technol 55(8-9), 75.

de Kreuk, M.K., Picioreanu, C., Hosseini, M., Xavier, J.B., van Loosdrecht, M.C. (2007b) Kinetic model of a granular sludge SBR: influences on nutrient removal. Biotechnol Bioeng 97(4), 801-815.

de Kreuk, M.K., Kishida, N., Tsuneda, S., Loosdrecht, M.C.M. (2010) Behavior of polymeric substrates in an aerobic granular sludge system. Water Res 44(20), 5929-5938.

Derlon, N., Wagner, J., Ribeiro da Costa, R.H.R., Morgenroth, E. (2016) Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume. Water Res 105, 341-350.

DWA (2009) Merkblatt DWA-M 210: Belebungsanlagen mit Aufstaubetrieb (SBR), Activated sludge plants with filling operation (SBR), Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e. V., Hennef.

DWA (2011a) Ermittlung und Bewertung der biologischen und chemischen Phosphorelimination bei der Simultanfällung, Determination and Assessment of Biological and Chemical Phosphorus Removal during Simultaneous Precipitation. KA - Korrespondenz Abwasser, Abfall 5, GFA Verlag, 456-464.

DWA (2011b) Merkblatt DWA-M 202: Chemisch-physikalische Verfahren zur Elimination von Phosphor aus Abwasser, Chemical-physical processes for the elimination of phosphorus from wastewater, Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e. V., Hennef.

DWA (2014) Merkblatt DWA-M 368: Biologische Stabilisierung von Klärschlamm, DWA Deutsche Vereinigung für Wasserwirtschaft, Biological stabilization of sewage sludge, Abwasser und Abfall e. V., Hennef.

DWA (2016) Arbeitsblatt DWA-A 131 DWA-Regelwerk: Bemessung von einstufigen Belebungsanlagen, Design of single-stage activated sludge plants, DWA Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e. V., Hennef.

Fang, HHP, Xu, LC, Chan, KY (2002) Effects of toxic metals and chemicals on biofilms and biocorrosion. Water Res 36(19), 4709–16.

Fang, F., Liu, X.-W., Xu, J., Yu, H.-Q., Li, Y.-M. (2009) Formation of aerobic granules and their PHB production at various substrate and ammonium concentrations. Bioresour Technol 100, 59-63.

Flemming, H.C., Leis, A. (2002) Sorption properties of biofilms, in Encyclopedia of Environmental Microbiology., ed. by Flemming, H.C. and Bitton, G. John Wiley and Sons Ltd., New York, 2958–2967.

Flowers, J.J., He, S., Yilmaz, S., Noguera, D.R. and McMahon, K.D. (2009) Denitrification capabilities of two biological phosphorus removal sludges dominated by different "Candidatus Accumulibacter" clades. Environ Microbiol Rep 1(6), 583-588.

Foley, J., de Haas, D., Yuan, Z., Lant, P. (2010) Nitrous oxide generation in full-scale biological nutrient removal wastewater treatment plants. Water Res 44(3), 831-844.

Fox, B.G., Thorn, R.M.S., Anesio, A.M., Reynolds, D.M. (2017) The in situ bacterial production of fluorescent organic matter; an investigation at a species level. Water Res 125, 350-359.

Gao, M., Liu, L., lianga, H., Wu, W.M. (2011) Comparison of four enhancement strategies for aerobic granulation in sequencing batch reactors. Journal of Hazardous Materials 186(1), 320–327.

Gao, M., Yang, S., Wang, M., Wang, X.H. (2016) Nitrous oxide emissions from an aerobic granular sludge system treating low-strength ammonium wastewater. J Biosci Bioeng 1228(5), 601-605.

Giesen, A. and Thompson, A. (2013) Aerobic granular biomass for cost-effective, energy efficient and sustainable wastewater treatment, in: 7th European Waste Water Management Conference.

Gobi, K. and Vadivelu, V. (2014) Aerobic dynamic feeding as a strategy for in situ accumulation of polyhydroxyalcanoate in aerobic granules. Bioresour Technol 161, 441-445.

Gobi, K. and Vadivelu, V. (2015) Polyhydroxyalkanoate recovery and effect of in situ extracellular polymeric substances removal from aerobic granules. Bioresour Technol 180, 169-176.

Gonzalez-Martinez, A., Munoz-Palazon, B., Rodriguez-Sanchez, A., Maza-Marquez, P., Mikola, A., Gonzalez-Lopez, J., Vahala, R. (2017) Start-up and operation of an aerobic granular sludge system under low working temperature inoculated with cold-adapted activated sludge from Finland. Bioresour Technol 239, 180-189.

Guo, X., Wang, X., Liu, J. (2016) Composition analysis of fractions of extracellular polymeric substances from an activated sludge culture and identification of dominant forces affecting microbial aggregation. Sci Rep 6, 28391.

Guo, N., Zhang, J., Xie, H.-J., Tan, L.-R., Luo, J.-N., Tao, Z.-Y., Wang, S.-G. (2017) Effects of the Food-to-Microorganism (F/M) Ratio on N_2O Emissions in Aerobic Granular Sludge Sequencing Batch Airlift Reactors. Water 9(7), 477.

Haslinger, J., Lindtner, S., Krampe, J. (2016) Operating costs and energy demand of wastewater treatment plants in Austria: benchmarking results of the last 10 years. Water Sci Technol 74(11), 2620-2626.

Hogendoorn, A. (2013) Enhanced digestion and alginate-like-exopolysaccharides extraction from Nereda sludge, Master thesis, Sanitary Engineering Section, Department of Water Management Faculty of Civil Engineering and Geosciences Delft University of Technology, Delft.

Hu, Z., Wentzel, M.C., Ekama, G.A. (2002) Anoxic growth of phosphate-accumulating organisms (PAOs) in biological nutrient removal activated sludge systems. Water Res 36(19), 4927–4937.

Hu, Z., Zhang, J., Xie, H., Li, S., Zhang, T., Wang, J. (2011) Identifying sources of nitrous oxide emission in anoxic/aerobic sequencing batch reactors (A/O SBRs) acclimated in different aeration rates. Enzyme Microb Technol 49(2), 237-245.

Huang, W., Cai, W., Huang, H., Lei, Z., Zhang, Z., Tay, J.H. and Lee, D.J. (2015) Identification of inorganic and organic species of phosphorus and its bio-availability in nitrifying aerobic granular sludge. Water Res 68, 423-431.

Hobus, I., Kolisch, G., Gengnagel, D. (2020) Kläranlage der Zukunft - Verfahrenskonzepte und Strategien für den Wupperverband. Sewage treatment plant of the future - process concepts and strategies for the Wupper Association. Wiener Mitteilungen 252, Wien.

IPCC – Intergovernmental Panel on Climate Change (2007) Climate Change 2007 – Impacts, Adaptation and Vulnerability. 4th Assessment Report.

Ishii, S.K., Boyer, T.H. (2012) Behavior of reoccurring PARAFAC components in fluorescent dissolved organic matter in natural and engineered systems: a critical review. Environ Sci Technol 46(4), 2006-2017.

Jahn, L., Schaar, H., Svardal, K., Krampe, J. (2017) Granulares Belebtschlammverfahren in kontinuierlich durchflossenen Belebungsanlagen, Granular activated sludge process in continuous-flow plants. Bundesministerium für Nachhaltigkeit und Tourismus, Wien.

Jahn, L., Svardal, K., Krampe, J. (2019) Comparison of aerobic granulation in SBR and continuous-flow plants. J Environ Manage 231, 953-961.

Jiang, H.L., Tay, J.H., Tay, S.T. (2004) Changes in structure, activity and metabolism of aerobic granules as a microbial response to high phenol loading. Appl Microbiol Biotechnol 63(5), 602-608.

Jin, B., Wilén, B.-M., Lant, P. (2004) Impacts of morphological, physical and chemical properties of sludge flocs on dewaterability of activated sludge. Chemical Engineering Journal 98(1-2), 115-126.

Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S., van Loosdrecht, M.C. (2009) Nitrous oxide emission during wastewater treatment. Water Res 43(17), 4093-4103.

Kim, I.S., Kim, S.M., Jang, A. (2008) Characterization of aerobic granules by microbial density at different COD loading rates. Bioresour Technol 99(1), 18-25.

Kjelleberg, S., Hermansson, M. (1984) Starvation-induced effects on bacterial surface characteristics. Applied and Environmental Microbiology 48, 497-503.

Knoop, S. and Kunst, S. (1998) Influence of temperature and sludge loading on activated sludge settling, especially on Microthrix parvicella. Water Sci Technol 37(4-5), 27-35.

Kopp, J. and Dichtl, N. (1998) Influence of Surface Charge and Exopolysaccharides on the Conditioning Characteristics of Sewage Sludge. Chemical Water and Wastewater Treatment V, 285-296.

Kopp, J. (2001) Wasseranteile in Klärschlammsuspensionen: Messmethode und Praxisrelevanz, Water content in sewage sludge suspensions: Measuring method and practical relevance, Institut für Siedlungswasserwirtschaft, Band 66, Technische Universität Braunschweig.

Kopp, J. (2017) Optimierung der Klärschlammentwässerung, Optimization of sewage sludge dewatering. Oral Presentation, 49. Aargauische Klärwärtertagung, Suhr, Department Bau, Verkehr und Umwelt, Kanton Argau.

Kristensen, G.H., Jørgensen, P.E., Nielsen, P.H. (1994) Settling Characteristics of Activated Sludge in Danish Treatment Plants with Biological Nutrient Removal. Water Sci Technol 29(7), 157-165.

Kromidas, S. (2011) Handbuch Validierung in der Analytik, Manual Validation in analytics. Wiley-VCH, ISBN: 9783527298112.

Laspidou, C.S., Rittmann, B.E. (2002) A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. Water Res 36(11), 2711–2720.

Layer, M., Adler, A., Reynaert, E., Hernandez, A., Pagni, M.,Morgenroth, E., Holliger, C., Derlon, N. (2019) Organic substrate diffusibility governs microbial communitycomposition, nutrient removal performance and kinetics ofgranulation of aerobic granular sludge. Water Res X 4(1), 100033.

Leenen, E.J.T.M. (1996) Nitrification by Artificially Immobilized Cells: Model and Practical System. Ph.D thesis, Wageningen Agricultural University, Wageningen.

Lehmann, C. and Kasper, M. (2017) Nereda - Leistungsfähiges biologisches Abwasserbehandlungsverfahren für die Schweiz. Nereda - Efficient biological wastewater treatment process for Switzerland, Aqua and Gas 1.

Lemaire, R., Meyer, R., Taske, A., Crocetti, G.R., Keller, J. and Yuan, Z. (2006) Identifying causes for N_2O accumulation in a lab-scale sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal. J Biotechnol 122(1), 62-72.

Li, X.Y., Yang, S.F. (2007) Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. Water Res 41(5), 1022–1030.

Li, A.J., Yang, S.F., Li, X.Y. and Gu, J.D. (2008) Microbial population dynamics during aerobic sludge granulation at different organic loading rates. Water Res 42(13), 3552-3560.

Li, J., Ma, L., Wei, S. and Horn, H. (2013) Aerobic granules dwelling vorticella and rotifers in an SBR fed with domestic wastewater. Separation and Purification Technology 110, 127-131.

Li, J., Ding, L.B., Cai, A., Huang, G.X. and Horn, H. (2014) Aerobic sludge granulation in a full-scale sequencing batch reactor. Biomed Res Int 2014, 268789.

Li, J., Cai, A., Ding, L., Sellamuthu, B. and Perreault, J. (2015) Aerobic sludge granulation in a Reverse Flow Baffled Reactor (RFBR) operated in continuous-flow mode for wastewater treatment. Separation and Purification Technology 149, 437-444.

Li, D., Lv, Y., Zeng, H. and Zhang, J. (2016) Enhanced biological phosphorus removal using granules in continuous-flow reactor. Chemical Engineering Journal 298, 107-116.

Lin, Y.M., Liu, Y. and Tay, J.H. (2003) Development and characteristics of phosphorus-accumulating microbial granules in sequencing batch reactors. Appl Microbiol Biotechnol 62(4), 430-435.

Lin, Y., de Kreuk, M.K., van Loosdrecht, M.C. and Adin, A. (2010) Characterization of alginate-like exopolysaccharides isolated from aerobic granular sludge in pilot-plant. Water Res 44(11), 3355-3364.

Lin, Y.M., Nierop, K.G.J., Girbal-Neuhauser, E., Adriaanse, M. and van Loosdrecht, M.C.M. (2015) Sustainable polysaccharide-based biomaterial recovered from waste aerobic granular sludge as a surface coating material. Sustainable Materials and Technologies 4, 24-29.

Liu, H. and Fang, H.H.P. (2002) Extraction of extracellular polymeric substances. Journal of Biotechnology 95(3), 249-256.

Liu, Y. and Tay, J.H. (2002) The essential role of hydrodynamic shear force in the formation of the biofilm and granular sludge. Water Res 36(7), 1653-1665.

Liu, Y. and Tay, J.H. (2004) State of the art of biogranulation technology for wastewater treatment. Biotechnol Adv 22(7), 533-563.

Liu, Y., Yang, S.F. and Tay, J.H. (2004) Improved stability of aerobic granules by selecting slow-growing nitrifying bacteria. J Biotechnol. 108(2), 161-169.

Liu, Y., Liu, Q.S. (2006a) Causes and control of filamentous growth in aerobic granluar sluge. Biotechnol Adv 24(1), 115-127.

Liu, Y. Q. and Tay, J. H. (2006b) Variable aeration in sequencing batch reactor with aerobic granular sludge. J Biotechnol 124(2), 338–346.

Liu, Y.Q., Moy, B., Kong, Y.H. and Tay, J.H. (2010) Formation, physical characteristics and microbial community structure of aerobic granules in a pilot-scale sequencing batch reactor for real wastewater treatment. Enzyme Microb Technol 46(6), 520-525.

Liu, H., Li, Y., Yang, C., Pu, W., He, L. and Bo, F. (2012) Stable aerobic granules in continuous-flow bioreactor with self-forming dynamic membrane. Bioresour Technol 121, 111-118.

Liu, Y.Q., Zhang, X., Zhang, R., Liu, W.T. and Tay, J.H. (2016) Effects of hydraulic retention time on aerobic granulation and granule growth kinetics at steady state with a fast start-up strategy. Appl Microbiol Biotechnol 100(1), 469-477.

Lochmatter, S., Gonzalez-Gil, G. and Holliger, C. (2013) Optimized aeration strategies for nitrogen and phosphorus removal with aerobic granular sludge. Water Res 47(16), 6187-6197.

Lochmatter, S., Maillard, J. and Holliger, C. (2014) Nitrogen Removal over Nitrite by Aeration Control in Aerobic Granular Sludge Sequencing Batch Reactors. Int. J. Environ. Res. Public Health 2014, 11(7), 6955-6978.

Long, B., Yang, C.Z., Pu, W.H., Yang, J.K., Jiang, G.S., Dan, J.F., Li, C.Y. and Liu, F.B. (2014) Rapid cultivation of aerobic granular sludge in a pilot scale sequencing batch reactor. Bioresour Technol 166, 57-63.

Maurer, M., Abramovich, D., Siegrist, H., Gujer, W. (1999) Kinetics of biologically induced phosphorus precipitation in waste-water treatment. Water Res 33(2), 484-493.

McHugh, D. J. (1987) Production, properties and uses of alginates. In: McHugh, D. J. (ed.) Production and Utilization of Products from Commercial Seaweeds - FAO fisheries technical paper 288. Rome: Food and Agriculture organization of the United Nations.

McSwain, B.S., Irvine, R.L. and Wilderer, P.A. (2004) The effect of intermittent feeding on aerobic granule structure. Water Sci Technol 49(11-12), 19-25.

McSwain, B.S., Irvine, R.L., Hausner, M. and Wilderer, P.A. (2005) Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. Appl Environ Microbiol 71(2), 1051-1057.

McSwain B.S., Irvine R.L. (2008) Dissolved oxygen as a key parameter to aerobic granule formation. Water Sci Technol, 58, 781–787.

Meng, F., Liu, D., Pan, Y., Xi, L., Yang, D. and Huang, W. (2019) Enhanced amount and quality of alginate-like exopolysaccharides in aerobic granular sludge for the treatment of salty wastewater. BioResources 14(1), 139-165.

Mennerich, A., Müller, M., Krüger, B. and Sporys, F. (2001) Influence of activated sludge SRT on anaerobic excess sludge digestion Fachhochschule Nordostniedersachsen, U.o.A.S., Department Water and Environmental and Engineering, H.-M.-S., D 29556 Suderburg, Germany (eds).

Mikkelsen, L.H. and Keiding, K. (2002) Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. Water Res 36(10), 2451–2462.

Mino, T., van Loosdrecht, M. and Heijnen, J. (1998) Microbiology and biochemistry of the enhanced biological phosphate removal process. Water Res 32(11), 3193-3207.

Mishima, K. and Nakamura, M. (1991) Self-Immobilization of aerobic activated sludge- A pilot study of the aerobic upflow sludge blanket process in municipal sewage treatment. Water Sci Technol 23(4-6), 981-990.

More, T.T., Yadav, J.S., Yan, S., Tyagi, R.D., Surampalli, R.Y.(2014) Extracellular polymeric substances of bacteria and their potential environmental applications. J Environ Manage 144, 1–25.

Morgenroth, E., Sherden, T., van Loosdrecht, M.C.M., Heijnen, J.J. and Wilderer, P.A. (1997) Aerobic granular sludge in a sequencing batch reactor. Water Res 31(12), 3191–3194.

Morgenroth, E. (1998) Enhanced Biological Phosphorus Removal in Biofilm Reactors. Berichte aus Wassergüte- und Abfallwirtschaft, Band 145, Technische Universität München.

Mosquera-Corral, A., de Kreuk, M.K., Heijnen, J.J. and van Loosdrecht, M.C. (2005) Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor. Water Res 39(12), 2676-2686.

Moussa, M.S., Hooijmans, C.M., Lubberding, H.J., Gijzen, H.J. and van Loosdrecht, M.C. (2005) Modelling nitrification, heterotrophic growth and predation in activated sludge. Water Res 39(20), 5080-5098.

Moy, B.Y.P., Tay, J.H., Toh, S.K., Liu, Y. and Tay, S.T.L. (2002) High organic loading influences the physical characteristics of aerobic sludge granules. Lett Appl Microbiol 34(6), 407-412.

Muda, K., Aris, A., Salim, M.R., Ibrahim, Z., van Loosdrecht, M.C., Ahmad, A. and Nawahwi, M.Z. (2011) The effect of hydraulic retention time on granular sludge biomass in treating textile wastewater. Water Res 45(16), 4711-4721.

Muda, K., Aris, A., Mohd, R. and Ibrahim, Z. (2012) Textile Wastewater Treatment Using Biogranules Under Intermittent Anaerobic/Aerobic Reaction Phase. Journal of Water and Environment Technol 10(3), 303-315.

Ni, B.J. and Yu, H.Q. (2008) Growth and storage processes in aerobic granules grown on soybean wastewater. Biotechnol Bioeng 100(4), 664-672.

Ni, B.J., Xie, W.M., Liu, S.G., Yu, H.Q., Wang, Y.Z., Wang, G. and Dai, X.L. (2009) Granulation of activated sludge in a pilot-scale sequencing batch reactor for the treatment of low-strength municipal wastewater. Water Res 43(3), 751-761.

Nielsen, P.H. and Jahn, A. (1999) Microbial extracellular polymeric substances: characterization, structure and function. Wingender, J., Neu, T.R. and Flemming, H.-C. (eds), pp. 49–72, Springer-Verlag, Berlin Heidelberg.

Niermans, R., Giesen, A., van Loosdrecht, M.C., Buin, B.d. (2009) Full-scale Experiences with Aerobic Granular Biomass Technology for Treatment of Urban and Industrial Wastewater. WEFTEC 2014: Session 202 through Session 209.

Nor Anuar, A., Ujang, Z., van Loosdrecht, M.C. and de Kreuk, M.K. (2007) Settling behaviour of aerobic granular sludge. Water Sci Technol 56(7), 55-63.

Oliva, J., Bernhardt, A., Reisinger, H., Domenig, M. and Krammer, H.-J. (2009) Klärschlamm – Materialien zur Abfallwirtschaft, Sewage sludge - materials for waste management. Umweltbundesamt, Wien.

Ortwein B. (2016) AirPrex®-ein Verfahren zur Schlammoptimierung mit der Option der Phosphat: Rückgewinnung AirPrex®: a sludge optimization process with the option of phosphate recovery. in Innovationsforum THERMOLYPHOS, 4. – 5. Oktober 2016 in Halle (Saale), ed. by cnp-TechnologyWater and Biosolids GmbH, Hamburg.

Palmeiro-Sánchez, T., Val del Río, A., Mosquera-Corral, A., Campos, J.L. and Méndez, R. (2013) Comparison of the anaerobic digestion of activated and aerobic granular sludges under brackish conditions. Chemical Engineering Journal 231, 449-454.

Parravicini, V., Valkova, T., Haslinger, J., Saracevic, E., Winkelbauer, W., Tauber, J., Svardal, K., Hohenblum, P., Clara, M., Windhofer, G., Pazdernik, K. and Lampert, C. (2015) ReLaKO-Reduktionspotential bei den Lachgas-Emissionen aus Kläranlagen durch Optimierung des Betriebes. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, Wien.

Peng, B., Liang, H., Wang, S. and Gao, D. (2018) Effects of DO on N_2O emission during biological nitrogen removal using aerobic granular sludge via shortcut simultaneous nitrification and denitrification. Environ Technol, 1-17.

Pijuan, M., Werner, U., Yuana Z. (2011) Reducing the startup time of aerobic granular sludge reactors through seeding floccular sludge with crushed aerobic granules. Water Res 45 (16), 5075-5083.

Pronk, M., Abbas, B., Al-zuhairy, S.H.K., Kraan, R., Kleerebezem, R. and van Loosdrecht, M. (2015a) Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge. Appl Microbiol Biotechnol 99(12), 5257-5268.

Pronk, M., de Kreuk, M.K., de Bruin, B., Kamminga, P., Kleerebezem, R. and van Loosdrecht, M.C. (2015b) Full scale performance of the aerobic granular sludge process for sewage treatment. Water Res 84, 207-217.

Pronk, M., Giesen, A., Thompson, A., Robertson, S. and van Loosdrecht, M. (2017) Aerobic granular biomass technology: advancements in design, applications and further developments. Water Practice and Technology 12(4), 987-996.

Qin, L., Liu, Y. and Tay, J.H. (2004) Effect of settling time on aerobic granulation in sequencing batch reactor. Biochem Eng J 21(1), 47-52.

Qin, L., Liu, Y. and Tay, J.H. (2005) Denitrification on poly-beta-hydroxybutyrate in microbial granular sludge sequencing batch reactor. Water Res 39(8), 1503-1510.

Quan, X., Zhang, M., Lawlor, P.G., Yang, Z. and Zhan, X. (2012) Nitrous oxide emission and nutrient removal in aerobic granular sludge sequencing batch reactors. Water Res 46(16), 4981-4990.

Ren, T.T., Liu, L., Sheng, G.P., Liu, X.W., Yu, H.Q., Zhang, M.C. and Zhu, J.R. (2008) Calcium spatial distribution in aerobic granules and its effects on granule structure, strength and bioactivity. Water Res 42(13), 3343-3352.

Rocktäschel, T., Klarmann, C., Helmreich, B., Ochoa, J., Boisson, P., Sorensen, K.H. and Horn, H. (2013) Comparison of two different anaerobic feeding strategies to establish a stable aerobic granulated sludge bed. Water Res 47(17), 6423-6431.

Rosenwinkel, K.H., Kroiss., H., Dichtl., N., Seyfried, C.F. and Weiland, P. (2015) Anaerobtechnik-Abwasser-, Schlamm- und Reststoffbehandlung, Biogasgewinnung, Springer Verlag, Braunschweig.

Rosman, N.H., Nor Anuar, A., Chelliapan, S., Md Din, M.F. and Ujang, Z. (2014) Characteristics and performance of aerobic granular sludge treating rubber wastewater at different hydraulic retention time. Bioresour Technol 161, 155-161.

Sarma, S.J., Tay, J.H. and Chu, A. (2017) Finding Knowledge Gaps in Aerobic Granulation Technology. Trends Biotechnol 35(1), 66-78.

Sawabe, T., Oda, Y., Shiomi, Y. and Ezura, Y. (1995) Alginate degradation by bacteria isolated from the gut of sea urchins and abalones. Microbial ecology 30, 193-202.

Schalk-Otte, S., Seviour, R.J., Kuenen, J.G. and Jetten, M.S. (2000) Nitrous oxide (N_2O) production by alcaligenes faecalis during fast and famine regimes. Water Res 34(7), 2080-2088.

Schönberger, R. (1990) Optimierung der biologischen Phosphatelimination bei der kommunalen Abwasserreinigung, Optimization of biological phosphate elimination in municipal wastewater treatment. Berichte aus der Wassergüte und Gesundheitsingenieurwesen, Band 93, Technische Universität München.

Schreiber, F., Loeffler, B., Polerecky, L., Kuypers, M.M. and de Beer, D. (2009) Mechanisms of transient nitric oxide and nitrous oxide production in a complex biofilm. ISME J 3(11), 1301-1313.

Sepúlveda-Mardones, M., Campos J.L., Magrí, A., Vidal, G. (2019) Moving forward in the use of aerobic granular sludge for municipal wastewater treatment – an overview. Submitted manuscript to Reviews in Environmental Science and Bio/Technology.

Sheng, G.P., Yu, H.Q.(2006) Characterization of extracellular polymeric substances of aerobic and anaerobic sludge using three-dimensional excitation and emission matrix fluorescence spectroscopy. Water Res 40(6), 1233–1239.

Sheng, G.P., Li, A.J., Li, X.Y. and Yu, H.Q. (2010) Effects of seed sludge properties and selective biomass discharge on aerobic sludge granulation. Chemical Engineering Journal 160(1), 108-114.

Skinner, S.J., Studer, L.J., Dixon, D.R., Hillis, P., Rees, C.A., Wall, R.C., Cavalida, R.G., Usher, S.P., Stickland, A.D. and Scales, P.J. (2015) Quantification of wastewater sludge dewatering. Water Res 82, 2-13.

Stewart, P.S. (2003) Diffusion in Biofilms. Journal of Bacteriology 185(5), 1485-1491.

Su, K.-Z. and Yu, H.-Q. (2005) Formation and Characterization of Aerobic Granules in a Sequencing Batch Reactor Treating Soybean-Processing Wastewater. Environ Sci Technol (5), 2818-2827.

Świątczak, P. and Cydzik-Kwiatkowska, A. (2017) Performance and microbial characteristics of biomass in a full-scale aerobic granular sludge wastewater treatment plant. Environ Sci Pollut Res 25(2), 1655-1669.

Tallec, G., Garnier, J., Billen, G. and Gousailles, M. (2008) Nitrous oxide emissions from denitrifying activated sludge of urban wastewater treatment plants, under anoxia and low oxygenation. Bioresour Technol 99(7), 2200-2209.

Tay, J.H., Liu, Q.S. and Liu, Y. (2001) The effects of shear force on the formation, structure and metabolism of aerobic granules. Applied Microbiology and Biotechnology 57(1-2), 227-233.

Tay, S.T., Moy, B.Y., Maszenan, A.M., Tay, J.H. (2005) Comparing activated sludge and aerobic granules as microbial inocula for phenol biodegradation. Applied Microbiology and Biotechnology 67, 708–713.

Thomé-Kozmiensky, K.J. (1998) Klärschlammentsorgung, Sewage sludge disposal. TK Verlag Thomé-Kozmiensky, Neuruppin, ISBN-13: 978-3924511876.

Thwaites, B.J., Reeve, P., Dinesh, N., Short, M.D. and van den Akker, B. (2017) Comparison of an anaerobic feed and split anaerobic-aerobic feed on granular sludge development, performance and ecology. Chemosphere 172, 408-417.

Thwaites, B.J., Short, M.D., Stuetz, R.M., Reeve, P.J., Alvarez Gaitan, J.P., Dinesh, N. and van den Akker, B. (2018) Comparing the performance of aerobic granular sludge versus conventional activated sludge for microbial log removal and effluent quality: Implications for water reuse. Water Res 145, 442-452.

Val del Rio, A., Morales, N., Isanta, E., Mosquera-Corral, A., Campos, J.L., Steyer, J.P. and Carrere, H. (2011) Thermal pre-treatment of aerobic granular sludge: impact on anaerobic biodegradability. Water Res 45(18), 6011-6020.

van den Akker, B., Reid, K., Middlemiss, K. and Krampe, J. (2015) Evaluation of granular sludge for secondary treatment of saline municipal sewage. J Environ Manage 157, 139-145.

van Dijk, E.J.H., Pronk, M. and van Loosdrecht, M.C.M. (2018) Controlling effluent suspended solids in the aerobic granular sludge process. Water Res 147, 50-59.

van Loosdrecht, M.C.M., Hooijmans, C.M., Brdjanovic, D. and Heijnen, J.J. (1997) Biological phosphate removal processes. Appl Microbiol Biotechnol 48(3), 289-296.

von der Emde, W., Sadzik, P. (1982) Untersuchungen über die Eindick- und Entwässerungseigenschaften von Schlämmen. Studies on the thickening and dewatering properties of sludges. Wiener Mitteilungen 47, 1–19.

Verawaty, M., Tait, S., Pijuan, M., Yuan, Z. and Bond, P.L. (2013) Breakage and growth towards a stable aerobic granule size during the treatment of wastewater. Water Res 47(14), 5338-5349.

Wagner, J., Weissbrodt, D.G., Manguin, V., da Costa, R.H., Morgenroth, E. and Derlon, N. (2015) Effect of particulate organic substrate on aerobic granulation and operating conditions of sequencing batch reactors. Water Res 85, 158-166.

Wang, Z.W., Liu, Y. and Tay, J.H. (2005) Distribution of EPS and cell surface hydrophobicity in aerobic granules. Appl Microbiol Biotechnol 69(4), 469-473.

Wang, Z.W., Liu, Y. and Tay, J.H. (2006) The role of SBR mixed liquor volume exchange ratio in aerobic granulation. Chemosphere 62(5), 767-771.

Wang, S.G., Liu, X.W., Gong, W.X., Gao, B.Y., Zhang, D.H. and Yu, H.Q. (2007a) Aerobic granulation with brewery wastewater in a sequencing batch reactor. Bioresour Technol 98(11), 2142-2147.

Wang, Z.W., Liu, Y. and Tay, J.H. (2007b) Biodegradability of extracellular polymeric substances produced by aerobic granules. Appl Microbiol Biotechnol 74(2), 462-466.

Wang, Y., Jiang, F., Zhang, Z., Xing, M., Lu, Z., Wu, M., Yang, J., Peng, Y. (2010) The long-term effect of carbon source on the competition between polyphosphorus accumulating organisms and glycogen accumulating organism in a continuous plug-flow anaerobic/aerobic (A/O) process. Bioresour Technol 101(1), 98-166.

Wang, X.H., Jiang, L.X., Shi, Y.J., Gao, M.M., Yang, S. and Wang, S.G. (2012) Effects of step-feed on granulation processes and nitrogen removal performances of partial nitrifying granules. Bioresour Technol 123, 375-381.

Wang, S., Ma, X., Wang, Y., Du, G., Tay, J.-H., Li, J. (2019) Piggery wastewater treatment by aerobic granular sludge: Granulation process and antibiotics and antibiotic-resistant bacteria removal and transport. Bioresour Technol 273, 350-357.

Wei, D., Wang, B., Ngo, H.H., Guo, W., Han, F., Wang, X., Du, B. and Wei, Q. (2015) Role of extracellular polymeric substances in biosorption of dye wastewater using aerobic granular sludge. Bioresour Technol 185, 14-20.

Welles, L., Lopez-Vazquez, C.M., Hooijmans, C.M., van Loosdrecht, M.C.M. and Brdjanovic, D. (2014) Impact of salinity on the anaerobic metabolism of phosphate-accumulating organisms (PAO) and glycogen-accumulating organisms (GAO). Appl Microbiol Biotechnol 98(17), 7609–7622.

Wentzel, M.C., Dold, P.L., Ekama, G.A. and Marais v., G.R. (1985) Kinetics of biological phosphorus release. Water Sci Technol 17(11-12), 57-71.

Wingender, J., Neu, T.R. and Flemming, H.-C. (1999) Microbial Extracellular Polymeric Substances, Characterization, Structure and Function. Wingender, J., Neu, T.R. and Flemming, H.-C. (eds), Springer Verlag Berlin Heidelberg, 1-19, ISBN: 9783642642777.

Winkler, M.K., Bassin, J.P., Kleerebezem, R., de Bruin, L.M., van den Brand, T.P. and van Loosdrecht, M.C. (2011) Selective sludge removal in a segregated aerobic granular biomass system as a strategy to control PAO-GAO competition at high temperatures. Water Res 45(11), 3291-3299.

Winkler, M.K. (2012a) Magic Granules: Segregation of biomass in aerobic granular sludge. Thesis. Department Environmental Biotechnology, Delft University of Technology, Delft.

Winkler, M.K., Bassin, J.P., Kleerebezem, R., Sorokin, D.Y. and van Loosdrecht, M.C. (2012b) Unravelling the reasons for disproportion in the ratio of AOB and NOB in aerobic granular sludge. Appl Microbiol Biotechnol 94(6), 1657-1666.

Winkler, M.K.H., Bassin, J.P., Kleerebezem, R., van der Lans, R.G.J.M. and van Loosdrecht, M.C.M. (2012c) Temperature and salt effects on settling velocity in granular sludge technology. Water Res 46(12), 3897-3902.

Winkler, M.K., Kleerebezem, R., Strous, M., Chandran, K. and van Loosdrecht, M.C. (2013) Factors influencing the density of aerobic granular sludge. Appl Microbiol Biotechnol 97(16), 7459-7468.

Winkler, M.K., Le, Q.H. and Volcke, E.I. (2015) Influence of Partial Denitrification and Mixotrophic Growth of NOB on Microbial Distribution in Aerobic Granular Sludge. Environ Sci Technol 49(18), 11003-11010.

Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L. and Siegrist, H. (2012) Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions. Water Res 46(4), 1027-1037.

Zeng, R.J., Lemaire, R., Yuan, Z. and Keller, J. (2003a) Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. Biotechnol Bioeng 84(2), 170-178.

Zeng, R.J., Yuan, Z. and Keller, J. (2003b) Enrichment of denitrifying glycogen-accumulating organisms in anaerobic/anoxic activated sludge system. Biotechnol Bioeng 81(4), 397-404.

Zeng, J., Gao, J.M., Chen, Y.P., Yan, P., Dong, Y., Shen, Y., Guo, J.S., Zeng, N. and Zhang, P. (2016) Composition and aggregation of extracellular polymeric substances (EPS) in hyperhaline and municipal wastewater treatment plants. Sci Rep 6, 26721.

Zhang, L., Feng, X., Zhu, N. and Chen, J. (2007) Role of extracellular protein in the formation and stability of aerobic granules. Enzyme and Microbial Technology 41(5), 551-557.

Zhang, F., Li, P., Chen, M., Wu, J., Zhu, N., Wu, P., Chiang, P. and Hu, Z. (2015) Effect of operational modes on nitrogen removal and nitrous oxide emission in the process of simultaneous nitrification and denitrification. Chemical Engineering Journal 280, 549-557.

Zheng, Y.-M., Yu, H.-Q. and Sheng, G.-P. (2005) Physical and chemical characteristics of granular activated sludge from a sequencing batch airlift reactor. Process Biochemistry 40(2), 645-650.

Zou, J., Tao, Y., Li, J., Wu, S. and Ni, Y. (2018) Cultivating aerobic granular sludge in a developed continuous-flow reactor with two-zone sedimentation tank treating real and low-strength wastewater. Bioresource Technology 247, 776-783.

DIN EN ISO 11732:2005-05: Water quality - Determination of ammonium nitrogen - Method by flow analysis (CFA and FIA) and spectrometric detection (ISO 11732:2005); German version EN ISO 11732:2005.

VDI (2016). VDI 4630: Vergärung organischer Stoffe - Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche, Fermentation of organic substances - Substrate characterization, Sampling, Substance data collection, Fermentation tests.