Chapter 4
Aerobic Granular Sludge Technology

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4.1 Introduction

Most wastewater treatment systems, such as the traditional activated sludge process, require large areas for their installation, which is mainly due to the need for large settling tanks and the low concentration of solids in the aeration basins. Considerable excess sludge production, high sensitivity to fluctuations in the applied load, and relatively low volumetric conversion capacities (0.5–2.0 kgCOD/m^3 day) are some disadvantages of the conventional processes.

The performance of the activated sludge-based processes is strongly dependent on the settling characteristics of the biomass present in the reactor. The microbial flocs must be separated from the treated wastewater in settling tanks and one part returned to the reactor. However, in some cases, poor settling flocs may exist, resulting in the washout of the sludge from the clarifiers. This of course reduces the quality of the treated effluent. In most cases, this loss of sludge is caused by the excessive growth of filamentous microorganisms, which may adversely affect the sludge sedimentation properties. The reactor configuration and the operation strategy can also influence the sludge characteristics. In the specific case of certain wastewaters, the formation of flocs with good sedimentation properties may be difficult to be accomplished.

The substantial increase in the number of inhabitants, in most cases concentrated in densely populated urban areas, has increased the need to improve the existing wastewater treatment plants or to construct new systems which are compatible with

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the increasing amount of wastewater generated. In general, the availability of space for these constructions is limited, and thus it is imperative that these new installations occupy the smallest area possible.

In this context, there has been significant evolution in the wastewater treatment sector in response to greater demands regarding the quality of the treated effluent and the need to minimize the space used, to simplify the operation and to reduce the investment costs. Meanwhile, research on the development of new forms of biomass agglomerates has intensified, aiming to facilitate and improve the biomass retention in biological treatment processes, which is crucial to reduce the footprint of the treatment plants. As the biomass acts as a biocatalyst for the degradation of a wide range of pollutants, retaining a high solids concentration in the reactor leads to a greater treatment capacity. Thus, it is very important that the biomass has good sedimentation properties to ensure proper functioning of the biological wastewater treatment process.

In recent years several biofilm reactors in which the biomass is immobilized in fixed or moving carrier materials have been developed. Examples of such reactors include the trickling filter or biofilter, rotating biological contactor (RBC), and moving bed biofilm reactor (MBBR) (described in Chap. 3). As advantages, these processes exhibit a reduced area for plant installation, in some cases dispensing with the need for settling tanks (or allowing a reduction in their dimensions), and the ability to withstand high volumetric and organic loads.

Aerobic granulation, also known as aerobic granular sludge technology, is an even more recent innovation within biofilm reactors for wastewater treatment. Considered as a particular type of biofilm comprised of self-immobilized cells, aerobic granular sludge constitutes a promising technology which does not require a material support for biofilm growth. The first patent was filed by HEIJNEN and VAN LOOSDRECHT (1998).

It is interesting to point out that granular sludge technology was initially developed for strictly anaerobic systems in 1980. The formation of anaerobic granules was most frequently carried out in upflow anaerobic sludge blanket (UASB) reactors, in which high biodegradable organic matter removal from domestic and industrial wastewaters can be obtained (LETTINGA et al. 1980). The limitations associated with anaerobic granulation, which include a long reactor start-up, relatively high operation temperature requirement, strong dependence on the concentration of organic matter, and low efficiency for the removal of nutrients (nitrogen and phosphorus), motivated the development of aerobic granulation. Due to the numerous advantages of this process, it has become a focus for discussion among engineers dealing with the environmental issues (ADAV et al. 2008a, b).

In the past 10 years, many studies have been carried out to investigate aerobic granules, both to better understand the formation of these compact microbial structures and investigate the possibility of making aerobic granulation technology a new compact system for wastewater treatment.

Aerobic granulation could represent a solution for the operation of some reactors where flocculent sludge with poor settling features predominates. As no material support is required, the initial investment may be reduced. In addition, the formation of an oxygen gradient and the presence of a wide range of microorganisms within the aerobic granules allow the simultaneous removal of organic matter, nitrogen, and phosphorus. In this context, aerobic granulation has been the subject of several studies, mostly conducted in laboratory scale, with the first applications in pilot and industrial scale appearing recently.

4.2 General Characterization of Aerobic Granular Sludge Technology

Aerobic granules consist of highly compacted microbial aggregates containing millions of microorganisms per gram of biomass. Many different bacterial species with specific function in the degradation of a variety of pollutants present in complex wastewaters are harbored in such compact microbial structures (LIU and TAY 2004). Aerobic granules can also be considered as "mini-ecosystems," comprised of a mixed microbial population among which the desirable organisms can be selected through the application of specific operational conditions (DE KREUK et al. 2005a).

At the IWA workshop called "Aerobic Granular Sludge," carried out in Munich, Germany, in 2004, it was established that aerobic granules should be considered as aggregates of microbial origin, which do not coagulate under reduced hydrodynamic forces and which have a faster settling velocity than activated sludge flocs (DE KREUK et al. 2005a). At this workshop it was also determined that, for an aggregate to be considered an aerobic granule, it must have a structure in which the position of the microorganisms is not rapidly changed, as in the case of activated sludge flocs. In addition, besides settling rapidly, the aggregate comprised of biomass and extracellular polymers must be formed without the need for a material support and must have a diameter of at least 0.2 mm. The classification of granules should be carried out using sieves, which enable expressing the amount of granules present within the overall biomass. The granulation process can be considered complete when the amount of granules corresponds to 80% of the solids present in the reactor (DE KREUK et al. 2005a).

With an external spherical shape and a diameter which can vary between 0.2 and 5.0 mm, the granules density is much higher than that of activated sludge flocs (ADAV et al. 2008a). This property shown by the granules leads to other several interesting characteristics of these microbial aggregates (ADAV et al. 2008a; LIU and TAY 2004; BEUN et al. 1999; DE KREUK and VAN LOOSDRECHT 2004):

- Excellent sedimentation ability, facilitating the separation of the treated effluent from the granular sludge.
- Regular shape, smooth and almost round.
- They are visible and form a separate phase in the liquid during the aeration and sedimentation phases.
- They enable high biomass retention in the reactor, increasing the capacity to deal with high organic loads.

- The microbial structure is dense and strong.
- Both aerobic and anoxic (anaerobic) zones are present within the granules, which allows different biological processes to be carried out in the same system.
- They can operate with high flow rates.
- They are less vulnerable to the toxicity of chemical compounds and heavy metals compared with suspended sludge.
- There is no need for a support material (required in other biofilm systems), reducing the investment costs.
- They allow a reduction in the running cost of the treatment plant of at least 20% and in the space required of 75%.

The attractiveness of granular sludge reactors is highlighted by the fact that these systems can retain a large quantity of microorganisms, allowing the rapid transformation of pollutants and leading to improvements in the performance and stability of the reactor. Consequently, large volumes of wastewaters can be treated in compact reactors. Since they are larger and have a greater density compared with activated sludge flocs, the granules rapidly settle which facilitates the separation of the treated effluent from the biomass.

The aerobic granules are preferentially cultivated in sequencing batch reactors (SBRs), where the operation can be divided into temporal cycles. These, in turn, are characterized by the phases of filling, reaction (aerobic, anoxic, or anaerobic), sedimentation, and treated effluent (supernatant) discharge. The working principle of SBRs facilities the retention of high concentrations of granular sludge in the bulk and dispenses with the need for settling tanks to separate the biomass from the treated effluent as well as sludge return to the biological reactor.

In discontinuous systems, the excellent capacity for the sedimentation of the aerobic granular sludge, besides improving the separation of the biomass from the treated wastewater, allows that a longer period of each operation cycle can be allocated to the reaction phase. In SBRs used to cultivate aerobic granules, the cycle periods are generally a few hours (3–6 h). In each cycle a certain quantity of influent wastewater is added to the reactor, and the reaction phase is then started, whether it is aerobic, anoxic, or anaerobic, during which conversions occur. The end of each cycle is characterized by rapid settling of the granules (retaining them in the reactor), this step representing a primary criterion of the design (BEUN et al. 1999). Only particles of a certain size and density, able to rapidly sediment out, are retained in the reactor. On the other hand, particles with slower settling velocities, such as microbial flocs, are washed out of the system, allowing only the development of aerobic granules (LIU and TAY 2002; BEUN et al. 1999). After the settling period, the drainage/emptying phase begins, in which the upper part of the reactor content is removed as clarified effluent. Thus, the removal of organic matter and nutrients (N and P) and the sludge sedimentation step occur within the same reactor, resulting in a single tank containing a high concentration of granular sludge, which, consequently, allows high volumetric conversion rates (BEUN et al. 1999).

The physical step of settling is responsible for biomass selection, exerting a "selection pressure" (LIU and TAY 2002). Thus, the time allocated to sedimentation

(a) (b) (c)

Fig. 4.1 Bubble column sequencing batch reactor used to cultivate aerobic granules. (**a**) Filling, (**b**) reaction, (**c**) sedimentation and effluent withdrawal

has a considerable influence on the aerobic granulation process and represents a key criterion of the design (BEUN et al. 1999). Short settling times lead to suspended biomass being washed out, and only the denser granules are retained (QIN et al. 2004). Figure 4.1 shows the different phases of the cycle in a lab-scale bubble column sequencing batch reactor, used to cultivate aerobic granules. As can be observed, the height/diameter ratio of the reactor is high (120 cm/6.5 cm = 18.5). As will be discussed later, this characteristic is important for the application of the aerobic granulation technology, considering that the settling velocity is one of the criteria for the selection of granules and to minimize the presence of poor settling microbial flocs.

In Fig. 4.1c, it can be observed that the treated effluent (supernatant) which is being drained is almost free from suspended solids, confirming the rapid and efficient sedimentation of aerobic granular sludge. In the case of the reactor shown in Fig. 4.1, the time allocated to the sedimentation of the granules is only 3 min. Small flocs are washed out of the reactor in every cycle, and thus the concentration of solids in the reactor, represented mainly by granules, increases during the operation.

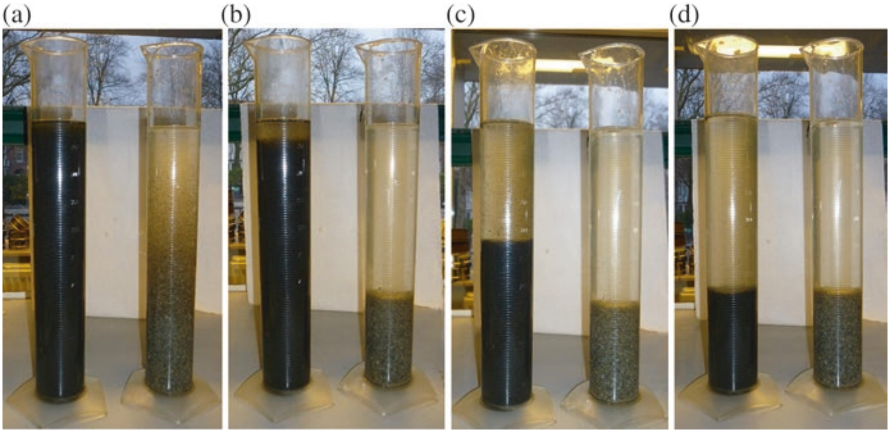

Fig. 4.2 Comparison of sedimentation over time of flocculent sludge and aerobic granules. (**a**) Start, (**b**) 30 s, (**c**) 12 min, (**d**) 20 min

The settling characteristics of activated sludge flocs and aerobic granular sludge is compared in Fig. 4.2, which illustrates their sedimentation at different time intervals. As can be observed, after only 30 s, the granules had completely settled out. The activated sludge flocs, however, required 20 min to reach the same level of sedimentation as the granular sludge.

Considering that the diffusion limitation increases when the biomass growth is no longer in suspension but instead in the form of biofilms/granules, the latter type of biomass always grows more slowly compared with biomass in suspension. Therefore, it is plausible to assume that bacteria preferentially grow in suspension rather than in the form of biofilms or granules since in the latter cases there are diffusion limitations associated with all of the components involved. Thus, only by avoiding the accumulation of suspended cells or flocs, through the application of reduced sedimentation times, will suitable granules be formed (BEUN et al. 1999, 2002). It is important to remember that slow-growing bacteria with a low substrate to cell yield coefficient ($Y_{x/s}$), such as nitrifying bacteria, show a greater tendency to grow in the form of biofilms/granules as compared to fast-growing aerobic heterotrophic bacteria (BEUN et al. 2002).

One parameter which can be varied in order to control the sedimentation step is the minimum settling velocity (v_{min}), which can be obtained by dividing the sedimentation height by the sedimentation time, the latter being selected and fixed as required. Bearing in mind that in most cases the granules have high settling velocities, the time allocated to the settling step is short, allowing more time for the degradation processes in the reaction period. This characteristic certainly makes the discontinuous operation mode (e.g., in SBRs) more attractive.

One factor to be highlighted relates to the size of the granules. As expected, the larger the particles, the greater their settling velocity will be. Thus, in this case, in particular, the cultivation of larger granules is favorable. However, it should be taken into consideration that diffusion effects may be present depending on the diameter of the granule. When the aim is, for instance, to create anoxic conditions inside the granules in order to allow denitrification, larger granules tend to be recommended. In larger granules the diffusion of oxygen to the interior of the granule is limited, especially when microbial populations situated in the outer layers of the granular biomass reduce the dissolved oxygen concentration. However, the control of the granular particles size is not trivial and depends on many operating parameters.

As previously noted, the granular sludge settling step in sequencing batch reactors is not troublesome since the granule settling velocity is much higher than that presented by flocculent biomass. However, as in any treatment process, there are obstacles to be overcome. In the case of the operation of granular sludge reactors, the main technical drawback is the instability of the aerobic granules, which can originate from the growth of filamentous bacteria, reducing the sedimentation capacity and leading to biomass washout from the reactor. This of course may limit the application of this technology to wastewater treatment. However, as will be observed in some studies involving aerobic granules reported in the literature, the growth of filamentous organisms at low or moderate levels does not lead to problems. In fact, these organisms may contribute to the stabilization of the granular structure, serving as a support for the formation of these microbial agglomerates.

While the settling velocity is one of the key operational strategies for granule selection and reduction of the amount of smaller particles in suspension, the growth rate of the microorganisms represents one of the main factors responsible for the density of the granules or biofilms. Organisms with high growth rates form less dense granules compared to those with low growth rates (VILLASEÑOR et al. 2000). Nitrifying microorganisms, for instance, form much denser biofilms than heterotrophs under the same conditions. Thus, a decrease in the bacterial growth rate contributes to increase the density of the biofilms/granules (VAN LOOSDRECHT et al. 1995) and, as will be observed later in this chapter, leads to the development of stable granules even under low oxygen conditions (DE KREUK and VAN LOOSDRECHT 2004). To achieve these objectives, some operational conditions must be ensured. Greater details regarding this issue will be provided in the section describing the factors affecting aerobic granulation (Sect. 4.4.4).

The formation of aerobic granules is also strongly influenced by shear forces, as described in Sect. 4.4.4. The hydrodynamics of the system is directly related to the development of dense aggregates, such as biofilms. From the microbiological point of view, biofilms and granular sludge can be considered to be the same, although they have obvious differences from the technical point of view. Thus, hypotheses created in an attempt to characterize biofilms are useful to gain a better understanding of the conditions required for the formation of stable aerobic granules with the desired characteristics.

Due to its distinct and special characteristics, aerobic granulation technology has been recently applied to the treatment of highly concentrated wastewaters containing organic compounds, nitrogen, phosphorus, toxic substances, and xenobiotics (JIANG et al. 2002; MOY et al. 2002; TAY et al. 2002b; LIN et al. 2003; ADAV et al. 2007a, b; ADAV and LEE 2008).

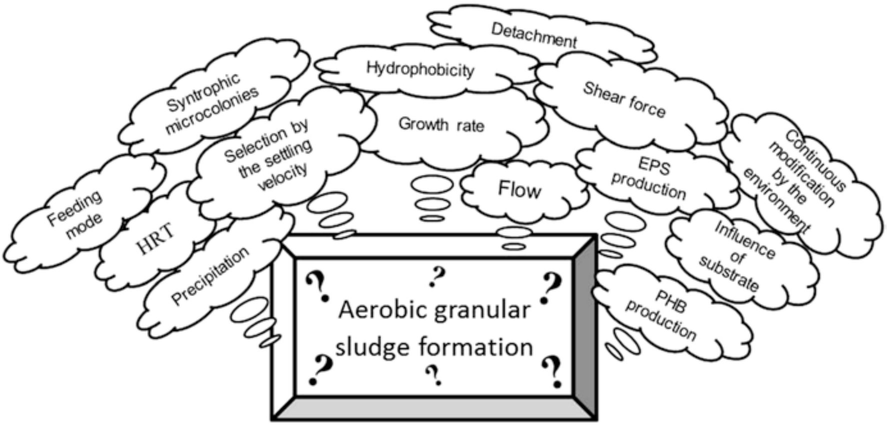

Fig. 4.3 Different ideas and hypotheses related to the formation of aerobic granular sludge (adapted from DE KREUK 2006)

Before presenting some studies involving the various applications of aerobic granules (Sect. 4.4.7), it is important to describe the aerobic granulation process and the different phases involved in the gradual transformation of flocculent sludge into granules (Sect. 4.4.3). The main factors which influence aerobic granulation, some case studies involving the formation of aerobic granular sludge, and the conversion processes which occur within the aerobic granules are addressed in Sects. 4.4.4, 4.4.5, and 4.4.6, respectively.

4.3 Formation of Aerobic Granules

Many different ideas and hypotheses were suggested to explain the formation of aerobic granules, as illustrated in Fig. 4.3. Several studies have proposed mechanisms to describe the granulation process, although there is still no consensus with respect to the process by which flocculent sludge is converted into granules.

Biogranulation basically involves interactions between cells, associated with biological, physical, and chemical phenomena which are related to the formation of stable and contiguous multicellular associations. In order for the bacterial cells present in a culture to aggregate, several conditions must be fulfilled (LIU and TAY 2004).

TAY et al. (2001a) described aerobic granulation as a self-immobilization process which does not require the use of a support material. Later LIU and TAY (2002) proposed the following stages for the aerobic granulation process:

1. Contact between microorganisms with the formation of aggregates via hydrodynamic, diffusion, gravitational, and/or thermodynamic forces
2. Stabilization of multicellular contact resulting from initial attraction, comprised of physical (van der Waals, attraction of opposite charges, thermodynamic

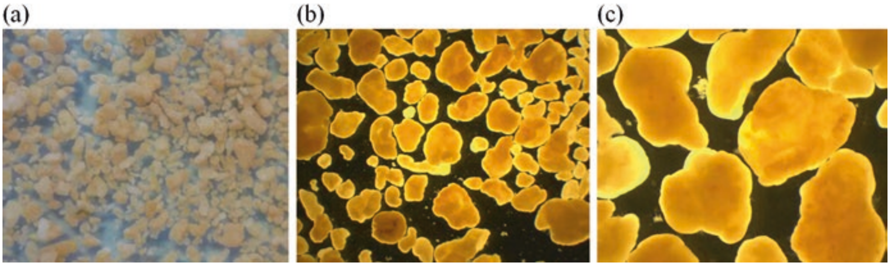

Fig. 4.4 Aerobic granules formed in a bubble column SBR. (**a**) Original size, (**b**) magnification 7.5×, (**c**) magnification 20×

forces, surface tension, hydrophobicity), chemical (ionic coupling, interparticle bonding), and biochemical interactions (fusion of cell membrane, cell receptor attraction, dehydration of cell surface)

3. Maturation of the cellular aggregates through the production of extracellular polymers and increase in cell groupings and metabolic changes, which facilitate interaction between cells and result in an organized microbial structure
4. Formation and stabilization of the tridimensional structure of the microbial aggregate in the steady state by way of hydrodynamic shear forces

Thus, aerobic granulation consists of a gradual process involving the transformation of the activated sludge into compact aggregates, which later acquire the form of granular sludge and finally mature granules (TAY et al. 2001a). In this context, rather than the simple random agglomeration of bacteria, the formation of biogranules consists of a microbial evolution process. In Fig. 4.4 the structure of mature granules can be observed, at different magnifications, cultivated in a lab-scale bubble column SBR, fed with synthetic wastewater containing acetate as a carbon source.

In general, the structures of biofilms/granules consist of highly complex ecosystems, in terms of their microbiology and morphology. In these systems, the structure influences the activity which, in turn, affects the structure. Due to the complexity of the structures of biofilms and granules, it is difficult to identify causes and effects of issues involving morphogenesis (VAN LOOSDRECHT et al. 1995). In addition, it should be noted that, from the engineering perspective, the events which occur during microbial granulation can be identified and, to a certain extent, understood. In contrast, the mechanisms associated with events which occur at the molecular or genetic level require a much more in-depth understanding.

Although several theories regarding the factors which are crucial for the development of granules have been extensively discussed, in most studies involving granular sludge, the possible contribution of protozoans and fungi in the formation of granules and their interactions with bacteria have been neglected. However, these eukaryotic organisms have a variety of important functions in activated sludge systems (conversion of biomass and water clarification) (FRIED and LEMMER 2003), and they are involved in the formation and structuring of the biofilms which play a role outside the field of wastewater treatment systems (HARTMANN et al. 2007).

In this context and taking into consideration preliminary observations of the granules in which stalked ciliates and fungi were present in significant numbers, WEBER et al. (2007) investigated the function of ciliated protozoans and fungi in the structural formation of microbial granules originating from conventional activated sludge. The authors analyzed the structure and development of different types of aerobic granules, cultivated in three laboratory-scale sequencing batch reactors (SBRs). These systems were fed, respectively, with wastewater rich in particulates containing malt and obtained from a mixture of barley powder and water (SBR_1), with wastewater from a brewery plant (SBR_2) and with a synthetic medium (SBR_3).

A detailed view of the architecture and composition of the granules has been obtained by applying scanning electron microscopy (SEM), optical microscopy, and confocal laser scanning microscopy (CLSM). The microscopic observations revealed that the granules consisted of bacteria, extracellular polymeric substances (EPS), protozoans, and, in some cases, fungi. The development of the granular sludge, from activated sludge until the obtainment of mature granules, was divided into three phases. During the first phase, stalked ciliated protozoans of the subclass Peritrichia started to be present in a great quantity in the activated sludge flocs, forming new stalks. These protozoans then began to proliferate and form large colonies while, at the same time, their stalks were colonized by bacteria. The colonization was intensified by the movement of their cilia, which provides a continuous flow of nutrients in the direction of the bacterial cells responsible for biofilm formation. After a few days, many ciliate cells got fixed to the surface of the microbial flocs. Most of the ciliate colonies formed had the shape of a tree and were comprised of the genera *Opercularia* and *Epistilys* (WEBER et al. 2007).

During the second phase, the sludge flocs became grouped, and a large growth of ciliates was observed. Concomitantly with the formation of voluminous flocs, a central zone appeared which consisted of the remains of the ciliates stalks and bacteria responsible for the production of EPS. The ciliate stalks served as a support for the development of the granules, since the bacteria used them as a substrate for their growth. The flocs which aggregated were considered to be precursors to the granules (WEBER et al. 2007).

Subsequently, with the start of the third phase, the ciliates were also colonized by bacterial cells and became embedded in the expanding biofilm. After a certain period, they were completely covered by bacteria and could no longer survive. Some cells of stalkless free-swimming ciliates appeared and left the biofilm. In this phase, compact bacterial granules were formed, which were gradually colonized by the free-swimming ciliates which managed to survive. These formed new stalks and colonies, which were used as a substrate for microbial growth. This study in particular emphasized the importance of ciliates in the formation of the structure of the granules, since they serve as a base for the growth of the microbial biofilm (WEBER et al. 2007).

As regards the fungi, their role in the granulation process is related to the fact that their filaments (hyphae), together with the ciliate stalks, act as a support for the bacterial growth, thus increasing the area available for bacterial colonization (WEBER et al. 2007). BEUN et al. (1999), as will be mentioned in Sect. 4.4.5, also noted the possible contribution of fungi as a support for the formation of granules, particularly during the initial phases of the granulation process.

WEBER et al. (2007) also reported that the mature granules formed were composed of several microbial layers, comprised of distinct bacterial species responsible for different processes, such as nitrification and denitrification. A dense central region was observed within these granules containing bacterial cells and EPS and a freely structured external portion consisting of ciliates and bacteria or fungal filaments (WEBER et al. 2007).

The exopolymeric material excreted by the microorganisms has been detected in significant quantities both in aerobic and anaerobic granules, forming a tridimensional matrix in which bacteria and other particulate material are jointly present (GROTENHUIS et al. 1991). These extracellular polymeric substances (EPS), which are comprised of polysaccharides, proteins, nucleic acids, humic acids, and lipids, contribute to the cellular adhesion and the formation of bioaggregates (biofilms and sludge flocs) and play an important role in the aerobic granulation process. They are advantageous in several aspects related to the survival of microbial cells under a great variety of circumstances. Furthermore, they provide stable operation of the aerobic granular sludge processes as they are strongly related to the stability of the granules (SCHMIDT and AHRING 1994; LIU et al. 2004b; NIELSEN and JAHN 1999). The importance of EPS is also highlighted considering that the blocking of its synthesis could hinder or even totally prevent microbial aggregation, as reported by CAMMAROTA and SANT'ANNA (1998) and by HWANG et al. (2006).

It should be noted that the polysaccharides are the only components of the exopolymeric material which are synthesized extracellularly for a certain specific function, while the proteins, lipids, and nucleic acids are present in the extracellular polymeric network due to the excretion of intracellular polymers or as a result of cell lysis (DURMAZ and SANIN 2001). Although present in all types of microbial aggregates, such as flocs and biofilms, exopolymeric materials are present in anaerobic and aerobic granules in greater quantity (TAY et al. 2001a). The composition of EPS is variable and is related to the microbial species present and their complexity, to the physiological state of the bacteria, and to the operational conditions in which the development of the granules occurs.

From the microbiological point of view, the extracellular polymeric substances can help to stabilize the membrane structure and serve as a protective barrier. LIU et al. (2004a) proposed the hypothesis that these exopolymers unite the cells and other particulate material in aggregates, facilitating their interaction and providing an extensive surface area for microbial binding, thus acting as biological "glues." Exopolymers are strongly associated with the cohesion and adhesion of cells, performing a crucial function in the microbial physiology and in maintaining the integrity of the structure of communities of immobilized cells. In addition, they can be involved in the regulation of the energy metabolism of bacteria. High shear forces stimulate bacteria to secrete more exopolymeric material, thus contributing to the formation of granules with strong compact structures. Under normal operating conditions, there is no need for the microorganisms to secrete excessive quantities of EPS. The increase in the production of EPS observed in biogranules is

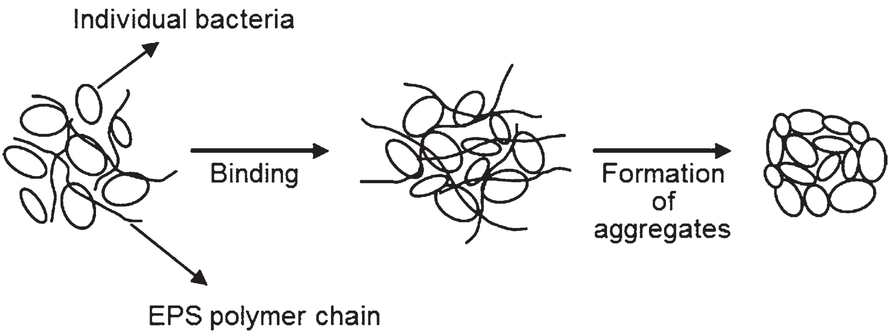

Fig. 4.5 Schematic illustration of the biogranulation process aided by the presence of EPS, evidenced through the formation of points of binding between microflocs and filaments (adapted from LIU et al. 2004a)

induced by the stressing operating conditions. In this context, several operational parameters can stimulate the bacteria to secrete greater amounts of these substances. These include the reactor configuration, the substrate composition, the substrate loading rate, the hydraulic retention time, hydrodynamic forces, the settling time in sequencing batch reactors, and the temperature (LIU and TAY 2004; LIU et al. 2004a, b).

Since EPS accumulate on the cell surface as capsule material, some cell characteristics, such as the hydrophobicity, charge density, binding site, and morphology, can be affected. It has been proposed that the extracellular polymeric material can reduce the negative charge of the cell surfaces, reducing the repulsion between neighboring microbial cells, favoring binding between them and with other inert particulate matter, and promoting their sedimentation in aggregates rather than as individual cells (SHEN et al. 1993; SCHMIDT and AHRING 1994). The hydrophobic interaction is recognized as being important to the biogranulation process, considering that most bacteria maintain contact with hydrophobic surfaces and with other cells, but do not bind to hydrophilic surfaces (GHIGO 2003). Figure 4.5 shows a schematic diagram of the biogranulation aided by the presence of extracellular polymeric substances, which act as agents for binding between neighboring cells and between cells and inert particulate matter.

Since it is responsible for the structural integrity of the aerobic granules, the exopolymeric material has been considered as not being easily biodegraded by the microorganisms which produce it, even in the absence of a substrate (SUTHERLAND 1999). Other studies have shown that during the period when an external substrate was not available, there was the induction of EPS degradation by the producer microorganisms, which resulted in the detachment of bacteria (ZHANG and BISHOP 2003; RUIJSSENAARS et al. 2000).

Although there is no consensus regarding the role of the different components which comprise the EPS matrix in the structural stability of the aerobic granules and despite the controversy regarding this subject, an interesting study was carried out by ADAV et al. (2008b). These authors sought to identify the individual contribution of the different components of EPS in terms of the structural stability of aerobic granules fed with wastewater containing phenol as the only carbon source. The granules were cultivated in a sequencing batch reactor, with an operation cycle of 6 h (5 min of filling, 320 min of aeration, 30 min of settling, and 5 min of effluent withdrawal) and with a high height/diameter ratio ($H/D = 24$). The granules had an average diameter of 900 μm (ADAV et al. 2008b).

The functions of the proteins, α- and β-polysaccharides, and lipids were evaluated via their selective hydrolysis using specific enzymes for each component of the EPS (proteinase K for proteins, lipase for lipids, α-amylase for α-polysaccharides, and β-amylase for β-polysaccharides), and the structural changes in the granules were observed using in situ fluorescence labeling and confocal laser scanning microscopy (CLSM). The concentration of proteins, carbohydrates, and lipids in the control granules (without the addition of enzymes) were 240.0 ± 13, 61.0 ± 9.4, and 51.1 ± 7.8 mg/gVSS, respectively. The protein/carbohydrate ratio of the granules fed with phenol was approximately 3.9 (ADAV et al. 2008b).

The authors observed from the results obtained in the fluorescence labeling of the control granules with different labels specific for each EPS component that the proteins and dead cells were distributed mainly in the center of the granule, while the active cells and the α-polysaccharides were located in the external regions of the granular biomass. β-Polysaccharides were located both in the center and in the external regions of the granules (ADAV et al. 2008b).

The selective enzymatic hydrolysis of the proteins, lipids, and α-polysaccharides had a minimal effect on the tridimensional structural integrity of the granules and did not lead to their disintegration. However, the selective hydrolysis of β-polysaccharides caused the fragmentation of the granules, generating small particles of 10–80 μm. These results reinforced the conclusions proposed by WANG et al. (2005), who stated that β-polysaccharides comprise the most important components in terms of the granule stability. These components of the extracellular polymeric matrix were attributed the function of acting as the main support of the granule structure, in which proteins, lipids, α-polysaccharides, and cells are interconnected, ensuring the mechanical stability and structural integrity of the granules. ADAV et al. (2008b) also stated that adequate mechanical resistance can be obtained using limited quantities of β-polysaccharides, a statement supported by the fact that the granules are stable even when the protein/polysaccharide ratio is high (around 4).

4.4 Factors Affecting Aerobic Granulation

The aerobic granulation process is affected by several operational parameters, notably the time allocated to the settling of the granules in sequencing batch reactors, growth rate of microorganisms, feed strategy, dissolved oxygen concentration, aeration intensity, reactor configuration, substrate composition/concentration, temperature, and pH. Thus, it should be possible to successfully cultivate aerobic granules if certain favorable conditions are established (LIU and TAY 2004).

The main factors which affect the formation of aerobic granules will be described below. The sequence in which these factors are presented represents, to a certain extent, the order of their importance in the formation of the aerobic granular sludge. It should be noted that in some cases the factors which significantly influence the aerobic granulation may be interrelated, with one factor affecting another. Thus, a global analysis of all of the factors should be carried out without separating them as if they act independently in the granular sludge formation process. In fact, their combination appears to be a crucial factor in the obtainment of stable granules.

4.4.1 Settling Time

The mechanism involved in the formation of aerobic granules in sequencing batch systems is not completely understood; however, most studies reported in the literature indicate that the settling time is one of the main factors related to the formation of dense stable granules. As mentioned in Sect. 4.4.2, reduced settling times lead to a strong "selection pressure" on the biomass present in the reactor. In fact, when long sedimentation periods are applied, flocs with poor settling characteristics are not effectively removed from the reactors. In this condition they may become dominant and therefore hinder the aerobic granulation. Thus, it can be considered that aerobic granules are formed when the settling times are below a certain critical value.

Some studies have indicated that reduced settling times, as selection pressure, contribute to enhancing aerobic granulation (JIANG et al. 2002; LIN et al. 2003; HU et al. 2005). TAY et al. (2002b) evaluated the granulation of nitrifying bacteria under different settling times and observed the importance of keeping them short in order to obtain the granules. WANG et al. (2007) observed that the granule stability can be improved with a gradual increase in the selection pressure.

Seeking to demonstrate the importance of the selection pressure and the effect of the settling time on the mechanism involved in the formation of aerobic granules, LIU et al. (2004b) operated four SBRs (SBR_1–SBR_4) of 2.5 L, with a height and diameter of 127 and 5 cm, respectively. The operation cycles of each reactor were similar, lasting for 4 h. Nevertheless, the settling time was different in each system. In the first phase, in order to evaluate the effect of the settling time on the formation of granules, this time was maintained at 20, 15, 10, and 5 min in SBR_1–SBR_4, respectively. The biomass concentrations were 5.3, 4.9, 5.5, and 5.4 g/L in SBR_1–SBR_4, respectively. In the reactor with the shortest settling time (SBR_4), excellent formation of granules was observed, with the absence of sludge flocs. However, in the other reactors, the percentages of granules formed were only 10% in SBR_1, 15% in SBR_2, and 35% in SBR_3 (LIU et al. 2004b).

After reaching a period of granule stabilization, the settling time was reduced from 20 to 5 min in SBR_1, 15 to 2 min in SBR_2, and 10 to 1 min in SBR_3. This procedure led to a high loss of biomass (washout). However, after reestablishing equilibrium in the reactors, the sludge flocs were completely replaced by aerobic granules. The authors also observed that the shorter the settling time applied, the lower the sludge volumetric index (SVI) obtained and the greater the hydrophobicity of the cell surface, contributing to the formation of stable granules (LIU et al. 2004b).

In the same context, QIN et al. (2004) evaluated the effect of the settling time on the granulation process in an SBR. The authors observed that the aerobic granules were successfully cultivated and became dominant only when the reactors were submitted to settling times of less than 5 min. On the other hand, at longer settling times, a mixture of granules and flocculent sludge was obtained.

4.4.2 Bacterial Growth Rate

As briefly mentioned in Sect. 4.4.2, the formation of dense stable aerobic granules is based, among other factors, on a reduction in the bacterial growth rate. One way to obtain this characteristic in systems fed with easily biodegradable substrates is to convert them into intracellular polymers, such as polyhydroxyalkanoates (PHA). One of the best ways to achieve this conversion is to submit the granular sludge reactors to two characteristic regimes: feast (with substrate available) and famine (with substrate completely consumed) (DE KREUK and VAN LOOSDRECHT 2004). The combination of these regimes is known as feast-famine. The feast-famine regime, which, in fact, represents a type of microbial selection, is easily obtained in reactors which operate in discontinuous mode, such as SBRs. This characteristic explains the preference for the use of these systems in the cultivation of aerobic granules, in agreement with the information given in Sect. 4.4.2.

During the feast phase, high concentrations of easily biodegradable substrates are present in the liquid medium, and they are stored as intracellular compounds by the microorganisms. When the feast phase is anaerobic, polyphosphate-accumulating organisms (PAOs), which are responsible for the biological removal of phosphate, or glycogen-accumulating organisms (GAOs) are selected depending on the availability of phosphorus in the influent (BRDJANOVIC et al. 1998; DE KREUK and VAN LOOSDRECHT 2004). On the other hand, when the feast regime is aerobic the growth of other heterotrophic microorganisms is favored. The stored substrates can be used by the microorganisms during the famine period (when no external substrate is available) for growth and maintenance. The growth rate during the famine regime is generally lower than during periods when highly biodegradable external substrates are present. Thus, the formation of intracellular polymers favors the development of dense stable aerobic granular sludge (BEUN et al. 2002), although this stability is not guaranteed under reduced oxygen concentration conditions (MOSQUERA-CORRAL et al. 2005). To ensure this stability, other operational conditions need to be ensured, as evidenced by DE KREUK and VAN LOOSDRECHT (2004). It is important to note that when the selection of bacteria such as PAOs and GAOs occurs in anaerobic-aerobic systems, the settling time (described in Sect. 4.4.1) becomes a less important factor, due to the fact that these bacteria have an inherent tendency to form microbial aggregates (DE KREUK et al. 2005a).

PICIOREANU et al. (1998) postulated that stable biofilms are formed when the ratio between the biomass growth rate and the diffusion transport is low (weak substrate and oxygen gradients). When this ratio is high, that is, when the gradients are strong, structures in the form of irregular flocs are predominant. This supposition was described through a characteristic G (growth) number, as shown in Eq. (4.1):

$$G = L_{\mathrm{Y}}^{2} \cdot \frac{\mu_{\mathrm{m}} C_{\mathrm{xm}}}{D_{\mathrm{S}} C_{\mathrm{So}}} \tag{4.1}$$

where G represents parameters which considerably affect the structures of the biofilms and granules, C_{So} is the concentration of soluble nutrients in the bulk, D_{S} corresponds to the diffusion coefficient, C_{xm} represents the maximum biomass density in the biofilm, μ_{m} is the maximum specific microbial growth rate, and L_{Y} corresponds to the biofilm thickness or granule radius.

A low G value results in more stable and homogeneous biofilms or better granule formation. Therefore, stable formation of granules at low oxygen concentrations should occur when the microorganisms have a low growth rate (PICIOREANU et al. 1998). This hypothesis has been demonstrated experimentally in the work of DE KREUk and VAN LOOSDRECHT (2004), as described in Sect. 4.4.5.

4.4.3 Feeding Strategy

As mentioned in Sect. 4.4.2, the adoption of the feast-famine regime is directly related to the selection of microorganisms with a lower growth rate, which are able to convert easily biodegradable substrates into intracellular polymers. These microorganisms lead to the development of dense stable aerobic granules, even at low dissolved oxygen concentrations (DE KREUK and VAN LOOSDRECHT 2004).

Although some studies have shown that periods without substrate are not a prerequisite for aerobic granulation (LIU and TAY 2008; LIU and TAY 2007), increases in the hydrophobicity due to a lack of carbonaceous material have been reported (CHEN and STREVETT 2003; KJELLEBERG and HERMANSSON 1984). During the operation of an SBR, periods of aeration with no substrate available lead to the development of more hydrophobic bacteria, facilitating microbial adhesion. This appears to be a strategy of the microorganisms in response to the lack of substrate, and under these conditions their surface characteristics change (KJELLEBERG and HERMANSSON 1984; TAY et al. 2001a). From the thermodynamic point of view, an increase in the hydrophobicity of the cell surfaces leads to a reduction in the excess Gibbs energy at the surface, which results in greater interaction between cells and dense stable structure. In addition, cells present in colonies submitted to periods without feed can form fibrils which strengthen the intercellular interaction and communication (VARON and CHODER 2000). In this context, it is clear that periods in which substrate is not available favor the formation of granules with a high capacity for microbial aggregation, resulting in more dense, compact, and resistant granular biomass structures.

In contrast to the strategy of adopting the feast-famine regime, there is evidence in the literature that a long period of aeration with no substrate available can lead to a reduction in the granule stability (WANG et al. 2006). McSWAIN et al. (2004) obtained an improvement in the aerobic granulation performance when using an intermittent feeding system, with several filling phases. Under these conditions, the formation of dense compact aerobic granules was favored.

4.4.4 Dissolved Oxygen and Aeration Intensity

Aerobic granules can be formed under reduced dissolved oxygen conditions, such as 0.7–1.0 mg/L (DANGCONG et al. 1999), or at above 2 mg/L (TAY et al. 2002a; BEUN et al. 1999, 2002; DE KREUK and VAN LOOSDRECHT 2004). In some cases it has been reported that the stability of aerobic granules is negatively affected by a reduction in the dissolved oxygen concentration (DE KREUK and VAN LOOSDRECHT 2004). Greater details regarding the influence of this parameter are given in the description of case studies involving the formation of aerobic granular sludge (Sect. 4.4.5).

The aeration intensity and the upflow air velocity are related to the hydrodynamics and the shear forces (ADAV et al. 2008a; BEUN et al. 1999). These, in turn, can stimulate the production of extracellular polysaccharides (TAY et al. 2001b), which, as mentioned in Sect. 4.4.3, are related to the cohesion and adhesion of the cells. Consequently, they are important agents responsible for the structural integrity of communities of immobilized cells, as observed by TAY et al. (2001c).

The formation of aerobic granules can therefore be favored when they are submitted to high rupture forces, represented, in this case, by high aeration intensities and upflow air velocity. In addition, the development of filamentous bacteria able to reduce the excellent sedimentation capacity of the granular sludge is minimized.

ADAV et al. (2007b) compared the granulation processes in three identical reactors fed with wastewater containing phenol and submitted to different aeration intensities (1–3 L air/min). At low air flows (1 L/min), granules were not formed. On the other hand, at the highest air flow tested (3 L/min), mature stable granules were obtained (1.0–1.5 mm) with compact inner core. At intermediate air flows (2 L/min), large granules developed (3.0–3.5 mm) with abundant filaments. The same authors stated that intermediate aeration levels are not able to provide sufficient oxygen or to break the filaments present in abundance, leading to possible decrease in the performance of SBRs (ADAV et al. 2007b).

In a study carried out by TAY et al. (2001a), the effect of shear forces on the aerobic granulation process was evaluated. The authors began the operation of an SBR fed with glucose as the carbon source submitted to two surface air velocities (0.008 and 0.025 m/s). At the lowest air velocity applied, only soft flocs were formed and granules were not observed. On the other hand, when the system was submitted to an air velocity of 0.025 m/s, granules with a regular shape were obtained. The authors also added that, according to the laws of thermodynamics, shear forces caused by upflow aeration can force the dense aggregates periodically submitted to conditions where substrate is lacking (considered to be an important factor in aerobic granulation as discussed in Sect. 4.4.3) to form regular-shaped granules, which have a minimum of free energy at the surface.

LIU et al. (2005) submitted two airlift sequencing batch reactors to different upflow air velocities (2.2 and 3.3 cm/s, respectively). In the reactor in which the lower velocity was used, there was a decrease in the SVI value from 103.5 to 47.2 mL/g after the appearance of the first granules. However, a few days later, filamentous organisms appeared which led to an increase in the SVI to 170 mL/g and, consequently, biomass washout from the reactor. On the other hand, in the reactor in which an upflow velocity of 3.3 cm/s was applied, the reduction in the SVI was greater, dropping to 26.5 mL/g. In this reactor the quantity of filamentous bacteria was very low, and the granules formed had a well-defined structure.

4.4.5 Reactor Configuration

Another factor which influences the formation of aerobic granules is the reactor configuration, which affects the flow of liquid and the microbial aggregation inside the reactor (BEUN et al. 1999). Aerobic granulation, when carried out in reactors with the format of a column and upward flow, differs from the process carried out in perfectly mixed reactors, mainly due to the hydrodynamic properties of each system, which modify the interactions between the flow and the microbial aggregates. The reactors in the format of a column, with a high height/diameter ratio, promote a long circular flow trajectory, allowing the microbial aggregates to be constantly subjected to hydraulic friction and high turbulence (also provided by air upflow), and regular-shaped granules tend to be formed. On the other hand, in perfectly mixed reactors, the microbial aggregates move with flows dispersed in all directions and are subjected to variable shear forces, upflow trajectories, and random collisions. These conditions favor the formation of granules with irregular shapes and sizes (LIU and TAY 2002).

Considering that the settling velocity is an important criterion for granule selection, the high height/diameter ratio of column reactors is advantageous. Firstly, this ensures a long circular trajectory which allows hydraulic friction between aggregates, and, secondly, when associated with the absence of a secondary settling tank, this characteristic results in a compact reactor.

WINKLER et al. (2011) and BASSIN et al. (2012a) observed segregation of the biomass along the sludge bed of granular sludge reactors with a high height/diameter ratio aimed at the simultaneous removal of nitrogen and phosphorus. Through fluorescence in situ hybridization (FISH) analysis, the authors observed that at the bottom of the sludge bed, there was a greater amount of polyphosphate-accumulating organisms (PAOs), while at the top, glycogen-accumulating organisms (GAOs) were dominant. These latter organisms only compete with the PAOs for organic matter and do not contribute to phosphorus removal. Thus, their presence in the reactor is undesirable. Through the selective removal of sludge from the top of the reactor to control the solids retention time, it was possible to reduce the quantity of GAOs and allow PAOs to become dominant. With this approach it was possible to obtain good phosphorus removal efficiencies even with the system operating at 30 °C, which is favorable for the development of GAOs. Both studies highlight that the biomass segregation along the sludge bed, which apparently is more commonly observed in reactors with high height-diameter ratio, offers an additional possibility for influencing the competition between different microorganisms with the aim of obtaining the desired microbial population.

DE KREUK and VAN LOOSDRECHT (2004) compared two granular sludge reactor configurations (sequencing batch airlift reactor, SBAR, and sequencing batch bubble column, SBBC reactor), which are the most commonly used systems for the cultivation of aerobic granules. In SBBC reactors combined with a pulse feeding regime under aerobic conditions, instable granules were formed (BEUN et al. 2000; LIU and TAY 2002). The stability achieved by the SBAR is probably due to the fact that in this type of reactor, the local shear forces are stronger. When the feeding was carried out under anaerobic conditions in an SBBC reactor, stable sludge granules were formed, although the reactor start-up period was longer. These observations indicate that on reducing the maximum growth rate of the microorganisms through the selection of PAOs and GAOs, which induced the complete conversion of acetate into PHB, the effect of the shear force on the granule formation appears to become less important. Thus, the characteristics of the granules formed in the SBBC reactor and the SBAR were similar. However, the fact that it took longer to obtain granulation in the SBBC reactor compared with SBAR is an indication that the hydrodynamic forces in the latter reactor are more favorable. Further information regarding a comparison between these two reactor configurations is given in Sect. 4.4.5 (Table 4.1).

It should be emphasized that the possibility of using a bubble column reactor, rather than an airlift reactor, in large scale, has significant economic advantages, considering that the former is easier and cheaper to construct. In addition, the effluent discharge can be more easily carried out in an SBBC reactor, since in the case of SBAR, the presence of a riser requires more attention to allow the same discharge in the riser and the downcomer tubes. If this design detail is not taken into consideration, the granular sludge could be washed out of the reactor (DE KREUK and VAN LOOSDRECHT 2004).

4.4.6 Substrate Composition and Concentration

Various substrates have been used to cultivate aerobic granules, and the main ones include glucose, acetate, phenol, starch, ethanol, molasses, sugar cane, and other synthetic components (LIU and TAY 2004; TAY et al. 2002a; BEUN et al. 1999; ZHENG et al. 2005; ADAV et al. 2007a, b). Details regarding the cultivation of granules with real wastewater have also been reported (SCHWARZENBECK et al. 2004;

Table 4.1 Comparison of different reactor technologies (SBAR, SBBC, and BASR) used in different studies involving granular sludge (adapted from BEUN et al. 2000)

SBAR	SBBC	BASR
(Sequencing batch airlift reactor)	(Sequencing batch bubble column)	(Biofilm airlift suspension reactor)
• Discontinuous system feeding	• Discontinuous system feeding	• Continuous system feeding
• Substrate: acetate	• Substrate: ethanol	• Substrate: acetate
• No secondary settling tank required	• No secondary settling tank required	• No secondary settling tank required
• Requires riser	• Does not require riser	• Requires riser and three-phase separator
• Does not require support	• Does not require support	• Requires support
• Selection variable: settling time	• Selection variable: settling time	• Selection variable: HRT
• Detachment mainly controlled by hydrodynamic conditions	• Detachment mainly controlled by hydrodynamic conditions	• Detachment mainly controlled by carrier concentration
• Nitrification and denitrification occur	• Nitrification and denitrification occur	• Denitrification does not occur
• Biomass concentration: 4 g/L	• Biomass concentration: 3 g/L	• Biomass concentration: 0.7 g/L
• Biomass density: 48 g/L	• Biomass density: 12 g/L	• Biomass density: 15 g/L
• Average granule diameter: 1.0 mm	• Average granule diameter: 2.0 mm	• Average diameter of biomass particles: 0.35 mm (carrier diameter: 0.26 mm)
• Specific surface area of granules in reactor: 785 m^2/m^3	• Specific surface area of granules in reactor: 340 m^2/m^3	• Specific surface area of granules in reactor: 1700 m^2/m^3

ARROJO et al. 2004; CASSIDY and BELIA 2005; INIZAN et al. 2005; TSUNEDA et al. 2006; FIGUEROA et al. 2008). Based on these studies, it can be observed that most wastewaters containing sufficient amounts of biodegradable organic matter are successfully treated by means of aerobic granular sludge.

TAY et al. (2001a) evaluated the characteristics of granules cultivated in two sequencing batch reactors, one fed with glucose (SBR_1) and the other with acetate (SBR_2) as carbon source. Both reactors were inoculated with activated sludge originating from a wastewater treatment plant. The SVI of the sludge used as the inoculum was high, corresponding to 230 mL/g. This value is associated with a significant presence of filamentous bacteria. After 1 week of operation, it was observed that the filaments gradually disappeared from the reactor fed with acetate, while they remained in that fed with glucose. The SVI values for the compact aggregates formed in SBR_1 and SBR_2 were 190 and 178 mL/g, respectively.

After 2 weeks of operation, the formation of round granules was observed in both reactors. Filamentous bacteria continued to predominate in the reactor fed with glucose. In the reactor fed with acetate, these bacteria disappeared completely. Nonetheless, the formation of aerobic granules had a similar pattern in the two reactors. The SVI of the granules in this stage of the aerobic granulation process decreased to 115 mL/g in SBR_1 and to 114 mL/g in SBR_2 (TAY et al. 2001a).

After 3 weeks of operation, mature granules were obtained in both reactors. The granules fed with glucose had a soft external surface, which was probably related to the predominance of filamentous bacteria. The SVI of the granules obtained in this stage of the granulation process lays within the range of 51–85 mL/g in SBR_1 and between 50 and 80 mL/g in SBR_2. The average diameters of the granules cultivated in SBR_1 and SBR_2 were 2.4 and 1.1 mm, respectively (TAY et al. 2001a).

Differences were observed in the microbial diversity of the granules depending on whether they were fed with glucose or acetate. In the former case, the granules consisted mainly of bacteria which had a round shape, particularly their inner core, and some rod-shaped bacteria with some filaments. In the latter case, the microbes were predominantly represented by rod-shaped bacteria. In relation to the settling velocity, an important parameter for aerobic granulation, values of 35 and 30 m/h were obtained for the granules cultivated in SBR_1 and SBR_2, respectively. The high settling velocities associated with granular sludge are strongly related to the dense microbial structure of these microbial aggregates.

The concentration of substrate may also affect the growth of filamentous bacteria. In general, granules cultivated in SBRs receive constant concentrations of organic substrates in terms of chemical oxygen demand (COD) (DANGCONG et al. 1999; BEUN et al. 1999; TAY et al. 2001a). After the formation of mature granules, the biomass concentration in the reactor is typically in the range of 10 to 20 g/L. In systems operating in batch regime, the relation between the initial substrate concentration and the initial biomass concentration (S_0/X_0) can be used to describe the substrate availability to the microorganisms (LIU and LIU 2006). LIU and LIU (2006) demonstrated that during the operation of an SBR with granular sludge, the concentration of biomass increased, resulting in a decrease in the S_0/X_0 ratio. Under these conditions of low substrate availability and a high biomass concentration, the authors observed a significant increase in filamentous microorganisms.

4.4.7 Sludge Used as Inoculum

The quality of the sludge inoculated into the reactors aimed at the formation of aerobic granules is related to its macroscopic characteristics, settleability, surface properties (hydrophobicity and charge density), and microbial activity (LIU and TAY 2004). In most studies, the aerobic granules were from inoculum originating from activated sludge systems. The characteristics of the bacterial community present in the inoculum is essential for the granulation process. Hydrophilic bacteria tend to bind less to the microbial flocs in comparison to hydrophobic bacteria, which constitute most of the free bacteria in the effluent of treatment plants (ZITA and HERMANSSON 1997). The hydrophobicity of cells influences the granulation process, as described in Sect. 4.4.3. With a higher number of hydrophobic bacteria in the inoculum, the granules form more rapidly and with better settling characteristics (WILEN et al. 2007).

4.4.8 Temperature

The formation of stable granules is affected by the temperature. In general, most studies on aerobic granular sludge technology have been carried out at ambient temperature (20–25 °C). DE KREUKI et al. (2005b, c) investigated the short- and long-term effect of temperature variations on the stability of aerobic granules and on the conversion processes which occur within the granular structure in a sequencing batch reactor. During the reactor start-up, when the temperature applied was 8 °C, granules with an irregular structure were formed, and growth of filamentous bacteria was observed, leading to a substantial washout of biomass and operational instability. DE KREUK et al. (2005c) also observed that when the reactor start-up was carried out at 20 °C, it was possible to operate the system in a stable manner, even when the temperature was further decreased to 15 °C and subsequently to 8 °C. The results obtained by these authors stress the importance of carrying out the start-up of granular sludge reactors, in pilot or full scale, in periods of high temperature, such as in summer. The denitrification capacity and the nutrient removal rate were reduced at low temperatures (DE KREUK et al. 2005c), as it will be detailed in the applications of aerobic granules (Sect. 4.4.7).

4.4.9 pH

The pH of the reactor significantly affects the microbial growth rate. Oxidation at high organic loads produces sufficient quantities of CO_2 to reduce the pH in non-buffered solutions (McSWAIN et al. 2004). Fungi grow well at low pH and can contribute significantly to the initial granulation, as noted in Sect. 4.4.3 (McSWAIN et al. 2004; BEUN et al. 1999). These organisms are able to release protons in exchange for NH_4^+ in solution, thus contributing to decreasing the pH value (*apud* ADAV et al. 2008a). YANG et al. (2008) observed that aerobic granulation at pH 4.0, in the presence of fungi, favored the formation of granules of approximately 7 mm. On the other hand, at pH 8.0, conditions under which the granulation was controlled by bacteria, the granule size reached only 4.8 mm. Despite these findings, the effects of pH on aerobic granulation are still not completely understood (ADAV et al. 2008a, b).

4.4.10 Addition of Divalent Cations

Aerobic granulation can be influenced by the addition of divalent cations, such as iron and calcium. According to some researchers, high concentrations of these cations can accelerate the formation of granules, increase their stability and resistance, and improve their settling characteristics (LIU and TAY 2004; DE KREUK et al. 2005a).

Divalent cations adhere to negatively charged groups on the surface of bacteria and molecules of extracellular polysaccharides, acting as binding points. These, in turn, increase the microbial agglomeration (LIU and TAY 2004).

JIANG et al. (2003) observed that the addition of calcium to the feed medium in concentrations of 100 mg Ca^{2+}/L enabled the formation of granules to occur more rapidly (16 days) in comparison with cases where this cation was not added (32 days). Furthermore, it was observed that granules fed with calcium had better settling characteristics and a higher content of polysaccharides (JIANG et al. 2003).

4.5 Case Studies Involving the Formation of Aerobic Granules

In this section, some studies related to the formation of aerobic granular sludge will be discussed. It should be noted that some of them represent the first investigations in this field of study, and thus they are described in greater detail. In addition, the studies described below demonstrate the influence of some factors which affect the aerobic granulation process, a topic discussed in Sect. 4.4.4.

BEUN et al. (1999) cultivated granules in a bubble column SBR, with a volume of 2.25 or 2.5 L. The internal diameter of the column was 5.6 cm and the total height was 150 cm, which provides a high height/diameter ratio. As mentioned in Sect. 4.4.5, high height/diameter ratios tend to favor the formation of granules with a regular shape, which is related to the hydrodynamic forces present in the reactor. The inoculum used was sludge from a conventional SBR accomplishing COD removal. The experiments were conducted at ambient temperature (20 ± 2 °C), and air was introduced by means of a small bubble diffuser located at the base of the column.

The reactor was fed with synthetic wastewater with a COD of 0.83 g/L, total Kjeldahl nitrogen (NKT) of 0.04 gN/L and total phosphorus of 0.16 gP/L. The cycle was 3 or 4 h. Of this total, periods of filling (with aeration) and settling were 2 min. The discharge period was 1 min, and the aeration period was the remaining time, that is, 177 min for the 3 h cycle and 237 min for the 4 h cycle. The main parameter chosen to select biomass with a high settling velocity was the sedimentation time. The settling velocity varied between 12 and 24 m/h (BEUN et al. 1999).

After the inoculation of the reactor with 10 mL of cells in suspension originating from the aforementioned SBR (start-up period), observations carried out directly in the reactor and by microscopy revealed that pellets of filamentous fungi (filamentous granules) predominated in the reactor, and these functioned as an immobilization matrix on which the bacteria could grow and form colonies. The fact is that the fungi easily formed mycelial filaments which settle easily and could be removed from the reactor. Bacteria do not have this property when in suspension, being washed out of the reactor, which explains the predominance of filamentous fungi during the reactor start-up period (BEUN et al. 1999).

The granules formed were not stable, due to the shear force (mainly caused by the relatively high surface air velocity of 0.041 m/s), and they broke into pieces within a few days, with the release of filaments from the surface of the pellets, which then became more compact. The pellets grew up to a diameter of 5–6 mm and then underwent lysis probably due to the oxygen limitation in their internal parts (BEUN et al. 1999).

Thus, washout of most of the biomass occurred (notably of the filaments which were not able to settle rapidly and stay in the reactor), leading to a new granulation stage. The granules formed in this second stage were not generally filamentous and consisted mainly of bacteria. In this stage, colonies of bacteria remained in the reactor since they were large enough to settle rapidly. These colonies, in turn, later grew forming granules (BEUN et al. 1999). Clearly, this proposed mechanism was based particularly on experiments with this reactor, which was inoculated with a small quantity of suspended sludge, only capable of settling slowly. If the inoculum had consisted of flocs and/or small granules, the mechanism would be different.

The morphology of the granules was analyzed through an evaluation of some parameters which are commonly used to characterize this type of microbial agglomeration, such as the form factor (0 = line, 1 = circular) and the aspect ratio, that is, how round a particle is (minimum Feret diameter/maximum Feret diameter; 0 = line, 1 = circular; Feret diameter, maximum distance between two points along the boundary selected). The values obtained for these two parameters during the whole operational regime were around 0.45 and 0.79, respectively, these being independent of the settling velocity, surface air velocity, organic load applied, and HRT (BEUN et al. 1999).

The pH value fluctuated around 6.5. The carbon source (ethanol) was consumed constantly at the maximum rate until its concentration was negligible, although nitrification did not occur. After the ethanol had been completely consumed, CO_2 continued to be produced due to the conversion of compounds stored within the cells (BEUN et al. 1999).

During the period considered ideal for the formation of stable granules, the reactor was submitted to an organic load of 5 kgCOD/(m^3 day), and the total cycle time was 3 h. The cell retention time increased during these periods from 1.8 to 3.4 days, mainly due to the increase in the sludge bed volume. Under these conditions, the formation of granules with an average diameter of 3.3 mm and a density of 11.9 gSSV/$L_{granules}$ was observed. At high organic loads, as expected, higher biomass growth was observed. When the other parameters (minimum settling velocity, surface air velocity and HRT) were ideal, biomass accumulated in the system and its concentration increased. The organic load did not directly affect the granulation under the conditions tested, although it did influence the final shape of the granules (BEUN et al. 1999).

It was observed that a short HRT (6.75 h) and a high shear rate were favorable for the granulation process. Also, it was verified that the minimum settling velocity (24 m/h) could be applied only when the organic load was low (2.5 COD/(m^3 day)). At this load, less biomass accumulated in the reactor, and thus a higher minimum settling velocity could be applied without the granules exerting an influence on each other during sedimentation. However, with an increase in the organic load to 5 kgCOD/(m^3 day), the quantity of granules increased, and the sedimentation was adversely affected. Under these conditions, the biomass was not able to settle below the treated effluent discharge level, and thus it was partially washed out of the reactor during the effluent withdrawal stage. Under these circumstances, reducing the minimum settling velocity, that is, increasing the settling time, led to improved sedimentation and to a greater accumulation of biomass in the reactor. According to the authors, another way to avoid washout of the biomass would be to counterbalance the effect of the increased organic load by applying a high shear rate, allowing the formation of more compact granules and stable operation (BEUN et al. 1999).

DANGCONG et al. (1999) observed the aerobic granulation process in a sequencing batch reactor (SBR) operating in laboratory scale fed with a synthetic medium containing sodium acetate as the organic substrate and ammonium chloride as the nitrogen source. The reactor was inoculated with activated sludge containing filamentous bacteria. The seed sludge SVI was in the range of 250–300 mL/g. Oxygen concentration was initially maintained at 3.5–4.0 mg/L. The HRT was 8 h and the biomass retention time was 20 days. The total cycle was 4 h (0.5 h of filling, 0.75 h of reaction, 2.5 h of sedimentation, and 0.25 h of supernatant removal). The reactor was maintained at 25 °C under stirring at 400 rpm to ensure good mixing and oxygen transfer.

The authors observed that after 20 days, the filamentous bacteria disappeared and the SVI decreased to 100–150 mL/g. The dissolved oxygen concentration in the reactor was then maintained at lower values within the range of 0.7–1.0 mg/L. Under these conditions, microbial flocs were formed and the SVI increased to 150–200 mL/g. However, the flocculent sludge was gradually converted to granules. After 1 month of operation at low DO concentration, almost all of the biomass was in the form of granules. At this stage of the process, the removal efficiencies for COD, N-NH_4^+, and nitrogen were 95, 95, and 60%, respectively. Filamentous bacteria disappeared, and the SVI was 80–100 mL/g, even though the DO concentration was less than 1.0 mg/L. The reactor maintained the same characteristics for 3 months of operation (DANGCONG et al. 1999).

The granular morphology analyzed by microscopy revealed that the granules were spherical, in contrast to flocculent sludge. The diameter of the granules varied between 0.3 and 0.5 mm. These values are small compared with the diameter of the granules formed in reactors operating under anaerobic or anoxic conditions (2–3 mm). In the case of this research, in particular, the shear forces caused by the stirring and aeration did not allow the formation of granules with large diameters. Nonetheless, the structure of the granules, particularly their inner core, was very similar to that of granules cultivated under anoxic conditions. This finding suggests that the internal region of the granules might have been subjected to anoxic conditions due to the low DO concentration in the liquid. This hypothesis is reinforced by the fact that 60% of the influent ammonium nitrogen was denitrified to nitrogen gas (DANGCONG et al. 1999).

The authors also observed a high COD removal rate (2.16 gCOD/(gSS day)) and high nitrification activity (0.24 gN-NH_4^+/(gSS day)). The organic and nitrogen loads were 1.5 kgCOD/(m^3 day) and 0.18 kgN-NH_4^+/(m^3 day), respectively. Even operating under this organic load and DO concentration of 0.7–1.0 mg/L, the ammonium fed to the system was completely nitrified. The authors also observed that the COD removal or even the carbon oxidation activity increased with an increase in the air flow, although the DO concentration was maintained constant (less than 1% of air saturation). These results suggest that the granular sludge activity was strongly influenced by the oxygen supply, but not by the DO concentration of the bulk (DANGCONG et al. 1999).

BEUN et al. (2000) cultivated aerobic granular sludge in a 3 L sequencing batch airlift reactor (SBAR). The HRT applied was 5.6 h and the organic load was 2.3 kgCOD/(m^3 day). The duration of each cycle of the SBAR was 3 h. Of this total, 3 min corresponded to feeding, 173 min to aeration, 2 min to settling, and 5 min to the effluent withdrawal. The reactor was fed with a synthetic medium containing sodium acetate as carbon source and ammonium chloride as nitrogen source. The reactor was inoculated with sludge originating from an activated sludge system. The reactor operation was initiated twice using different inocula. The first operational period had a duration of 41 days (Experiment 1), while the other lasted 55 days (Experiment 2).

In Experiment 1, 30 days were required to reach the steady state, while in Experiment 2, the time necessary to reach stable operation was 10 days. The difference in the duration of the start-up periods is probably related to the inoculum used. It should be noted that Experiment 1 was carried out to describe the start-up period of the reactor and the initial development of the granules. The aim of Experiment 2 was to describe the stability of the granules under certain conditions since this reactor was operated for a longer time (55 days) (BEUN et al. 2000).

The sludge inoculated into the reactor in Experiment 1 consisted of a mixture of filaments and small particles. After 30 days of operation, the diameter of the granules was approximately 1.2 mm, and they had a smooth surface. During this period, the presence of flocculent biomass was not observed visually. From the start-up to 30 days of operation, the concentration of biomass in the reactor increased from 2 to 6 g/L, and the solids retention time (SRT) increased from 2 to 30 days, later reducing to 17 days. The increase in the SRT was caused by a decrease of filamentous and flocculent sludge. The selection of granules was achieved by applying a short settling time in the reactor with a high height/diameter ratio. Sludge particles with low settling velocities were washed out of the reactor with the treated effluent, and only granules with good settling characteristics (minimum settling velocities of 16.2 m/h) were retained in the system. This allowed a reduction in the effluent biomass concentration (BEUN et al. 2000).

During Experiment 2, the biomass concentration in the reactor remained stable at 4 g/L, and the solids retention time was, on average, 9 days. The average diameter of the granules obtained in the steady state was 1.0 mm. The biomass density had a stable value of 48 $g/L_{granules}$ (BEUN et al. 2000).

Through the determination of the acetate concentration during one representative SBAR operation cycle, it was observed that this organic substrate was consumed in 21 min (feast period). The concentration profiles of NH_4^+, NO_3^+, and NO_2^- showed that both nitrification and denitrification occurred in the reactor. During the feast period, the concentrations of NO_3^- and NO_2^- decreased, due to the occurrence of denitrification with acetate as carbon source. In the period without acetate available (famine period), the concentrations of NO_3^- and NO_2^- initially increased (nitrification was more rapid than denitrification) and later decreased again (denitrification with endogenous substrate as carbon source) (BEUN et al. 2000).

It should be noted that BEUN et al. (2000) did not expect denitrification to occur, since the reactor was operated only under aerobic conditions. During the famine period, the concentration of dissolved oxygen in the SBAR was almost 100% of air saturation. In general, in aerated and continuously fed airlift reactors, denitrification does not occur (TIJHUIS et al. 1994 *apud* BEUN et al. 2000). The reactors were fed with the same influent and submitted to the same loads as the SBAR used in this research, although they require the introduction of phases without aeration to allow the denitrification process to occur. In the SBAR, significant denitrification occurs even under conditions which are not ideal for this process. These observations are probably related to the fact that the feeding of the reactor was discontinuous rather than continuous (BEUN et al. 2000).

To exemplify the effect of the reactor configuration on the formation of granular sludge and highlight important design criteria, Table 4.1 shows a comparison between the SBAR described in BEUN et al. (2000), the bubble column reactor used by BEUN et al. (1999), and the biofilm airlift suspension reactor (BASR) operated by TIJHUIS et al. (1994). The values for some parameters obtained for each reactor in the steady state (submitted to an organic load of 2.5 kgCOD/(m^3 day) and a surface air velocity of 86.4 m/h) are given in Table 4.1.

In comparison with SBBC, much denser granules with a smaller diameter were obtained in SBAR when the same substrate load and aeration intensity were applied. In SBBC, the distribution of the hydrodynamic forces along the column was more homogeneous compared with SBAR. In the latter reactor, the hydrodynamic forces are greater at the bottom of the reactor where the liquid and particles change the direction of the flow. The stronger hydrodynamic forces concentrated in certain regions of the SBAR probably result in the formation of denser granules with smaller diameters. Also, in the SBBC the stratification of the granules was observed. In the upper part of the reactor, less biomass in the form of granules will be present in comparison with the lower part. This leads to more rapid growth of the granules at the top of the reactor which, in turn, promotes the formation of less dense granules with a larger diameter, and, subsequently, higher detachment and instability are observed (BEUN et al. 2000).

In the same way, on comparing the granules formed in the SBAR with those formed in the continuously fed BASR, denser granules with a larger diameter were obtained in the SBAR. In the case of the BASR, the development of granules is related to the biofilm fragments originating from the rupture of the biofilm particles. The reason for smaller diameters obtained in the BASR may be related to the presence of carriers, which promote a stronger shear force and consequently greater detachment. The granules of the BASR are probably less dense because this reactor was continuously fed, in contrast to the SBAR, which was operated discontinuously (BEUN et al. 2000). As mentioned in Sect. 4.4.2, the adoption of a feast-famine regime obtained through operation in discontinuous mode favors the development of microorganisms with a lower growth rate, which consequently promotes the development of dense granules. Further details regarding the comparison of these different reactor configurations will be provided below in the description of the study carried out by BEUN et al. (2002).

BEUN et al. (2002) cultivated aerobic granular sludge in a sequencing batch airlift reactor (SBAR), with a volume of 3 L, for a period of 140 days. The ratio between the height of the riser and the diameter was high (90 cm/6.25 cm = 14.4), as commonly observed in reactors aimed at the cultivation of aerobic granules. The synthetic medium fed to the reactor had an acetate concentration of 18.3 Cmmol/L, which corresponds to an organic load of 2.5 kgCOD/(m^3 day), and the HRT was 5.6 h. The temperature of the reactor was maintained at 20 °C, and the pH was fixed at 7.0 ± 0.2. The reactor operating cycle comprised 2 min of feeding, 170 min of aeration, 3 min of settling, and 5 min of discharge/drainage of supernatant (treated effluent). The inoculum used was activated sludge originating from a wastewater treatment plant with nutrient (N and P) removal.

In general, it was observed that the acetate (source of organic carbon) was completely consumed within a few minutes (feast period). When no substrate remained, endogenous respiration occurred (famine period). The period of transition from the feast to famine phase was observed through a rapid increase in the dissolved oxygen (DO) concentration in the reactor. During the feast period, the DO concentration in the reactor was relatively low (75% of air saturation) due to the consumption of oxygen during acetate metabolism. After the complete consumption of the substrate, the DO concentration became almost 100% of air saturation (BEUN et al. 2002).

In the first 12 days of operation, the settling period was gradually decreased from 5 to 4 min and then to 3 min, the value applied for the rest of the reactor operation. On the first day of operation, the feast period was approximately 100 min, decreasing to 60 min on the third day. From the third to the fourth day, the feast period increased again due to the washout of large quantities of biomass. The reactor walls became completely covered with biomass during the first 4 days. On the sixth day, the biomass fixed on the walls was removed (BEUN et al. 2002).

Small granules were formed in the reactor within 1 week after the inoculation with activated sludge originating from a conventional wastewater treatment plant. The granulation process then began, and the growth of biomass on the reactor walls decreased. The concentration of biomass in the reactor began to increase considerably, and thus the duration of the feast period decreased once again (BEUN et al. 2002).

It should be noted that in attempting to explain the formation of sludge granules at the beginning of the reactor operation, one possibility is that they originate from the growth of biofilm on the reactor walls, although during the reactor cleaning, the biofilm was completely removed. Considering the high liquid circulation rate, small pieces of the biofilm could be broken off, constituting the initial material for the formation of granules (BEUN et al. 2002).

The selection of only dense granules from a mixture of granules, filaments, and flocs was achieved due to the different settling velocities of the different biomass agglomerates: granules (biomass with high settling velocity) and filaments and flocs (biomass with low settling velocity). This strategy was successful considering that a rapid transition from the presence of sludge in the form of flocs to granular sludge occurred. Another hypothesis which could explain the formation of granules is that flocs with high settling velocity originating from the inoculum may represent the beginning of the granulation process (BEUN et al. 2002). It is interesting to note that in this study, there was no intermediate phase with the predomination of fungi, as observed in a previous study carried out by BEUN et al. (1999).

When the steady state was reached (at around day 37 of operation), the average granule diameter was 2.5 mm, biomass density was 60 gVSS/L, and the settling velocity was greater than 10 m/h. Also, the settling time was chosen so that only particles with a settling velocity greater than 10 m/h would be retained in the reactor. The rest of the biomass which did not settle sufficiently rapidly was washed out with the treated effluent (BEUN et al. 2002).

The biomass consisted of both heterotrophic and nitrifying microorganisms, which were able to withstand some disturbances, such as sudden increases in pH and fluctuations in the DO concentration. After these operational instabilities, the granules generally partially broke up. However, they rapidly returned to their state before the disturbance (BEUN et al. 2002).

The authors also observed that during the operational period (at around day 80), the biomass concentration and density in the reactor decreased due to the deterioration of the granules caused by a decrease in the DO concentration to 50% of air saturation between days 66 and 71 of operation, and thus smaller granules were washed out of the reactor. A low sludge production was observed which was associated with the sludge age of 50 days (BEUN et al. 2002).

BEUN et al. (2002) also cut the granules with the aid of a cutting blade in order to examine the internal structure under a light microscope, and they observed the presence of two layers in the granule: one central layer, with a diameter of around 1.7 mm, and one external layer approximately 0.4 mm thick. The latter had a denser structure in relation to the central part of the granule, which had a softer, more gelatinous structure, besides being transparent. Large empty cavities resulting from complete lysis inside the biomass were not observed in the center of the granules. The depth of oxygen penetration was calculated as between 17 μm (during the feast period) and 20 μm (during the famine period) (BEUN et al. 2002).

The authors also carried out a comparison between the SBAR aimed at the formation of granules and a continuously fed turbulent system (BASR), operated by TIJHUIS et al. (1994). This comparison had been previously described in BEUN et al. (2000), however, in less detail. In the BASR, granules (which, in principle, are biofilms without a material support) were formed from biofilm fragments originating from the rupture of the biofilm particles. The most notable difference between the two systems is related to the biomass density, which was much higher in the SBAR compared with the BASR, as previously highlighted in relation to the work reported in BEUN et al. (2000). As mentioned above, the reason for this considerable difference in the density may be attributed to the way in which the reactors were fed, with continuous feeding in the BASR and intermittent feeding in the SBAR. One point to be taken into consideration is that when there is substrate present in the SBAR (feast period), the acetate penetration into the granules is greater than 500 μm, bearing in mind that the concentration of this substrate in the liquid is high due to the pulse feeding. On the other hand, in the BASR with continuous feeding, the concentration of acetate in the liquid is always low (<0.1 Cmmol/L), which does not allow complete penetration of acetate to the interior of the biofilm (penetration < 20 μm). Thus, the microorganisms present deep within the biofilm will be deprived of acetate, which does not occur in the SBAR, where the cells present in the center of the granules can assimilate acetate and grow (using both oxygen and nitrate as electron acceptors). It is this growth inside the granules of the SBAR which makes the biomass increase, in contrast to the situation in the BASR (BEUN et al. 2002).

As previously mentioned, the electron acceptor required for the conversion of acetate can be O_2 or NO_3^-. In both reactors (SBAR and BASR), O_2 does not completely penetrate the biofilm. In the BASR, the O_2 penetration depth is around 80 μm, which is greater than the penetration of acetate. The O_2 penetration depth in the SBAR during the feast period is around 20 μm, which is less than the depth of acetate penetration. This value can be even lower in cases where the DO concentration in the bulk is decreased. The microorganisms located deeper within the particle than this depth can use NO_3^- as an electron acceptor. When this is not available, cell lysis occurs in the central region of the biofilm giving it a less dense structure, and it finally breaks into pieces (as observed when applying a DO concentration of 50% of air saturation) (BEUN et al. 2002).

The authors also highlight the importance of the influence of the shear force on the biofilm structure, which is determined by the balance between the biomass growth and the shear force (VAN LOOSDRECHT et al. 1995). Considering the total number of particles in the reactor, detachment should be, in principle, greater in the BASR (filled with basalt as the support material, probably with a high frequency of collisions between the particles), since the number of particles in this reactor is ten times higher than that in the SBAR, which would lead to the formation of a less dense biofilm than in the BASR. However, as previously mentioned, the specific growth rate of the biomass in the SBAR is lower than in the BASR, which leads to the formation of a denser biofilm. The balance between these two factors is obviously favored in the SBAR compared with the BASR, since the biomass density in the former system is much higher than that in the latter (BEUN et al. 2002).

GARRIDO et al. (2001) observed that the settling properties of the sludge obtained from an industrial-scale sequencing batch reactor (SBR_{ind}) showing high COD and nitrogen removal from wastewater originating from a laboratory which analyzes industrial dairy products were unsatisfactory. This conclusion was based on the fact that the sludge volume index (SVI) did not fall below 100 mL/gTSS and the zone settling velocity (ZSV) was around 0.3 m/h. Based on the results obtained by GARRIDO et al. (2001) and ARROJO et al. (2004) studied the possibility for the formation of aerobic granular sludge in two sequencing batch reactors (SBR_1 and SBR_2), in order to improve the biomass settling characteristics. Both reactors SBR_1 and SBR_2 were fed with the same industrial wastewater fed to SBR_{ind} and inoculated with the sludge from this latter reactor.

The two reactors were operated under similar conditions during most of the experimental period. However, in the case of SBR_1, an anoxic phase with a duration of 10–30 min was included at the beginning of each cycle, by sparging with nitrogen. The temperature varied between 15 and 20 °C, and the dissolved oxygen was between 0 and 8 mg/L. With no automatic control of the pH, this parameter varied between 7.4 and 8.5. SBR_1 was initially fed with a synthetic medium up to day 27 of operation. This synthetic medium was then gradually replaced with industrial effluent, maintaining a reasonably constant COD. After 48 days of operation, this reactor was fed only with industrial effluent. The operation of SBR_2 began 50 days later, and this reactor was fed only with industrial effluent. The duration of the operational cycle was 3 h (3 min of filling, 171 min of anoxic and aerobic reaction, 1 min of settling, 0.5–3 min of drainage, and a rest period of 2–4.5 min) for both reactors, with a volume exchange ratio of 50% (ARROJO et al. 2004).

In both systems, the poor settling flocculent sludge was almost completely washed out in the first 7 days, since the operational strategy adopted included a short settling period and rapid effluent removal. Only microbial agglomerates with settling velocities higher than 9 m/h were retained in the reactors. Three weeks after the reactor start-up, the formation of small aggregates with an average diameter of 1.05 mm was observed. Flocs in suspension gradually disappeared from the reactor, and the settling properties of the aggregates obtained were good, as were the SVI (60 mL/gSSV) and ZSV (20 m/h). Granules with similar morphology (distinct from the flocculent sludge used as the inoculum) developed in the two systems, and the size distribution was 0.25 and 4.0 mm. The biomass concentration was around 0.2 gTSS/L at the beginning of the experiments and increased to 3 gTSS/L after 50 days of operation. This parameter later reached a stable concentration of 5–6 gTSS/L. The percentage of volatile suspended solids (VSS) in relation to the total suspended solids (TSS) varied from 87 to 95% (ARROJO et al. 2004).

The experimental results showed that the use of industrial wastewater did not appear to adversely affect the development of granules or the accumulation of biomass. The biomass density was around 10–15 $gVSS/L_{granules}$ in both reactors, values similar to those reported by BEUN et al. (1999) (a study discussed earlier in this section), that is, 11.9 $gVSS/L_{granules}$.

MOSQUERA-CORRAL et al. (2005), seeking to find better conditions for the removal of nitrogen in an aerobic granular sludge system, studied the short- and long-term effects of a reduction in the oxygen concentration on the performance of a sequencing batch airlift reactor (SBAR). The reactor was operated in successive cycles of 3 h, with 3 min of filling, 169 min of aeration, 3 min of settling, and 5 min of effluent withdrawal. The short period of settling combined with the height of the reactor contributed to the selection of particles with a settling velocity higher than 12 m/h.

For a period of 150 days, the SBAR was operated under the same conditions as those described in BEUN et al. (2000), the only difference being the COD/N ratio, which was changed from 14 to 8. The oxygen concentration employed was 100% of air saturation. The granules, with an average diameter of 0.6 mm, were formed after 30 days of operation. The biomass concentration in the reactor stabilized at around

5 gVSS/L, the solids retention time reached a stable value of around 25 days, and the maximum density of the granules was 53 gTSS/$L_{granule}$. A high density combined with a regular granule shape resulted in a low value for SVI_5, which fluctuated between 48 and 75 mL/gTSS (MOSQUERA-CORRAL et al. 2005).

After 150 days of operation, the oxygen concentration was reduced to 40% of air saturation aiming to increase the nitrogen removal efficiency, as indicated by the model developed by BEUN et al. (2001) and discussed in Sect. 4.4.7. Ten days after the reduction in the O_2 concentration, filamentous structures were observed on the surface of the granules. Starting from day 174 of operation, the granules began to disintegrate, resulting in a reduction in their size and form factor (<0.52). The settling of the granular sludge was considerably affected by its disintegration, with substantial biomass washout from the reactor occurring. The biomass concentration decreases from 5 to 3.5 gVSS/L, and the solids retention time decreased to 8 days. The SVI_5 increased to 100 mL/gTSS, a value considered to be high for the settling conditions applied in the SBAR, and this resulted in the washout of most of the biomass. The lower oxygen penetration depth of the granule when the DO concentration was reduced from 100 to 40% of air saturation may have led to a reduction in the production of extracellular polymeric substances (EPS) in the inner region of the granules, weakening and rupturing their structure and leading to a decrease in their density. Under these circumstances, the settling properties of the granular sludge were adversely affected, leading to biomass washout from the reactor (MOSQUERA-CORRAL et al. 2005).

The SBAR operation was then restarted with new inoculum applying an oxygen concentration of 40% saturation. The first granules were formed after 10 days of operation, and they were small and unstable, being frequently washed out with the effluent. The average biomass concentration was 0.144 gTSS/L, and the SVI_5 was 200 mL/gTSS. Since it was not possible to obtain stable granules under these conditions, the reactor operation was stopped.

The results obtained by MOSQUERA-CORRAL et al. (2005) highlighted the difficulty associated with obtaining stable granules in the presence of low oxygen concentrations, and to achieve this objective, the concentration of this element needs to be increased. However, it is clear that in order to save energy and achieve good denitrification efficiencies, a low O_2 concentration needs to be applied.

The hypothesis raised by PICIOREANU et al. (1998) (discussed in Sect. 4.4.2) is in agreement with the results obtained by MOSQUERA-CORRAL et al. (2005), since low oxygen concentrations lead to an increase in the diffusion limitation and, consequently, to a stronger concentration gradient in the granule. If the proposed explanation discussed by these authors, in which stable biofilms are formed when the ratio between the biomass growth rate and diffusive transport is low (weak substrate and oxygen gradients), is correct in relation to the factors responsible for controlling the granule structure, then the selection of organisms with low growth rates would lead to the formation of stable granular sludge even in the presence of low dissolved oxygen concentrations. In order to reduce the growth rate of the organisms in aerobic granules, easily biodegradable substrates, such as acetate, should be converted into substrates which are slowly degraded, for instance, intracellular stored polymers (e.g., polyhydroxyalkanoates—PHA).

In the studies previously described in this section, such as that reported in BEUN et al. (2002), a period of feeding in pulse form was applied, in which the substrate was able to penetrate the granule completely, being partially used for rapid growth (40%) and partially stored as intracellular polymer (30–70%). This period was followed by a long phase of aeration in which the organisms grow using the polymers stored intracellularly at a low growth rate. As noted in Sect. 4.4.2, the period with substrate available for growth is called the feast phase, and the period in which the organisms use internally stored substrate is known as the famine phase. A reduction in the oxygen concentration leads to an increase in the duration of the feast phase (substrate present) and, consequently, to a longer period in which the growth rates are higher. In addition, it promotes a stronger O_2 gradient inside the granules which is not desirable when the formation of stable granules is required, as mentioned by PICIOREANU et al. (1998). Both the increase in the period with higher growth rates and the stronger gradient inside the granules lead to the growth of filaments and unstable granular sludge (DE KREUK and VAN LOOSDRECHT 2004).

According to DE KREUK and VAN LOOSDRECHT (2004), in order to obtain stable aerobic granules even under conditions of low oxygen concentrations, the bacterial growth rate needs to be reduced during the total cycle of sequencing batch reactors. This can be achieved through the selection of different types of bacteria which completely convert the easily biodegradable substrates into intracellular stored polymers which are slowly degraded, rather than only 60%, as in the study carried out by BEUN et al. (2002).

This conversion can be carried out by polyphosphate-accumulating organisms (PAO) and glycogen-accumulating organisms (GAO), which are able to convert all of the acetate into PHA (such as polyhydroxybutyrate, PHB) under anaerobic conditions. It is in this context that DE KREUK and VAN LOOSDRECHT (2004), based on previous studies involving theoretical concepts of biofilm morphology which indicate that the biofilm structure is dependent on the shear forces, microorganism growth rate, and substrate diffusion into the biofilm (VAN LOOSDRECHT et al. 1995; PICIOREANU et al. 1998), demonstrated that the selection of PAOs and GAOs in aerobic granules leads to the formation of stable granular sludge, even under low oxygen conditions. To this aim, the authors modified the feeding phase, which was no longer aerobic and in the pulse form, but instead a long period under anaerobic conditions followed by the aerobic reaction phase. In this way, the conditions required for the selected bacteria (PAOs and GAOs) to store all of the substrate inside the cells, without the occurrence of growth, were created, which consequently allowed the formation of stable granules.

Two 3 L reactors were operated, one being an SBAR and the other an SBBC. Both were operated in sequencing batch mode with the only difference being the presence of a riser in the former. In the first experiment, the oxygen concentration was not controlled, and thus it had values close to 100% of air saturation. In the second experiment, the dissolved oxygen concentration used was 40 or 20% of air saturation. Both reactors were submitted to successive cycles of 3 h. Of this total, 60 min was allocated to filling from the reactor base (plug-flow regime through the granule bed under anaerobic conditions), 112 min to aeration, 3 min to settling (to maintain particles with settling velocity greater than 12 m/h in the reactor), and 5 min to the

Table 4.2 Main characteristics of the granules obtained during the different stages of the experiment (DE KREUK and VAN LOOSDRECHT 2004)

Granule characteristics	Long feeding period[a]					Pulse feeding regime[b]	
	SBAR				SBBC	SBAR	
	DO 100%	DO 40%	DO 20%	DO 20%	DO 40%	DO 100%	DO 40%
Dominant organisms	PAO	PAO	PAO	GAO	PAO	Heterotrophs	
Stability	Stable	Stable	Stable	Stable	Stable	Stable	Stable
Local shear	Yes	Yes	Yes	Yes	No	Yes	Yes
Average diameter (mm)	1.3	1.1	1.3	1.2	1.1	1.6	5.0
Density ($gSSV/L_{biomass}$)	89	87	78	108	90	53	13
Solids concentration ($gVSS/L_{reactor}$)	8.5	12	16.5	15	13	5.1	0.9
SVI_8	24	20	14	17	19	50	200
Cell retention (days)	40	67	70	71	63	8	<5

[a]DE KREUK and VAN LOOSDRECHT (2004)
[b]MOSQUERA-CORRAL et al. (2005)

effluent discharge. The HRT and the applied organic load were 5.6 h and 1.6 kgCOD/(m^3 day), respectively. The feeding medium was composed of sodium acetate as a carbon source, NH_4Cl as a source of ammonium nitrogen, and KH_2PO_4/K_2HPO_4 as a source of phosphorous (DE KREUK and VAN LOOSDRECHT 2004).

Table 4.2 summarizes the main characteristics of the granules obtained during the different stages of the experiment carried out by DE KREUK and VAN LOOSDRECHT (2004) employing a long anaerobic feeding period. In addition, for comparison purposes, the properties of the granules obtained by MOSQUERA-CORRAL et al. (2005), who applied pulse feeding, are described.

As can be observed in Table 4.2, in contrast to the results obtained in previous studies in which a completely aerobic feast/famine regime was employed, DE KREUK and VAN LOOSDRECHT (2004) obtained stable granules even at low oxygen concentrations (40 and 20% of air saturation). Furthermore, the characteristics of these granules were similar to those formed at high dissolved oxygen concentrations (100% of air saturation). Filaments were not observed on the surface, and the sludge settleability was not affected, as shown by the low SVI_8 values. The solids retention time and the solids concentration increased with a decrease in the oxygen concentration (DE KREUK and VAN LOOSDRECHT 2004).

Due to the alternating periods of anaerobic feeding (presence of acetate) and aerobic reaction and the availability of phosphorus in the feeding influent, the selection of polyphosphate-accumulating organisms (PAOs) occurred. The acetate was completely converted into intracellular stored polymers (PHB) during the anaerobic feeding period, and the phosphate was released to the liquid medium. During the aerobic period, growth occurred through the use of stored PHB, while the phosphate in the liquid medium was converted to polyphosphate inside the cells.

Therefore, another advantage associated with the selection of PAOs is the possibility to remove phosphate from the wastewater. Ammonium was nitrified during the aeration period, and the nitrate formed was used as an electron acceptor in the anoxic zones located inside the granules. Thus, under DO conditions of 20% of air saturation, removal efficiencies of 100% for COD, 99% for phosphorus, and 90% for nitrogen were observed.

DE KREUK and VAN LOOSDRECHT (2004) also observed, through FISH analysis, that the biomass was mainly composed of PAOs. In contrast, when phosphate was no longer added to the feeding medium, the PAO population disappeared and was replaced by GAOs. These organisms, as previously mentioned, are also able to store acetate under anaerobic conditions. The energy required by GAOs to consume acetate is produced by glycogen hydrolysis. Glycogen reserves are replenished during the aerobic phase from stored PHB, which is also used for cell growth and maintenance. Despite the changes which occurred in the dominant populations, the characteristics of the granules did not show significant modifications.

Another point of interest in relation to the work of DE KREUK and VAN LOOSDRECHT (2004) should be mentioned here. The long feeding period under anaerobic conditions, besides allowing the complete conversion of the substrate (acetate) into intracellular stored polymers (PHB), improving the granule stability under low oxygen conditions, permitting the removal of phosphorus, and promoting better efficiencies for simultaneous nitrification/denitrification (SND), can simplify the operation of sequencing batch aerobic granular sludge reactors. When the duration of the feeding phase of N reactors represents 1/N of the cycle time, a continuous influent flow can be obtained. The plug-flow regime through the granular sludge bed allows a simultaneous feeding/discharge period. Approximately 90% of the void volume of the settled bed can be simultaneously replaced with the addition of influent, and thus the effluent discharge phase becomes unnecessary. In this operation mode, the influent and effluent flows become continuous, which greatly facilitates the design of the pre- and posttreatment installations (DE KREUK and VAN LOOSDRECHT 2004). It also resolves some difficulties encountered on scaling up the operation, such as the need for excessively large pumps or equalization tanks.

4.6 Conversion Processes in Aerobic Granules

Granular sludge technology has the great advantage that it allows the removal of COD and nutrients (nitrogen and phosphorous) in a single reactor. The operation of this reactor, as described in Sect. 4.4.2, is preferentially carried out in the sequencing batch mode. In order to obtain the simultaneous removal of these pollutants, some operational conditions must be ensured.

The mechanisms involved in nutrient removal with aerobic granules are basically the same of that observed for the conventional activated sludge process. The main difference is that, in the former technology, biological processes do not occur in different tanks but instead simultaneously in different regions inside the granules.

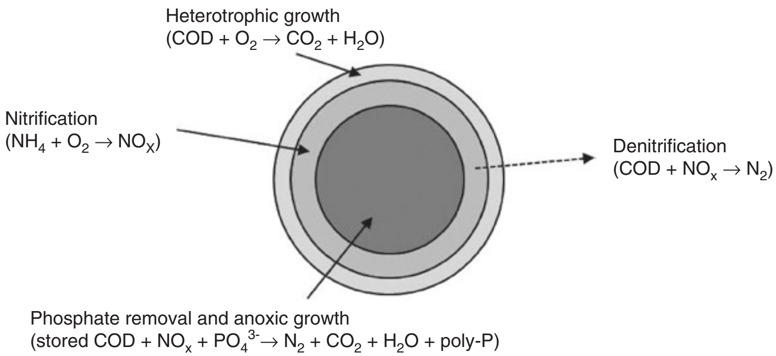

Fig. 4.6 Schematic representation of the different layers of the aerobic granules (adapted from DE KREUK 2006)

In this regard, the measurement and estimation of the capacity of specific conversions is complicated compared to suspended biomass. The most appropriate experimental conditions and methods to determine specific ammonium, nitrite, and phosphate uptake rates under normal operation of aerobic granular sludge reactors have been previously evaluated and are described by Bassin et al. (2012b). These authors proposed methodologies that may serve as an experimental frame of reference for investigating the metabolic capacities of microbial functional groups in aerobic granular sludge processes.

The simultaneous nitrification/denitrification process (SND) is an important mechanism which occurs in aerobic granules. The occurrence of this process is related to the presence of an external aerobic zone in the biofilm/granule for nitrification and an internal anoxic zone for denitrification (POCHANA and KELLER 1999). The distribution of heterotrophic microorganisms in the granules has a strong influence on the process. Since the removal of organic matter and nitrification/denitrification occur simultaneously in aerobic granular systems, there is competition between the heterotrophic and autotrophic microorganisms for space and oxygen. The former are located in the external layers since they have high growth rates, while the latter, mostly slow-growing organisms, are confined to deeper regions, where oxygen availability is limited (VAN LOOSDRECHT et al. 1995). Figure 4.6 shows a schematic representation of the structure of an aerobic granule comprised of an aerobic layer and an anaerobic/anoxic layer, as well as the different processes which occur in these layers.

During the feast regime, the concentration of the external carbon source is high, and this substrate will be completely dispersed in the interior of the granules and will be stored anaerobically (by PAOs or GAOs), aerobically, or anoxically (by other heterotrophic organisms) in the form of intracellular polymers (e.g., polyhydroxyalkanoates—PHA). The dissolved oxygen, used for growth and substrate storage, is rapidly consumed by the heterotrophic microorganisms located in the external layers of the granules. The dissolved oxygen is also consumed by autotrophic microorganisms which, being dependent on this element for nitrification, are

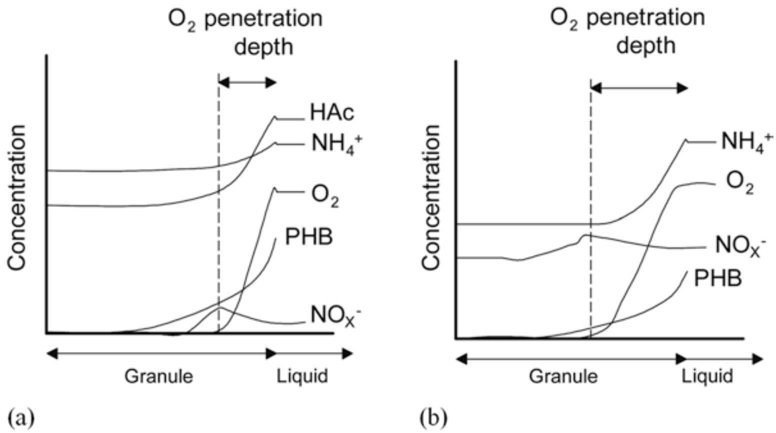

Fig. 4.7 Concentration profiles for acetate (HAc), PHB, NH_4^+, NO_x^- ($NO_2 + NO_3$), and O_2 inside the granules during the feast (**a**) and famine (**b**) periods of a sequencing batch reactor (SBR)

located in the aerobic layers of the granules, converting ammonium into nitrite and nitrate. During the feast period, the penetration of oxygen into the granules is limited. The storage of substrate (acetate) in the form of PHA and the growth occur aerobically in the external layer or anaerobically/anoxically inside the granules. It should be noted that during the feast phase of the operation cycle in an SBR, denitrification of NO_x (NO_2 and NO_3) compounds remaining from the last cycle can occur through the use of the external carbon available during this period. This process is indicated by the dotted line in Fig. 4.6.

During the period when the external carbon source is exhausted (famine phase), only the substrate stored inside the cells, in the form of PHA, is available to the granules. This substrate is used by the microorganisms for growth, which occurs much more slowly compared with the feast period (BEUN et al. 2001). The penetration of oxygen during the famine phase is greater, due to the reduction in the respiration rate of heterotrophic organisms during this period, although it continues to be limited due to the increase in the nitrification rate (THIRD et al. 2003). Heterotrophs have a lower requirement for oxygen in the famine phase, during which they grow using intracellularly stored PHA. Consequently, oxygen is no longer limiting for nitrifying bacteria (BEUN et al. 2001), and nitrification rate increases. The nitrate produced during nitrification can be simultaneously denitrified inside the granules using the stored substrate (PHA) as a carbon source (electron donor). Good nitrogen removal efficiencies are achieved when the aerobic and anoxic regions are well balanced during the aerobic phase (BEUN et al. 2001). The volume of these regions is influenced by the oxygen concentration in the bulk and by the granule size. Fig. 4.7 shows the theoretical concentration profiles for acetate (HAc), PHB, NH_4^+, NO_x^- ($NO_2 + NO_3$), and O_2 inside the granules during the feast (a) and famine (b) periods of a sequencing batch reactor (SBR).

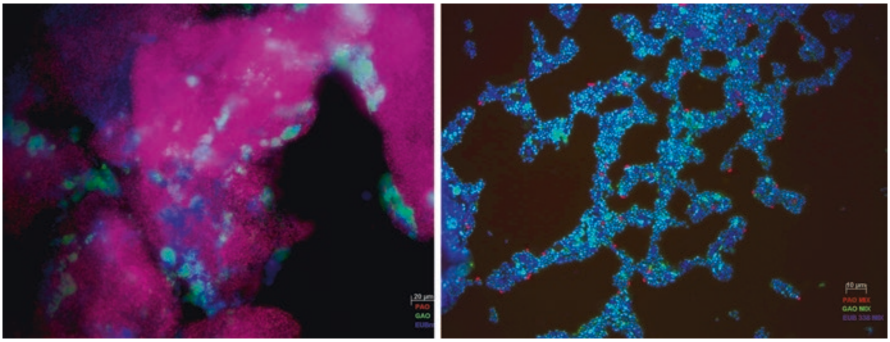

Fig. 4.8 Predominance of PAOs and GAOs in aerobic granules cultivated in a bubble column reactors. The *red* represents PAOs (PAOmix) and the *green* represents GAOs (GAOmix). The *red* and *green* are slightly modified due to the superposition of *blue*, which represents the entire bacterial population (EUBmix)

Studies on the formation of aerobic granules, described in Sect. 4.4.5, present differences regarding the operation mode of the reactors. This led to the formation of granules with different properties. Also, the operational conditions employed influenced the microbial conversion processes.

To exemplify this, when phosphorus removal is to be achieved, the discontinuous granular sludge reactor must be fed, under anaerobic conditions, for a relatively long period, favoring the development of PAOs. The adoption of a long anaerobic feeding regime, together with the creation of conditions for the growth of PAOs (and eventually GAOs), results in the formation of dense stable granules even in the presence of low dissolved oxygen concentrations (DE KREUK and VAN LOOSDRECHT 2004), as discussed in Sect. 4.4.5. In addition, regarding the application of aerobic granulation technology on a large scale, it can be assumed that sequencing batch reactors fed with a pulse regime in which the influent wastewater is added almost instantaneously, represent an economically and technically unrealistic option.

In order to illustrate the presence of the abovementioned organisms, Fig. 4.8 shows the presence of polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) in aerobic granules cultivated in different bubble column reactors, in laboratory scale, fed with acetate as carbon source, NH_4Cl as nitrogen source, and KH_2PO_4/K_2HPO_4 as phosphorus source. The detection of these microorganisms was carried out by fluorescence in situ hybridization (FISH), using the specific probes PAO 462/PAO 651/PAO 846 (represented by PAOmix in Fig. 4.8) and GAOQ431/GAOQ989 (represented by GAOmix in Fig. 4.8) (CROCETTI et al. 2000, 2002). Figure 4.8a shows the predominance of PAOs, as observed by FISH of biomass samples enriched with white colored granules. On the other hand, the dominance of GAOs in other granular sludge sample is illustrated in Fig. 4.8b. This sludge sample, in particular, was obtained in a reactor where phosphate was suppressed from the influent medium to allow the enrichment of GAOs.

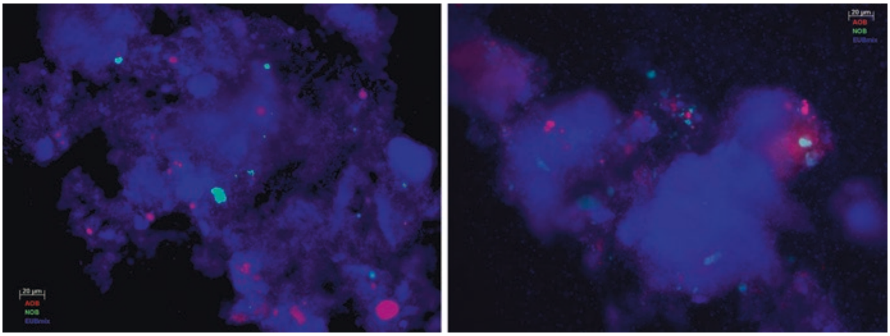

Fig. 4.9 Detection of AOB and NOB in aerobic granular sludge achieving simultaneous COD, nitrogen, and phosphate removal. The *red* indicates AOB, whereas the *green* indicates NOB. The *red* and *green colors* are slightly modified due to the superposition of *blue*, which represents the entire bacterial population (EUBmix)

The presence of GAOs in enhanced biological phosphorus removal (EBPR) systems is undesirable, since these microorganisms compete with PAOs for the substrate (volatile fatty acids) under anaerobic conditions but do not contribute to the removal of P-PO_4^{3-}. Operational strategies which favor the development of PAOs to the detriment of GAOs should be taken into consideration when the aim is to increase the phosphorous removal capacity (OEHMEN et al. 2006). Further details regarding the factors affecting the competition between these two microbial populations can be found elsewhere (LOPEZ-VAZQUEZ et al. 2008, 2009).

It should be noted that the application of the biological phosphorous removal can also simplify the simultaneous nitrification/denitrification (SND) process. In most biofilm systems consisting of heterotrophic and autotrophic organisms, the former dominate in the external layers since they compete more successfully with nitrifiers for dissolved oxygen and space in the biofilm. This distribution is not favorable for SND in continuous systems, bearing in mind that most of the organic components will be consumed in the external aerobic regions and thus cannot be used as electron donors for the denitrification in the interior of the biofilm. In addition, this type of system is more sensitive to oxygen concentrations, since heterotrophic organisms grow much more rapidly than nitrifying organisms. This does not occur when polyphosphate-accumulating organisms are selected, as these bacteria can coexist with nitrifiers in the same external layer of the granules due to their similar growth rates (BRDJANOVIC et al. 1998), as observed by DE KREUK et al. (2005b).

Figure 4.9 shows the occurrence of nitrifiers, both ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), in aerobic granules cultivated in a laboratory-scale bubble column reactor, as observed by FISH. In this case, the probes used were Nso1225/Nso190/Neu653 (specific for AOB), Ntspa662/Nit1035 (specific for NOB), and EUB 338mix for general bacteria. The references for the probes are given in Chap. 6.

In Fig. 4.9 it can be observed that population of nitrifying bacteria (AOB/NOB) represents a very small part of the total bacterial population. This result is consistent with the fact that in most wastewater treatment plants, the nitrifying autotrophic microorganisms correspond to a small part of the total bacterial population. However, despite their almost negligible contribution, these microorganisms are responsible for most of the dissolved oxygen consumption. In systems with on-line monitoring of the DO concentration, an increase in its concentration is clearly observed when the nitrification process ends, that is, when all of the ammonium has been oxidized to nitrate.

It should be noted that FISH analysis was carried out only in order to confirm the presence or absence of the abovementioned microbial groups and the position of the microorganisms illustrated in Fig. 4.9 does not correspond to their location inside the granules. In this case, a procedure in which the granules were crushed was adopted in order to facilitate the hybridization of cells to be later observed by FISH. The observation of the actual position occupied by the microorganisms in the granular sludge can be carried out with a confocal laser scanning microscope, which allows optical sections of the material to be analyzed, which are then grouped to construct tridimensional images. In the specific case of aerobic granules, successive optical sections allow the exact location of the microbial consortiums responsible for the different conversion processes to be determined.

In contrast to the nitrifiers and some heterotrophic bacteria, the capacity of PAOs to use nitrate as electron acceptor (in this case they are referred to as denitrifying polyphosphate-accumulating organisms, DPAOs), combined with their ability to store organic compounds (PHB) within the cell, enables their presence at the center of the granules (maintained under anoxic conditions).

It is also important to consider that to favor the simultaneous nitrification/denitrification process, the oxygen concentration must be selected so as to facilitate the occurrence of both processes. Low oxygen concentrations lead to an increase in the anoxic volume of the granules, rich in organisms able to store polymers, increasing the denitrifying capacity. Under these conditions, however, the aerobic external layer in which the autotrophic organisms are active is reduced, which may lead to a decrease in the nitrification efficiency. Also, in many cases, it is not possible to maintain stable operation when the oxygen concentration is lowered to below a certain critical value, notably due to the disintegration of the granules and their consequent washout from the reactor (MOSQUERA-CORRAL et al. 2005). On the other hand, high oxygen concentrations lead to a decrease in the anoxic regions inside the granules due to the complete penetration of oxygen, consequently reducing the denitrifying capacity.

Thus, it is clear that the ratio between the volumes of the aerobic layer and the anoxic central layer of the granules is important and determines the efficiency of the SND process. The greater the concentration of dissolved oxygen, the greater the oxygen penetration depth under the same oxygen consumption rate, which leads to a smaller anoxic volume inside the granules. Therefore, the size of the granules is an important variable in terms of the reactor operation, although it is difficult to control. Fig. 4.10 represents schematically the aerobic volume (external region) and

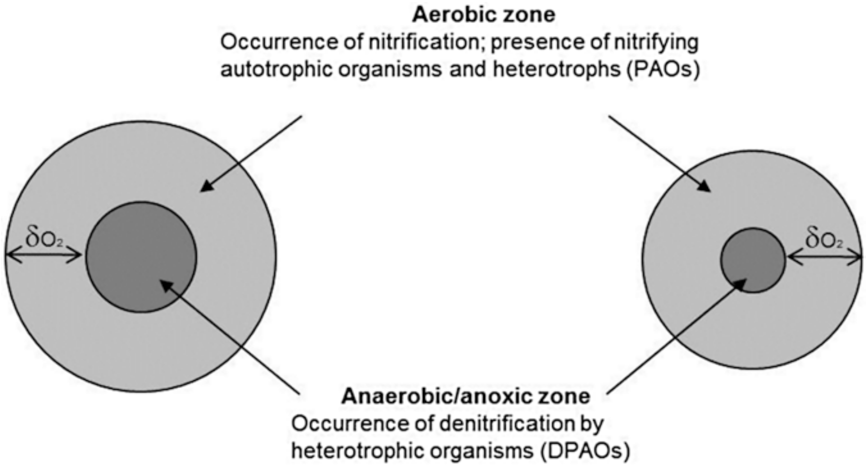

Fig. 4.10 Reduction in the anaerobic or anoxic zone with a decrease in the granule diameter at constant dissolved oxygen concentration (adapted from DE KREUK et al. 2005b)

the anoxic volume (internal region) of granules with different diameters submitted to the same oxygen concentration.

DE KREUK et al. (2005b) observed that granules with smaller diameters lead to a decrease in the nitrogen removal efficiency, indicated by an increase in the nitrate concentration in the effluent. On the other hand, ammonium oxidation was not affected by the different sizes of the granules. The highest nitrogen removal was obtained with granules larger than 1.3 mm. However, for diameters larger than 1.7 mm, the granules began to disintegrate, forming small irregular-shaped particles, with a small effective anoxic zone. In fact, the process conditions are not the only factors which influence the granule diameter. The interior of large granules tends to destabilize due to endogenous respiration, and the granules break into small fractions which can grow again to form new granules (DE KREUK et al. 2005b). In Sect. 4.4.7, the influence of the oxygen concentration on the SND process as assessed in some literature studies will be discussed.

Table 4.3 shows the nitrification and nitrogen removal efficiencies obtained by different researchers, who employed different operating conditions. It can be observed that the results obtained are closely related to parameters such as the granule size, oxygen concentration, and COD/N ratio. The low nitrogen removal efficiencies obtained by QIN and LIU (2006) and MOSQUERA and CORRAL et al. (2005) in comparison with those obtained by KIM et al. (2004) are associated with the fact that the former authors used higher dissolved oxygen concentrations, a condition which is not favorable for denitrification. Certain special circumstances, which are dependent on the granule diameter, DO concentration and COD/N ratio, can lead to the formation of microbial aggregates which are rich in substrate, with an interior region where no oxygen is available due to diffusion limitation. In this case, ideal conditions for denitrification are created.

Table 4.3 Nitrification and nitrogen removal efficiencies in granular sludge systems submitted to different operating conditions

Authors	COD/N	DO (mg/L)	T (°C)	Particle diameter (mm)	Organic load (gCOD/(L day))	Nitrogen load (gN/(L day))	Nitrification efficiency (%)	Nremoval efficiency (%)
KIM et al. (2004)	21	≥2	25	1.0–2.0	2.5	0.12	97	97
QIN and LIU (2006)	4.4–13.3	≥4	25	0.8–4.6	2	0.15–0.45	≥99	24–32
MOSQUERA and CORRAL et al. (2005)	8.3	8	20	1.6	1.6	0.19	100	16
TSUNEDA et al. (2003)	0	≥2	–	0.36	0	Up to 1.5	100	–
KIM and SEO (2006)	0	≥2	20–25	0.3–0.5	0	Up to 2.5	100	–

The aforementioned operational aspects are only some of the factors which strongly influence the treatment of wastewaters by aerobic granules. In fact, many other factors must be taken into consideration when aerobic granules are used for the treatment of a certain wastewater. The composition of the different substrates and their gradients along the structure of the granules, the operating temperature, the reactor configuration, and the pH comprise a significant group of factors which affect the diffusion coefficient, conversion rates, granule size, spatial distribution of the biomass, and density. These, in turn, strongly influence one another, and the effect of each one individually is difficult to be determined experimentally.

Computational models can be used to efficiently evaluate the relative importance of the individual parameters in the biofilms/granules and provide the information on the main factors affecting the treatment performance and the distribution of the different microbial populations in the granules. These models can also serve as a tool to improve the design of aerobic granular sludge reactors. The work by BEUN et al. (2001), which will be described in Sect. 4.4.7, provides an example of the use of models together with experimental data to investigate the effects of some operational parameters.

It should be mentioned here that the assessment of the conversions which occur in the granules by means of mass balances can be complicated, particularly due to the fact that the reactors used operates in sequencing batch mode. In these discontinuous systems, interpreting the results and reporting concentrations should be carried out with caution. For instance, the effluent which is not removed because it is below the effluent discharge port dilutes the influent. In addition, the nitrite or nitrate which remains from the previous cycle is firstly denitrified directly, using external organic carbon originating from the feed. The amount of nitrogen removed must therefore be related to the nitrogen concentration present in the influent, the nitrogen remaining from the previous cycle, and the quantity removed during the aeration phase. The nutrient balance of these reactors should ideally take into consideration the composition of the gas formed during the process.

Bassin et al. (2011) have studied an important factor which can influence the nitrogen mass balance calculations in aerobic granular sludge processes. These authors observed the occurrence of an ammonium adsorption phenomenon inside the granules in laboratory- and pilot-scale aerobic granular sludge reactors. Adsorption tests have shown that ammonium adsorption in aerobic granular sludge can be considerably higher than that occurring in activated sludge and Anammox granular sludge. For mass balancing over a treatment plant, this is no real problem. Under steady-state conditions, adsorption does not make a difference to the ammonium effluent concentrations. However, a significant error can be introduced in the calculations when ammonium adsorption is neglected in granular sludge bioreactor systems that are characterized by strongly variable ammonium concentrations as a function of place (plug-flow systems) or time (batch systems).

4.7 Application of Aerobic Granules in Wastewater Treatment: From Laboratory Studies to Full-Scale Experiences

Granular sludge technology can be applied for the removal of a wide variety of organic compounds and nutrients, such as nitrogen and phosphorus. The great advantage associated with the use of aerobic granules combined with operation of the reactors in sequencing batch mode is that the treatment can be carried out in a single tank. As mentioned in Sect. 4.4.6, the COD removal, nitrification, and denitrification processes can be carried out simultaneously due to the presence of aerobic, anoxic, and/or anaerobic regions in the granules.

BEUN et al. (2001) investigated the effect of the dissolved oxygen (DO) concentration on the performance of a sequencing batch airlift reactor (SBAR) containing granular sludge, particularly with regard to the nitrogen removal. The authors used both experimental data and results obtained from the model developed. The latter were used not only to describe the effect of low DO concentrations on nitrogen removal but also to predict the effect of several process conditions on the nitrogen removal efficiency.

The reactor was operated for 142 days. On day 42, a small quantity of nitrifying sludge was added to accelerate the accumulation of nitrifying microorganisms in the reactor. The feed consisted of sodium acetate as a carbon source (18.3 Cmmol/L) and ammonium chloride as a source of ammonium (40 mgN-NH_4^+/L). The performance of the SBAR was described by a model obtained in Aquasim (*apud* BEUN et al. 2001). The model was used to simulate the processes which occurred in the reactor under the same conditions applied in the laboratory experiments and to obtain some information on the physical distribution of the autotrophic and heterotrophic biomass within the granules (BEUN et al. 2001).

It was observed that the nitrification, denitrification, and COD removal can occur simultaneously in a granular sludge sequencing batch reactor. During the feast phase (period with acetate available), the concentration of NH_4^+ decreased slowly, and the amount of NO_x ($NO_2 + NO_3$) remaining from the previous cycle was denitrified with acetate. After the acetate had been completely consumed (beginning of the famine period), the NH_4^+ concentration immediately began to decrease more rapidly in comparison to the feast regime, due to the occurrence of the nitrification process. The NO_x concentration increased during this period. From the nitrogen mass balance, it was observed that denitrification occurred during the famine period. When all of the NH_4^+ had been converted, the NO_x concentration did not decrease. This implies that, after this period, denitrification no longer took place. According to the authors, denitrification did not continue due to the complete penetration of oxygen into the granules after the nitrification had been completed. At DO concentration corresponding to 100% of air saturation, it was observed that 87% of the NH_4^+ was removed via nitrification, and the rest was used for biomass growth. Around 70% of the NO_x formed by nitrification was denitrified (BEUN et al. 2001).

In order to improve the denitrification efficiency, from day 66 to 71 of reactor operation, the DO concentration was reduced to 50% of air saturation 40 min after the beginning of each cycle until its end. This concentration was reached by mixing air and nitrogen gas. With this procedure an increase in the denitrification was observed, although ammonium was detected in the effluent due to DO limitation (BEUN et al. 2001).

On days 79, 100 and 142, the DO concentration was decreased to a constant value of 20% saturation 40 min after the beginning of a certain cycle until its end. The NH_4^+ concentration in the effluent increased, and the NO_x concentration in the effluent decreased. Under these conditions, 83% of the NH_4^+ was converted to NO_x by nitrification, and 100% of the NO_x formed was denitrified. It was clearly demonstrated that on decreasing the DO concentration to 20% of air saturation, the nitrification rate decreased and the denitrification potential increased substantially (BEUN et al. 2001).

Bearing in mind that the model provided a good description of the experimental data obtained at DO of 100% and 20% of air saturation, it was used to evaluate the effect of DO on the nitrification and denitrification, varying the concentration of this parameter within this range (20–100%). The nitrogen balance during one cycle was used to calculate the nitrogen removal efficiency of the reactor. The authors clearly observed that the increase in the DO concentration contributed to improving the nitrification efficiency and reducing the NH_4^+ concentration, although it led to a decrease in the denitrification efficiency, increasing the NO_3^- concentration. The maximum nitrogen removal was obtained at DO of 40% air saturation (BEUN et al. 2001).

In another simulation, the performance of the SBAR at DO of 40% air saturation was studied in greater detail, since under this condition the maximum nitrogen removal percentage was observed. The feast period was more than twice as long as the period of operation with DO of 100% air saturation, and the oxygen penetration during this period was low. In the case of acetate, there was complete penetration in the granules, regardless of the DO concentration. Since oxygen was not present in the center of the granule during the feast period, and with the availability of NO_3^- originating from the previous cycle, the acetate could be stored in the central region of the granules, under anoxic conditions, in the form of PHB (BEUN et al. 2001). Although the concentration of PHB was low in the center of the granules compared with the external layers, the presence of NO_3^- in the famine period and the low oxygen concentration promoted the occurrence of denitrification in the central region of the granules, where stored polymers were used as carbon source (BEUN et al. 2001).

At DO of 40% air saturation, NH_4^+ was not completely removed during the cycle, the concentration of NO_3^- in the effluent was lower than during SBAR operation at 100% air saturation, and the nitrogen removal efficiency was 80%. At this DO concentration, the depth of oxygen penetration was small, and the occurrence of nitrification was limited to the external layers of the granules. An internal anoxic zone was established in the center of the granules, favoring denitrification. The authors clearly demonstrated that the DO concentration has a notable effect on the nitrogen removal. With an increase in the DO concentration, nitrification was favored. On the other hand, the same increase in DO had an adverse effect on denitrification. Thus, an ideal balance between nitrification and denitrification needs to be established through controlling the DO concentration in the reactor to ensure maximum nitrogen removal (BEUN et al. 2001).

In another simulation, the nitrification was considered to be inactive during the operational cycle, and the effect of nitrification on the oxygen penetration depth was observed. In the simulation without the occurrence of nitrification, the amount of nitrate remaining from the preceding cycle was denitrified during the feast period. It was also observed that, even during the feast period of the standard operational cycle (DO of 100% air saturation), the removal of NH_4^+ occurred mainly due to biomass growth, given that the ammonium removal rate was the same as that of the cycle in which nitrification was neglected by the simulation. This finding is associated with the fact that during the feast phase, the fast-growing heterotrophic microorganisms responsible for the degradation of acetate dominate the competition with nitrifiers for oxygen. From the beginning of the famine phase of the standard cycle, NH_4^+ removal by nitrification increased (BEUN et al. 2001).

During the feast period, the penetration depth was relatively small for the simulations both with and without nitrification. Once the acetate had been completely consumed in the simulation with nitrification, the depth of oxygen penetration was 350 μm while NH_4^+ was present in the liquid medium. Under these circumstances, the oxygen was used for nitrification. When ammonium had been totally consumed, the oxygen penetrated completely into the interior of the granules. In the simulation in which the occurrence of nitrification was neglected, when acetate was no longer available (end of feast period), the oxygen was able to completely penetrate into the granular biomass, since it was not used for nitrification. These results obtained by the model suggest that the activity of nitrifying bacteria is responsible for the fact that the oxygen does not reach the center of the granules, favoring the occurrence of denitrification in this region (BEUN et al. 2001).

The authors also investigated the effect of the storage and degradation of PHB on the denitrification, by performing a simulation in which the accumulation and degradation of this compound are considered to be inactive during the operation cycle of an SBAR. In the standard simulation (with PHB), growth of the granules occurred throughout the cycle. During the feast period, the biomass grew due to acetate degradation, while during the famine period, the growth was on PHB (BEUN et al. 2001). The duration of the feast period in the simulation without PHB was almost twice that of the standard simulation with PHB. The growth of the granules occurred only during the feast phase, although the quantity of biomass produced was much greater in this period than during the whole cycle of the standard simulation. The removal of NH_4^+ was greater under conditions without PHB compared with the feast period under conditions with PHB due to the higher biomass growth rate of heterotrophs in the former case, although nitrification did not occur during this period. After all of the acetate had been consumed (beginning of famine period), the removal of NH_4^+ was attributed only to nitrification, and from this period onward biomass growth did not occur (BEUN et al. 2001).

With regard to the role of PHB in denitrification during the famine period, in the simulation without this intracellular polymer, it was observed that all of the NH_4^+ removed was converted into NO_3^-, that is, denitrification did not occur. In the famine period of the simulation with PHB, 50% of the NH_4^+ was nitrified to NO_3^-, indicating that the denitrification process occurred. In the latter case, the PHB stored in the center of the granule can be used as a carbon source for denitrification when an external carbon source (acetate) is no longer available, so long as NO_3^- (originating from nitrification) is present as an electron donor and not oxygen (BEUN et al. 2001).

BEUN et al. (2001) also noted the fact that, in systems with continuous operation, no storage and degradation of organic substrate occurred, since its concentration in the reactor is always low, preventing the occurrence of feast and famine periods. According to the authors, in these systems the acetate penetration depth is always much lower than the respective depth for oxygen, and thus anoxic conditions need to be introduced to promote denitrification which, in turn, requires the reactor operation mode to be adjusted. In addition, an external carbon source should be added for the denitrification process, given that intracellular polymers are not available in these continuous systems (BEUN et al. 2001).

As regards the distribution of the microbial community in the structure of the granules, the authors also speculate that in a system fed in a discontinuous form, for instance, SBAR, the organic substrate (in this case acetate) completely penetrates the biofilm or granule due to the high concentration in the liquid medium, particularly at the beginning of the feast period. Since the oxygen is present only in the external layers of the biofilms, it is expected that nitrifying bacteria will be located in these regions where oxygen is available, and that the acetate is stored in the form of PHB by the heterotrophic population, under anoxic conditions, in the interior regions of the biofilms/granules (BEUN et al. 2001).

The stratification of the granule structure was also predicted by the simulation carried out by BEUN et al. (2001). The autotrophic biomass was located mainly in the external region of the granules. The location of the heterotrophic community was less well defined. These organisms were present both in the central regions of the granules (using acetate for growth during the feast period and using NO_3^- as an electron acceptor) and in the external layers of the granules (using the oxygen available as an electron acceptor).

The simulation at DO of 40% of air saturation showed that, in the long term, the DO influenced the microbial distribution in the granular sludge. Under these conditions, a greater fraction of the autotrophic biomass was located in the aerobic external region of the granules in comparison with condition in which DO corresponded to 100% air saturation. This situation is explained considering that the nitrifying biomass needs oxygen for the conversion of NH_4^+. The heterotrophic population can use either oxygen or NO_3^- as the electron acceptor and can thus be present also in the center of the granules. It was observed that the exact location of the autotrophic biomass influenced the nitrogen removal and the distribution of these organisms is, in turn, affected by the concentration of dissolved oxygen in the reactor (BEUN et al. 2001).

DE KREUK and VAN LOOSDRECHT (2004) showed that the selection of polyphosphate-accumulating organisms and glycogen-accumulating organisms (through adopting an anaerobic feeding regime) promoted an improvement in the stability of aerobic granules at low oxygen concentrations. Based on this previous study, DE KREUK et al. (2005b) studied the factors which are important in order to obtain the simultaneous removal of nitrogen and phosphorus in aerobic granular sludge reactors.

The operational conditions used by DE KREUK et al. (2005b) were the same as those applied in an earlier study (DE KREUK and VAN LOOSDRECHT 2004), as described in Sect. 4.4.5. In the search to identify the main conversion processes which occurred at different dissolved oxygen concentrations, the authors observed that after 52 days of operation under saturated oxygen conditions, complete consumption of acetate occurred during the anaerobic period. After 65 days, phosphate removal of 95% was achieved, considering that the initial concentration was 20 mgP/L. On days 65 and 233 of operation, the average concentration of phosphate released into the liquid medium during the anaerobic feeding phase was 86 mgP/L, while the effluent concentration was only 0.4 mgP/L. The ratio between the phosphate released and the acetate consumed was 0.44 P-mol/C-mol, which is close to that obtained at pH 7 for a culture highly enriched with PAOs, i.e., 0.55 (SMOLDERS et al. 1994 *apud*).

During the first 2 days after the reactor start-up, ammonium-oxidizing organisms were inhibited with allylthiourea (ATU) in order to suppress nitrification. As a consequence, the presence of nitrate was prevented during the anaerobic feeding period, and therefore the development of PAOs was not affected. The complete oxidation of ammonium was observed 39 days after the addition of ATU had been stopped, although it took around 100 days for all of the nitrite to be oxidized. After 154 days of operation, ammonium and nitrite were no longer detected in the effluent. Due to the operation at oxygen saturation of 100%, incomplete denitrification occurred, and total nitrogen removal amounted to 34% (27% due to the biomass growth) (DE KREUK et al. 2005b).

The concentration of oxygen was lowered to 40% of air saturation in order to improve the denitrification efficiency, decreasing the oxygen penetration depth and, as a consequence, the aerobic volume of the granules. This change did not affect the phosphate removal, which remained at around 97%. The acetate continued to be totally consumed during the anaerobic period, while the average ratio between the phosphate released and acetate consumed showed no variation (0.45 P-mol/C-mol). The denitrification efficiency increased slowly during this period, with concentrations below 5 mgN-NO_3/L being measured in the effluent 64 days after operation at DO of 40% air saturation. Ammonium and nitrite were completely oxidized, and the average nitrogen removal was 98% (DE KREUK et al. 2005b).

Subsequently, the authors observed that a change in the morphology of the granules occurred, and they changed from spherical to irregular-shaped particles, with fissures in the direction of their centers. One of the consequences was a reduction in the nitrogen removal efficiency, which dropped to values between 50 and 70%. In order to improve the removal of nitrogen, the dissolved oxygen concentration was further decreased, from 40 to 20% of air saturation. The phosphate removal remained high (94% on average) during the first 90 days of operation under these conditions. However, later the phosphate concentration began to increase, probably due to the low biomass yield resulting from the high solids retention time applied (71 days) (DE KREUK et al. 2005b). In order to completely remove the phosphate, the sludge age needs to be maintained at relatively low values. If this condition is not respected, there will not be efficient removal of phosphate, since the only way of removing this nutrient is by removing biomass rich in polyphosphate (i.e., polyphosphate-accumulating organisms) in appropriate proportions.

The concentrations of nitrogen compounds ($N\text{-}NH_4^+$, $N\text{-}NO_2^-$, and $N\text{-}NO_3^-$) did not change immediately after the decrease in oxygen concentration from 40 to 20% of air saturation. After 30 days of operation under this condition, a drop in the nitrogen efficiency was observed as a result of the reduction in the external aerobic layer where nitrifiers were present, although the capacity of these microorganisms was reestablished over time. During operation in the steady-state at DO of 20% of air saturation, in which the granule size was greater than 1.3 mm, the anoxic volume containing denitrifying polyphosphate-accumulating organisms (DPAOs) was large enough to allow denitrification. The highest nitrogen removal efficiency obtained was 94% (DE KREUK et al. 2005b).

DE KREUK et al. (2005b) also evaluated the short-term effect of both an increase and a decrease in the dissolved oxygen concentration on the performance of the reactor. The experiments were carried out during operation under steady-state conditions at DO of 40% of air saturation. This concentration was modified during the cycle to 100, 40, and 10% air saturation. The phosphate consumption rate decreased with a reduction in the oxygen concentration, due to the reduced oxygen penetration depth, resulting in an increase and decrease in the anoxic and aerobic volumes, respectively. It was also observed that, in the absence of nitrate (obtained through the inhibition of nitrification by allylthiourea), the phosphate consumption rate decreased by around 45% in relation to the maximum obtained during the operation at 100% air saturation with nitrification/denitrification. The highest nitrogen removal efficiency recorded in this experiment was obtained at 40% air saturation, which corresponds to the long-term DO concentration in which the granules were cultivated.

The same authors also observed that with high phosphate concentrations in the influent (19.6 $mgP\text{-}PO_4^{3-}/L$; COD/P = 20.2) and with a low substrate to cells conversion factor (0.25 gVSS/gCOD), the value calculated for the P content in the granules was 0.20 gP/gVSS. The amount of P in the sludge reported in the literature is usually lower, varying from 0.02 to 0.14 (FALKENTOFT 2000). Thus, it was assumed that the phosphate removal could be partially attributed to precipitation occurring inside the granules. This can be confirmed by the increase in the fixed solids (ash) content of the granules enriched with PAOs (representing 30–41% of the total solids) under anaerobic feeding conditions, compared to that observed for the granular biomass from the reactor operated under aerobic conditions and subjected to pulse feeding (where ash corresponded to 6% of the total solids). In addition, most granules were white, although some were light brown. These latter granules had a fixed solids content of 17.2%, while for the white granules, the corresponding value was 50.4%, suggesting the presence of precipitates in the white biomass (DE KREUK et al. 2005b). It should be noted that the precipitation inside the granules may be advantageous since, besides favoring the removal of phosphate, it makes the granules heavier, increasing their settling velocity.

MOSQUERA-CORRAL et al. (2005), as discussed in Sect. 4.4.5, evaluated the best operating conditions for the removal of nitrogen in aerobic granular systems. To this aim, they observed the short- and long-term effects of a decrease in the oxygen concentration on the performance of a sequencing batch airlift reactor (SBAR). The authors obtained complete removal of ammonium after 120 days of operation at DO of 100% air saturation. Under these conditions, the average nitrogen removal efficiency was 16%.

When the lowest oxygen concentration (40% air saturation) was applied, the denitrification efficiency increased, and the NO_x compounds (NO_2 and NO_3) were almost completely denitrified during the first days of operation under these conditions. These results are related to an increase in the anoxic volume of the granules with the decrease in the oxygen concentration, increasing the denitrification capacity. In contrast, the nitrification efficiency was reduced, and thus ammonium was present in the effluent. This decrease could be related to the competition, for oxygen, between the ammonium-oxidizing microorganisms and the heterotrophic microorganisms in the external layer of the granules. An interesting result obtained by MOSQUERA-CORRAL et al. (2005) was that the aerobic biomass volume in the external layer of the granules reduced to a lesser degree than the ammonium oxidation rate. When the oxygen concentration was reduced from 100 to 50% air saturation, for instance, the aerobic volume decreased by 27% while the ammonium oxidation rate dropped by 47%. These data indicate a structure composed of several layers within the aerobic zone with the heterotrophic organisms growing further toward the outside than the nitrifying bacteria. According to VAN LOOSDRECHT et al. (1995), this stratification is mainly caused by the differences between the growth of the two populations of microorganisms and due to the competition for oxygen. The decrease in the nitrification rate did not result in an overall reduction in nitrogen removal. The average nitrogen removal was 63% at an oxygen concentration of 40% air saturation, indicating that most of the nitrite and nitrate was denitrified under these conditions.

MOSQUERA-CORRAL et al. (2005), as also described in Sect. 4.4.5, restarted the operation of the system with new inoculum and applied an oxygen concentration of 40% of air saturation. Under these conditions, the volumetric acetate consumption rate was very low due to the long feast period of 160 min. Nitrification was not observed since the nitrifying bacteria were not able to develop in this unstable system. The formation of stable granules was not possible in this system, and therefore the reactor operation was stopped.

In experiments carried out to study the short-term effect of a decrease in the dissolved oxygen concentration on nitrogen removal, MOSQUERA-CORRAL et al. (2005) observed that reducing the DO from 100 to 50, 40, 20, or 10% of air saturation did not affect the acetate consumption rate, since the duration of the feast phase was similar at all DO concentrations tested. The ammonium oxidation decreased, and the nitrate production increased when the oxygen concentration was lowered. At oxygen concentrations below 20% of air saturation, the ammonium concentration in the effluent increased, indicating that complete nitrification was not achieved. NO_x compounds were denitrified during the feast phase using acetate as the carbon source. During the famine phase of the cycles with high oxygen concentration (40–100% of air saturation), denitrification did not occur. On the other hand, at low DO concentrations, this process did occur during the famine phase, using PHB stored intracellularly as electron donor. The nitrogen removal percentage increased with a decrease in the oxygen concentration, reaching a maximum value of 34.5% when employing a DO level of 10% air saturation.

The main biological conversions taking place in laboratory-scale aerobic granular sludge sequencing batch reactors have been evaluated by Bassin et al. (2012a). Two bioreactors carrying out simultaneous COD, nitrogen, and phosphate removal were operated at different temperatures (20 and 30 °C). In contrast to previous studies on this topic, Bassin et al. (2012a) not only described the general performance in terms of nitrogen and phosphate removal but also investigated the importance of specific subpopulations of PAOs (PAO clade I and II) on phosphate and nitrogen conversions. PAO clade I use both nitrite and nitrate as electron acceptors for denitrification, whereas PAO clade II is only able to use nitrite, since these microorganisms lack the nitrate reductase enzyme. This particular work aimed to link the nitrogen and phosphate conversions to the microbial community structure. Complete nitrification/denitrification and phosphate removal were achieved in both systems submitted to low dissolved oxygen concentration (less than 2 mg/L).

The authors observed that a considerable fraction of the phosphate removal was coupled to denitrification (denitrifying dephosphatation). This particular step is carried out by denitrifying polyphosphate-accumulating organisms (DPAOs), which use intracellular polymers stored under anaerobic conditions as electron donors for denitrification and do not require the addition of an external carbon substrate to carry out this process (Kuba et al. 1993; Kerrn-Jespersen and Henze 1993). Besides an efficient use of the incoming COD, the use of oxidized forms of nitrogen (e.g., nitrite or nitrate) rather than oxygen as electron acceptors for phosphate uptake also leads to energy savings due to the lower aeration requirement.

Denitrifying glycogen-accumulating organisms (DGAOs) were observed to be the main microbes responsible for the reduction of nitrate to nitrite. A significant fraction of the nitrite was further reduced to nitrogen gas while being used as an electron acceptor by denitrifying polyphosphate-accumulating organisms (PAO clade II) for anoxic phosphate uptake. An improved operational strategy to control the PAO-GAO competition in aerobic granular sludge reactors and favor the development and growth of PAOs under unfavorable conditions for the P removal process (e.g., high temperatures) is also described in the work conducted by Bassin et al. (2012a).

Previous studies focusing on granular sludge reactors, without being concerned with the intracellular storage of polymers, are also published in the literature. One example is an investigation by QIN and LIU (2006), in which microbial granules with excellent settling properties were cultivated in four sequencing batch reactors

(SBR_1–SBR_4) operated under alternating aerobic-anaerobic conditions and submitted to different nitrogen loads. The cycle time was 6 h (5 min of filling, 230 min of aerobic reaction, 119 min of anaerobic reaction, 2 min of sedimentation, and 4 min of effluent discharge) and the HRT was 12 h. The dissolved oxygen (DO) concentration was greater than 50% air saturation during the aerobic phase. In the anaerobic stage, nitrogen gas was used. The solids retention time was approximately 20 days after the granule formation.

The reactors were inoculated with activated sludge originating from a municipal wastewater treatment plant and were fed, from the bottom, with synthetic medium containing ethanol as the only carbon source, ammonium chloride, sodium bicarbonate, and other required nutrients. The influent COD was maintained constant at 500 mg/L, while the incoming ammonium concentration of the four reactors was gradually increased from 37.5 to 112.5 mgN/L. These values led to a gradual increase in the nitrogen load from 0.15 (in SBR_1) to 0.45 kg/(m^3 day) (in SBR_4) (QIN and LIU 2006).

Granules were observed in the reactors after 40 days of operation under alternating aerobic-anaerobic conditions. These had a spherical shape and a compact structure. The biomass concentration in the reactors was significant, varying from 3.0 gVSS/L in SBR_1 to 5.5 gVSS/L in SBR_4, indicating that the granulation promoted better biomass retention in the reactors. The SVI decreased from 230 mL/g (activated sludge inoculated into the reactor) to 30–12 mL/g (granular sludge submitted to increasing nitrogen loads from SBR_1 to SBR_4). These SVI values are still lower than those obtained during the granular sludge reactor operation under exclusively aerobic conditions, where the typical values varied between 50 and 100 mL/g (DANGCONG et al. 1999; TAY et al. 2001b). The settling velocity of the granules cultivated in the four reactors was greater than 60 m/h, which is much higher than that shown by conventional activated sludge (2–5 m/h) (*apud* QIN and LIU 2006).

In an experiment without the addition of an external carbon source during the anaerobic phase, QIN and LIU (2006) observed that 95% of the influent COD was removed during the first hour of the aerobic phase. During this period the ammonium concentration slowly decreased. After the COD had been completely removed, the ammonium concentration began to decrease rapidly due to nitrification. During the aerobic phase, ammonium was completely converted into nitrite and nitrate. In the subsequent anaerobic phase, the denitrification was observed at very reduced levels (partial denitrification). Approximately 13–27 mg/L N-NO_x was denitrified when the external carbon source was not available, which represent less than 10% of the N-NO_x formed by nitrification.

Based on the results obtained without the addition of an external carbon source and seeking to achieve complete denitrification, ethanol was used as the external carbon source in a mass ratio of 2:1 ethanol/N-NO_x at the beginning of the anaerobic (anoxic) phase. At this condition, almost all of the nitrite and nitrate produced in the aerobic phase by nitrification were rapidly denitrified into N_2 in the anaerobic phase (QIN and LIU 2006).

In contrast to the abovementioned studies, in which different processes occurred in different regions (aerobic and anoxic/anaerobic) within the granules due to the presence of an oxygen concentration gradient and the availability of intracellular polymers as a carbon source for denitrification, in the study by QIN and LIU (2006), the situation was similar to that at most wastewater treatment plants. Thus, in this case, the advantages of granular sludge technology (e.g., saving of organic matter for the denitrification by the use of stored substrate in the form of intracellular polymers) did not apply (QIN and LIU 2006).

The respirometric activities of ammonium-oxidizing and nitrite-oxidizing bacteria were inferred by the specific oxygen uptake rates (SOURs) associated with the specific ammonium ($SOUR_{NH4}$) and nitrite ($SOUR_{NO2}$) oxidation rates, respectively. The activity of the heterotrophic bacteria was determined by SOUR associated with carbon oxidation ($SOUR_H$). It was observed that both in the absence and presence of an external carbon source in the anaerobic phase, the activity of the ammonium-oxidizing bacteria increased with an increase in the applied nitrogen load, while the nitrite-oxidizing bacteria showed no significant variation under these conditions. In the absence of an external carbon source, it was also observed that the activity of the heterotrophic population decreased when the microbial consortium was enriched with nitrifiers, making heterotrophs less dominant. The supply of external carbon stimulated the activity of heterotrophs and denitrifiers (which were comprised mostly of facultative heterotrophic bacteria), especially when high organic loads were applied (QIN and LIU 2006).

The activity of the denitrifying bacteria was determined through the specific NO_x reduction rate, which varied from 12.0 to 25.3 mgN/(gVSS h) when the reactors were fed with an external carbon source at the beginning of the anaerobic phase. These values are an order of magnitude higher than those obtained during the operation of the reactors without the addition of an external carbon source, which varied from 1.9–3.5 mgN/(gVSS h). The results showed that the activity of denitrifying bacteria was essentially determined by the availability of the external carbon source in the anaerobic phase (QIN and LIU 2006).

Due to their large surface area, high degree of porosity, and good settling capacity, the aerobic granules play an important role in the treatment of toxic chemical compounds and heavy metals. GAI et al. (2008) studied the mechanism of Cu^{2+} adsorption by granular sludge. These authors observed that 70% of the copper was adsorbed through ion exchange with Na^+, Ca^{2+}, and Mg^{2+}, which were released by the granules. XU and LIU (2008) proposed that ion exchange, binding with EPS, and chemical precipitation are the main mechanisms involved in the biosorption of Cd^{2+}, Cu^{2+}, and Ni^{2+} by aerobic granules. These authors also observed that alcohol, carboxylate, and ether groups actively participate in the capture of the metals. NANCHARAIAH et al. (2008) cultivated aerobic granules in wastewaters containing nitrilotriacetic acid. The chelating agent significantly accelerated the granulation process, while the granules formed removed this compound almost completely. ZHU et al. (2008) cultivated granules in an SBR with a high height/diameter ratio, aiming at the degradation of 4-chloroaniline. Mature granules were obtained in 90 days, and the specific degradation rate of 4-chloroaniline varied from 0.14 to 0.27 g/(gVSS day).

Most of the reactors employed to cultivate aerobic granules were fed with synthetic wastewater. As previously mentioned in this section, studies have focused mainly on issues related to COD and nutrient (N and P) removal under different operating conditions. There have been few research studies in which aerobic granulation was applied to the treatment of industrial wastewaters.

As mentioned in Sect. 4.4.5, ARROJO et al. (2004) evaluated the formation of aerobic granules in two sequencing batch reactors (SBR_1 and SBR_2) fed with industrial wastewater originating from a laboratory where the analysis of dairy products is carried out. In SBR_1 an anoxic phase was included at the beginning of each cycle, while SBR_2 was submitted only to aerobic conditions. The same authors also evaluated the nitrogen removal process. At the beginning of the experiment in SBR_1 (submitted to organic and nitrogen loads of 1 gCOD/(L day) and 0.1 gN-NH_4^+/(L day), respectively), the nitrogen removal was related to the assimilation of ammonium for biomass growth, since nitrification did not occur during this period. Nitrification activity was only observed after 1 month of operation. When the ammonium concentration was doubled due to changes in the composition of the wastewater (after day 150 of operation), denitrification began to occur in both systems. Thus, nitrification and denitrification were the main mechanisms involved in the nitrogen removal after this period. The nitrogen removal efficiency reached 80% on day 188 of operation in both reactors. The organic and nitrogen loads applied in both systems were between 1 and 7 COD/(L day) and 0.1 and 0.7 gN/(L day), respectively.

The nitrogen removal efficiency was 70% in both units even though SBR_2 was only operated under aerobic conditions. The influent COD was between 500 and 1800 mg/L, and the ammonium concentration varied from 30 to 180 mgN-NH_4^+/L throughout the operational period. The COD removal efficiencies were between 85 and 95%. The ammonium and nitrate concentrations in the effluent varied from 0 to 20 mgN-NH_4^+/L and 0 to 40 mgN-NO_3^-/L, respectively. Similar values were obtained by GARRIDO et al. (2001), who treated industrial wastewater in a 28 m^3 SBR containing flocculent biomass. However, it should be taken into consideration that the organic and nitrogen loads applied were greater in the SBR with granular sludge (around five times greater considering the maximum values applied), and even so the effluent characteristics, in terms of nitrogen concentration and COD, were similar. Such results highlight the advantages of aerobic granulation when compared with conventional activated sludge technology (ARROJO et al. 2004).

The concentrations of the compounds were measured during the operating cycles (particularly on day 260 of operation when the organic and nitrogen loads were 5 gCOD/(L day) and 0.4 gN-NH_4^+/(L day), respectively). The results revealed that almost all of the biodegradable organic matter and the nitrate (remaining from the previous cycle) were completely removed in both systems during the first 10 min of the cycle. Nitrate was consumed via denitrification, while the biodegradable organic compounds were partially oxidized aerobically, partially used as electron donors for denitrification, and partly accumulated in the form of biomass. Ammonium was oxidized to nitrate during the aerobic period, particularly after the anoxic period in SBR_1 and throughout the cycle in SBR_2, immediately after the biodegradable COD had been completed consumed. Even when organic loads of 5–7 gCOD/(L day) were applied, the occurrence of nitrification was observed (ARROJO et al. 2004).

The specific denitrification activity was 0.28 and 0.30 gN/(gVSS day) in SBR_1 and SBR_2, respectively, while the specific nitrification activity was approximately 0.033 gN/(gVSS day) in SBR_2 and 0.023 gN/(gVSS day) in SBR_1. In the case of SBR_2, the fact that the granules presented denitrifying activity under aerobic conditions may be explained by the fact that during the first few minutes of the cycle, a large quantity of biodegradable organic matter was fed to the system. Thus, during this period, the diffusion of this organic matter fraction to the interior of the granules was greater in comparison to the diffusion of oxygen. Also, the dissolved oxygen present during the filling period of SBR_2, in a concentration of 3 mg/L, was probably consumed by the external layer of the granules. Thus, the internal layers, maintained under anoxic conditions, received sufficient carbon source and nitrate to allow the denitrification process to be carried out (ARROJO et al. 2004). These findings have also been reported in BEUN et al. (2001), as mentioned earlier in this section.

An important parameter to be considered in this study was the total suspended solids (TSS) concentration in the industrial wastewater fed to the reactors (SBR_1 and SBR_2), which varied from 200 to 900 mg/L and contained a large fraction of VSS (in the range of 75–97%). The suspended solids concentration in the influent was partially removed in both SBRs. The TSS content of the effluent was lower than that of the influent when the reactors were fed with industrial wastewater. TSS concentrations were in the range of 50 to 800 mg/L (ARROJO et al. 2004).

The presence of TSS in the effluent of the SBRs was related to the TSS in the influent, to the detachment of small pieces of granules and to the occurrence of small flocs which were washed out of the reactor. Effluent TSS content was strongly influenced by both the duration of the discharge period and the relation between the particulate COD and the biomass (CODp/VSS) in the systems. The TSS concentration in the effluent increased when the CODp/VSS ratio was greater than 0.12 gCODp/gVSS. In order to decrease the solids content of the effluent, the authors applied a strategy in which the discharge period was reduced. A significant reduction in the TSS concentration of the effluent occurred, that is, from 450 to 200 mgTSS/L and then to 150 mgTSS/L, when the discharge was gradually reduced from 3 to 1 min and then to 0.5 min, respectively. This was related to the higher washout of the small suspended biomass aggregates when the duration of this phase was reduced, decreasing the growth of small flocs in the reactors. Thus, the strategy employed was shown to have a positive effect on the effluent quality in terms of solids concentration (ARROJO et al. 2004).

On day 296 of operation, both reactors began to be fed with the same synthetic wastewater, free of solids, which was used during the start-up of SBR_1. The TSS concentration of the effluent was around 200 mgTSS/L, which is lower than the value observed when the reactors were fed with industrial wastewater and when the discharge time was 3 min (450 mgTSS/L). This observation indicates that the solids content of the effluent is not dependent only on the biomass washout from the reactor but also on the solids content of the influent wastewater (ARROJO et al. 2004).

The results obtained by these authors should certainly be taken into consideration on applying the granular sludge technology. Biomass washout, which in some cases can occur, may be the consequence of the high solids concentration in the influent. ARROJO et al. (2004) noted that the presence of suspended solids in the effluent may be one of the main obstacles in the development of SBRs with granules at full scale. The TSS removal efficiency is a crucial aspect which must be taken into consideration when applying granular sludge technology, since pollutants are often found in the form of colloids or particles. SCHWARZENBECK et al. (2004) used aerobic granules for the treatment of wastewater containing malt and a high content of particulate organic matter (0.9 gTSS/L) and observed that the particles with average diameters of less than 25–50 μm were removed with 80% efficiency. The removal efficiency of particles smaller than 50 μm was only 40%. The authors observed that the capacity of the granular sludge to remove particulate organic matter was due to its incorporation by the biofilm matrix and the metabolic activity of the protozoan population which covered the surface of the granules. However, INIZAN et al. (2005) observed that the aerobic granules were not able to remove suspended solids present in wastewaters originating from the pharmaceutical industry.

In this context, the advantages of granular SBRs, such as a high conversion capacity and good granule sedimentation properties, can frequently be counteracted by the presence of suspended solids in the effluent. This factor can represent a paradox since granular SBRs are generally described in the literature as systems which can improve the bioreactor performance in relation to the retention of solids. In some situations, a posttreatment process (e.g., filtration membranes, surface filters, secondary settling tanks) can be added to remove the TSS content in order to comply with environmental legislation, although this procedure will reduce the economic attractiveness of aerobic granulation technology.

CASSIDY and BELIA (2005) operated a granular sludge reactor fed with slaughterhouse wastewater containing total COD of 7685 mg/L, soluble COD of 5163 mg/L, total Kjeldahl nitrogen of 1057 mg/L, and volatile suspended solids content (VSS) of 1520 mg/L. The authors obtained a COD and phosphorus removal efficiency of 98%, while both the removal of nitrogen and VSS reached 97%. To achieve this performance, CASSIDY and BELIA (2005) submitted the reactor to DO of 40% air saturation, a condition considered to be ideal for nitrogen removal, according to BEUN et al. (2001). Also, in order to maintain the granule stability under conditions of limited DO concentration, the authors followed the recommendations of DE KREUK et al. (2005b) and included an anaerobic feeding period. TSUNEDA et al. (2006) observed a nitrogen removal rate of 1.0 kgN/(m^3 day) and a nitrogen removal efficiency of 95% in a system containing autotrophic granules, applied in the treatment of wastewater originating from a metal refinery containing 1.0–1.5 gN-NH_4^+/L and up to 22 g/L of sodium sulfate.

Aerobic granulation technology has been applied not only in laboratory scale. A research study in pilot scale was carried out in the Netherlands in order to demonstrate the applicability of aerobic granulation technology to municipal wastewater treatment. The hydraulic capacity of the plant was 5 m^3/h, the main installation being comprised of two parallel granular sludge sequencing batch reactors ($GSBR_1$ and $GSBR_2$), with a diameter and a height of 6 and 0.6 m, respectively. The pretreatment of the pilot plant consisted of a primary settling tank, optionally followed by a pressurized sand filter (DE BRUIN et al. 2005).

The influent had an average COD of 560 mg/L, suspended solids of 225 mg/L, Kjeldahl nitrogen of 58.4 mg/L and total phosphorus of 10 mg/L. $GSBR_1$ was always operated as a bubble column reactor, while $GSBR_2$ was initially operated as an airlift reactor and later transformed into the bubble column configuration. The cycle time of the reactors varied from 2.5 to 4 h. The feeding period, under anaerobic conditions, was 50–60 min, and the settling time was 20–30 min. The remainder of the cycle referred to the aerobic phase (DE BRUIN et al. 2005).

The pilot plant was inoculated with activated sludge originating from a conventional treatment plant. Initially, the granulation process showed good progress, which resulted in granules with good settling properties. However, the granulation did not continue due to the low operating temperatures (around 5 °C) and the inadequate control of the aeration, which led to high concentrations of nitrate in the effluent and low phosphate removal efficiencies. Thus, aeration control was implemented. Moreover, the reactor walls were insulated and the influent flow preheated in order to control the process temperature (DE BRUIN et al. 2005).

The reactor start-up was carried out with the new modifications aiming to obtain high phosphate removal and nitrification efficiencies. The average removal of phosphate was 65%, and nitrification was incomplete. Since the ammonium concentrations in the effluent were high and the settling characteristics of the sludge deteriorated, the organic load, in terms of COD, was reduced by 50%. Under this condition, complete nitrification was achieved and greater concentrations of nitrate in the effluent were observed. At the same time, phosphate removal reduced considerably, and the granulation process did not improve (DE BRUIN et al. 2005).

Based on the results obtained, some operational conditions were modified. It was observed that during the start-up period of the reactors, it was not possible to maintain complete phosphate removal and complete nitrification at the same time. Bearing in mind that the selection of organisms with a low growth rate, in this case polyphosphate-accumulating organisms (PAOs), was considered to be a prerequisite for granulation, the COD was increased once again, and the beginning of aerobic granulation was observed. In approximately 3 months, around 30% of the sludge in $GSBR_1$ consisted of granules. However, due to an operational failure, 75% of the sludge was lost from both reactors (DE BRUIN et al. 2005).

The reactors were again inoculated with the same activated sludge as used before, and this time rapid granulation was observed. This enhancement of the granulation process was attributable to the sample collection method. With the previously used method, many of the granules were crushed/broken during their collection. One month after start-up, the granulation in $GSBR_1$ was complete, and the SVI_{30} was only 55 mL/g. The granulation in $GSBR_2$ proceeded more slowly and corresponded to 50% after 1 month of the reactor start-up. The SVI_{30} of the sludge present in this reactor was around 70 mL/g. As the granulation process proceeded, the sludge washout decreased which resulted in the accumulation of biomass in the reactors (DE BRUIN et al. 2005).

TAY et al. (2005) studied the development of aerobic granules in a pilot-scale sequencing batch reactor, inoculated with pre-cultivated granular sludge in a small reactor in the format of a column. The authors observed that the granules inoculated disintegrated within the first days of operation, although granules were subsequently formed. On the other hand, granules which were maintained in the laboratory-scale reactor did not disintegrate. The results obtained were related to the different hydrodynamic conditions of the reactors in pilot and laboratory scale. Factors such as the reactor diameter and the wall effect, as well as the size and location of the air diffusers, may have influenced the hydrodynamic conditions in the reactors which, in turn, determined the granule properties (TAY et al. 2005).

In general, the results obtained show the potential for the application of granules in larger scales, since it was demonstrated that granules can be formed with municipal wastewater and significant levels of nutrient removal can be achieved (DE BRUIN et al. 2005). In addition, the research in pilot scale highlighted the difficulty in obtaining successful granulation when scaling up from laboratory to larger scales. It was clear that greater care and better control of the operational conditions are required in order for the formation of aerobic granules to be carried out satisfactorily, which can lead to good reactor performance.

A study on the applicability showed that the granular sludge process is a very promising technology from the economic point of view (DE BRUIN et al. 2004). Based on the total annual costs, granular sludge reactors operating in sequencing batch mode (GSBR) with pretreatment and posttreatment can be more attractive than the conventional activated sludge process, with potential savings varying within 6–16%. One study on the sensitivity showed that the GSBRs are less sensitive to the price of land and more sensitive to the flow of water originating from rain. Due to the potential application of high volumetric loads, the area occupied by GSBR systems represents only 25% of the area used by conventional flocculent sludge systems. However, granular sludge systems involving only primary treatment may not reach the established effluent discharge standards, mainly due to the presence of solids in the effluent resulting from the washout of biomass with unsatisfactory settling properties. With stricter standards being imposed with regard to effluent discharge, the activated sludge plants must incorporate a posttreatment stage, such as sand filtration, or be transformed into bioreactors with membranes. In this case, GSBR systems with primary and posttreatment may represent an attractive alternative (DE BRUIN et al. 2004).

Data on the application of aerobic granular sludge (AGS) in full-scale installations are still scarcely reported in the literature. Few works have provided detailed information on this issue so far. Pronk et al. (2015) reported the implementation of the technology for the treatment of domestic sewage in the Netherlands. This work provided interesting data related to the operation of the plant. The AGS plant was operated in parallel with the existing activated sludge reactor. The AGS reactor treats 28,600 m^3/day of sewage, which corresponds to 41% of the total influent flow. Average influent COD and BOD_5 during the field tests were reported to be 506 and 224 mg/L, respectively.

The AGS reactor was operated in sequencing batch mode with simultaneous feeding and effluent withdrawal (fill-and-draw operation), reaction and final sludge settling, sludge withdrawal, and idle periods. Nitrogen removal was mainly obtained by simultaneous nitrification and denitrification (SND). However, non-mixed periods under anoxic conditions obtained by a recycle from top to bottom of the reactor were used as a strategy to improve nitrogen removal performance. During aeration, dissolved air concentration was kept between 1.8 and 2.5 mg/L. The cycle time was set at 6.5 h at dry weather conditions, and it is reduced to 3 h at rainy weather conditions by shortening the aeration period. The AGS reactor was inoculated with surplus sludge (1 g/L) coming from other full-scale AGS plant located in Epe, the Netherlands. No granules were present in the seed biomass, which presented a SVI_{30} and SVI_5 of 140 and 90 mL/g, respectively (Pronk et al. 2015).

The authors reported the development of a stable and robust granule bed with more than 8 g/L of biomass after 5 months of start-up period. SVI_5 and SVI_{30} were found to be 45 mg/L and 35 mL/g, respectively. More than 80% of the granular biomass was larger than 0.2 mm and more than 60% larger than 1 mm. Effluent nitrogen and phosphorus concentrations lower than 7 mgN/L and 1 mgP/L were achieved during both summer and winter, complying with the environmental regulations. Maximum nitrogen and phosphorus volumetric conversion rates were observed to be 0.17 and 0.24 kg/(m^3 day). The energy usage was around 60% lower than in conventional activated sludge reactors, while the volume required for the AGS plant was 33% smaller than the existing activated sludge plant (Pronk et al. 2015).

Li et al. (2014) also reported successful granulation in a full-scale SBR treating 50,000 m^3/day of domestic sewage in Yancang wastewater treatment plant, located in Haining, China. The wastewater has the following characteristics: COD of 200–600 mg/L, BOD of 50–105 mg/L, ammonium of 28–40 mg/L, and total phosphorus of 2–4 mg/L. The AGS reactor was inoculated with sludge from the secondary settler of an oxidation ditch. The full-scale AGS plant was divided in four tanks, operated in fill-and-draw mode. The wastewater was fed from the top of the reactors. The volume exchange ratio varied from 50 to 70%.

After inoculation, the SBR cycle consisted of 40 min filling, around 240 min aeration, 60 min settling, and 30 min effluent withdrawal. Subsequently the settling time was reduced to 40–50 min. After around 330 days of operation, the SVI_{30} of the granular biomass reached only 47.1 mL/g, while the diameter of the granules was 0.5 mm. X-ray fluorescence analysis has shown that metal (e.g., iron) and other inorganic ions (calcium, silicon, phosphorus) present in the raw wastewater precipitated in the sludge and acted as a core, enhancing granulation. The authors also evaluated the polysaccharide concentration in the biomass. The amount of polysaccharide in the full-scale SBR biomass was higher than in the continuous flow activated sludge-based reactors (i.e., anaerobic-oxic reactor and oxidation ditch). They reported that these EPS components could have mediated cohesion and adhesion of cells, contributing to maintain a stable structure of aerobic granules. Moreover, the cyclic operation of the SBR consisting of a periodic feast-famine regime, short settling time, and no return sludge was favorable for the development of fast-settling particles (Li et al. 2014).

(a) (b)

(c)

Fig. 4.11 Full-scale demonstrations of aerobic granular sludge technology. In (**a**), the granular reactors are displayed in the bottom of the picture (Provided by Royal Haskoning DHV). (**a**) Nereda® WWTP Garmerwolde, The Netherlands. Operation since 2013. (**b**) Nereda® WWTP Frielas, Portugal. Operation since 2014. (**c**) Nereda® WWTP Deodoro, Brazil. Operation since 2016

Many full-scale aerobic granular sludge treatment plants have been implemented in Europe, Africa, Australia and South America. Figure 4.11 shows three examples of wastewater treatment plants designed by the company Royal HaskoningDHV, which is current market leader in applying aerobic granular sludge process for industrial and municipal application. Since 2005 they have full-scale applications with their Nereda® aerobic granular sludge technology. Current (2016) project portfolio amounts to approx. 30 ranging from smaller industrial applications up to 2.4 M PE municipal wastewater treatment plants and the amount of references is increasing rapidly.

4.8 Final Considerations and Future Perspectives

The aerobic granulation technology, when compared with conventional processes which make use of flocculent biomass, offers many advantages from the technical-operational point of view. The settling characteristics of granular sludge are by far superior to those of conventional activated sludge, which leads to a shorter time required for the settling of particles, lower SVI values, and a greater biomass concentration in the reactor. The latter characteristic favors the stability of the biological system, making it less vulnerable to shock loads of organic or toxic compounds, and also increases the treatment capacity of the bioreactor.

The cultivation of granular sludge is mostly carried out in sequencing batch reactors (SBRs). The mode of operation of these reactors is easier when combined with the use of biomass in the form of granules. The filling and treated effluent discharge phases are to be carried out simultaneously, particularly when a plug-flow regime is adopted, with an upward flow through the settled sludge bed. Considering that the settling of granules is rapid, a longer period can be allocated to the reaction phase, that is, to the actual biological treatment. Another positive characteristic of the use of aerobic granules in an SBR is the reduction in the area required for the installation of the biological treatment system since, in most cases, a secondary settling tank is not needed. The greater retention of biomass in the system, which allows an increase in the volumetric conversion rates, also contributes to reducing the volume of the granular sludge reactor, reducing even further the space requirement.

With the use of aerobic granules, the removal of organic compounds and nutrients (nitrogen and phosphorus) can be carried out simultaneously. The presence of different regions within the granules, both aerobic and anoxic/anaerobic, allows both heterotrophic and autotrophic nitrifiers to coexist in their structure, favoring a high microbial diversity. The stratification of the granule structure means that aerobic heterotrophic and autotrophic microorganisms are present in the external layer, below which there is a layer with a reduced level of oxygen where facultative anaerobic bacteria are located. Finally, in the central region of the granules, anaerobic/anoxic microorganisms predominate due to the absence of oxygen. In fact, the distribution of aerobic and anaerobic/anoxic regions is strongly influenced by the dissolved oxygen concentration and the diameter of the granules. However, the control of the latter remains a challenge.

While most of the heterotrophic microorganisms are responsible for the degradation of organic matter, some of them, such as polyphosphate-accumulating organisms (PAOs), may also be present. These are responsible for phosphate removal and may also play an important role in the denitrification process (in this case being known as denitrifying PAOs, DPAOs) taking place in the anoxic zone of the granules. The DPAOs use compounds stored intracellularly (polyhydroxyalkanoates, PHA) as a carbon source. Some operational conditions of SBRs must be ensured for the selection of PAOs, such as the application of a feeding period under anaerobic conditions. This procedure also induces the formation of stable granules (important for preventing their washout from the reactor) even at low dissolved oxygen concentrations, since it favors the development of bacteria with a lower growth rate. Moreover, it contributes to reduce the investment and running costs and facilitate operation in larger scales.

Studies on granular sludge systems for the treatment of industrial wastewater have indicated that the use of this technology is suitable for obtaining good removal efficiencies for organic matter and nutrients (N and P). However, in some cases, the use of a pre- or posttreatment is recommended to ensure that the wastewater complies with the discharge limits imposed by the environmental regulators, particularly considering that the effluent may contain high concentrations of suspended solids.

The most important aspect of the aerobic granulation process is the start-up period, in which the formation of granules takes place. When granules originating from other systems in operation are used as the inoculum, the time required for aerobic granulation to occur can be minimized. Another drawback associated with aerobic granulation relates to the uncertainty regarding criteria such as the stability and robustness of the granules, characteristics which can often be affected due to operation under certain specific conditions.

Although several studies have been carried out with respect to aerobic granulation technology, some issues have not been fully explored in this research area. A better understanding of the mechanisms involved in the formation of the granules is one point to be considered. Greater attention should be given to the oxygen concentration gradients present in the granular sludge, which are determined by the distribution and activity of different microbial populations inside the granules. These directly influence the conversion processes which occur within the granules. Since the oxygen concentration profiles are the result of different factors which influence one another, such as the diffusion coefficients, conversion rates, granule size, pore size and distribution, and permeability and density, it is difficult to perform experimental studies to investigate the effect of each factor individually. In this regard, the development of mathematical models certainly represent a valuable tool to provide the understanding of important factors which affect the reactor performance and the distribution of microbial populations responsible for the biodegradation processes. Furthermore, such models are also useful for optimizing the process and for the scaling-up, design, and control of reactions in pilot/industrial scale.

Increasing the scale of granular sludge systems alters the hydrodynamics, which play an important role in the formation and stability of aerobic granules. Thus, additional studies in pilot/industrial scales should be conducted. Another promising issue to be investigated is a detailed research at the microbiological level in order to elucidate the relation between certain microorganisms and the stability of the granular sludge. Furthermore, the exopolymers produced by granular biomass is also of particular interest for better understanding the formation of these aggregates. The influence of the granule structure on the conversion processes and the mass transport involved in these processes also need to be studied in greater depth.

Finally, laboratory and pilot-scale research on aerobic granular sludge for the treatment of several types of wastewaters should be encouraged as it consists of an important step for full-scale implementation and expansion of the technology worldwide.

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