Bioresource Technology 270 (2018) 678–688

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

Aerobic granular sludge: Cultivation parameters and removal mechanisms

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ARTICLE INFO

Keywords:
Aerobic granules
Cultivation
Microbial groups
Long-term operation

ABSTRACT

Aerobic granular sludge (AGS) has been the focus of many investigations, and the main parameters responsible for AGS formation are hydrodynamic shear force, short periods and feast-famine cycles. However, some other parameters are associated with AGS maintenance after long periods of operation. This review evaluates the parameters responsible for AGS formation and maintenance and some reference values are proposed. In addition, some discussions are addressed about the main metabolic pathways that AGS uses for the removal of some compounds, such as nutrients, organic matter, dyes, recalcitrant compounds, among others. Finally, the main microbial groups present in the AGS and their respective functions are discussed. It is also highlighted that many parameters that are taken as reference currently for AGS cultivation and maintenance can be optimized for energy savings, implementation costs, among others, as well as a greater recovery of resources during wastewater treatment, within the scope of the biorefinery concept.

1. Introduction

Granular sludge was originally described in anaerobic sewage treatment systems in 1980 (Lettinga et al., 1980). In aerobic systems, the first observation occurred in a bubble-column lab-scale reactor in 1997 (Morgenroth et al., 1997). About two decades after the discovery, aerobic granular sludge (AGS) has been the focus of several works on treatment of domestic and high organic load wastewaters, bioremediation/biotransformation of toxic aromatic pollutants (including phenol, toluene, pyridine), treatment of industrial effluents (textile, dairy, brewery), adsorption of heavy metals, recovery of high value added products, and others.

Liu and Tay (2004) defined aerobic granules as highly packaged microbial aggregates, containing millions of microorganisms per gram of biomass, in which the different bacterial species have specific functions in the degradation of pollutants present in the wastewater, and grow in the absence of a support material. Aerobic granules have a strong compact microbial structure, good settling capacity and high biomass retention, with the ability to handle with high organic loading rates (Zheng et al., 2006).

When compared to the activated sludge system, the AGS technology presents a 20–25% reduction in operation costs, 23–40% less electricity requirement and a 50–75% reduction in space requirements (Adav et al., 2008; Bengtsson et al., 2018; Nereda®, 2017). In relation to the other compact treatment options, such as membrane bioreactor (MBR), the AGS process had an estimated electricity usage that was 35–70% lower (Bengtsson et al., 2018). With regard to emerging technologies, until now there is no comparative data between AGS and membrane aerated biofilm reactor (MABR), but, when compared to activated sludge systems, greater energy savings ($> 90\%$) are observed for MABR systems. However, the advantages associated with operational ease, flexibility and lower costs have favored the greater dissemination of AGS technology in domestic sewage plants (Donohue, 2017; Bengtsson et al., 2018).

The comparison among AGS, anaerobic granules and conventional activated sludge is shown in Table 1.

Due to the oxygen penetration gradient, there are different layers in the same granule. According to the oxygen penetration range, it is possible to have aerobic/anoxic or aerobic/anoxic/anaerobic zones in the granule, resulting in a variety of microbial populations in AGS. Some authors have even suggested that the granule is formed by “mini ecosystems” (Liu and Tay, 2004).

The aerobic granulation process is affected by several operational parameters, such as substrate composition, organic loading rate (OLR), hydrodynamic shear force, feast-famine regime, feeding strategy, dissolved oxygen concentration, reactor configuration, solids retention time, sequential batch reactor (SBR) cycle time, settling time and volume exchange ratio. Although all these factors influence granules properties, only the factors associated with the selective pressure on sludge particles seem to contribute to the formation mechanism of

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https://doi.org/10.1016/j.biortech.2018.08.130
Received 16 July 2018; Received in revised form 29 August 2018; Accepted 30 August 2018
Available online 31 August 2018
0960-8524/

Table 1
Comparison among AGS, anaerobic granules and conventional activated sludge.

Parameters	AGS	Anaerobic granules	Activated sludge
Settling velocity	10–90 m/h	< 20 m/h	2–10 m/h
Size	0.2–5 mm	0.2–2.0 mm	< 0.2 mm
Degree of compactness and density	High	High	Low
Shape	Regular and spherical	Regular and spherical	Irregular and filamentous
Layers	Aerobic, anoxic and anaerobic	Facultative and obligate anaerobic	Aerobic
Tolerability	High	Low	Low
EPS production	High	Middle	Low

Bengtsson et al., 2018; Nancharaiah and Reddy 2018; Rico et al., 2017; Simon et al., 2009.

granules (Liu et al., 2005).

Some studies indicated that granulation could be obtained even though the granules have not presented stability after long periods of operation. Therefore, there is an optimum window of conditions for successful development of cultivated granules and for granule stability in long-term operation (Lee et al., 2010).

2. This review

The proposed review article aims to present in a logical and structured way information on AGS cultivation and maintenance parameters, and reference values were defined. Additionally, some discussions about the main metabolic pathways that AGS uses (biosorption, bioaccumulation or biodegradation) for the removal of some compounds are addressed. Moreover, the main microbial groups present in the AGS and their respective functions are discussed.

3. Microbial groups

Due to the variety of microbial populations, it is necessary to manipulate the operational conditions to select the desired species for a given process. The slow-growing organisms have been shown to positively influence the density and stability of biofilms (de Kreuk and van Loosdrecht, 2004; Liu et al., 2004a).

According to Bernet and Spérandio (2009), compared to the growth of heterotrophic microorganisms, nitrifying bacteria growth is slower even under optimum cultivation conditions. The maximum specific growth rate for heterotrophic microorganisms is in the range of 4–13.2 day^{-1}, whereas, for the nitrifying ones, the range is 0.62–0.92 day^{-1}. Anammox bacteria have the lowest growth rate among bacteria that may be present in the granule, with values close to 0.065 day^{-1} (Isaka et al., 2006).

In addition to slow-growing microorganisms, de Kreuk and van Loosdrecht (2004) have suggested that polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) assist in the formation and stabilization of AGS. Therefore, the presence of slow-growing bacteria (nitrifying, Anammox) and PAOs and GAOs is reported to be beneficial for the cultivation of stable granules.

3.1. Selection of PAOs/DPAOs and GAOs/DGAOs

de Kreuk and van Loosdrecht (2004) verified that the use of PAOs or GAOs indeed provided the conditions for obtaining stable granular sludge. Considering phosphorus removal, the main PAOs are: *Accumulibacter* spp., *Rhodocyclus* spp. and *Enterobacter* spp. The competition of this group occurs with the genus *Competibacter* (GAO), since both consume the same substrate, volatile fatty acids (VFAs), and have similar metabolism. However, GAOs are not able to accumulate phosphorus, therefore decreasing the system efficiency for nutrients removal (Bassin et al., 2012).

Carta et al. (2011) showed that the denitrifying polyphosphate-accumulating organisms (DPAOs) and denitrifying glycogen-accumulating organisms (DGAOs) are also considered slow-growing organisms and leads to full granulation. These organisms consume internal storage polymers, such as polyhydroxyalcanoates (PHA), in order to reduce nitrite or nitrate, and, in the case of DPAOs, contribute to phosphate uptake as well.

DPAOs and DGAOs are generally reported to be from the families Comamonadaceae, Sphingomonadaceae, Hyphomicrobiaceae, Rhodobacteriaceae, Xanthomonadaceae and Rhodocyclaceae, especially of the genera *Thauera*, *Zoogloea*, *Meganema*, *Devosia* and *Stenotrophomonas* (Weissbrodt et al., 2013). In addition, the families of Rhodocyclaceae, Xanthomonadaceae and Sphingomonadaceae were also related to extracellular polymeric substances (EPS) production in AGS systems (Szabó et al., 2017).

The main selection strategy for these microorganisms consists of a long anaerobic feeding period followed by an aerobic reaction period (PAOs and GAOs) or an anoxic reaction period (DPAOs and DGAOs). Additionally, ordinary heterotrophic organisms (OHOs) present lower growth rates for slowly biodegradable storage polymers (PHA, glycogen) than for easily biodegradable substrate (Carta et al., 2001).

The application of a feast/famine regime to a SBR system decreases the maximum growth rate of fast-growing microorganism (OHO), specifically during the famine phase. The anaerobic uptake of easily biodegradable substrate, prior to the aerobic/anoxic reaction phase, and/or substrate conversion by relatively slow-growing bacteria during the aerobic or anoxic period are essential conditions to achieve a stable granulation (Pronk et al, 2015).

He et al. (2018) speculated that a low chemical oxygen demand to nitrogen (COD/N) ratio and a high dissolved oxygen (DO) concentration could favor accumulation of GAOs over PAOs. In fact, Wang et al. (2018) elucidated by pyrosequencing that the reduction of the COD/N ratio favored accumulation of GAOs and DPAOs whereas inhibited the growth of PAOs.

Aeration also affects the selection of PAOs and GAOs. Interestingly, both reduction in aeration intensity (He et al., 2017) and aeration time (He et al., 2018) could inhibit the growth of GAOs whereas stimulate the enrichment of PAOs. He et al. (2018) observed that GAOs showed a significant decrease by reducing the aeration time from 120 min to 60 min.

3.2. Selection of AOB, NOB and Anammox

Liu et al. (2004a) investigated the feasibility of improving the stability of aerobic granules through selecting slow-growing nitrifying bacteria. The enriched nitrifying population in aerobic granules was responsible for the observed low growth rate of aerobic granules. It seems that the COD/N ratio is also an important factor in selecting nitrifying bacteria in aerobic granules. The study demonstrated that the selection of slow-growing nitrifying bacteria by controlling the COD/N ratio would be a useful strategy for improving the stability of aerobic granules.

Luo et al. (2014) evaluated the impact of the COD/N ratio on the abundance of ammonia-oxidizing bacteria (AOB) (*Nitrosomonas*) and nitrite-oxidizing bacteria (NOB) (*Nitrospira* and *Nitrobacter*). The authors observed that NOB decreased at a low COD/N ratio, whereas *Nitrosomonas* were abundant in the system. Regarding the competition between NOB, *Nitrospira* was favored over *Nitrobacter* at low nitrite concentrations.

He et al. (2017) observed that lowering the superficial gas velocity (SGV) from 0.17 to 0.11 and then to 0.04 cm/s, favored the enrichment of AOB while suppressed the accumulation of NOB.

An important family identified by Szabó et al. (2017) was Brocadiaceae, responsible for the anaerobic ammonium oxidation (Anammox). A high solids retention time (SRT) is essential for successful

Table 2
Microbial groups and main reactions involved in AGS systems (adapted from Metcalf and Eddy, 2016).

Groups	Reaction	Abbreviation	Electron donor	Electron acceptor	Products
Ordinary heterotrophic organisms	COD removal	OHO	Organic matter	O_2	CO_2, H_2O
Nitrifying organisms	Nitritation	AOB	NH_4^+	O_2	NO_2^-
	Nitratation	NOB	NO_2^-	O_2	NO_3^-
	Complete nitrification	COMAMMOX	NH_4^+	O_2	NO_3^-
Polyphosphate-accumulating organisms	Phosphorus removal	PAO	Organic matter	O_2	CO_2, H_2O
Glycogen-accumulating organisms Heterotrophic denitrifying organisms	COD removal	GAO	Organic matter	O_2	CO_2, H_2O
	Denitrification	DOHO	Organic matter	NO_2^-, NO_3^-	N_2, N_2O, NO, NO_2^-
	Denitrification and phosphorus removal	DPAO	Organic matter	NO_2^-, NO_3^-	N_2, N_2O, NO, NO_2^-, PO_4^{3-}
	Denitrification and glycogen accumulation	DGAO	Organic matter	NO_2^-, NO_3^-	N_2, N_2O, NO
Autotrophic anaerobic organisms	Anaerobic ammonium oxidation	Anammox	NH_4^+	NO_2^-	N_2, N_2O, NO^-

Anammox, anaerobic ammonium oxidation; AOB, ammonia-oxidizing bacteria; COMAMMOX, complete ammonia oxidizer; DGAO, denitrifying glycogen-accumulating organisms; DOHO, denitrifying ordinary heterotrophic organisms; DPAO, denitrifying polyphosphate-accumulating organisms; GAO, glycogen-accumulating organisms; NOB, nitrite-oxidizing bacteria; OHO, ordinary heterotrophic organisms; PAO, polyphosphate-accumulating organisms.

cultivation of Anammox at ambient temperatures. Given the low yield and growth rate of Anammox bacteria, heterotrophic growth should be minimized, to maintain a high fraction of Anammox bacteria in the sludge.

In aerobic granular sludge systems the competition between Anammox and NOB for nitrite and between AOB and NOB for oxygen is the major issue in selecting the desired population. Low oxygen concentrations have been proposed to favor AOB in preference to NOB. When oxygen is limited in AGS systems, all three groups of bacteria (AOB, NOB and Anammox) do not grow in suspension but are agglomerated in one compact granule. In this case, the minimum SRT required is therefore defined by the slow-growing microorganism, i.e. Anammox bacteria (Winkler et al., 2011).

The major functional microbial groups presented in the aerobic granules are shown in Table 2. The bacterial groups observed in different works on AGS and their respective functions are presented in Table 3.

4. Parameters for AGS cultivation

The "selection pressure" is a stress that induces a change in the behavior of a microbial population, being a key driving force for successful AGS cultivation.

Some examples of selection pressure generally accepted for AGS formation are (1) distinct feast-famine periods during operation, (2) short settling time, and (3) high aeration intensity (Qin et al., 2004). The values of the above mentioned parameters are directly related to the reactor used for AGS cultivation.

4.1. Operation mode

The AGS formation was obtained with best results in sequential batch reactors (SBR) (Liu et al., 2005). In this configuration, the granule cultivation occurs without the need of a secondary clarifier, and all phases (filling, anaerobic, anoxic and aerobic reaction, settling and decanting) take place in the same tank. Some SBR operate with simultaneous influent filling and effluent decanting, therefore both operations occur in a single phase.

However, AGS cultivation is also possible in continuous-flow systems, which were recently discussed elsewhere (Kent et al., 2018). The main continuous-flow configurations reported are: conventional activated sludge systems, membrane bioreactors (MBR) and systems with sequential reactors. Although continuous-flow systems presented satisfactory results in terms of granule formation, the sludge was unstable and easily disintegrable. Therefore, the use of SBR systems for AGS

Table 3
Bacterial groups and their functions in the AGS.

Bacteria	Function	Reference
Nitrospira defluvii	NOB	Winkler et al. (2012)
Nitrobacter	NOB	
Competibacter	PAO	
Accumulibacter	GAO	
Nitrosomonas europaea	AOB	
Azotobacter	Alginate production	Lin et al. (2013)
Pseudomonas	DOHO	
Tetrasphaera	PAO	Weissbrodt et al. (2013)
Xanthomonadaceae	OHO and EPS production	
Acidorovax	DOHO	
Rhizobiales	DOHO	
Beggiatoa spp.	OHO	Figueroa et al. (2015)
Chryseobacterium spp.	OHO	
Chloroflexi	OHO	
Thiothrixnivea	OHO	
Defluviicoccus	GAO	Franca et al. (2015)
Zoogloea sp.	DOHO	Wan et al. (2015)
Flavobacterium sp.	OHO	
Acinetobacter sp.	DOHO	
Rhodocyclaceae	EPS production	Szabó et al. (2017)
Xanthomonadaceae	EPS production	
Methylobacteriaceae	DOHO	
Nitrospiraceae	NOB	
Bradyrhizobiaceae	NOB	
Brocadiaceae	Anammox	
Comamonas	DOHO	He et al. (2017)
Dechloromonas	DPAO	
Azospira	DOHO	
Aquincola tertiaricarbonis	DGAO	Nancharaiah et al. (2018)
Paracoccus aminovorans	AOB	
Rhodoplanes elegans	PAO	
Paracoccus	Pyridine degradation	Liu et al. (2018)
Trichococcus	OHO	
Thauera sp.	PAO	
Bdellovibrio	EPS production	

Anammox, anaerobic ammonium oxidation; AOB, ammonia-oxidizing bacteria; DGAO, denitrifying glycogen-accumulating organisms; DOHO, denitrifying ordinary heterotrophic organisms; DPAO, denitrifying polyphosphate-accumulating organisms; EPS, extracellular polymeric substances; GAO, glycogen-accumulating organisms; NOB, nitrite-oxidizing bacteria; OHO, ordinary heterotrophic organisms; PAO, polyphosphate-accumulating organisms.

cultivation is recommended. In this review, the evaluated parameters refer to SBR systems.

4.2. Shear stress

As previously mentioned, hydrodynamic shear force, short settling periods and feast-famine feeding regimes are the main important triggering forces in SBRs (Nancharaiah and Reddy, 2018).

The aeration intensity, upflow air velocity and height/diameter (H/D) ratio are directly related to the shear stresses imposed on the system (Adav et al., 2008). In literature, shear stress is reported by its beneficial effects on granule structure, AGS metabolism (Tay et al., 2001), EPS production, granule performance and stability, as well as on the rapid AGS formation (Liu and Tay, 2002).

Liu and Tay (2002) observed that a higher H/D ratio can provide a longer circular flowing trajectory, which creates an effective hydraulic attrition for microbial aggregation. Many studies about AGS were mainly established on well-controlled lab-scale SBR with an H/D ratio over 10.

In relation to pilot and full scale applications, very different H/D ratios were observed. For instance, pilot reactors treating real wastewater in Netherlands and China, as well as treating synthetic wastewater in Spain, were designed for an H/D ratio of 8. On the other hand, the full scale wastewater treatment plant (WWTP) located in Gansbaai, South Africa, was designed with an H/D ratio lower than 1 (Li et al., 2014).

Thus, although high values of H/D ratio ensure the successful formation of aerobic granules, it is not viable the application on full scale plants. By decreasing the H/D ratio, it is necessary to increase the aeration to enhance the shear stress necessary for AGS formation, resulting in an increase of implementation and operation costs.

Aeration has been considered the main parameter of shear force control, and high aeration rate leads to a faster aerobic granulation due to the strong hydraulic shear force caused. Moreover, high aeration intensity is favorable for keeping the stability of aerobic granules not only by providing sufficient hydraulic shear force but also by inhibiting the overgrowth of filamentous bacteria and large granules (He et al., 2017; Adav et al., 2008; Li et al., 2011).

Some studies have applied high aeration intensity for both enhancing aerobic granulation and maintenance of granule stability during long-term operation, whereas a relatively low hydrodynamic shear stress favors the increase of the granule size. However, a high hydrodynamic shear stress typically requires substantial amounts of energy (Zhou et al., 2016).

In AGS reactors, shear force is typically quantified by dividing the aeration rate over the cross sectional area of the SBR tank, and is represented as up-flow superficial air velocity. Devlin et al. (2017) evaluated the impact of different organic loading rates on granulation in an SBR with a constant upflow air velocity (v_{air}). In the experiment, the authors observed that a v_{air} of 0.41 cm/s was sufficient for granule formation, being the optimum performance achieved when COD was lower than 300 mg/L. However, the same velocity was not suitable for granulation when the COD was higher than 600 mg/L, showing the possibility of v_{air} optimization as a function of the influent load.

According to above-mentioned experiments, it is observed that values of v_{air} higher than 2.5 cm/s resulted in the formation of dense, compact and mature granules.

It is important to mention the relation between aeration and DO. The limitation of DO diffusion into aerobic granules (due to their large and compact structure) is indispensable for the creation of different zones, which are necessary for the simultaneous nitrification/denitrification (SND) process.

The relations among DO concentration, microbial granule composition, AGS stability, nitrogen removal and resource savings were verified in some previous studies (Mosquera-Corral et al., 2005).

de Kreuk and van Loosdrecht (2004) reported that it was possible to form AGS at low DO concentrations when conditions that select slow-growing microorganisms were applied. However, other studies have pointed out that, although it is possible to obtain aerobic granules at low DO concentrations, they are not stable and disintegrate during long-term operation periods (Mosquera-Corral et al., 2005).

Mosquera-Corral et al. (2005) evaluated the optimization of nitrogen removal in a short-term AGS system by reducing the saturation DO concentration from 100% to 50%, 40%, 20% and 10%. The authors observed that the reduction did not influence the rate of acetate absorption in the aerobic period and the nitrogen removal was favored by DO concentrations decrease. In the same study, the authors assessed long-term effects at two saturation DO concentrations (100% and 40%) and found that nitrogen removal increased from 8% to 45% when oxygen saturation was reduced to 40%. However, the granules began to disintegrate, and there was biomass washout.

As presented in the topic of microbial groups, the DO concentration can be used to control a specific function group. However, in most of the studies, the difficulty of establishing an optimum DO concentration is remarkable, since this depends on specific factors, such as biomass concentration, granule size, type of substrate and the applied organic loading rate.

According to these results, it seems that stable granules can only be achieved when the DO concentration is greater than 50% of their DO saturation concentration. However, this parameter can be optimized in future investigations, aiming not only the formation of stable granules, but also energy savings and high N removals.

The advantages related to high shear rates are: (i) rapid formation of AGS, (ii) stability of the granules, (iii) increased hydrophobicity, (iv) inhibition of overgrowth of filamentous bacteria and large granules, and (v) EPS production. The main works that studied the effect of

Table 4
Effect of ascending air velocity on the formation and stabilization of AGS.

v_{air} (cm/s)	Size (mm)	SVI_{30} (mL/g)	EPS (mg/g VSS)	Biomass concentration (g VSS/L)	Stability	Influent	Reference
1.4	3.2	–	–	1.2	No	2.5 kg $COD/m^3{\cdot}d$	Beun et al. (1999)
4.1	2.1	–	–	2.0	Yes	Synthetic wastewater (ethanol)	
0.3	0.1	170	–	1.5	No	6 kg $COD/m^3{\cdot}d$	Tay et al. (2001)
1.2	0.39	62	85 (PS)	4.5	Yes	Synthetic wastewater (acetate)	
2.4	0.37	55	95 (PS)	6.0	Yes		
3.6	0.33	46	100 (PS)	6.9	Yes		
0.8	0.05	187	–	1.0	No	6 kg $COD/m^3{\cdot}d$	Chen et al. (2007)
1.6	0.35	73	–	2.5	No	Synthetic wastewater (acetate)	
2.4	0.7	41	31	7.1	Yes		
3.2	0.5	27	37	4.7	Yes		
0.6	3.0–3.5	60	300	–	No	1000–1500 mg COD/L	Adav et al. (2007)
1.8	1.0–1.5	50	370	–	Yes	Synthetic wastewater (phenol)	

EPS, extracellular polymeric substances; SVI_{30}, sludge volume index at 30 min; v_{air}, upflow air velocity.

upflow air velocity on the AGS formation process are presented in Table 4.

4.3. SBR cycles and settling time

Most of the SBR applied to AGS cultivation operate with a total cycle time between 4 and 12 h. Short cycles, lower than 4 h, for example, can result in biomass washout (in terms of volatile suspended solids) with the system supernatant (Liu and Tay, 2004). However, Tay et al. (2002) did not observe nitrifying granulation in SBR cycles higher than 24 h, possibly due to the low nitrogen load. For medium SBR cycles, particularly between 4 and 12 h, was cultivated nitrifying granules.

Alternating aerobic and anaerobic conditions favors the growth of beneficial microorganisms to granulation (PAOs, GAOs, nitrifying bacteria), since the proliferation of heterotrophic microorganisms is suppressed by the lack of soluble carbon source under aerobic conditions. Therefore, SBR cycles generally have an anaerobic period followed by an aerobic period. Some studies have evaluated aerobic filling, but this strategy did not allow the selection of PAOs and GAOs, for this is recommended anaerobic feeding (de Kreuk and van Loosdrecht, 2004). Recently, some studies have used the anaerobic/aerobic/anoxic sequence, aiming to improve the simultaneous nitrogen and phosphorus removal (He et al., 2016).

The settling time is a key factor for the formation of aerobic granules, being considered the main strategy of microbial selection (Beun et al., 1999; Qin et al., 2004, b). The SBR operation with a short settling time (< 10 min) allows a quick selection of microbial aggregates, causing the washout of light microbial flocs.

Qin et al. (2004) studied the relationship between granule formation and settling times (5, 10, 15 and 20 min). In the SBR incorporating 5-min settling time, granule formation was found to be excellent with flocculent sludge absence.

Studies indicate that short settling times are essential for AGS formation. The strategy of gradual reduction of the settling time is used for granule formation, but the strategies vary a lot in literature (Jiang et al., 2002; Liu et al., 2003; Yang et al., 2003; Hu et al., 2005). Usually SBR are operated with settling times between 2 and 10 min after granules formation (Adav et al., 2008).

4.4. Feast/famine period

The regime influence on granule formation is reported in literature, in which the lack of food, during the famine period, stimulates bacteria hydrophobicity, allowing the connection among them (Tay et al., 2001).

Studies have shown that, although a short period of starvation (famine) could accelerate granule formation, this condition had a negative impact on AGS stability (Liu and Tay, 2008). On the other hand, long famine periods can lead to the induction of excessive EPS consumption, higher energy expenses and decrease of effluent volume treated in the reactor (Wang et al., 2005).

Liu and Tay (2008) demonstrated that, when the feast period was 40% of the aeration time, the aerobic granulation process was accelerated. However, the long-term stability of the granules was compromised. Most of the studies pointed out that, when the feast period was equal to 20% of the aeration time, the granules formed were stable and resistant (Wang et al., 2017).

In a study that aimed at optimizing the feast and famine periods, López-Palau et al. (2012) showed that, when the feast period was equal to 33% of the aeration time, stable, dense and resistant granules were formed.

In summary, it was observed that a feast period lower than 40% of the aeration time resulted in the formation of granules with good settling velocity and good stability in long operating periods.

Most research on synthetic wastewater demonstrated that extracellular polymeric substances (EPS) is mainly produced during the feast phase of the sequencing batch reactor (SBR) cycle, following the feeding period in which the readily biodegradable COD is mainly degraded. A significant consumption, however, was observed during the famine phase in which substrate is present in limiting concentrations and bacteria utilize EPS as carbon and energy source for endogenous respiration (Corsino et al., 2015).

The EPS contribute mainly to the aerobic granules stability, and literature shows these polymers function as a “biological glue” for granule formation and stability (Wang et al., 2005; Chen et al., 2007; Adav et al., 2008). Nancharaiah and Reddy (2018) highlighted the importance of EPS in the granule stability (in terms of the PN/PS ratio), in the adsorption of metals and organic pollutants, as well as serving as carbon and electron source, especially in the famine period.

The cause of the granule breakage is likely related to the mineralization of their core (Lemaire et al., 2008), as well as to the clogging of their porosity that would limit the flow of nutrients and oxygen from the bulk into the inner layers (Lee et al., 2010). According to Lemaire et al. (2008) and Lu et al. (2012), the porosity clogging is related to an excess of extracellular polymeric substances (EPSs) production.

Therefore, if on the one hand a low EPSs content does not allow to obtain the granulation, on the other hand an excess of EPSs production could limit the aerobic granules maintenance in the long-term (Corsino et al., 2016).

In this way, it is important the control of EPS production and consumption during the RBS cycle. Due to the analytical difficulty of determining EPS, an indirect way of evaluating the production and consumption of polymers is through the duration of the feast and famine periods. The evaluation of the exogenous (feast) and endogenous (famine) phase can be done by monitoring the COD throughout the cycle.

5. Parameters for AGS maintenance

In addition to the key cultivation parameters, some additional factors are directly related to the stability of the granules (Adav et al., 2008).

Some parameters generally accepted for AGS maintenance are (1) influent wastewater composition (Liu et al., 2005), (2) food to microorganism (F/M) ratio and related factors (Liu et al., 2005; Wu et al. 2018), (3) SRT (Liu et al., 2005), and (4) pH and temperature (de Kreuk et al., 2005).

5.1. Influent wastewater composition

Several studies indicate the possibility of AGS formation using real wastewater. However, the granulation time was higher than 50 days (Wang et al., 2007). Therefore, AGS cultivation was analyzed for both domestic wastewater and acetate. In the presence of acetate, the formed granules were more stable and had a more compact and dense structure compared to the granules formed with domestic wastewater (Nor-Anuar et al., 2012). The formation of AGS with dairy wastewater resulted in a filamentous and unstable granule structure (Schwarzenbeck et al., 2005).

Because of the above-mentioned problems, Peyong et al. (2012) have suggested the external addition of soluble COD to the AGS cultivation when it is intended to treat effluents that are not favorable to the development of slow-growing bacteria. In addition to this strategy, some investigations used a pre-cultivated granular sludge as inoculum (Pijuan et al., 2011). This practice was also observed in real-scale wastewater treatment plants, in which the inoculum was grown in pilot systems (Li et al., 2014). This strategy reduces the reactor start-up time and allows the cultivation of stable granules prior to their application to real-scale systems.

Most of the investigations with AGS have been conducted with synthetic effluents, being the main carbon sources: glucose, acetate, ethanol and phenol. In the evaluation of the granule type formed from different substrates, it was observed that glucose produced filamentous granules, whereas acetate formed dense and resistant granules. The use of phenol as substrate resulted in the production of strong granules, but with long cultivation time (Adav et al., 2007).

Some recent works have adopted propionate as carbon source for aerobic granulation and observed that, although the granulation has been delayed, the granules formed had a strong and compact structure and could be used for long periods without disintegration (Lee et al., 2010).

Energy substrates, which are used by a wide variety of microorganisms (ex. glucose), impair the formation of VFA, as they are degraded by multi-step processes (with the formation of several intermediates), producing granules with a complex structure and a diversity of microorganisms, including filamentous bacteria. However, simple substrates, such as acetate and propionate, select simple and uniform microstructures, usually forming dense and compact granules (Moy et al., 2002).

Pronk et al. (2015) evaluated, in an AGS culture, the intermediates produced in the anaerobic period of a SBR. The author observed that methanol generated methane and CO_2, forming unstable and filamentous granules with predominance of methanogenic species in the granule core. The use of propionate and acetate increased the accumulation of polyhydroxyalkanoates (PHA), favoring PAOs and resulting in a stable granule.

Therefore, VFAs, such as acetate and propionate, formed the best granules, with acetate being the most recommended substrate (Lee et al., 2010). Although favoring the formation of granules, propionate may inhibit the activity of microorganisms when present at high concentrations (Garrity et al., 2007).

With respect to the influent COD/N ratio, high values can cause granule disintegration by growth of filamentous microorganisms. On the other hand, the ratio reduction to values close to 1, caused large changes in the microbial community and reduced EPS content, impacting on the nitrification, resistance, size and sedimentation capacity of the aerobic granule, with subsequent loss of granular biomass (Carrera et al., 2004; Yang et al., 2005).

Kocaturk and Erguder (2016) indicated that high COD/N ratio values, between 10 and 30, favored the growth of large granules with predominance of filamentous microorganism. However, low COD/N ratios, between 2 and 5, resulted in the formation of stable but small granules. In this study, the author observed that the optimal COD/N ratio was 7.5 in terms of removal efficiency and granule stability. The results are similar to those reported by Peyong et al. (2012) and Luo et al. (2014), who observed the formation of mature granules and a good system performance at COD/N ratios between 2 and 10.

5.2. F/M ratio and related factors

The F/M ratio has also been suggested as a relevant factor in aerobic granulation process. The literature reports that the F/M ratio is directly related to the composition and amount of EPS generated, granule stability, formation time, microbial diversity and pollutant removal efficiency (Wu et al., 2018; Li et al., 2011).

Tay et al. (2004) suggested that a low F/M ratio (0.33 g COD/g VSS·day) was important for the development of stable aerobic granules. Li et al. (2011) adopted an F/M ratio greater than or equal to 1.1 g COD/g VSS·day for the granule formation and an F/M ratio of 0.3 g COD/g VSS·day at the maturation stage.

Wu et al. (2018) studied the optimal F/M ratio in the granule stabilization process. The results indicated that F/M ratios between 0.4 and 0.5 g COD/g VSS·day showed a greater microbial diversity and better pollutant removal efficiencies.

Kang and Yuan (2017) observed granules disintegration due to the reduction of F/M ratio from 0.4 to 0.2 g COD/g VSS·day, with a value of 0.4 being suggested as optimum. When F/M ratio values are relatively low, granule stability is favored because of the relatively small size. However, very low values cause EPS production decrease, impairing granulation process.

The control of F/M ratio is done through two parameters: organic loading rates (OLR) and concentration of volatile suspended solids.

Long et al. (2015) investigated the stability of AGS at different OLR (4.8–18 kg COD/m^3·d) and observed that the granules could maintain stability when the OLR was lower than or equal to 15 kg COD/m^3·d, with granules of approximately 1.8 mm. When the OLR increased to 18 kg COD/m^3·d, the granules were disintegrated, and biomass washout occurred. Regarding OLR effect on the removal of organic matter and nutrients, these authors observed that high removals of COD and nutrients were obtained when an OLR lower than 12.6 kg COD/m^3·d was used.

Peyong et al. (2012) observed that low OLR values (0.54 kg COD/m^3·d) led to the disintegration of the aerobic granular biomass.

The results presented by Long et al. (2015) are in agreement with those reported by Thanh et al. (2009), in which the formation of mature granules was observed at an OLR up to 15 kg COD/m^3·d.

At low OLR values, filamentous bacteria are more abundant, causing granule disintegration. However, AGS instability at high OLRs has been attributed to three main aspects: increased granule size and consequent disintegration due to carbon penetration inability, hydrolysis and protein degradation of the granule nucleus (Zheng et al., 2006), and loss of the microbial self-aggregation capacity due to protein concentration reduction in the EPS (Adav et al., 2010; Zheng et al., 2006).

Therefore, OLR values between 0.5 and 10 kg COD/m^3·d can be considered as reference values for the formation and maintenance of mature granules.

In the SBR used for the growth of aerobic granules, the exchange ratio is the operative parameter of control of the influent OLR. In this way, it is possible to optimize a RBS with constant OLR and a variable volumetric exchange rate. In this situation, the RBS could receive different volumes of sewage, depending on the COD, with the OLR constant and consequently, F/M ratio too. In regions where COD shows variations throughout the year, the volumetric exchange rate of the reactor would be adjusted. The most laboratory research used exchange volume between 40 and 60%.

5.3. SRT

It was shown that SRT would not be a decisive factor for aerobic granulation in SBR (Li et al., 2008). However, studies have shown that it is an important parameter for the stabilization of the granules, considering that the floc-forming bacteria are slow-growing.

Zhu et al. (2013) demonstrated that a long SRT of granular sludge leads easily into the deterioration of aerobic granule, and an appropriate selective sludge discharge mode favors the stability of aerobic granular sludge process. Experiments conducted by Lin (2003) showed that microbial granules developed at a SRT of about 10 days were quite stable with a small granule size and absence of a fluffy outer growth.

It should be pointed out that, in most aerobic granular sludge SBRs, the SRT is not strictly controlled, but varies naturally with changes in sludge settleability under given selection pressures (Liu and Liu, 2006).

5.4. pH and temperature

Most of the aerobic granular sludge research was carried out at room temperature (20–25 °C). de Kreuk et al. (2005) observed that starting up a laboratory scale reactor at 8 °C resulted in outgrowth of filamentous organisms and irregular structures, leading to biomass washout. The reactor was unstable and the experiment had to be stopped because the biomass could not be retained in the reactor. For this, in cold regions it is preferably the start of a new system should take place in summer, when temperature is high and the sludge formation process is faster.

With respect to the pH, Yang et al. (2008) observed that pH of 3 caused rapid formation of fungi-dominating granules after 1 week of operation. However, degranulation shortly occurred because of the granules instability.

Similar results were obtained by Corsino et al. (2018a), in which was observed that under low pH, biological kinetic rates decreased proportionally to the applied OLR. Therefore, neutralization of pH is recommended, especially if high strength wastewater has to be treated.

6. AGS removal mechanisms

Due to the high bacterial diversity in the aerobic granule, several metabolic pathways of obtaining energy and carbon are presented, important characteristics for the stable removal of pollutants under different operating conditions.

The removal of organic and inorganic compounds by AGS can take place by the following processes: biosorption, bioaccumulation and biodegradation (Liu et al., 2005; Wang et al., 2010).

6.1. Nutrients and organic matter removal in AGS

The mechanisms of simultaneous nitrification, denitrification and phosphorus removal (SNDPR) by AGS is well reported in literature (Lu et al., 2016; He et al., 2016).

Thus, in the anaerobic/anoxic period, denitrification of residual nitrite and nitrate occurs, in addition to the processes of EPS hydrolysis, fermentation and VFA assimilation, with the release of phosphate. Organisms belonging to Chloroflexi phylum are filamentous bacteria and have been shown to be important in the hydrolysis of proteins and polysaccharides (Weissbrodt et al., 2013). Under aerobic conditions, a higher diversity of microorganisms starts to act in the granular biomass for the SNDPR processes, such as AOB, NOB, denitrifying ordinary heterotrophic organisms (DOHO), PAOs, DPAOs and DGAOs. The processes of organic matter and nutrients (N and P) removal in AGS are shown in Fig. 1.

6.2. Toxicity, metals and recalcitrant compound removal

The understanding of some removal mechanisms for toxic, recalcitrant and emerging pollutants in AGS can be found in literature, and it was observed that the removal of metals and some recalcitrant compounds occurred by biosorption and it was related to the EPS content (Kong et al., 2014).

The use of EPS in the biosorption process is an economical and useful approach, and can be an alternative to conventional methods, such as precipitation, coagulation, ion exchange, electrochemical and membrane processes, used for metal removal (Gutnick and Bach, 2000).

The mechanisms of dye removal in AGS systems have also been studied. The literature reports that, under anaerobic conditions and with the presence of a co-substrate (electron donor), azo dye reduction occurs, requiring four electrons to break each azo bond. The azo dye reduction by-products, aromatic amines, are usually removed under aerobic conditions, as they are used as carbon and nitrogen source. The final products can be nitrogen compounds, water and carbon dioxide (Sarvajith et al., 2018; dos Santos et al., 2007; dos Santos et al., 2004).

The main dye removal processes reported are biosorption and biodegradation. Wei et al. (2015) evaluated the process of removing methylene blue in AGS reactors and found that 9.4% of the dye was adsorbed on the EPS, and 80.7% was transformed/degraded by the sludge, showing the importance of both simultaneous processes. Although several investigations have studied dye removal in AGS systems, it is worth mentioning that the mechanisms and metabolic pathways used by the granule are still poorly reported in literature.

The AGS has shown a biodegradation mechanism of some compounds linked to the nitrogen removal. For instance, Jemmat et al. (2013) found that the removal of p-nitrophenol occurred simultaneously with ammonia oxidation in a co-metabolic process.

In a recent study, Liu et al. (2018) observed a similar mechanism when evaluating the removal of pyridine, whose degradation occurred via nitrite denitrification using pyridine biodegradation intermediates as electron donor substrates. Thus, the degradation process of pyridine, mainly mediated by the microorganism *Paracoccus* sp., occurred simultaneously with denitrification. According to the author, pyridine could be aerobically degraded in the aerobic layer. In addition, pyridine

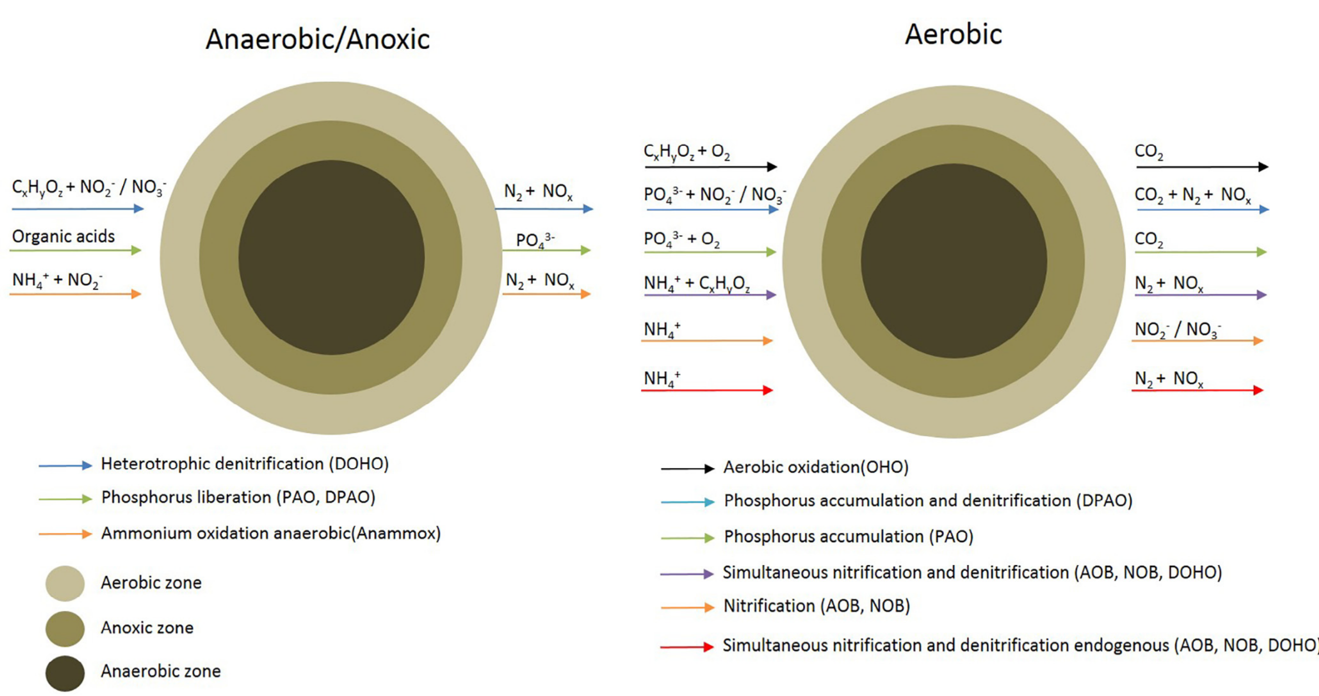

Fig. 1. Organic matter and nutrients (N and P) removal in AGS.

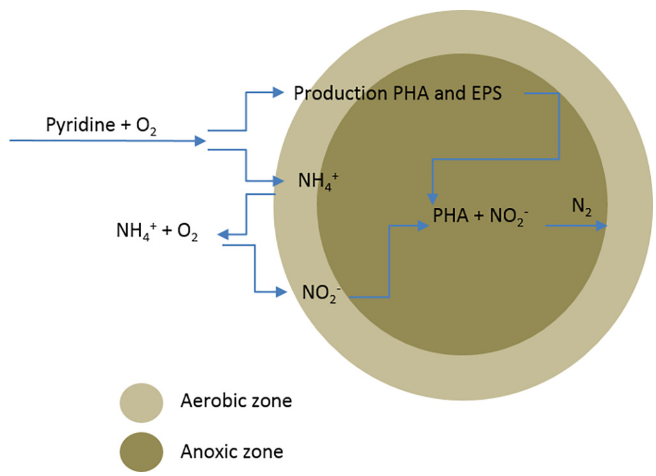

Fig. 2. Simultaneous pyridine biodegradation and nitrogen removal (adapted from Liu et al., 2018).

could be absorbed by aerobic granules due to the porosity and high surface area, followed by intra-particle diffusion. The adsorbed pyridine could be degraded further and stored as intracellular/extracellular polymers (Lopez-Vazquez et al., 2009). Nitrite produced from the last SBR cycle could be denitrified in the anoxic layer formed in the aerobic granules, using the biodegradation intermediates of pyridine, intracellular/extracellular polymers produced or pyridine itself as the electron donors (Liu et al., 2018).

Sarvajith et al. (2018), by using an AGS system for a yellow dye (YD) removal, observed that the color and nitrogen were removed simultaneously. Initially, the electron donor compound lactate was oxidized, and the electrons generated were used both to reduce the azo dye under anaerobic conditions and to support heterotrophic denitrification of the nitrite generated during the aerobic phase. In this same work, the author evaluated the impact of aerobic, anaerobic and microaerobic conditions on dyes removal and found that the color removal was higher under microaerobic conditions (during the anaerobic period), since the presence of small quantities of oxygen favored the occurrence of oxidative reactions. The mechanisms of pyridine removal associated to nitrogen removal are shown in Fig. 2.

Azizi et al. (2015), by evaluating the azo dye Acid Red 18 removal in AGS systems, observed that the azo dye reduction and subsequent color removal occurred in the anaerobic phase. The author also cited the possibility of dye reduction occurring during the aerobic period through anaerobic reactions inside the granule, using EPS as an electron source to promote dye reduction.

Removal of emerging micropollutants, such as estrogen, phenolic compounds, estradiol and other compounds, using AGS has also been studied, and some authors have observed significant changes in the bacterial composition of the granule when the system started to treat some pharmaceutical and personal care products (Amorim et al., 2018). However, it is known that the EPS can act as a protective layer on the granule surface, avoiding the penetration of toxic compounds and protecting the bacterial community (Liu et al., 2009).

Some studies that evaluated the removal of different compounds and their respective removal pathways are shown in Table 5.

7. Gaps of aerobic granular sludge

The addition of cations, bioaugmentation, metal removal and fast-growing techniques were considered the main research lines on aerobic granulation for the last 10 years. Currently, the research lines have aimed at the granular system optimization, the evaluation of AGS in the removal of compounds and recovery of resources, and the understanding of the various AGS mechanisms. Thus, considering recent discoveries and innovations, some of the main discussions that still require further clarification and elucidation are presented in the following items.

7.1. AGS mechanisms

As presented throughout this review, some authors presented the mechanisms of removal of pollutants in AGS systems, such as metals, dyes (mainly azo), recalcitrant compounds, among others. In the removal of metals, such as Cr^{4+}, the removal was verified by biosorption, with the possibility of desorption of up to 81% of the adsorbed metal (Chen et al., 2018). However, the authors did not specify whether the adsorption occurred by the EPS, the bacterial cavity or ion exchange with some ion. On the other hand, the mechanism was clearly demonstrated in the work carried out by Liu et al. (2015), in which it was observed that the removal of Pb^{2+}, Cd^{2+} and Zn^{2+} occurred by adsorption on EPS. The Ni^{2+} removal was associated with a significant release of Ca^{2+} ion by the ion exchange mechanism (Liu and Xu, 2007). The authors observed that only 14.2% of Ni^{2+} was removed by the EPS adsorption process, whereas the ion exchange was responsible for at least 68% of the metal removal.

Therefore, the following discussions are proposed: (i) mechanisms elucidation for simultaneous removal of nutrients and metals and the factors that impact this process; (ii) in terms of metals biosorption, it is important to evaluate the mechanisms used by AGS (ion exchange or adsorption on EPS) and the process impacts on the granule stability in long-term operation; (iii) in the metal removal process by adsorption, it should be studied how the operating conditions influence the maximum metal adsorption rate, considering that this parameter has presented considerable variations for the same type of compound. For example, for Cd^{2+}, Liu et al. (2004b), using AGS, observed an absorption rate of 625 mg Cd^{2+}/g VSS, whereas Liu et al. (2003) obtained values close to 173 mg Cd^{2+}/g VSS. In both studies, acetate was used as carbon source.

On color removal with AGS, Sarvajith et al. (2018) evaluated the simultaneous removal of ammonia and azo dye (yellow dye). However, it is not clear yet which dyes follow this pathway, which is the best substrate, the effect of DO concentration, optimal time of each SBR phase, which intermediates are formed etc. Therefore, the proposed discussions are: (i) the role of EPS as electron donor for dye reduction; (ii) the evaluation of the microaeration in the anoxic/anaerobic period of SBR aiming at the removal of different dyes and the impacts of this condition on the removal of organic matter and nutrients; (iii) the possibility of granules formation with controlled DO and optimum size that allow dye reduction during the aerobic period, taking place in the anaerobic layer of the AGS; (iv) evaluation of the maximum dye concentrations and intermediates generated that can act as inhibitors of the dye removal process; (v) evaluation of dye removal in SNDPR systems, observing functional group competitions and optimization strategies; (vi) evaluation of real-scale AGS systems with textile effluents and with other dye classes considered to be more recalcitrant, such as anthraquinone dyes; (vii) granulation at higher temperatures and in the presence of higher salinity levels, since several textile effluents are discarded with these characteristics.

Regarding the removal of emerging micropollutants, Amorim et al. (2018) observed that the presence of some drugs causes instability in the AGS bacterial communities, especially in the PAOs, AOB and NOB groups. In this line of research, there are still many issues to be evaluated, such as the stability of AGS in the removal of these compounds and the removal mechanisms used by the granular biomass.

7.2. System optimization

Some innovations in system design and new operational approaches have been proposed aiming at improving the AGS technology (formation and stability of the granule, energy savings, nutrient removal etc.).

For instance, He et al. (2017) evaluated the regulation of aeration

Table 5
Removal of metals, recalcitrant and toxic compounds in AGS.

	Compound	RE (%)	Mechanisms	Reference
Metals	U^{6+}	22	Biosorption	Nancharaiah et al. (2006)
	Ni^{2+}	68	Biosorption and ion exchange	Liu and Xu (2007)
	Zn^{2+}	95.3	Biosorption	Liu et al. (2015)
	Cd^{2+}	92.4	Biosorption	Liu et al. (2015)
	Pb^{2+}	90.6	Biosorption	Liu et al. (2015)
	Cu^{2+}	76.8	Biosorption	Wei et al. (2015)
Organic compounds and emerging pollutants	Bisphenol A	~92	ND	Balest et al. (2008)
	Estradiol	68	ND	Balest et al. (2008)
	4-Chloroaniline	99	Biodegradation	Zhu et al. (2008)
	Nitrobenzene	97–99	Biodegradation	Zhao et al. (2011)
	2-Fluorphenol	99	Biodegradation	Duque et al. (2011)
	p-Nitrophenol	99	Biodegradation	Suja et al. (2012)
	Paracetamol	> 90	Biodegradation	Hu et al. (2012)
	Fluoxetine	69	Biosorption	Moreira et al. (2015)
	Aniline	99	Biodegradation	Jang et al. (2017)
	Thiocyanate	99	Biodegradation	Tomar and Chakraborty (2018)
	Pyridine	99	Biodegradation	Liu et al. (2018)
	Phenol	99	Biodegradation	Tomar and Chakraborty (2018)
	Petroleum hydrocarbons	~90	Biosorption and biodegradation	Corsino et al. (2018b)
Dyes	Reactive blue 59	99	ND	Kolekar et al. (2012)
	Acid Red 14	> 92	Biodegradation	Franca et al. (2015)
	Methylene Blue	89	Biosorption and biodegradation	Wei et al. (2015)
	Acid Red 18	50	Biodegradation	Azizi et al. (2015)
	Yellow dye	85	Biodegradation and biosorption	Sarvajith et al. (2018)

ND, not determined; RE, removal efficiency.

intensity in the treatment of effluents with low OLR aiming at energy saving and improvement of the denitrification process. The results indicated improvement in the SNDPR process and predominance of AOB and PAOs. In the same line of optimization, He et al. (2018) evaluated the optimum aeration period for complete nitrification and phosphorus sequestration, while the remaining time used in the SBR cycle would be adjusted for the anoxic phase. In this approach, the reactor would be operated in anaerobic/aerobic/anoxic phases (A/O/A). The research showed that the reduction of the aeration period was favorable to granulation and improvement of SNDPR.

On the SNDPR mechanisms, the impact of sludge withdrawal on AGS-cultivating RBS has been evaluated. The first study related to the sludge withdrawal was published by Bassin et al. (2012), evaluating the impact of the selective removal of sludge from the reactor top and a fraction from the reactor bottom. The strategy favored PAOs in the competition with GAOs and allowed the removal of phosphorus values higher than 90% throughout the experiment.

In the same approach, Zhu et al. (2013) showed that the 10% sludge removal from the bottom of the reactor showed improvements in the removal of nitrogen and phosphorus. The authors verified that high sludge ages are related to the granule deterioration. Recently, Wu et al. (2018) proposed a method of withdrawing to maintain the optimal value of the food/microorganism ratio (F/M). Controlled withdrawal provided the formation of stable granules with better sedimentation capacity.

The latter work point out to the possibility of cycle optimization (aeration time and other SBR phases), upflow air velocity, sludge withdrawal in aerobic granular systems. However, it is necessary to understand the impacts of these changes on the granule stability over long periods of operation.

8. Conclusion

The AGS cultivation can be obtained from three parameters of easy control, upflow velocity (> 2.5 cm/s), sedimentation time (< 10 min) and alternation of feast and famine period in the same cycle. However, some parameters related to the stability of the granules can still be optimized, such as the reduction of the OD during the famine period and the equalization of the OLR through the exchange volume. The mechanisms of COD and nutrients removal are well elucidated, but the removal of some recalcitrant compounds needs a better investigation, since this knowledge is fundamental in decision making for system operation and design.

Acknowledgements

The authors would like to acknowledge the support obtained from the following Brazilian institutions: Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES; Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG; Instituto Nacional de Ciência e Tecnologia em Estações Sustentáveis de Tratamento de Esgoto – INCT ETEs Sustentáveis (INCT Sustainable Sewage Treatment Plants).

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