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Resource recovery in aerobic granular sludge systems: is it feasible or still a long way to go?

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HIGHLIGHTS

- Resource-recovery in aerobic granular sludge (AGS) systems was analyzed.
- C/N value plays an important role for the recovery of ALE, tryptophan and PHA.
- SRT effect is more pronounced for the recovery of ALE and tryptophan.
- Salinity could potentially be manipulated to increase ALE and phosphorus recovery.

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ABSTRACT

Lately, wastewater treatment plants are much often being designed as wastewater-resource factories inserted in circular cities. Among biological treatment technologies, aerobic granular sludge (AGS), considered an evolution of activated sludge (AS), has received great attention regarding its resource recovery potential. This review presents the state-of-the-art concerning the influence of operational parameters on the recovery of alginate-like exopolysaccharides (ALE), tryptophan, phosphorus, and polyhydroxyalkanoates (PHA) from AGS systems. The carbon to nitrogen ratio was identified as a parameter that plays an important role for the optimal production of ALE, tryptophan, and PHA. The sludge retention time effect is more pronounced for the production of ALE and tryptophan. Additionally, salinity levels in the bioreactors can potentially be manipulated to increase ALE and phosphorus yields simultaneously. Some existing knowledge gaps in the scientific literature concerning the recovery of these resources from AGS were also identified. Regarding industrial applications, tryptophan has the longest way to go. On the other hand, ALE production/recovery could be considered the most mature process if we take into account that existing alternatives for phosphorus and PHA production/recovery are optimized for activated sludge rather than granular sludge. Consequently, to maintain the same effectiveness, these processes likely could not be applied to AGS without undergoing some modification. Therefore, investigating to what extent these adaptations are necessary and designing alternatives is essential.

1. Introduction

Despite the great variability of sanitary conditions worldwide, domestic wastewater treatment is now an extremely mature technology from the perspective of human health and environmental impact (Batstone et al., 2015). This fact has promoted a paradigm shift: municipal wastewater is no longer perceived as a waste stream because it contains resources that can be recovered with innovative technologies in the so-called water resource factories (WRF), which feed into a circular economy (Kehrein et al., 2020). A great practical application example of this concept is the Energy and Raw Materials Factory, a collaborative network among Dutch water authorities, which focuses on the recovery of at least five resources from municipal wastewater: cellulose, bioplastics, phosphate, alginate-like exopolysaccharides (ALE) and biomass (van Leeuwen et al., 2018).

As several review articles from the last five years have pointed out (Table 1), aerobic granular sludge (AGS) has a great potential for

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Table 1
Summary of recent review papers regarding aerobic granular sludge.

Title	Contents	Reference
Aerobic granular processes: Current the research trends	This review presents recent studies of current research and development efforts in the formation, operation, and storage stability of aerobic granules, as well as the potential use of waste biomass as a resource for beneficial applications. PHA and phosphorus recovery, besides the utilization of aerobic granules as adsorbents, are briefly discussed.	Zhang et al. (2016)
An integrative review of granular sludge for the biological removal of nutrients and recalcitrant organic matter from wastewater	The authors examine the operational conditions and feeding characteristics impacting the granulation process, and the physical-chemical characteristics of granular sludge. They discuss the granulation process through the lens of microbial ecology. Some operational parameters affecting nutrients removal are reviewed, but not in detail. The authors mention that the latest AGS investigations target resource recovery.	Winkler et al. (2018)
Treatment of municipal wastewater with aerobic granular sludge	This article thoroughly discusses operational parameters associated with aerobic granules' formation and the efficient removal of carbon and nutrients by them. Resource recovery from AGS is presented amongst the challenges and future research directions of the field, with phosphorus and ALE recovery being mentioned.	Bengtsson et al. (2018)
Aerobic granular sludge technology: Mechanisms of granulation and biotechnological applications	The authors review AGS formation mechanisms and AGS application for nutrients and carbon removal. Special attention is given to the removal of toxic and recalcitrant pollutants by AGS, and studies regarding industrial wastewater treatment are summarized. The use of dewatered sludge as manure, the production of biomethane from the anaerobic digestion of AGS, the use of AGS as a cheap adsorbent, and the production of PHB and ALE are mentioned as alternatives of resource recovery from AGS.	Nancharaiah and Reddy (2018)
Aerobic granular sludge: Cultivation parameters and removal mechanisms	The parameters for AGS cultivation and maintenance, as well as their reference values, are defined. AGS's metabolic pathways for the removal of carbon, nutrients, metals, and toxic or recalcitrant compounds are addressed. The main microbial groups present in AGS and their respective functions are discussed. Resource recovery from AGS is mentioned as one of the main current research lines in the field.	Rollemberg et al. (2018)
Aerobic granular sludge process: a fast-growing biological treatment for sustainable wastewater treatment	The article discusses granulation mechanisms, the EPS matrix and the microbiology of aerobic granules, as well as the removal of nutrients and recalcitrant organic matter by AGS. It is suggested that future research should focus on improving AGS's resource recovery potential for widespread implementation in municipal and industrial wastewater treatment. ALE is mentioned as a resource with the potential of being applied as a raw material	Nancharaiah and Sarvajith (2019)
Various applications of aerobic granular sludge: A review	This article reviews the application of AGS for nutrients, phenolic compounds, and heavy metals removal from municipal, domestic and industrial wastewaters. It discusses the integration of aerobic granular bioflocs with membrane technology, microbial fuel cells, and microalgae to enhance wastewater treatment efficiency.	Purba et al. (2020)
Effect of feeding strategy and organic loading rate on the formation and stability of aerobic granular sludge	The impact of feeding strategy and OLR on AGS formation and stability are reviewed. Slow anaerobic feeding is considered the feeding strategy most suitable for a stable AGS formation. Application of a high OLR followed by a reduced OLR is recommended for achieving stable AGS formation with enhanced treatment performance.	Iorhemen and Liu (2020)

PHA: polyhydroxyalkanoates; AGS: aerobic granular sludge; ALE: alginate-like exopolysaccharides; EPS: extracellular polymeric substances PHB: polyhydroxybutyrate; OLR: organic loading rate.

resource recovery. This type of biomass is cultivated in sequencing batch reactors (SBR), in which a simultaneous removal of chemical oxygen demand (COD), phosphorus (P), nitrogen (N), and some recalcitrant compounds can occur in a single reactor (Nancharaiah and Reddy, 2018). In these SBR reactors, bubble-aeration, periodic feast-famine conditions, and aerobic starvation contribute to enhanced extracellular polymeric substances (EPS) production and microorganisms' aggregation. Simultaneously, short settling times are used to select and retain dense aggregates and granules in the reactor (Nancharaiah and Sarvajith, 2019). Such systems usually present better settleability and higher biomass concentration compared to activated sludge (AS) systems. As a result, a significant reduction in land area (~75%) and power consumption (30–50%) is achieved, also compared to AS systems (Rollemberg et al., 2020a).

Currently published AGS reviews (Table 1) commonly mention ALE, polyhydroxyalkanoates (PHAs), and phosphorus as products to be recovered from AGS. In addition, processes to recover the COD from AGS as energy and/or EPS, and the P content as struvite, have been recently modeled (Kehrein et al., 2020). These designs consider that the integration of a resource recovery technology into an AGS process likely implies trade-offs with other possible resource recovery technologies, as influent constituents, for instance, P and COD, can only be recovered once (Kehrein et al., 2020).

However, the manipulation of certain operational parameters might have singular effects on the production of each resource. This analysis is currently lacking from AGS literature as most of the reviews cover mainly granulation mechanisms and operational parameters for efficient removal of carbon and nutrients (Table 1). Little to no emphasis is given to the operational parameters affecting the recovery of resources from AGS per se, and no consideration is given to the possible trade-offs involving the optimization of production of different resources. It must be noticed that the extreme novelty of the research involving resource recovery from AGS is a compelling argument for the existence of these gaps. However, such discussion is key to facilitate and guide future work in the field. Therefore, the objective of the present paper is to summarize the current knowledge regarding the effect of operational parameters for the production of some resources in AGS systems. The chosen resources were ALE, tryptophan, phosphorus, and PHAs, as justified in the following paragraphs. Fig. 1 schematically presents the main potential applications of these four resources and where they can be found in aerobic granules.

This image summarizes the main potential applications of alginate-like exopolysaccharides (ALE), tryptophan, polyhydroxyalkanoates (PHAs), and phosphorus. In the center, an image of an AGS sample observed in an optical microscope is shown. A more detailed image of a single granule obtained with a scanning

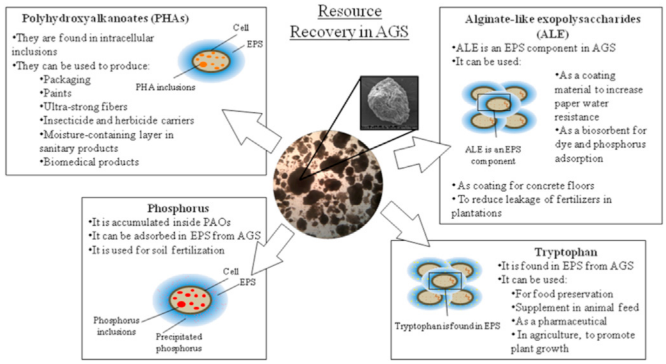

Fig. 1. Resource recovery possibilities in AGS systems.

electron microscope is also portrayed. Drawings from single cells and clusters of cells were depicted to show the location of each resource in the granular biomass.

The so-called alginate-like exopolysaccharides (ALE) are substances similar to alginate extracted from the AGS extracellular matrix that are identified based on their ability to form a gel in the presence of calcium (Ca^{2+}) and uronic acid residues (G and M) (Lin et al., 2008). ALE can be applied as a coating material to increase paper water resistance (Lin et al., 2015), as a biosorbent for dye and phosphorus adsorption (Ladnorg et al., 2019), and in both agriculture and civil construction (van Leeuwen et al., 2018). In 2016, the first ALE extraction plant for excess Nereda® sludge was built and patented as Kaumera Nereda® Gum, in Zutphen, The Netherlands (Royal HaskoningDHV, 2020). In December 2020, a second factory started the operation in Epe, The Netherlands (Royal HaskoningDHV, 2020). The term “Kaumera” is also used to represent the different biopolymers (including ALE) that can be extracted from AGS originated from the Nereda wastewater treatment process. So, depending on the AGS characteristics, a different biopolymer for a specific commercial application can be much more interesting to be produced (Royal HaskoningDHV, 2019). Despite this promising future, review papers regarding the influence of operational parameters over the production of ALE by AGS are lacking. Feng et al. (2021) has reviewed some key parameters affecting EPS production in granular sludge. Nevertheless, their paper does not focus exclusively on AGS systems, nor addresses operational parameters for the production of ALE specifically, what the present paper aims to do.

L-tryptophan is one of the nine amino acids essential to humans (Mustafa et al., 2018). It is used for food preservation due to its antioxidant properties (Huvaere and Skibsted, 2009), also being used as a supplement in animal feed (Favero Neto and Giaquinto, 2020). In addition, it can be applied as a pharmaceutical (Porter et al., 2005). Agricultural applications have also been investigated (Mustafa et al., 2018). Recently, AGS studies have shown that this biomass can produce tryptophan. Tryptophan was reported as one of the dominant organic materials in the EPS matrix of aerobic granules (Guo et al., 2020). Besides, a tryptophan production of 0.048 g g VSS^{-1} on a pilot-scale AGS system has been registered (Rollemberg et al., 2020b). However, due to the extreme novelty of these findings, this compound is hardly ever mentioned as a potential resource to be recovered from AGS. Nonetheless, since there is already some evidence of how AGS systems' operation can influence tryptophan production, this compound is included in the present review.

In AGS systems, phosphorus is removed from wastewater by polyphosphate accumulating organisms (PAOs) or is accumulated in the EPS matrix of granules (Huang et al., 2015). Excess sludge can be dehydrated and then directly applied to the soil (Yuan et al., 2012). It can also undergo anaerobic digestion, forming an appropriate liquor for phosphorus recovery through crystallization (Pastor et al., 2008), which might be carried out to produce struvite (Yang et al., 2017). Another alternative is the incineration of sludge, which generates P-rich ash with potential for struvite recovery (Kataki et al., 2016). In 2009, a Dutch wastewater treatment plant named Amsterdam West started the operation, designed with the activated sludge technology to treat the wastewater flow of 1 million population equivalents (van Nieuwenhuijzen et al., 2009). In this treatment, phosphate recovery was introduced for about 2000 m^3 of excess sludge per day, with an estimated production of struvite around 1000 tonnes per year (van der Hoek et al., 2017). Recovery of P from AGS is also a viable alternative (Kehrein et al., 2020), in which several reviews on AGS subject cover the topic of phosphorus removal (Table 1). Therefore, operational parameters affecting AGS phosphorus removal have been well summarized elsewhere. However, the present paper will again discuss this topic in order to analyze phosphorus removal strategies in comparison to strategies for the production of other resources by AGS.

PHAs are a family of biopolymers that have similar properties to plastics and can be used to produce packaging, paints, ultra-strong fibers, insecticides, herbicide carriers, moisture-containing layers in sanitary products (diapers, absorbents, etc.), besides having promising applications in biomedicine (Możejko-Ciesielska and Kiewisz, 2016). Even though the most common way to produce PHAs is through pure culture technology of natural or engineered microbial strains, PHA production by mixed microbial cultures (MMC) is increasingly considered due to the possibility to reduce the costs of the production process and to integrate PHA production in wastewater and organic waste biological treatments (Mannina et al., 2020). The PHARIO® process is currently an alternative to recover PHAs from activated sludge in a scalable and controllable manner, applicable to most conventional wastewater treatment plants (PHARIO, 2020). PHAs recovery in AGS systems is also a possibility, with the advantage of their high biomass retention, which increases PHA productivity if the same percentages of PHA accumulation of activated sludge systems are maintained (Wang and Yu, 2006). However, even though the operational parameters affecting PHAs production from MMC have been recently reviewed by Mannina et al. (2020) and Sabapathy et al. (2020), similar summaries have not yet been produced regarding PHA production from AGS, a gap that the present paper intends to cover.

2. Methods

The present research has been conducted mainly in the platforms Science Direct and PubMed. The main keywords utilized were aerobic granular sludge (AGS), resource recovery, alginate, alginate-like exopolysaccharides (ALE), tryptophan, enhanced biological phosphorus removal (EBPR), polyphosphate-accumulating organisms (PAO), and polyhydroxyalkanoate (PHA). 56% of the articles analyzed were published between 2016 and 2020, while 22% were published between 2011 and 2015, 12% between 2006 and 2010, and 9% before 2006.

3. Alginate-like exopolysaccharides (ALE)

Alginates are polysaccharides formed by various proportions of two monomers: β-D-manuronic (M) and α-L-guluronic (G) acids (Lin et al., 2010). Primarily due to its ability to form gels in contact with divalent cations (such as Ca^{2+} and Mg^{2+}), it can be used as a thickener in the food and textile industries, as a gelling agent for medicine encapsulation and dressing production, and as a filmforming agent for food preservation and papermaking (Cao et al., 2020; Moradali et al., 2018). In recent decades, alginate's use in advanced pharmaceutical and biomedical applications has been investigated due to its biocompatibility, non-toxicity, and versatile biological activity (Szekalska et al., 2016).

Its commercial production is exclusively from brown algae genera, such as *Ascophyllum*, *Laminaria,* and *Macrocystis*, which are found strictly in coastal regions of North America, Europe, Asia, and Australia (Gao et al., 2018; McHugh, 2003). The annual production is estimated to be around 30 thousand tonnes, with market values ranging between US$ 80–140 per kilogram (Murujew et al., 2019). Although not commercially competitive, the production of alginate by the bacteria genus *Azotobacter* and *Pseudomonas* under controlled conditions is also feasible, generating polymers with uniform characteristics and specific compositions (Remminghorst and Rehm, 2006).

AGS extracellular matrix contains ALE, which are substances similar to alginate that are identified based on their ability to form a gel in the presence of calcium (Ca^{2+}) and uronic acid residues (G and M) (Lin et al., 2008). However, its specific composition has not yet been elucidated. Based on the FT-IR spectrum, some studies suggest that ALE is basically composed of polysaccharides (Lin et al., 2010). Other researches propose that it actually consists of a polymeric complex, with both polysaccharides and proteins, in addition to alginate itself (Karakas et al., 2020; Meng et al., 2019; van Leeuwen et al., 2018). Therefore, complementary spectroscopic analyses, such as Nuclear Magnetic Resonance Spectroscopy (NMR), Raman Spectroscopy, and X-ray Photoelectron Spectroscopy (XPS), are necessary to elucidate the ALE structure in more detail.

Nevertheless, since about 15–25% of aerobic granules' dry weight is made of ALE, its recovery from excess AGS is a promising alternative for producing this high added value resource (Felz et al., 2016). Some studies have already demonstrated ALE's potential as a coating material to increase paper water resistance (Lin et al., 2015) and as a biosorbent for dye and phosphorus adsorption (Ladnorg et al., 2019). ALE can also be applied in agriculture due to its ability to retain water, reducing fertilizers' leaching and improving their absorption by crops. For civil construction, ALE can be used as a coating for concrete floors (van Leeuwen et al., 2018; Kaumera, 2020).

Initiatives that enable the sustainable recovery of ALE from full-scale AGS systems already exist. The public-private initiative National Alginate Research Program (NAOP) was created in The Netherlands to investigate and develop a sustainable and economically viable way of extracting ALE from Nereda® wastewater treatment plants (WWTP) sludge (van der Roest et al., 2015). In 2019, the first ALE extraction plant for excess Nereda® sludge started operating in Zutphen, The Netherlands (Dutch Water Sec, 2020). Field tests revealed that 18 kg of ALE could be produced from 80 kg of Nereda® granulated sludge (22.5% recovery), demonstrating the high potential for alginate recovery even in full-scale reactors (van Leeuwen et al., 2018). As previously mentioned, a second factory was built in Epe, The Netherlands, in 2019, and started the operation in December 2020. The EPE factory has been designed to produce 50 tonnes of Kaumera per year, in which the production will be increased according to the market demand until a limit of 100 tonnes of Kaumera per year (Dutch Water Sec, 2020). The Zutphen factory has a much higher capacity and is designed to produce up to 400 tonnes of Kaumera annually.

3.1. Operational parameters associated with ALE production in AGS systems

There are still few published studies on the impacts of AGS systems operating conditions on ALE biosynthesis (Table 2). Sections 3.1.1 to 3.1.5 summarize the main operational parameters associated with ALE production in AGS systems identified by researchers so far.

3.1.1. Organic loading rate

It has been reported that the rapid variation of organic loading rate (OLR), between 4.4, 5.2, 8.7 and 17.4 kg $COD \cdot m^{-3} \cdot d^{-1}$ (1250, 1500, 2500, and 5000 mg $COD \cdot L^{-1}$ in the reactor, respectively), influenced ALE production by AGS (Yang et al., 2014).

The experiments were conducted in two SBRs with different feeding strategies: RC (OLR was varied only after 30 days of operation) and RS (OLR was varied during the first 50 days of operation). In both systems, the peaks of ALE and cellular messenger cyclic diguanylate (c-di-GMP) coincided with the organic loading shocks. The authors suggest that the rapid increase in influent COD stimulated the release of c-di-GMP by the microbial community, which would trigger the biosynthesis of ALE.

3.1.2. Carbon to nitrogen ratio

Three SBRs were operated under different feed compositions: R9 (COD:N = 10; 500 mg $COD \cdot L^{-1}$); R10 (COD:N = 20; 1000 mg $COD \cdot L^{-1}$) and R11 (COD:N = 30; 1500 mg $COD \cdot L^{-1}$) (Rollemberg, 2020). R9 (243.6 mg $ALE \cdot g\ VSS^{-1}$) and R10 (289.7 mg $ALE \cdot g\ VSS^{-1}$) showed statistically significant differences in ALE content ($p \approx 0.04$), indicating that influent C:N ratios directly impact ALE synthesis. However, in R11, the ALE content was reduced (236.6 mg $ALE \cdot g\ VSS^{-1}$). AGS systems subject to high COD:N ratios (or high OLR) generally have an abundance of slow settleability filamentous organisms, which cause operational instability, as pointed out by the authors.

3.1.3. Salinity

There has been a report on ALE production by AGS fed with different NaCl concentrations (R1: 0 g $NaCl \cdot L^{-1}$; R2: 10 g $NaCl \cdot L^{-1}$; R3: 30 g $NaCl \cdot L^{-1}$) (Meng et al., 2019). R2 had the highest ALE yields (R1: 26.8 mg $ALE \cdot g\ VSS^{-1}$; R2: 49.8 mg $ALE \cdot g\ VSS^{-1}$; R3: 28.9 mg $ALE \cdot g\ VSS^{-1}$). The authors suggested that a moderate salinity (around 1% NaCl) triggers algC gene expression in Pseudomonas, activating the phosphomanomutase enzyme, which is part of the alginate biosynthesis mechanism (Moradali et al., 2018). Under excessive osmotic stress, however, this enzyme activation can be inhibited.

3.1.4. Granule size

Higher ALE content has been correlated with large granules dominating the system (diameter between 400 and 600 μm) after granulation is achieved (Schambeck et al., 2020). Similar results were reported in a study conducted with 5 SBRs with different cycle configurations. A generally greater and more stable ALE content was observed on mature granules (R1: 225.6 ± 19.5 mg $ALE \cdot g\ VSS^{-1}$; R2: 234.1 ± 13.4 mg $ALE \cdot g\ VSS^{-1}$; R3: 251.7 ± 16.8 mg $ALE \cdot g\ VSS^{-1}$; R4: 250.8 ± 24.2 mg $ALE \cdot g\ VSS^{-1}$; R5: 241.3 ± 22.6 mg $ALE \cdot g\ VSS^{-1}$) when compared with granules still in formation (R1: 213.7 ± 46.6 mg $ALE \cdot g\ VSS^{-1}$; R2: 232.0 ± 51.7 mg $ALE \cdot g\ VSS^{-1}$; R3: 238.1 ± 49.4 mg $ALE \cdot g\ VSS^{-1}$; R4: 227.4 ± 51.7 mg $ALE \cdot g\ VSS^{-1}$; R5: 190.3 ± 43.8 mg $ALE \cdot g\ VSS^{-1}$) (Rollemberg, 2020).

3.1.5. Sludge retention time

An SRT of 20 days reduced ALE content (203.9 ± 27.8 mg $ALE \cdot g\ VSS^{-1}$) when compared to AGS systems operated with SRT of 10 (237.5 ± 11.8 mg $ALE \cdot g\ VSS^{-1}$) and 15 days (244.1 ± 15.2 mg $ALE \cdot g\ VSS^{-1}$) or with no SRT control (Rollemberg, 2020). Possible explanations for such findings are the higher rate of endogenous respiration for long SRTs, which would induce EPS consumption as a carbon source, or the fact that a reduction of the SRT could benefit

Table 2
Operational parameters and ALE yields in AGS systems.

System configuration	Carbon source	Operational cycle	Aeration rate	COD/N	SRT	C, N and P removals	ALE yields	Reference
Lab-scale 2 L Exchange ratio: 50%	Synthetic wastewater: glucose + sodium acetate (600 mg COD·L^{-1})	Cycle: 4 h Feed: 2 min Anaerobic period: 30 min Aeration: 199 min Settling: 4 min Draw: 2 min and Idle: 3 min	3.0 L min^{-1} DO: 7–9 mg L^{-1}	6	20 d	COD: 90% TN: 99% TP: 78%	0.03–0.05 g ALE·g VSS^{-1}	Meng et al. (2019)
Lab-scale 2.75 L Exchange ratio: 54.5%	Synthetic wastewater: sodium acetate	Cycle: 3 h Anaerobic feed: 60 min Aeration: 112–115 min Settling: 5-3 min Draw: 5 min	4 L min^{-1} DO: 2.5 mg L^{-1}	–	–	COD: 89%	0.16 g ALE·g VSS^{-1}	Wang et al. (2017)
Lab-scale 4.78 L Exchange ratio: 57.9%	Synthetic wastewater: propionate (1250–5000 mg COD·L^{-1})	Cycle: 4 h Feed: 6 min Aeration: 208–223 min Settling: 20-5 min Draw: 6 min	8 L min^{-1} Air velocity: 2.65 cm s^{-1}	6–25	–	COD: 95%	0.11 g ALE·g VSS^{-1}	Yang et al. (2014)
Lab-scale 7.6 L Exchange ratio: 50%	Synthetic wastewater: sodium acetate (500 mg COD·L^{-1})	Cycle: 6 h Anaerobic feed: 60 min Anaerobic period: 60 min Aeration: 210–225 min Anoxic period: 10 min Settling: 20-5 min Draw: 1 min	– Air velocity: 1.2 cm s^{-1} DO: 1–3 mg L^{-1}	10	10 d	COD: 90% TN: 90% TP: 75%	0.23 g ALE·g VSS^{-1}	Rollemberg (2020)
Pilot-scale 110 L Exchange ratio: 50%	Municipal wastewater (513 mg COD·L^{-1})	Cycle: 6 h Anaerobic feed: 60 min Aeration: 234 min Anoxic period: 30 min Settling: 30min Draw: 6 min	– DO: no control	11	15 d	COD: 80% TN: 73% TP: 33%	0.24 g ALE·g VSS^{-1}	Schambeck et al. (2020)
Pilot-scale 140 L Exchange ratio: 40–60%	Municipal wastewater (461 mg COD·L^{-1})	Cycle: 6 h Feed: 1 min Anaerobic period: 60 min Aeration: 240–280 min Settling: 55-15 min Draw: 1 min and Idle: 3 min	– DO: 2–4 mg L^{-1}	11	12 d	COD: 95%	0.21 g ALE·g VSS^{-1}	Rollemberg et al. (2020b)

DO: Dissolved oxygen; COD: Chemical oxygen demand; TN: Total nitrogen; TP: Total phosphorus; ALE: alginate-like exopolysaccharides.

PAOs growth, allowing a greater production of ALE (Rollemberg, 2020). The alleged relation between PAOs and ALE production is discussed in section 3.2.

3.2. Association of ALE production with phosphorus removal

A study has shown that the highest ALE yields are associated with significant increases in phosphorus removal efficiency in AGS systems (Meng et al., 2019). Another report with AGS found a positive correlation between the ALE content in the maturation phase (i.e., post-granulation) and the removal of organic matter, ammonia, and phosphorus (Schambeck et al., 2020). In the same study, the presence of PAOs was also observed after granulation, when ALE's synthesis was higher. Therefore, multiple authors have recommended conducting further studies in-depth to assess the relationship between phosphorus removal and ALE production (Rollemberg, 2020; Schambeck et al., 2020; Wang et al., 2017).

There is also evidence that the higher removal of phosphorus observed in ALE-rich granules is related to the precipitation of struvite and not to the biological removal of phosphorus by PAOs. A study has shown that the ALE extracted from aerobic granules was capable of inducing the formation of struvite and precipitates similar to this latter compound, in which the ammonium ion (NH_4^+) is replaced by ions such as potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}) and iron (Fe^{3+}) (Lin et al., 2012). In the experiment, while the phosphorus accumulated in the biomass was 93 mg TP·g TSS^{-1}, the accumulated phosphorus in the form of struvite-like precipitates reached 72 mg TP·g TSS^{-1}. In addition, under an ammonia concentration of 10.4 mg N·L^{-1}, ALE was responsible for 60% of the ammonia adsorption in the form of struvite. Thus, the cultivation of granules with high ALE content can be an alternative to achieve high removals of N and P.

3.3. Extraction methods for ALE recovery

The extraction methods for EPS and ALE recovery are well reported in the literature, although they are usually considered costly. Felz et al. (2016) stated that, due to EPS′ complex structure, it is practically impossible to extract all its components by one single method. Therefore, the selected methods will influence both the composition and the yields of recovered polymers. These authors compared six different EPS extraction methodologies, using a sludge sample collected from a pilot-scale Nereda® reactor. Only the alkaline extraction method using sodium carbonate (Na_2CO_3) at high temperatures formed a gelatinous matrix, proving to be the most suitable technique for ALE recovery. Widely used as a methodology in scientific research (Karakas et al., 2020; Lin et al., 2010; Meng et al., 2019; Rollemberg et al., 2020b; Schambeck et al., 2020; Yang et al., 2014), this process is similar to the extraction of alginate from brown seaweed and is based on solubility properties of this compound. First, the insoluble alginate salts are converted to soluble sodium alginate by adding Na_2CO_3. Then alginic acid is precipitated by pH adjustments and collected. Finally, the acid is again converted to sodium alginate by adding a mixture of alcohol and water, in which the sodium salt does not dissolve (McHugh, 2003). A similar process is utilized in Nereda® plants to extract the Kaumera from the excess sludge (Tour Kaumera Nereda Gum, 2020). Initially, the AGS is thickened by gravity and homogenized in a buffer tank; then it goes through a belt thickener, is coagulated by the addition of a polymer, and heated until 80 °C. The pH is increased with the addition of hydroxide, separating the ALE from the sludge. In a sequence, the mixed liquor is cooled and centrifuged, and the supernatant is acidified so the ALE can flocculate as a gel, being finally separated by a new centrifugation. A summary of the current knowledge for ALE recovery in AGS systems is presented in Fig. 2.

The main factors associated with ALE production by AGS are summarized. Key findings were based on the following studies: (1) Yang et al. (2014), (2) Rollemberg (2020), (3) Meng et al. (2019), (4) Rollemberg (2020) and Schambeck et al. (2020), (5) Rollemberg (2020), (6) Meng et al. (2019) and Schambeck et al. (2020). OLR: Organic Loading Rate; COD: Chemical Oxygen Demand; N: Nitrogen; SRT: sludge retention time; c-di-GMP: cyclic diguanylate.

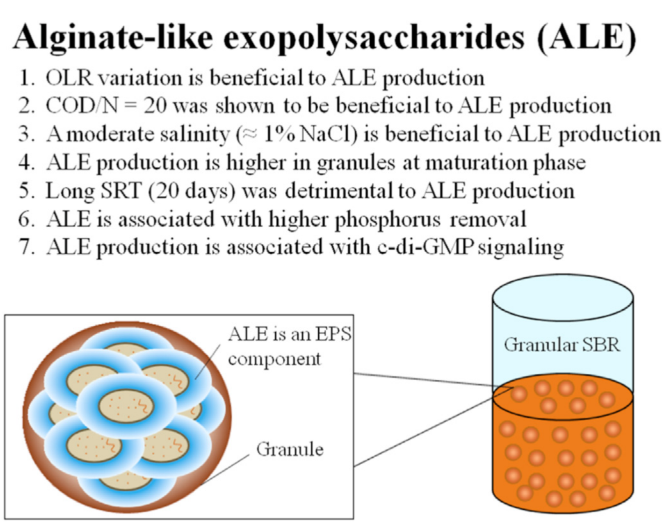

Fig. 2. Alginate-like exopolysaccharides (ALE) recovery in AGS systems.

4. Tryptophan

L-tryptophan is one of the nine amino acids essential to humans, being also essential for animals, plants, and some bacteria (Mustafa et al., 2018). In the food industry, it is used for food preservation due to its antioxidant properties (Huvaere and Skibsted, 2009) and as a supplement in animal feed (Favero Neto and Giaquinto, 2020). It can also be applied as a pharmaceutical since it promotes good results in treating psychological diseases like depression (Porter et al., 2005), anxiety (van Veen et al., 2009), and sleep disorder (Sangsopha et al., 2019). In addition, its exogenous application, which can be through a foliar spray, seed priming, or soil application, has been reported to be highly significant for normal plant growth and development (Mustafa et al., 2018). Recently, its commercial value was reported to be around US$ 80–120 per kilogram (Rollemberg, 2020).

This compound can be produced by three methods: 1) chemical synthesis from indole; 2) biochemical synthesis, in which the enzyme tryptophanase is produced by bacteria, isolated, and later used for the production of tryptophan from indole, pyruvate, and ammonia; and 3) microbial fermentation, in which bacteria capable of synthesizing tryptophanase produce tryptophan directly, using other substrates, such as glucose and xylose, in addition to those previously mentioned (Liu et al., 2019; 2009; Sano:Konosuke and Mitsugi, 1975; Snyder and Smith, 1944; Watanabe and Snell, 1972).

The microbial fermentation method mainly employs the species *Corynebacterium glutamicum* and *Escherichia coli*, the latter having the advantages of being easily genetically manipulated and growing fast in inexpensive media (Niu et al., 2019). In *E. coli*, the transport of glucose into the cell is carried out mainly through the glucose phosphotransferase system (PTS). However, the galactose transport system can also be carried out, in which the enzyme galactose permease (Gal P) is used. Both systems consume phosphoenolpyruvate (PEP), which, together with erythrose-4-phosphate (E4P), is considered a critical precursor of tryptophan production (Liu et al., 2019). After the substrate enters the cell, tryptophan production continues within it, as shown schematically in Fig. 3.

Recently, it has been reported that AGS can produce tryptophan, which is associated with the protein portion of EPS (D. Li et al., 2019; Li et al., 2020; Zhang et al., 2018a). A study has shown that, among the analyzed compounds, tryptophan was one of the dominant organic materials in the EPS matrix of aerobic granules (Guo et al., 2020). Hamza et al. (2018) and He et al. (2020) also report the presence of tryptophan in aerobic granules. A tryptophan production of 0.048 g g VSS^{-1} on a pilot-scale AGS has been obtained (Rollemberg et al., 2020b).

4.1. Operational parameters associated with tryptophan production in AGS systems

Even though published studies are still scarce, there is already some evidence of how the operation of aerobic granular SBRs can influence tryptophan production. For example, in reactors in which the operation favors the development of granules with a diverse microbial group and compact structure, it is possible to observe a higher concentration of this amino acid (Luo et al., 2014; Xiao and Zhou, 2020). When the granular systems are destabilized, leading to the formation of a floccular biomass, the concentration of tryptophan tends to decrease (Hamza et al., 2018). Sections 4.1.1 to 4.1.3 summarize the main operational parameters associated with tryptophan production in AGS systems that have been identified by researchers so far.

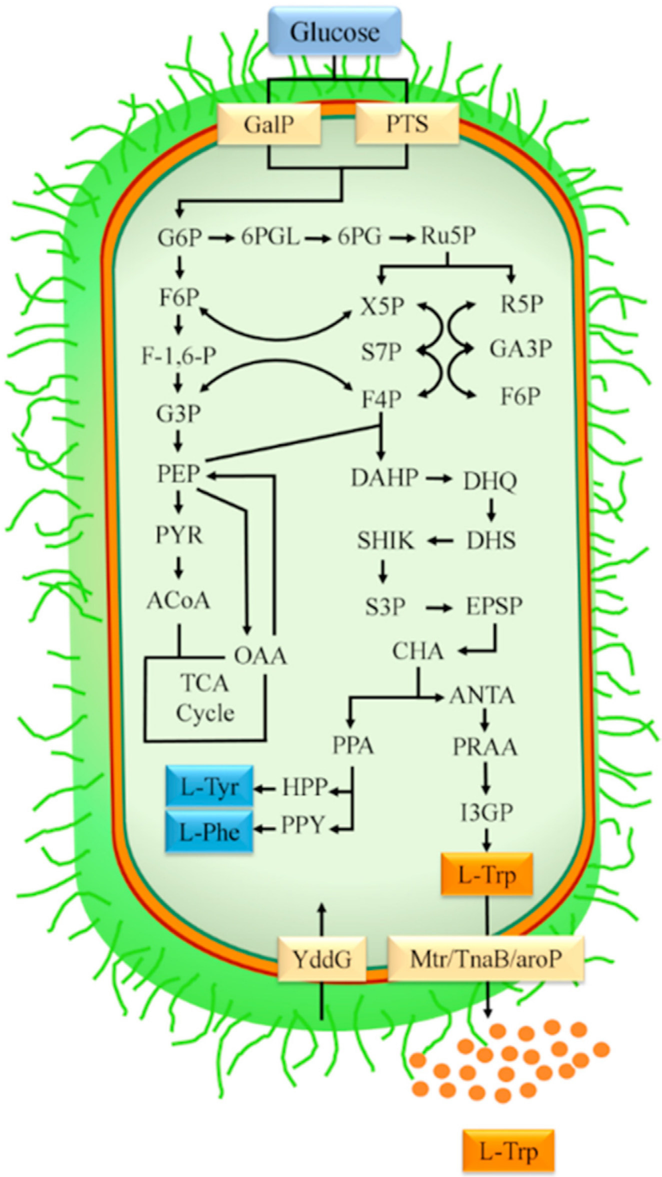

Fig. 3. Biosynthesis of L-tryptophan in *E. coli.* G6P: glucose-6-phosphate, F6P: fructose-6-phosphate, F1–6P: fructose-1,6-bisphosphate, G3P: glyceraldehyde-3-phosphate, 6PGL: 6-phosphogluconolactone, 6 PG: 6-phosphogluconate, 2D3D6PG: 2-dehydro-3-deoxy-D-gluconate-6-phosphate, RU5P: ribulose-5-phosphate, X5P xylulose-5-phosphate, R5P: ribose-5-phosphate, S7P: sedoeptulose-7-phosphate, E4P: erythrose-4-phosphate, PEP: phosphoenolpyruvate, PYR: pyruvate, ACoA: acetyl coezin A,OAA: oxaloacetic acid, DAHP: 3-deoxy-D-arabinoeptulosanate-7-phosphate, DHQ: 3-dehydroquitane, DHS: 3-desidroshikimato, SHIK: shikimato, S3P: shikimato-3-phosphate, EPSP: 5-enolpyruvyl-shikimato-3-phosphate, CHA: corismate, PPA: prephenate, HPP: 4-hydroxyphenylpyruvate, PPY: phenylpyruvate, ANTA: anthranilate, PRAA: N - (5-phosphoribosyl) -anthranilate, CPADRP: 1- (o-carboxyphenylamine) -1′-deoxyribulose 5′-phosphate, I3GP: (1S, 2R)-1-C- (indole-3-la)-glycerol-3-phosphate), L-Phe: L-phenylalanine, L-Tyr: L-tyrosine, L-Trp: L-tryptophan. Modified from Niu et al. (2019).

4.1.1. Carbon to nitrogen ratio

A COD/N ratio of 30 was reported to lower tryptophan yields (37.9 ± 22.7 mg g VSS^{-1}) in comparison with AGS reactors operated under COD/N ratios of 10 and 20 (51.8 ± 5.3 and 54.1 ± 8.0 mg g VSS^{-1}, respectively) (Rollemberg, 2020). Higher tryptophan production was also observed in a reactor operated with a C/N ratio of 7.5 compared to reactors operated with C/N of 15 and 30 (Yu et al., 2020). When the C/N ratio was gradually reduced from 15 to 5, tryptophan content in the AGS increased, while tryptophan production in the reactor operated under C/N of 15 was relatively constant (Zhang et al., 2018a). When the COD/N ratio is lower than 5, the higher was the presence of proteins and the lower was the number of polysaccharides in the EPS matrix. Such unbalance may lead to granule breakdown and biomass return to a flocculate state, although a decrease in tryptophan concentration was not found (Luo et al., 2014). In summary, a C/N ratio close to 5 seems to be ideal for tryptophan production to guarantee also the stability of the AGS systems.

The observed tryptophan accumulation under lower C/N ratios has been associated with a positive selection of the *Thauera* and *Paracoccus* genera, which are positively related to its production (Zhang et al., 2018a). These organisms, especially the *Thauera* ones, are related to the improvement of denitrification capacity since they are more abundant in granule's nuclei, together with *Nitrosomonas* and *Leadbetterella* genera. On the other hand, they are also associated with a reduction of nitrification capacity (Zhang et al., 2018a). Therefore, it is possible that operational strategies that promote tryptophan production would affect nitrogen removal in AGS systems.

4.1.2. Granule size

A lower tryptophan content was observed on mature granules (R1: 48.1 ± 2.1 mg g VSS^{-1}; R2: $46.6 \pm 2.$ mg·g VSS^{-1}; R3: 50.4 ± 2.8; mg·g VSS^{-1}; R4: 49.8 ± 4.3 mg g VSS^{-1}; R5: 34.5 ± 5.7 mg g VSS^{-1}) upon comparison with granules still in formation (R1: 58.9 ± 13.8 mg g VSS^{-1}; R2: 60.8 ± 14.2 mg g VSS^{-1}; R3: 63.5 ± 13.5; mg·g VSS^{-1}; R4: 62.9 ± 16.7 mg g VSS^{-1}; R5: 56.2 ± 15.8 mg g VSS^{-1}) (Rollemberg, 2020). These findings are reinforced by studies that report an increase in the relative abundance of *Thauera* sp. during the early formation stages of aerobic granules, followed by a decrease after maturation (Hamza et al., 2018), since these microorganisms have been associated with tryptophan production (Zhang et al., 2018a).

4.1.3. Sludge retention time

It has been reported that at an SRT of approximately 6 days, tryptophan formation is increased in AGS, while at an SRT of 12 days, a decreased production is observed (Zhang et al., 2018b). In addition, AGS systems operated with SRT of 10, 15, or 20 days showed no statistical difference between tryptophan yields (58.3 ± 4.6 mg g VSS^{-1}, 52.9 ± 5.7 mg g VSS^{-1} and 48.1 ± 7.0 mg g VSS^{-1}, respectively) (Rollemberg, 2020). The increase in tryptophan production seems to be related to the formation of larger granules of a more compact structure, which occurs at lower SRT (Zhang et al., 2018b) due to the higher secretion of EPS and its lower consumption during the famine period.

4.2. Association of tryptophan production with quorum sensing mechanisms

There is evidence that quorum sensing (QS) mechanisms can be exploited to stimulate EPS and tryptophan production. It is speculated that N-acyl homoserine lactones (AHL) molecules formed by 8-carbon chains, such as 3OH–C8- homoserine lactone (3OH–C8-HSL), can promote the secretion of tryptophan and protein-like substances by granular biomass (Zhang et al, 2018b, 2020).

4.3. Extraction methods for tryptophan recovery

Regarding the methods used to recover tryptophan from AGS, there are still no widely used processes, given the scarcity of published studies. Among the extraction methods, the thermal techniques, which consist of protein solubilization by exposing the sludge to elevated temperatures for a time long enough to promote the necessary chemical reactions, are highlighted (Harris and McCabe, 2015; Yang et al., 2014).

Regarding tryptophan quantification, it is possible to perform it visually through a three-dimensional excitation-emission matrix (3D-EEM) (Guo et al., 2020; Tu et al., 2012). High-performance liquid chromatography (HPLC) can also be used, also serving to purify the extracted tryptophan since it is a process that separates molecules by their polarity and hydrophobicity (Opiteck et al., 1998; Rollemberg et al., 2020b). A summary of the current knowledge to TRY recovery in AGS systems is presented in Fig. 4.

The main factors associated with tryptophan production by AGS are summarized. Key findings were based on the following studies: (1) Luo et al. (2014), Rollemberg (2020), Yu et al. (2020), and Zhang et al. (2018a); (2) Hamza et al. (2018), Rollemberg (2020), and Zhang et al. (2018a); (3) Zhang et al. (2018b); (4) Zhang et al. (2020, 2018b). COD: Chemical Oxygen Demand; N: Nitrogen; SRT: sludge retention time; AHL: N-acyl-homoserine lactones; QS: quorum sensing.

5. Phosphorus

Phosphorus is an essential nutrient for developing life, being one of the main compounds used in agricultural activities. Such a resource is finite, and the amount of mineral phosphorus is decreasing globally, requiring the exploration of new sources (Chrispim et al., 2019). When this compound reaches surface waters via different anthropogenic sources, such as raw wastewater (WW), which contains between 0.5 and 10 mg L^{-1} of phosphate, it may cause adverse effects on the environment, such as eutrophication and changes in pH and oxygen levels (Ramasahayam et al., 2014). Thus, the use of phosphorus contained in WW, especially through the discharged sludge, would redirect this element to productive uses, for which the supply of phosphorus is decreasing.

In this context, some techniques, such as chemical adsorption, chemical precipitation, and biological removal, have been used to remove phosphorus from WW (Ramasahayam et al., 2014). Among these, enhanced biological phosphorus removal (EBPR) processes are highlighted for being more economically and environmentally feasible when compared to physical-chemical processes (Bassin et al., 2012b).

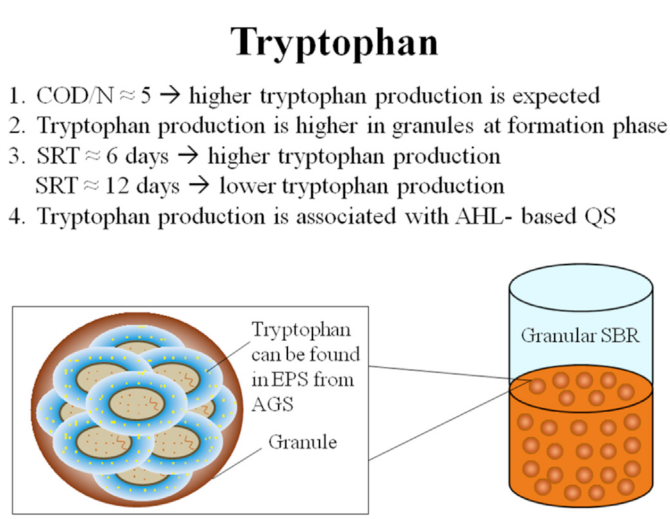

Fig. 4. Tryptophan recovery in AGS systems.

5.1. Phosphorus removal by aerobic granular sludge

In AGS, phosphorus is removed by EBPR, which is carried out by PAOs, in addition to being accumulated in EPS (Huang et al., 2015). Examples of PAOs are *Candidatus Accumulibacter Phosphatis*, *Candidatus Halomonas Phosphatis*, *Pseudomonas putida*, *Aeromonas* spp., and the genus *Rhodocyclus* (Yang et al., 2017). Usually, these organisms use volatile fatty acids (VFAs) as substrate. They are enriched in AGS systems by adopting a long period of anaerobic feeding, followed by an aerobic or anoxic period (Saito et al., 2004).

In the anaerobic period, PAOs absorb the soluble organic matter to synthesize PHAs using the energy from polyphosphate (poly-P) hydrolysis (Flowers et al., 2009). Under aerobic or anoxic conditions, PHAs will be oxidized to produce energy, part of which will be used to synthesize poly-P and store them as cellular inclusions (Wang et al., 2020). Thus, the removal of phosphorus from the system is carried out through sludge discharge, opening up the possibility of recovering this nutrient from the discarded biomass (Huang et al., 2015).

The configuration of the SBR cycle will serve not only to enable the metabolism of PAOs but also to disfavor ordinary heterotrophic organisms (OHO). When the sludge is subjected to anaerobic feeding, the conversion of simple organic matter into storage polymers (PHAs) by the accumulating organisms will decrease the amount of substrate available during the subsequent aerobic phase, decreasing the growth rate of OHO (Pronk et al., 2015). However, under these conditions, the group of glycogen-accumulating organisms (GAOs), which perform carbon conversions similar to PAOs, will also be favored (Kong et al., 2001). GAOs compete directly for the substrate with PAOs. However, they cannot remove phosphorus, accumulating glycogen instead of polyphosphate, which makes them undesirable when the objective is phosphorus removal (Bassin et al., 2012b).

5.2. Operational parameters associated with EBPR in AGS systems

Several reviews on AGS cover the topic of EBPR (see Table 1). Therefore, the operational parameters that affect this process have already been properly summarized elsewhere, especially for activated sludge systems. However, in order to compare phosphorus removal strategies to the strategies for ALE, tryptophan, and PHAs production by AGS systems, operational parameters associated with EBPR in AGS are once more summarized from sections 5.2.1 to 5.2.11. Hopefully, some new information is also presented.

5.2.1. pH

pH is a very important parameter in biological systems, for affecting microbial metabolism, competition between microorganisms for substrate, toxicity of compounds, amongst others. For instance, in EBPR systems investigated with the AGS technology, chemical profiles were consistent with shifts in populations from PAOs to GAOs when pH decreased from 7.5 to 7.0 and then to 6.5. However, the microbial community composition was not remarkably affected, which remained dominated by *Accumulibacter* PAOs (Ahn et al., 2009). These results seem to indicate that, similarly to AS-based EBPR systems, a pH close to 8 would favor PAOs over GAOs in AGS-based EBPR systems (Oehmen et al., 2005). Even though pH 7.5 has been associated with granule breakdown (Ahn et al., 2009), cultivation of granules with a pH between 7.8 and 8 has been achieved (Lashkarizadeh et al., 2016).

Phosphorus removal is also maintained when AGS is subjected to a shock of acid loads but is lost when these loads are basic, as demonstrated by Lashkarizadeh et al. (2016). The authors subjected mature granules to shocks of acidic (pH 6) and basic (pH 9) loads. In the first case, phosphorus removal dropped from 98% to zero, but it was reestablished after 5 days, reaching 99%. In the second case, in addition to the breakage of granules, which produced a flocculent sludge, the efficiency of phosphorus removal dropped from 98% to 50%, without showing any recovery during the experiment time.

5.2.2. Temperature

Temperature is also a key factor in selecting PAOs over GAOs (Weissbrodt et al., 2013). A report in an AS system has shown that below 20 °C *Accumulibacter* PAOs are favored over *Competibacter* GAOs due to the decrease in acetate uptake. In comparison, at 10 °C, glycogen's anaerobic conversion becomes the limiting metabolic process for GAOs (Lopez-Vazquez et al., 2009). In AGS systems, a temperature increase from 20 °C to 30 °C also severely and negatively affected the growth and activity of PAOs (Bassin et al., 2012a).

5.2.3. Salinity

When AGS was fed with alternating streams of demineralized water and a solution simulating seawater (NaCl = 28 g L^{-1}), *Candidatus Accumulibacter* PAOs showed a remarkable adaptation to the osmotic shocks, demonstrating a stable aerobic removal rate of 3.1 mg P·g $VSS^{-1}\cdot h^{-1}$ and phosphate release of 59.5 mg P·L^{-1} (de Graaff et al., 2020). However, under different constant saline conditions (0, 11, 22, and 33 g NaCl·L^{-1}), phosphorus release and uptake by aerobic granules were affected by salinity increase (Bassin et al., 2011). When salinity reached 22 g NaCl·L^{-1}, P uptake ratio decreased, whereas with 33 g NaCl·L^{-1}, no P release or uptake was observed, and the PAO microorganism *Candidatus* Accumulibacter phosphatis was no longer detected (Bassin et al., 2011). These findings indicate that maintaining a low salinity content (around 1%) is better for EBPR in AGS systems. Similarly, in AS-based EBPR systems, a low salinity content (up to 0.6% NaCl) is reported to favor PAOs while in concentrations above 0.6% NaCl GAOs are likely to prevail (Welles et al., 2014).

5.2.4. Carbon source

Among the various carbon sources, VFAs, especially acetate and propionate, are preferable in AS-based EBPR systems because they exist widely in WW and are necessary to produce PHAs (Shen and Zhou, 2016). Between these two, propionate is generally more advantageous to cultivate PAOs (Oehmen et al., 2004). When more complex substrates are used, such as sucrose, phosphorus removal tends to be negatively affected, as PAOs will only consume them after being fermented and converted to VFAs. Because of this, such substrates will end up being totally or partially consumed for nitrification, decreasing the carbon availability for PAOs (Guerrero et al., 2011).

In AGS-based EBPR systems, results are similar. At long-term low temperature (10 °C), phosphate removal was slightly higher (98.2%) when granules were fed with propionate rather than acetate (94.2%) (Wang et al., 2020). Another report showed that a mixture of glucose and acetate produced larger and more stable granules, with higher P removal capability and higher anaerobic P release than the mixture of glucose and propionate (Cai et al., 2016). In turn, granules formed with a mixture of glucose and butyrate had a stronger structure and higher P removal capacity than those cultivated with a mixture of glucose and valerate (Cai et al., 2019). Removal of total phosphorus was also higher when granules were cultivated with acetate (42%), compared to ethanol (31%) and glucose (21%) (Rollemberg et al., 2019).

5.2.5. Carbon to phosphorus ratio

In an AGS system cultivated with acetate, fractions of PAOs and GAOs were 70% and 6% under COD/P of 15, respectively, but reached 10% and 22% under COD/P of 100, respectively (Muszyński and Miłobędzka, 2015). In the same study, the ratio between phosphorus release and dissolved organic carbon uptake (P_{rel}/DOC_{upt}) during the anaerobic/anoxic period was also drastically lower (0.01 mol mol^{-1}) in the sludge grown with COD/P of 100, when compared to the value observed (0.45 mol mol^{-1}) with COD/P of 15.

5.2.6. Free ammonium

Free ammonia rather than ionic ammonium has been commonly thought to have a broad inhibitory effect on bacterial metabolism, including ammonia and nitrite oxidizing bacteria, and PAOs (Valdivelu et al., 2006, 2007). A study has shown that high concentrations of free ammonia could adversely affect AGS's settleability, morphology, and phosphorus removal capacity (Zheng et al., 2013a). The authors operated four reactors during 51 days, with R1 maintained under a fixed initial concentration of ionic ammonium (15 mg NH_4^+-N ·L^{-1}), while the others were subjected to different concentrations, reaching up to 200 (R2), 400 (R3) and 600 (R4) mg NH_4^+-N ·L^{-1}. The calculated free ammonia was 8.88, 17.76 and 26.64 mg N ·L^{-1} for reactors R2, R3 and R4, respectively. Under these conditions, the levels of PAOs in the sludge were 84.3% (R1), 67.6% (R2), 30.8% (R3), and 18.4% (R4), while the levels of GAOs were 11.0% (R1), 14.7% (R2), 34.2% (R3), and 14.5% (R4). Therefore, high concentrations of ammonium are harmful to PAOs and beneficial to GAOs. However, when very high values of this compound are reached, like in R4, both groups are suppressed.

5.2.7. Nitrite

It has been reported nitrite concentration lower than 5 mg N—NO_2^-·L^{-1} allowed phosphate uptake in AGS systems while higher concentrations inhibited the process (Coma et al., 2012). Furthermore, when aerobic granules were exposed to 10, 20 and 30 mg NO_2^-·L^{-1} (equivalent to 3, 6 and 9 mg N—NO_2^-·L^{-1}), phosphorus uptake and release rates were significantly diminished, and phosphorus removal rate decreased from 100% to 0—50%, even when the P concentrations were decreased by half (Zheng et al., 2013b). Before the nitrite addition, average PAOs and GAOs abundances were 80.3% and 11.0%, respectively, and after the initial addition of nitrite, abundances dropped to around 30% and 20%, respectively. Original proportions were recovered after nitrite dosage ceased. It is also important to mention that the nitrite added was almost entirely denitrified during the anaerobic feeding phase, indicating a competition between denitrifying heterotrophs PAOs and GAOs for the carbon source.

5.2.8. Nitrate

A fraction of PAOs, the so-called denitrifying phosphorus-accumulating organisms (DPAOs), can perform denitrification (Rollemberg et al., 2018). These organisms can be divided into two groups: group IA gathers DPAOs with the ability to couple nitrate reduction with phosphorus uptake, while group IIA gathers DPAOs that only use nitrite as an electron acceptor in addition to oxygen (Flowers et al., 2009). Thus, when the nitrate concentration is higher than the denitrifying capacity of ordinary heterotrophic denitrifying organisms (DOHO), there will be excess nitrate for DPAOs, which can use it to sustain a fast growth (He et al., 2010; Zheng et al., 2014). Therefore, nitrate may interfere with P bioaccumulation.

5.2.9. Trace metals

High Cr(VI) concentrations (≥0.5 mg L^{-1}) were shown to significantly inhibit P removal in AGS-based EBPR systems, while this phenomenon was not found at lower concentrations (≤0.4 mg L^{-1}) (Fang et al., 2015). Furthermore, the long-term exposure to Cr(VI) concentration between 0.3 and 0.8 mg L^{-1} promoted irreversible effects on granules' ability to remove phosphorus, as P removal efficiency could not be restored even after 16 days without Cr(VI) loading (Fang et al., 2015).

Iron interferes positively in phosphorus removal efficiency in AGS systems, being beneficial both in its cationic (Fe^{2+}) and zerovalent forms. Continuos dosage of 5 mg $Fe^{2+} \cdot L^{-1}$ promoted the early formation of granules and a stable P removal of 92% (Cai et al., 2018). Besides, supplementing AGS systems with 10 mg L^{-1} of nano-scale zerovalent iron promoted a small decrease of phosphate concentration in the effluent (Eljamal et al., 2020). These effects can be attributed to iron's capacity to adsorb phosphorus from the environment and to promote the development of PAOs (Cai et al., 2018; Eljamal et al., 2020).

5.2.10. Pharmaceuticals

Pharmaceuticals affect the biological phosphorus removal depending on the concentration, type, and combination of drugs, as well as the biological process itself (Katsou et al., 2016). In AGS-based EBPR systems spiked with 2 μg L^{-1} of sulfamethoxazole (SMX) for three months, the phosphorus removal was not affected and remained around 87% (Kang et al., 2018). In similar granular systems, the removal efficiency of phosphate was over 99% during the experiment with no noticeable disturbances when 500 μg L^{-1} of 17α-ethinylestradiol (EE2), 4-nonylphenol (NP), and carbamazepine (CBZ) were added to the influent (Kent and Tay, 2019). However, the anaerobic phosphorus release decreased slightly after CBZ was added to the feed, although regardless of the contaminants present, once the aeration phase of the cycle was underway, the ortho-phosphate was taken up by PAOs, leading to excellent phosphate removal. Another study showed that a dosage of nine chiral pharmaceuticals (alprenolol, bisoprolol, metoprolol, propranolol, venlafaxine, salbutamol, fluoxetine, and norfluoxetine), each at 1.3 μg L^{-1}, in an AGS system operated with anaerobic feeding phase, resulted in a P-release decrease during the anaerobic period, even though phosphate content at the effluent remained almost the same as before the spike of drugs (Amorim et al., 2014).

5.2.11. Sludge discharge

Two sludge discharge strategies (one designed to keep the fastest settling granules, and the other, the granules with intermediate velocity) provided different effects over phosphorus removal in AGS systems (Henriet et al., 2016). The first strategy decreased phosphorus removal, while the second one rapidly increased the efficiency, which stabilized above 90%. Selective removal of GAOs, attained through discharging the top of the AGS bed, also generated 100% phosphorus removal (Winkler et al., 2011).

5.3. Other phosphorus removal pathways in AGS systems

In addition to the phosphorus bioaccumulation within cells, a fraction of phosphorus is adsorbed by the EPS produced by the aerobic granules. For instance, the phosphorus present in EPS represented around 45.4% of the total AGS′ phosphorus in the anaerobic period, and 30% in the aerobic period (Wang et al., 2014a,b). Another study showed that microbial cells, EPS, and mineral precipitates contributed about 73.7%, 17.6%, and 5.3–6.4% to the total P of EBPR–AGS, respectively (Huang et al., 2015). They found that the inorganic P species were orthophosphate, pyrophosphate, and polyphosphate (polyphosphate being the major P species in the AGS, cells, and EPS). The organic P (OP) species identified were monoester and diester phosphates. In the core of the granule, hydroxyapatite ($Ca_5(PO_4)_3OH$) and calcium phosphate ($Ca_2(PO_4)_3$) were the dominant P minerals accumulated, and cells were mainly in the outer layer of AGS. At the same time, EPS were distributed in the whole granules (Huang et al., 2015). Furthermore, the precipitation of struvite in AGS can be induced biologically through the production of alginate, as discussed in a previous section (Lin et al., 2012). A summary of the current knowledge on the operational parameters for P recovery in AGS systems is presented in Fig. 5.

PAOs: Polyphosphate-accumulating organisms; GAOs: Glycogen-accumulating organisms; VFA: volatile fatty acids; COD: Chemical Oxygen Demand; P: Phosphorus. Key findings were based on the following studies: (1) Luo et al. (2014), Rollemberg (2020), Yu et al. (2020) and Zhang et al. (2018a); (2) Ahn et al. (2009), and Lashkarizadeh et al. (2016); (3) Bassin et al. (2012a); (4) Bassin et al. (2011); (5) Wang et al. (2020); (6) Muszyński and Miłobędzka (2015); (7) Zheng et al. (2013a); (8) Zheng et al. (2013b); (9) Fang et al. (2015); (10) Cai et al. (2018); (11) Amorim et al. (2014), Kang et al. (2018) and Kent and Tay (2019); (12) Winkler et al. (2011).

5.4. Alternatives for phosphorus recovery from AGS systems

Regarding the ways to utilize the phosphorus accumulated by AGS, the main alternatives are: 1) direct soil application of biomass, 2) biological release followed by chemical recovery, and 3) chemical release/chemical/thermochemical recovery. In the last two cases, biophosphates must be solubilized before recovery as inorganic compounds (Yang et al., 2017).

The direct application of dehydrated sludge, which has a moisture content between 75 and 88% (considering a normal filter or centrifuge solids product), to the soil, is a method that can be effective for soil fertilization as the use of mineral fertilizers can be carried out over a wide range of pH and soil buffers concentrations (Kahiluoto et al., 2015; Yuan et al., 2012). As chemical and biological contaminants can be transferred to the environment, the distribution of dry sludge onto the soil must be based not only on nutrient concentration but also on its contaminant content (Yuan et al., 2012). Other problems associated with this practice are the difficulty and high costs of transporting the large volume of sludge (Melia et al., 2017).

In the biological release method followed by chemical recovery, the sludge is initially submitted to an anaerobic digestion. During this process, the phosphorus accumulated in the form of poly-P inclusions and present in the biomass cell structure will be released into the liquid medium, forming an appropriate liquor for

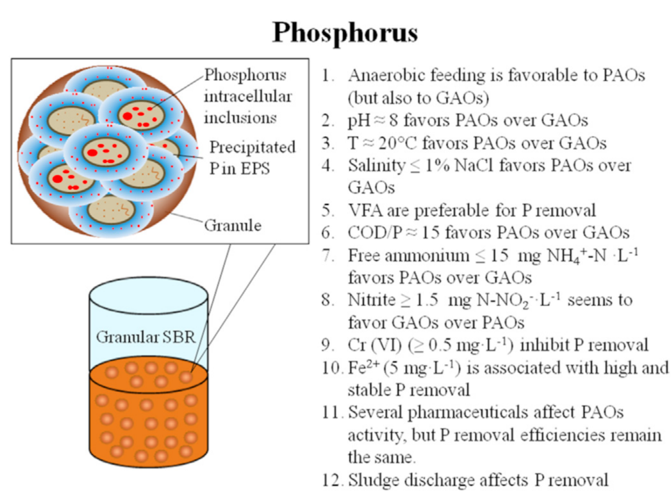

Fig. 5. Phosphorus removal/recovery in AGS systems.

phosphorus recovery through crystallization (Pastor et al., 2008). The crystallization can be carried out by co-precipitation with magnesium and ammonia to produce struvite, or co-precipitation with calcium or potassium (Yang et al., 2017). Since struvite has a slow nutrient releasing rate to the soil (Rahman et al., 2014), its application is more economically rational when the soil is rich in biota, which can process less accessible forms of P (Maroušek et al., 2019).

Lastly, a good example of the thermo-chemical recovery of phosphorus is the incineration of sludge, which generates P-rich ash with the potential for struvite recovery (Kataki et al., 2016). It is important to note that the direct application of ash to the soil is not recommended, as it may contain substances that are harmful to soil quality and the health of cultivated vegetables, such as Ca, Mg, Al, Fe, trace elements, among others, which may demand a previous removal before ashes application (Hong et al., 2005; Melia et al., 2017).

5.4.1. Feasibility of phosphorus recovery from AGS

There is evidence that the difference between the biomass morphology of AS and AGS has a substantial effect on phosphorus release, and therefore on phosphorus recovery. It has been reported that, despite the similar content of biological phosphorus in both types of biomass (AS: 3.7% of dry biomass; AGS: 2.9% of dry biomass), the efficiency of orthophosphate's release after anaerobic digestion of sludge was significantly higher for AS than for AGS (AS: 51.3–56.1%, AGS: 32.8–37.5%). (Cydzik-Kwiatkowska and Nosek, 2020). Due to the compact and multi-layered structure of granules, the phosphorus precipitated in their core are hardly ever released without their disintegration, a process that seems not to occur properly during anaerobic digestion, as cell lysis is less visible for AGS than for AS (Cydzik-Kwiatkowska and Nosek, 2020).

However, it is still unclear to what extent these results were affected by the uncontrolled pH since AGS fermentation has been reported to be way more efficient under acidic conditions (pH 4: 81% of TP released after 6 days; pH 10: 14% of TP released after 6 days; uncontrolled pH: 17% of TP released after 6 days) (Zou et al., 2018). Nevertheless, in a recently modeled configuration for P recovery of an operating AGS WWTP, even more conservative values for phosphorus release were adopted than the ones previously reported. The authors assumed that, after anaerobic digestion, 80–90% of the loaded P would continue in the digestate, remaining unavailable for the subsequent struvite precipitation (Kehrein et al., 2020). Therefore, the estimated fraction of influent P recovered as struvite (9–10%) would be much smaller than the P fraction recovered as ash (65–68%) through sludge incineration.

Furthermore, a new assessment framework to identify potential roadblocks/hurdles during the commercialization of recovered phosphorus was recently proposed (B. Li et al., 2019). This analysis has shown that only struvite crystallization and composting are ready for commercialization, while other technologies require significant investment in research and development. In addition, another feasibility analysis determined to what cost struvite could be supplied from The Netherlands to the West African ports of Dakar, Abidjan, and Lomé (Vries et al., 2017). The authors assumed that struvite had an estimated price of around € 55 per tonne and could potentially replace two mineral P fertilizers: Di Ammonium Phosphate (DAE) and Phosphate rock (PR). The comparison of prices was made considering the percentage of P_2O_5 of each fertilizer. DAE prices were also decreased according to its extra nitrogen content when compared to struvite. Struvite costs were estimated between US$ 520–540 per tonne of P_2O_5, while corrected DAE prices were around US$ 600 per tonne of P_2O_5 in 2016, and PR prices were close to US$ 350 per tonne of P_2O_5 in the same year.

6. Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) are a family of biopolymers that have similar properties to plastics. The most common PHAs are the homopolymer poly-3-hydroxybutyrate (PHB) and copolymers of 3-hydroxybutyrate (HB) with 3-hydroxyvalerate (HV) (Puyol et al., 2017). According to Możejko-Ciesielska and Kiewisz (2016), PHAs can be used to produce packaging, paints, ultra-strong fibers, insecticide and herbicide carriers, and moisture-containing layers in sanitary products (diapers, absorbents etc.). However, the authors point out that its most promising application is in biomedicine, especially in the case of PHB and of poly (HB-co-HV), which have been considered for the manufacture of cardiovascular products and drug carriers, in addition to its potential use for injuries treatment and orthopedics field.

Kumar et al. (2020) report that the industrial production of PHAs can be based on plants or bacteria's activity, the second being the least costly alternative. The authors explain that when there is an excess of carbon in the medium and a limitation of nitrogen, phosphorus, and oxygen, the bacteria will accumulate carbon as PHA, i.e., a form of energy reserve for starvation periods. In general, manufacturers feed the pure bacterial cultures with sugars (glucose and sucrose) or VFAs, generating PHA yields of at least 60% in cell dry weight (CDW), with some that report percentages superior to 90% (Możejko-Ciesielska and Kiewisz, 2016). The annual production of individual companies varies on a wide range, between 50 and 50 thousand tonnes of PHA, in which the market price varies from 1.50 to 5 € per kg of PHA (Kumar et al., 2020).

6.1. PHAs production by mixed microbial cultures

The disadvantages observed in the production of PHAs by pure cultures, such as the need to maintain sterile conditions and to use synthetic substrates, led to the development of an alternative in three steps using mixed microbial cultures (MMC) (Dionisi et al., 2004). Traditionally, the first part is the production of a liquor rich in VFAs through sludge fermentation (Step I). This liquor will serve as feed for an SBR inoculated with activated sludge (AS) (Step II), whose cycle is usually composed of feeding, aeration, and withdrawal. In this reactor, the PHA-accumulating biomass will be selected by creating periods with carbon availability (feast) followed by long periods with no carbon (famine). Finally, the Step III is to stimulate the enriched biomass to accumulate the most considerable amount of PHA possible, which is done by maintaining a saturated carbon medium, a regime called feed-on-demand. Then, the enriched sludge goes on to PHA extraction and purification. The common AS can have a PHA accumulation potential (PAP) as low as 9% (g PHA·g sludge dry weight^{-1}) (Lee et al., 2015). Therefore, the most important phase of PHA production by mixed cultures is the selection of biomass able to store a large amount of these polymers.

Researchers have identified several operational parameters with influence over the production of PHAs by MMC. Firstly, the ratio between the feast period and the total cycle should not exceed 20% (Dionisi et al., 2007). In order to maintain such a proportion, the OLR should not be too high (Dionisi et al., 2006). Some authors also suggest that the best parameter to ensure that the biomass develops a high PAP is the absence of PHA at the end of cycles (Step II, biomass accumulation reactor previously described) because when PHA is present, the PHA yields (Step III, PHA production reactor previously described) are lowered even though the optimal feast/cycle ratio is maintained (Pittmann and Steinmetz, 2014).

There is evidence that the pH on biomass accumulation reactor should be kept between 7 and 8 (Pittmann and Steinmetz, 2014), while in the PHA production reactor, pH closer to neutral should be maintained (Lee et al., 2015). Increasing temperature from 15 °C to 30 °C, the biological metabolism will be higher, making the feast phase shorter, the famine phase longer, and likely the PHA yields will be higher (Johnson et al., 2010b). However, lower PHA yields at 30 °C than at 15 °C has also been reported (Pittmann and Steinmetz, 2014).

In addition, the carbon source will have a significant influence on PHA composition. While acetic acid is associated with the exclusive production of 3HB, propionic acid enables the production of both monomers 3HB and 3HV, whose proportion would be determined by the ratio of the acids present in the feed (Janarthanan et al., 2016). Whether carbon or nitrogen is limiting and to what extent this limitation occurs is also an important aspect to be controlled. When the ratio of carbon uptake to nitrogen uptake is lower than 33 C mol·N mol^{-1}, there is a high nitrogen limitation, which may affect the production of PHAs. In contrast, ratios greater than 48 C mol·N mol^{-1} indicate carbon limitation and are generally beneficial (Johnson et al., 2010a).

Furthermore, in the vast majority of studies that use activated sludge for the production of PHAs, nitrification is inhibited by the addition of thiourea or allylthiourea to prevent competition for oxygen between the PHA-accumulating heterotrophic organisms and the nitrifying autotrophic organisms (Wang et al., 2019). However, recently, some researchers have selected PHA-accumulating organisms while simultaneously removed nitrogen from the inlet stream (Basset et al., 2016).

6.2. The PHARIO process

Currently, the PHARIO process is a promising alternative to adapt existing WWTP for PHAs recovery from activated sludge in a scalable and controllable way. This concept assumes that most of the sewage treatment plants are capable of producing biomass with high PHA accumulation potential (PAP), sometimes requiring small modifications (PHARIO, 2020). Therefore, the accumulation SBR from the traditional three steps MMC PHA production is dispensed. The fermenter for VFA production is then fed with primary sludge from the WWTP, preferentially combined with organic waste. The accumulation reactor is fed with the surplus activated sludge, producing PHA-rich biomass, which is acidified to conserve the PHA and then dewatered in centrifuges or belt filter press. The dewatered PHA-rich biomass is dried in a thermal dryer to a dry matter content of at least 90%. PHA is then extracted using solvents like butanol. Through solvent cooling, the PHA can be recovered and the solvent reused. The residual matter not recovered is incinerated similarly as the original sludge would have been (Bengsston et al., 2017).

The applicability of the process was determined by an evaluation of activated sludge samples from 15 wastewater treatment plants (WWTP) in The Netherlands (Bengsston et al., 2017). The report showed that four of them (27%) had a PHA accumulation potential (PAP) of at least 0.38% g PHA·g VSS^{-1}, whereas, generally, a PAP above 0.40% g PHA·g VSS^{-1} is considered a threshold for the economic viability of integrating PHA production with the WWTP. The results showed no need for special conditions concerning SRT, loading rates, or temperature to achieve a high PAP on existing WWTP. The enrichment is also feasible with different types and concentrations of wastewater, although sometimes it is not occurring. The crucial factor is the existence of a contact zone between the readily biodegradable COD (RbCOD) and the sludge, which ensures that some of the biomass periodically experiences a relatively high concentration of RbCOD. Process modifications that are relatively minor and simple can create such feast-stimulating zones. Similarly, in WWTP with the UCT/MUCT process, a well-defined contact zone in the initial part of the anoxic volume may be suggested to generate a stronger selective pressure for high PAP. With these modifications, it was estimated that most of the plants could potentially supply biomass for a PHA production between 1000 and 6000 tonnes per year. Some feedstocks from the region could provide the necessary VFA, decreasing the production costs. Considering all the 15 WWTP, the total potential is in the order of 25,000 tonnes of PHA per year.

6.3. PHA production by aerobic granular sludge

AGS has also been investigated as a viable biomass for PHAs production. The main advantage of granular systems is the high biomass retention that can be achieved in the SBR, which increases PHA productivity if the same percentages of PHA accumulation of activated sludge are maintained (Wang and Yu, 2006). The literature regarding this PHA production alternative from AGS is still scarce when compared to that dealing with activated sludge (Fang et al., 2009; Gobi and Vadivelu, 2015a; 2014; Karakas et al., 2020; Qin et al., 2005; Vjayan and Vadivelu, 2017; Wang et al., 2014a,b; Wang and Yu, 2006; Wosman et al., 2016). The production generally does not include fermenters or accumulation reactors. The operational cycles of the SBRs are generally shorter, include a sedimentation step and may include an anaerobic step when EBPR is targeted. Nitrification inhibition is not performed since there is evidence that nitrifying organisms help to maintain the AGS stability (Liu et al., 2004).

6.4. Operational parameters associated with PHAs production in AGS systems

Concerning the AGS technology, there are still few published studies on the impact of operating conditions on PHAs biosynthesis (Table 3), which is presented in Sections 6.4.1 to 6.4.5.

6.4.1. Organic loading rate

Unlike AS, aerobic granules appear to withstand higher OLR not only without losing their ability to accumulate PHA but also increasing its production. For instance, Gobi and Vadivelu (2015a,b) assessed the effect of different OLRs (0.91, 1.82, 2.73 and 3.64 kg COD·m^{-3}·d^{-1}) on PHA production, using Palm Oil Mill Effluent (POME) as substrate. They verified that the PHA yields increased from 66% to 87% g PHA·g CDW^{-1}, for the ORLs of 0.91 and 3.64 kg COD·m^{-3}·d^{-1}, respectively. However, AS submitted to 1200 mg VFA·L^{-1} and 2000 mg VFA·L^{-1}, which were equivalent to OLRs of 0.6 and 1 kg VFA·m $^{-3}$·d^{-1}, had PHA yields in the accumulation reactor of 25% and 1.8% g PHA·g CDW^{-1}, respectively. This behavior is likely associated with substrate diffusion resistance observed in AGS. Because of the protection created by the AGS structure, carbon concentration within these clusters should be lower than the one in the mixed liquor, which depends on the OLR applied.

6.4.2. Carbon to nitrogen ratio

Regarding the proportion of carbon and nutrients in the feed, nitrogen limitation, if extreme, will also be detrimental to the retention of biomass in granular systems, similar to what is observed in the case of activated sludge. In AGS systems, it has been reported that an increase in COD/N ratio up to 60 was able to stimulate PHB production up to 25% mg PHB·mg $MLVSS^{-1}$ (Fang et al., 2009). However, when COD/N reached 90, despite the higher PHB yields (46% mg PHB·mg $MLVSS^{-1}$), filamentous organisms' growth quickly caused biomass loss, undermining the main advantage of granular sludge over activated sludge, in other words, its greater biomass retention.

Other researchers were able to produce stable aerobic granules

Table 3
Operation parameters and PHA yields at various carbon sources for granular sludge systems.

Carbon source	Seed Sludge	Nitrification Inhibition	Cycle	HRT	Feast/ Cycle or Feast/ Aeration	Removal efficiency	Aeration Rate	PHA yields	PHA composition	Reference
Ethanol	Activated sludge	None	Cycle: 6 h Feed: 5 min Aerobic reaction: 230 min Anaerobic reaction: 119 min Settling: 2 min Draw: 4 min	12 h	–	>95% COD Nitrogen removal was close to 100% when ethanol was supplied in the anaerobic phase	Air during aerobic reaction: 4 L min^{-1} Nitrogen gas during anaerobic reaction: 1L·min^{-1}	15–20% g PHA·g CDW^{-1}	–	Qin et al. (2005)
Sodium Acetate	Activated sludge	None	Cycle: 6 h Start aeration: 5 min Feed: 4 min Aeration: 333 –347min Settling: 15, 10, 5 and 1 min Draw: 3 min	12 h	Feast/ Cycle: 0.17	>90% COD (Stage II)	3.3 L min^{-1}	40 ± 4.6% g PHB·g $MLVSS^{-1}$ (Stage II)	–	Wang and Yu (2006)
Sodium Acetate	Anaerobic granular sludge from UASB treating citrate-producing wastewater	None	Cycle: 6 h Feed: 5 min Aeration: 330 min Settling: 2 min (5min after day 60) Draw: 3 min Idle: 20 min	12 h	–	>90% COD through almost the entire experiment	–	Unstable performance with a maximum value of 45% g PHB·g $MLVSS^{-1}$	–	Fang et al. (2009)
Palm oil mill effluent (POME)	AS from an aerobic pond of a POME treatment plant	None	Cycle: 6 h Feed: 20 min Aeration: 330 min Settling: 1.5 min Draw: 8.5 min	24 h	Feast/ Aeration: 0.15 –0.36	90% COD	3 L min^{-1}	68.3% g PHA·g CDW^{-1}	PHB-co-PHV, with 55% HB and 45% HV	Gobi and Vadivelu (2014)
Palm oil mill effluent (POME) OLR of 0.91, 1.82, 2.73 and 3.64 kg COD·m^{-3}·d^{-1} depending on the reactor	Aerobic granular sludge	None	24 h cycle Phases duration were not informed	4 d	–	–	1, 2, 3 and 4 L min^{-1} Air velocity: 0.4; 0.9; 1.3; 1.7 cm s^{-1}	87% g PHA·g CDW^{-1} for OLR of 3.64 kg COD·m^{-3}·d^{-1} and AR of 2 L min^{-1}	PHB-co-PHV 80% HB and 20% HV for all the OLRs studied with AR of 2 L/min HB 89-69% e HV 11–31% for all the ARs studied with OLR of 1.82 kg COD·m^{-3}·d^{-1}	Gobi and Vadivelu (2015a)
Palm oil mill effluent (POME)	Aerobic granular sludge	None	Cycle: 8 h Aeration: R1: 8 h of 2 L min^{-1} R2: 2 h of 2 L min^{-1} followed by 6 h of 1 L min^{-1} R3: 2 h of 2 L min^{-1} followed by 6 h of 0.5 L min^{-1} R4: only 2 h of 2 L min^{-1}	0.67 days	Feast/ Cycle: R1: 0.25 R2: 0.19 R3: 0.13 R4: 0.06	>90% COD	0.5, 1 and 2 L min^{-1} Air velocity: 0.3, 0.5 and 1 cm s^{-1}	R1: 56% g PHA·g CDW^{-1} R2, R3 and R4: 64–66% g PHA·g CDW^{-1}	P3 (HB-co-HV) 65–79% HB and 35-21% HV	Vjayan and Vadivelu (2017)

(continued on next page)

Table 3 (*continued*)

Carbon source	Seed Sludge	Nitrification Inhibition	Cycle	HRT	Feast/ Cycle or Feast/ Aeration	Removal efficiency	Aeration Rate	PHA yields	PHA composition	Reference
Phenol	Activated sludge	None	Cycle: 4 h Feed: 10 min Aeration: 197 −225 min Settling: 30-2 min Draw: 3 min (Days 28 −90)	8 h	–	>99% Phenol Around 95% COD	5 L min^{-1} Air velocity 3 cm s^{-1}	R2 - above 50% g PHA·g MLSS^{-1} from days 26–64, being above 70% on day 26[a]	–	Wosman et al. (2016)
Raw wastewater (R1) or wastewater without settable solids (R2)	-	None	60 min anaerobic feeding followed by almost 300min of aeration	-	-	89 ± 5% COD and 70 ± 16% TN for both reactors R1: 75 ± 13% TP R2: 78 ± 20% TP	–	R1: 10.8% g PHA·g CDW^{-1} on day 436 R2: 9.3% g PHA·g CDW^{-1} on day 449[a]	-	Karakas et al. (2020)

HRT: Hydraulic retention time; PHA: Polyhydroxyalkanoate; COD: Chemical oxygen demand; PHB: Polyhydroxybutyrate PHV: Polyhydroxyvalerate; HB: Hidroxybutyrate; HV: Hydroxyvalerate; CDW: Cell dry weight; MLVSS: Mixed liquor volatile suspended solids; TP: Total phosphorus; TN: Total nitrogen.

[a] The PHA was extracted at the end of the cycle.

with a COD/N ratio of 72 (Wang and Yu, 2006). To accomplish this, two stages of operation were necessary: stage I (first 70 days) for the selection of PHB-accumulating biomass and stage II (day 71–155) for granule formation. In stage I, three strategies were used to increase the PHB accumulation capacity: the decrease in aeration rate (from 0.2 to 0.04 m^3 h^{-1}), which was reversed due to the loss of biomass, followed by an increase in the inlet COD from 800 to 1600 mg L^{-1}, and finally, a decrease in ammonia concentration so that the COD/N ratio was increased from 48 to 72. All subsequent strategies caused an increase in PHB yields, reaching 43.1 ± 2.0% g PHB·g MLVSS^{-1} on day 70. Only then granulation was achieved by gradually reducing the settling time to 1 min. PHB accumulation was stable during the second stage, with an average of 40 ± 4.6% g PHB·g MLVSS^{-1}. In summary, an increase of COD/N up to 70 in granular systems seems advantageous for PHA production, with significant loss of stability when COD/N reaches 90.

6.4.3. Granule size

Another important aspect of being considered in PHB production by AGS is the granule size, which may cause substrate diffusion limitation. SBRs inoculated with bigger granules (R1: 0.35–0.5 mm; R4: >2 mm) presented lower PHA yields (R1: 68% g PHA·g CDW^{-1}; R4: 60% g PHA·g CDW^{-1}) since the scarcity of substrate inside the granules would prevent the bacteria located in that region to produce PHA (Gobi and Vadivelu, 2015a). In contrast, another study found that an increase in granule size (R1: 1030 μm; R4: 1300 μm) and density, which was expressed in terms of Sludge Volume Index - SVI (R1: 41 ± 4 mL g^{-1}; R2: 37 ± 3 mL g^{-1}; R3: 26 ± 3 mL g^{-1}; R4: 20 ± 2 mL g^{-1}), increased PHA yields (R1: 56% g PHA·g CDW^{-1}; R2, R3, and R4: 64–66% g PHA·g CDW^{-1}) and decreased the time required to reach the peak of PHA during the cycle (R1: 2 h; R2: 1.5; R3: 1.0; R4: 0.5 h) (Vjayan and Vadivelu, 2017). This behavior was directly associated with the aeration rates (R1: 2 L min^{-1} for 8 h; R2: 2 L min^{-1} for 2 h and 1 L min^{-1} for 6 h; R3: 2 L min^{-1} for 2 h and 0.5 L min^{-1} for 6 h; R4: only 2 L min^{-1} for 2 h), which seemed to affect the spatial distribution of microbial communities within the granules. The PHA-accumulating organisms were located in the granules' outermost layer, while in the innermost layer, PHA accumulation was not observed. Therefore, the lower the aeration rate, the thinner the layer of PHA-accumulating organisms, which facilitated their access to the substrate, allowing the acceleration of PHA accumulation.

6.4.4. Aeration rate

It has been reported that a higher aeration rate (1, 2, 3 and 4 L min^{-1}) lowered the time required to reach the peak of PHA formation (2 h for aeration rates of 1 and 2 L min^{-1} and 1 h for aeration rates of 3 and 4 L min^{-1}), but did not affect the PHA yields (75% g PHA·g CDW^{-1} for all reactors) (Gobi and Vadivelu, 2015a). However, another study showed that when the aeration rate is decreased only after the famine phase ends, not only the time to reach the peak is decreased, but PHA yields are increased (Vjayan and Vadivelu, 2017).

This can be explained by the fact that, during the feast period, a lower concentration of dissolved oxygen (DO) is limiting to PHA production, but if the decrease in DO occurs only at the end of the cycle, the organisms are stimulated to accumulate PHB more quickly in order to start consuming it in the short period after the COD drops and before the decrease in aeration rate, since during this time DO concentration will be the highest. This aeration strategy also has the advantage of not being harmful to granule structure since, even without 6 h aeration, intact granules were obtained (Vjayan and Vadivelu, 2017). However, aeration rates should not be too low since biomass loss with a decrease of aeration rate from 0.2 to 0.04 m^3 h^{-1} is reported (Wang and Yu, 2006).

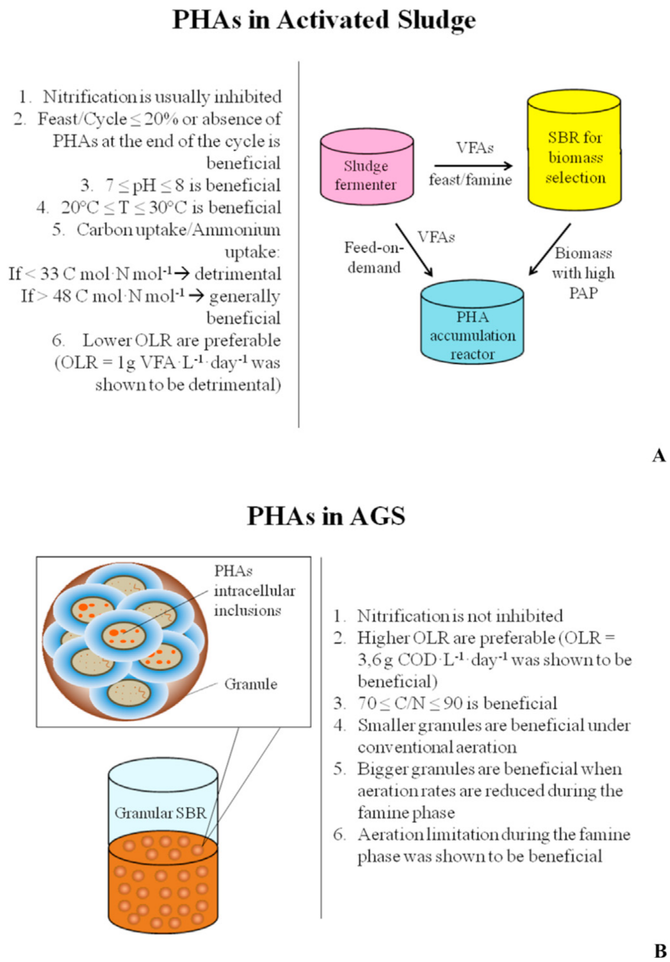

Fig. 6. Polyhydroxyalkanoates (PHAs) recovery in activated sludge (A) and aerobic granular sludge (AGS) systems (B).

6.5. Microbial community associated with PHA production in aerobic granules

The present authors are unaware of studies that have investigated the microbial community associated with PHA production in aerobic granules. Although results obtained with activated sludge (Janarthanan et al., 2016) indicate how this community is possibly delineated, definitive conclusions cannot be drawn, as the morphological differences between flocculent and granular sludge are substantial. It is reasonable to assume that PAOs and GAOs, PHA producers notoriously abundant in granules grown in EBPR SBRs, might play an important role in PHA-accumulating AGS systems. However, in activated sludge systems, PAOs are reported do not be required for achieving high PAP, and WWTP with A2O or UCT/MUCT process configurations showed generally lowered PAPs (Bengsston et al., 2017).

6.6. Extraction methods for PHA recovery

Lastly, regarding the extraction, quantification, and composition identification of PHAs, Gobi and Vadivelu (2015b) explain that the process starts with the breakage of cells, followed by the dissolution of their PHA content via chloroform addition, with the subsequent polymer precipitation by methanol addition and weigh of the solid precipitate. These authors compared four techniques of cell lysis, using the solvents sodium hypochlorite (NaOCl), sodium hydroxide (NaOH), acetone (C_3H_6O), and sodium chloride (NaCl). They found that the PHA yields increased with the solvent's ability to remove EPS. The best results were obtained with NaOCl in the proportion of 12.5 mL to 1 g of biomass, which produced 100% EPS removal and PHA yield of 89% g PHA·g CDW^{-1}. Furthermore, the identification of polymer composition is usually carried out through gas chromatography, using standard samples of the PHAs of interest (Albuquerque et al., 2010; Fauzi et al., 2019). A summary of the current knowledge for PHA recovery in AS and AGS systems is presented in Fig. 6.

The main factors associated with polyhydroxyalkanoates (PHAs) production by activated sludge (AS) and aerobic granular sludge (AGS) are summarized. Key findings were based on the following studies: (A1) present article; (A2) Dionisi et al. (2007) and Pittmann and Steinmetz (2014); (A3) Pittmann and Steinmetz (2014); (A4) Johnson et al. (2010b); (A5) Johnson et al. (2010a); (A6) Pittmann and Steinmetz (2014); (B1) present article; (B2) Gobi and Vadivelu (2015a); (B3) Fang et al. (2009) and Wang and Yu (2006); (B4) Gobi and Vadivelu (2015a); (B5) Vjayan and Vadivelu (2017); (B6) Vjayan and Vadivelu (2017). ORL: Organic loading rate; VFA: volatile fatty acids; C: carbon; N: nitrogen.

7. Effects of the operational strategies for resource recovery in AGS systems

A comparison between factors affecting the production of ALE, tryptophan, phosphorus, and PHA in AGS systems can be found in Table 4. As it can be drawn from it, the main trade-offs regard in the proportion of carbon to nitrogen in the influent stream and sludge retention time.

While a COD/N ratio of 20 is preferable for ALE production (Rollemberg, 2020), tryptophan yields increase with C/N ratios close to 5 (Luo et al., 2014; Rollemberg, 2020; Yu et al., 2020; Zhang et al., 2018a) and PHA yields are greater at COD/N ratios close to 70 (Fang et al., 2009; Wang and Yu, 2006). Considering the parameter SRT, tryptophan is favored by SRTs close to 6 days (Zhang et al., 2018b), while ALE production, although never tested under SRTs that low before, is known to be decreased when the SRT is higher than 15 days (Rollemberg, 2020).

On the other hand, raising salinity up to 1% of NaCl would be a way of optimizing ALE production (Moradali et al., 2018), without undermining phosphorus removal (Bassin et al., 2011), if not increasing it, since ALE induces biological precipitation of phosphorus in AGS (Lin et al., 2012) and is possibly associated with PAOs enrichment (Schambeck et al., 2020). Furthermore, phosphorus and PHA recovery can theoretically be optimized simultaneously in PAOs-rich granules, if the P releasing stage required to produce a P-rich liquor for struvite precipitation is combined with a PHA accumulation phase.

8. Knowledge gaps

Overall, Table 4 demonstrates the necessity to expand scientific knowledge on the influence of operational parameters over resource recovery in AGS systems, especially in regards to ALE, tryptophan, and PHA production. Investigations considering the effects of carbon to phosphorus ratio, pH, temperature, carbon source, free ammonium, nitrite, trace metals, and pharmaceuticals should be conducted. The impact of aeration rates on ALE and tryptophan production has also not been clarified yet.

Furthermore, the relationship between ALE secretion and PAOs activity must be elucidated. The interactions between quorum sensing systems governing ALE and tryptophan production in AGS are also an interesting subject to be investigated since there is

Table 4
Comparison between key operational parameters to the production of ALE, Tryptophan, Phosphorus and PHA in AGS.

	ALE	Tryptophan	Phosphorus	PHA
OLR	Shock loads (Yang et al., 2014)			Higher OLR (up to 3.64 kg $COD \cdot m^{-3} \cdot d^{-1}$) Gobi and Vadivelu (2015a)
C/N	COD/N = 20 (Rollemberg, 2020)	C/N = 5 (Luo et al., 2014; Rollemberg, 2020; Yu et al., 2020; Zhang et al., 2018a)		COD/N = 70 (Fang et al., 2009; Wang and Yu, 2006)
C/P			COD/P = 15 (Muszyński and Miłobędzka, 2015)	
pH			8 (Ahn et al., 2009; Lashkarizadeh et al., 2016)	
Temperature			20 °C (Bassin et al., 2012a)	
Salinity	1% NaCl (Moradali et al., 2018)		• ≤ 1% NaCl (Bassin et al., 2011) • Shock loads (de Graaff et al., 2020)	
Granule size	Matured granules (Rollemberg, 2020; Schambeck et al., 2020)	Granules in formation (Hamza et al., 2018; Rollemberg, 2020; Zhang et al., 2018a)		Smaller granules are beneficial under conventional aeration (Gobi and Vadivelu, 2015a) Bigger granules are beneficial when aeration rates are reduced during the famine phase (Vjayan and Vadivelu, 2017)
Aeration rate				Reduced or null aeration during the famine phase increases yields (Vjayan and Vadivelu, 2017)
SRT	15 days (Rollemberg, 2020)	• 6 days • 12 days were detrimental (Zhang et al., 2018b)		
Carbon source			Volatile fatty acids (Wang et al., 2020)	
Free ammonium			≤15 mg NH_4^+-N·L^{-1} (Zheng et al., 2013a).	
Nitrite			<1.5 mg N–NO_2^-·L^{-1} (Zheng et al., 2013b)	
Trace metals			Up to 0.4 mg Cr(VI)· L^{-1} are not detrimental Fang et al. (2015) 5 mg $Fe^{2+} \cdot L^{-1}$dosage is beneficial (Cai et al., 2018)	
Quorum Sensing	c-di-GMP (Yang et al., 2014)	AHL (Zhang et al, 2018b, 2020)		
Observation	Associated with high P removal		Several pharmaceuticals can affect PAOs activity, but P removal efficiencies remain the same. (Amorim et al., 2014; Kang et al., 2018; Kent and Tay, 2019) Selective discharge of GAOs-rich sludge is beneficial (Winkler et al., 2011)	

COD: Chemical oxygen demand; C: Carbon; N: Nitrogen; P: Phosphorus; OLR: Organic loading rate; c-di-GMP: Cyclic diguanylate; AHL: N-acyl homoserine lactones; PAOs: Polyphosphate accumulating organisms; GAOs: Glycogen accumulating organisms.

evidence that AHL signals production is affected by altered c-di-GMP levels (Schmid et al., 2017). Regarding PHA, it is necessary to describe how the microbial community associated with its production in aerobic granules looks like. It is not yet known, for example, if there is a difference in PHA yields between AGS enriched with GAOs or PAOs. It is also necessary to assess which sludge discharge strategies are most beneficial to ALE, tryptophan, and PHA production.

In terms of industrial applications of resource recovery in AGS systems, tryptophan has the longest way to go. So far, researchers have managed only to extract and identify tryptophan in AGS, but purification methods are lacking. Because of this, tryptophan recovered from AGS has not been applied yet. It is still to be determined whether tryptophan extracted from AGS could be purified to the point of replacing conventionally produced tryptophan. Less stringent uses, such as agricultural applications, must also be evaluated. Phosphorus and PHA recovery from AGS must be validated and optimized still since most of the experience is on activated sludge, and the biomasses are very different both in terms of P uptake during wastewater treatment and P release during the digestion process. Therefore, ALE industrial production from AGS could be considered the most mature process.

Therefore, investigating to what extent these adaptations are necessary and designing alternatives is essential. Lastly, a deeper economic analysis comparing the production of resources based on traditional and AGS-based methods is needed.

9. Conclusions

This article summarized the operational parameters affecting the production of ALE, tryptophan, phosphorus, and PHAs in AGS-based systems, as described in scientific literature so far. The carbon to nitrogen ratio was identified as a parameter that plays an important role for the optimal production of ALE, tryptophan, and PHA. The sludge retention time effect is more pronounced for the production of ALE and tryptophan. Additionally, salinity levels in the bioreactors can potentially be manipulated to increase ALE and phosphorus yields simultaneously. Regarding industrial applications, tryptophan has the longest way to go. On the other hand, ALE production/recovery could be considered the most mature process if we take into account that existing alternatives for phosphorus and PHA production/recovery are optimized for activated sludge rather than granular sludge. Consequently, to maintain the same effectiveness, these processes likely could not be applied to AGS without undergoing some modification. Therefore, investigating to what extent these adaptations are necessary and designing alternatives is essential.

Sample Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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