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Review article

Challenges of aerobic granular sludge utilization: Fast start-up strategies and cationic pollutant removal

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ABSTRACT

Aerobic granular sludge (AGS) is a self-aggregated microorganism consortium with pollutant removal properties. The aim of this work is to study and review the application of aerobic granules for water treatment with special focus on new applications and methodologies. Carbon-nitrogen containing pollutants are the classic targets of AGS technology. Carbon and nitrogen removal of AGS are classified as a biodegradation process. More recently, the AGS granules have been studied as sorbent materials for wastewater treatment. In particular, the sorption of cationic pollutants has been studied through biosorption and bioaccumulation mechanisms without distinguishing when one or the other process is involved. AGS conformation made them suitable for complex wastewater treatment. Indeed, several studies have demonstrated the removal of polyvalent cationic pollutants even with higher capacity than conventional sorbent materials. However, this was achieved almost exclusively for synthetic substrates, with single cation evaluation and using in some cases only qualitative measures. For successful industrial AGS application in complex substrates, it is necessary to evaluate and demonstrate the technology in real industrial conditions and reduce the currently long start-up times which limits its utility. Two new strategies have been proposed: autoinducer molecules and the production of artificial granular from common active sludge with commercial alginate. Finally, the increase of research on AGS cations assimilation properties will allow a new point of view, where granules will be materials for the recovery of valuable metals from industrial wastewater streams.

1. Introduction

The anaerobic granular sludge was discovered in 1976 being the first granular biofilm conformation reported [1]. After the discovery of anaerobic granular sludge, the aerobic granular sludge (AGS) was described in the 1990s [2,3]. The AGS is a microbial consortium auto-immobilized in microspheres of typically 0.5–4 mm in diameter [1,4,5]. More precisely, the ratio between sludge volume index (SVI) after 30 and 10 min has been defined as the parameter to characterize the granular sludge [6]. More recently, the ratio of SVI 30/SVI 5 close to 1 is a widely used parameter to define the presence of granular sludge [7,8].

During the period 2005–2014, the research activities on AGS exploded with 57 science publications per year (Fig. 1). During these

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years, the AGS technology was studied as a type of process used in the treatment of wastewater. Thanks to its biodegradation ability, the biological wastewater treatment have placed AGS technology as the forefront of industrial innovation and research [1,9]. The popularity of aerobic granular sludge technology over classical activated sludge (AS) technology is explained by a reduction in sludge volume, better settleability, minimal loss of active sludge, ability to maintain high sludge concentration and smaller footprint during wastewater treatment (Table 1).

During the period 2016–2021, the research activities on AGS experimented a new increase with 203 science publications per year (Fig. 1). Investigations were related to extracellular polymeric substances (EPS) and its role in the process performance [10]. Besides, new research focused on the sorption and accumulation properties of AGS. Thus, AGS processes can also be a source of valuable products when sludge is used for cations recovering (including valuable metals). The sorption of recalcitrant aromatic compounds, cationic metal, dyes and nuclear waste have been reported in AGS technology [1,10]. From this group, the cationic metals removal has received more attention due to their economic and environmental importance. The possibility of reuse the AGS excess such adsorbent material contributes to the environmental and economic aspects of this technology. The most common cations studied have been lead, manganese, chromium, magnesium, calcium, iron, zinc, and copper. In most cases, these cations have been studied exclusively on synthetic substrates, and little is known about the mass transfer mechanisms that govern the sorption process. This work analyzes and discusses the possibility of using AGS with real industrial effluents to recover important cations.

Different reactor designs have been tested to form and maintain AGS, but to date, full-scale aerobic granulation has been used most with the sequencing batch reactor (SBR) design [11]. However, some full-scale AGS plants with a continuous process using the hydrocyclone separator have been reported. For example, the continuous systems proposed by S:Select® are based on the selection of AGS with hydrocyclone at sufficiently high pressure to achieve a stable wastewater treatment system [12]. Further reactor design optimization and improvement of operating conditions are obviously needed [13]. Even more important, granulation of the sludge and maturation (to achieve enough nutrient removal) can take several months. Then, the main problem of this technology is the long start-up times which restrict its application at industrial scale. Two new potential alternatives that could be used are, (1) molecular inductors to improve the removal ability and produce a fast start-up [1], and (2) artificial aerobic sludge granulation: immobilization with alginate matrix for common active sludge [14]. Molecular inductors are responsible for the cellular communication that allows the production and lysis of granular sludge [1,15]. On the other hand, alginate-like molecules are the main polymers in EPS and are responsible for structure and sorption properties of AGS [9].

This review aims to identify the process and product aspects of AGS related to its biological mechanisms. In the same way, the start-up issue was analyzed and two new strategies to overcome it are discussed (artificial aerobic sludge granulation and microbial communication). Finally, the industrial advantages of AGS technology was analyzed with a view in future applications including its use for complex substrates with a high variation in nutritional and cations concentration parameters such as landfill leachate. The production of AGS and the potential removal of the most significant cations were analyzed in landfill leachate.

2. Pollutant removal mechanisms of aerobic granular sludge

AGS pollutant removal mechanisms are include three strategies: biodegradation, bioaccumulation, and biosorption [1,9,13]. Fig. 2 explains the three mechanisms that allow the removal of pollutants. The granule can be divided into three oxygen-dependent zones: aerobic, anoxic and anaerobic zones. The anoxic and anaerobic zones are described in the absence of free oxygen, but in the anoxic zone metabolic reactions depend on a bound oxygen electron acceptor. For example, denitrification reaction uses nitrite and nitrate as electron acceptor; thus, it occurs in the anoxic zone. The biodegradation mechanism is associated to the nutrient pollutant removal process. On the other hand, biosorption and bioaccumulation allow the removal of different sorbate compounds, then they are responsible for the reuse of AGS such as a product for example to recover important metals. The importance of these three mechanisms is discussed in the following chapters.

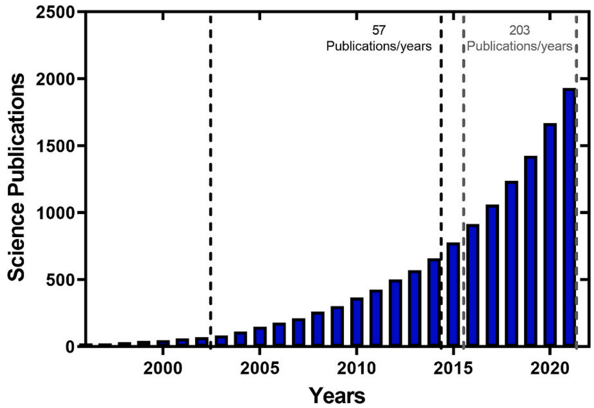

Fig. 1. Bibliometric analysis of scientific papers published in indexed journals with the topic "aerobic granular sludge". Data obtained from web of science.

Table 1
Differences between the activated sludge and aerobic granular sludge (Nancharaiah and Reddy, 2018; Winkler et al., 2018).

Parameter	Activated sludge	Aerobic granular sludge
Average size	Irregular; Small, ~0.20 mm	Spherical shape; >0.20 mm
Settling velocity	~10.0 m/h	>10.0 m/h
Sludge volume index	SVI 5 ≠ SVI 30	SVI 5 = SVI 30
Microenvironments	Minimum possibilities for anaerobic zones	Aerobic, anaerobic and anoxic zones.
EPS	Low EPS content	High EPS content
Tolerance to toxic compounds	Low	High

EPS: extracellular polymeric substances; SVI 5: Sludge volume index (SVI) by 5 min; SVI 30: Sludge volume index (SVI) by 30 min.

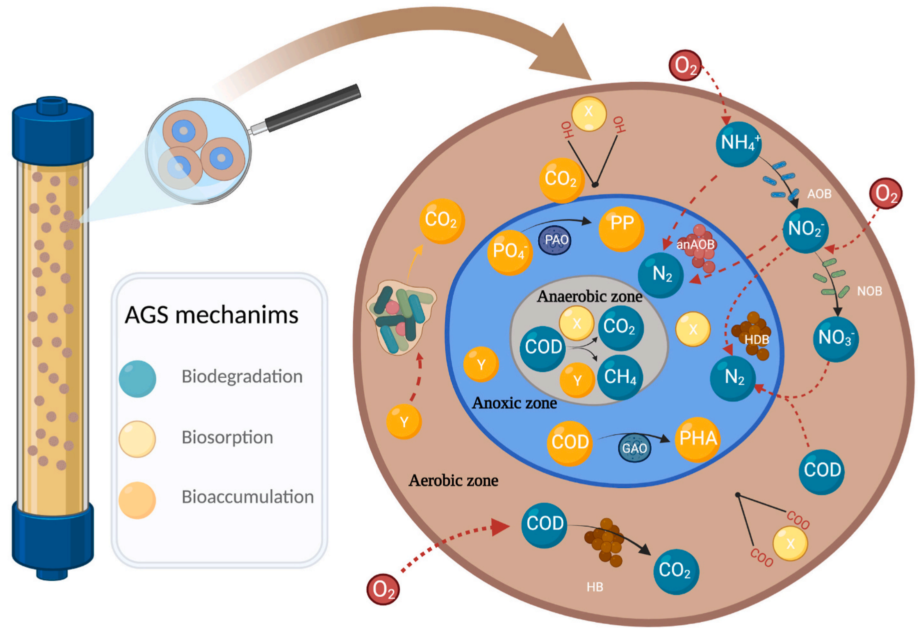

Fig. 2. Aerobic granular sludge mechanisms: biodegradation biosorption and bioaccumulation, adapted from Refs. [9,39,91]. COD: Chemical oxygen demand; X: Sorbate used in biosorption; Y: Sorbate used in bioaccumulation; AOB: Ammonia-oxidizing bacteria; NOB: Nitrite-oxidizing bacteria; anAOB: Anammox bacteria; HDB: Heterotrophic Denitrifying bacteria. Created with BioRender.com.

Table 2
Operational conditions of different AGS systems reported in the literature.

#	Ref.	medium	Volume	H/D	OLR	NLR	HRT	SRT	Oxygenation	Temperature	agitation
			(L)		(kg COD/m^3 d)	(kg N/m^3 d)	(h)	(d)		(°C)	
1	[21]	real wastewater	1.50	5.50	1.72	0.18	6.00	66.0	6.50–8.00 mg O_2/L	18.0	bubbling
2	[2]	synthetic wastewater	31.4	5.00	0.69	0.04	13.3		2.00 mg O_2/L		bubbling
3	[3]	synthetic wastewater	2.25	26.8	7.50	0.14	6.75	3.40		20.0	bubbling
4	[4]	real wastewater	14.0	5.30	2.60	0.03	19.2		300 Nl/h		pumping
5	[17]	real wastewater	1.80	4.50	3.60	0.24	6.00		4.00–7.60 mg O_2/L	27.0	bubbling
6	[5]	real wastewater	4.00	6.90	9.00	1.80	8.00			27.0	bubbling
7	[18]	real wastewater	10.0	1.30	3.84	1.39	10.0		6.00–8.00 L/min		bubbling
8	[19]	real wastewater	4.00	8.00	0.80	0.06	48.0	30.0	7.50 L/min	20.0	bubbling
9	[8]	synthetic wastewater	5.00	3.80	1.22	0.20	16.0	54.0	2.00–3.00 L/min	20.0	bubbling
10	[7]	real wastewater	1.75		1.80	0.22	8.00	15.0	7.00 L/min	25.0	bubbling
11					3.22	0.45	8.00	5.90			
12	[20]	real wastewater	71	5.71	1.50	0.08	12.0		5.00–8.00 mg O_2/L		bubbling
13	[22]	real wastewater	115.5	10	0.9	0.09	5.6	5.6	8.00 mg O_2/L	29	bubbling

H/D: ratio of height to diameter; OLR: Organic load rate; NLR: Nitrogen load rate; HRT: Hydraulic retention time; SRT: Sludge retention time.

2.1. Biodegradation mechanism

Biodegradation is the emblematic biochemical process that decomposes any biodegradable compound [13]. Substrate gradients and shear stress phenomena produce ecological niches in the aerobic granular sludge [16] (Fig. 2). This is the main advantage of AGS compared to anaerobic granular sludge as the aerobic granules are composed of both free and non-free oxygen areas [1]. Similar to any other mixed microbial culture, the microorganisms in aerobic granules can share the biodegradation pathways, where a degradation product generated by one organism can be utilized by others to achieve complete degradation [1,13].

Table 2 and Table 3 show different operational conditions and results of AGS reactors. The AGS technology has been recently evaluated for the treatment of several industrial wastewaters: textile [4], rubber [17], livestock [5], dairy [18], brewery [19], landfill leachate [8], petroleum wastewater [20], fish canning effluent [7,21] and domestic wastewater [22] are some of them. In the reactor design, the most common ratio of height to diameter used is 5.00, but also high values such as 26.8 showed high pollutant removal capacities (Table 2). In addition, almost exclusively bubble column reactors have been used in the works analyzed. Hydraulic retention time (HRT) are typically in the range of hours, although for real wastewater treatment it can reach values of up to 2 days [19]. Few works reported the operational temperature, but usually environmental temperature values are lower than 24.0 °C (Table 2). Common reaction cycles comprise feeding, aeration, sedimentation and discharge phases (Table 3 and Fig. 3). Nevertheless, the most recent reports also consider an additional anaerobic phase (Table 3 and Fig. 3) [[5,7,8,19,22]]. Anaerobic degradation plays an important role in controlling the C/N ratio during aerobic phase in AGS reactor [23]. Since oxygen competition between aerobic heterotrophs and nitrifying bacteria can inhibit nitrogen removal on substrates with a high organic matter concentration. Thus, in complex substrates, such as landfill leachate, with a high variation in nutrients, organic matter removed without oxygen can contribute to nitrogen removal.

Fig. 4 summarize the biodegradation data of the AGS reactor from the literature. Most AGS systems operate with OLR and NLR values lower than 4.00 kg COD/m^3 d (Fig. 4a) and 0.50 kg N/m^3 d (Fig. 4b), respectively. The highest values have been reported for livestock and dairy wastewater [5,18] (Fig. 4). The organic removal (OLR) has been mostly over 50.0% (Fig. 4a). On the other hand, few nitrogen removal values are higher than 80.0% (Fig. 4b). Operations with higher OLR are associated with granules of greater diameters (see tendency in Fig. 4c). Then, for operation with high organic load it should be considered larger granular sizes.

AGS also can be suitable for nitrogen removal. The ecological niche advantages of AGS act also favouring the partial nitrification-ANaerobic AMMonium Oxidation (Anammox) process. The partial nitrification (PN)-Anammox (A) granular sludge can be considered as a type of AGS, but with only autotrophic metabolism (Fig. 2). The PN-A is the most promising method for autotrophic nitrogen removal from wastewater with nitrogen removal values higher to 89.0% [24,25]. In the external layers, nitrite can be obtained from the oxidation of ammonium to nitrite (PN); and in internal layers the Anammox reactions oxidize ammonium using nitrite as an electron acceptor, thus producing gaseous nitrogen [26,27]. Certainly, the type of nutrient removal in a granular biological reactor defines the type of oxygenation and the necessary operating strategy. Our group has demonstrated the ideal operating conditions in parameters such as oxygen [24], type of agitation [28], size of granular sludge [29] and appropriate operational strategies for several industrial substrates [23,25,30].

Table 3
Operational results and reaction cycle of different AGS systems reported in the literature.

#	Ref.	Reaction cycle	Organic removal (%)	Nitrogen removal (%)	Granular Diameter (mm)
1	[21]	3-h cycles: 3 min of feeding, 171 min of aeration, 1 min of sedimentation and 5 min of discharge.	90.0	40.0	3.40
2	[2]	6-h cycles: 13 min of feeding, 325 min of aeration, 0 min of sedimentation and 12 min of discharge.	87.0		2.35
3	[3]	4-h cycles: 2 min of feeding, 237 min of aeration, 2 min of sedimentation and 1 min of discharge.	100	4.38	3.30
4	[4]		72.1	65.7	0.50
5	[17]	3-h cycles: 5 min of feeding, 150 min of aeration, 15 min of sedimentation, 5 min of discharge and 5 min idle.	98.4	92.7	2.00
6	[5]	4-h cycles: 10 min of feeding, 185 min of aeration, 20 min of anaerobic phase, 15 min of sedimentation, 5 min of discharge and 5 min idle.	74.0	73.0	4.10
7	[18]		87.0	66.0	
8	[19]	12-h cycles: 40 min of anaerobic feeding, 676 min of aeration, 1.5 min of sedimentation and 2 min of discharge.	87.0		
9	[8]	8-h cycles: 30 min of feeding, 90 min of anaerobic phase, 330 min of aeration, 5 min of sedimentation and 12 min of discharge.	52.0	99.0	
10	[7]	4-h cycles: 5 min of feeding, 227 min of aeration, 1 min of sedimentation and 7 min of discharge.	80.0	50.0	0.94
11		4-h cycles: 80 min of feeding, 152 min of aeration, 2 min of sedimentation and 6 min of discharge.	85.0	30.0	1.38
12	[20]	6-h cycles: 13 min of feeding, 319 min of aeration, 18 min of sedimentation and 10 min of discharge.	95.0	35.0	0.46
13	[22]	4-h cycles: 2 min of feeding, 90 min of anaerobic phase, 134 min of aeration, 20 min of sedimentation and 4 min of discharge.	80.0	83.0	≥0.2 mm

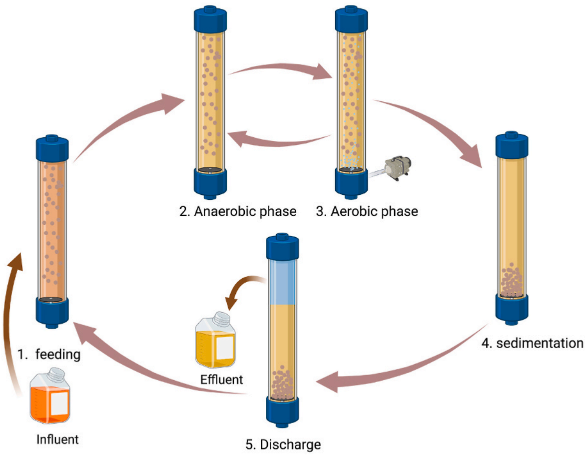

Fig. 3. Stage of the reaction cycles in the AGS reactor. The position of the anaerobic phase and the aerobic phase can change depending on the authors. Created with BioRender.com.

2.2. Biosorption mechanism

Biosorption is a physicochemical process by which both living and dead microbial cells adsorb different contaminants without expending energy from metabolic activities [1].

One of the first uses of the term biosorption described the elimination of calcium and magnesium ions by tannin resin, black acacia bark (*Acacia mollissima*) in 1935 [31]. However, the "biosorption" was popularized by Volesky who created Sorbex, Inc. a company dedicated to the biosorption removal and recovery of heavy metals for industrial solutions [31]. Nowadays, the concept is used for physical and chemical sorption of any material of biological origin.

Biosorption mechanisms include sorption, ion exchange, and complexation/coordination. It is fast and reversible, and the biosorbent properties are basically analogous to conventional ion exchange resins [32]. However, the AGS has a complex structure with many functional groups (e.g., carboxyl, phosphate, hydroxyl, amino, thiol), which can interact with a broad range of sorbate species to varying degrees of strength and are influenced by both physical and chemical factors (Fig. 2). In fact, depending on the system and given conditions, biosorption can be a mechanistically highly complex process [32]. Precipitation and crystallization are other mechanisms that may occur adding an extra layer of complexity to sorption and/or desorption in AGS. They can lead to very high uptake capacities but this may inhibit desorption. Multiple factors affect biosorption: solution pH, solution ionic strength, temperature, agitation speed, pollutant concentration and interactions with other pollutants including competition for binding sites. The various mechanisms involved in biosorption are likely to operate simultaneously to varying degrees. Furthermore, because of the diversity of functional groups and their presence on biosorbents, the stability and predictability of the biosorption process remain problematic [13].

Successful biosorption with AGS has been reported for dyes, nuclear waste, recalcitrant aromatic compounds, and cationic metals. These last one has been widely assayed (see Table 4). The effect of initial pH, biosorption kinetics, biosorption equilibria and the effect of the sludge concentration has been investigated. Different kinetics and isotherm mathematical models have used to model sorption capacity. The most used biosorption kinetic models have been a pseudo-first-order kinetic model [33] and a pseudo-second-order [34] expressed as Equation (1) and Equation (2), respectively:

$$q_t = q_e\left(1 - \frac{1}{e^{k_1 t}}\right) \qquad 1$$

$$q_t = t\left(\frac{1}{k_2 {q_e}^2} + \frac{1}{q_e}\right)^{-1} \qquad 2$$

Where q_t and q_e (mg/g) are the adsorption capacities of sorbate at equilibrium and contact time t (min), and k_1 (1/min) and k_2 (g/mg min) are the pseudo-first-order and pseudo-second-order rate constants.

The most used biosorption isotherm models have been the Langmuir model [35] and a Freundlich model [36], although a number of different equations have also been proposed. It is well known that the Langmuir model assumes that adsorption occurs via a monolayer on the surface of adsorbents, and there is no interaction between the adsorbates (Equation (3)) [35]. The Freundlich model assumes that multilayer adsorption occurs on the heterogeneous surface of the adsorbent (Equation (4)) [36]. The models can be expressed such as:

$$q_e = q_{\max}\left(\frac{K_{eq}C_e}{1 + K_{eq}C_e}\right) \qquad 3$$

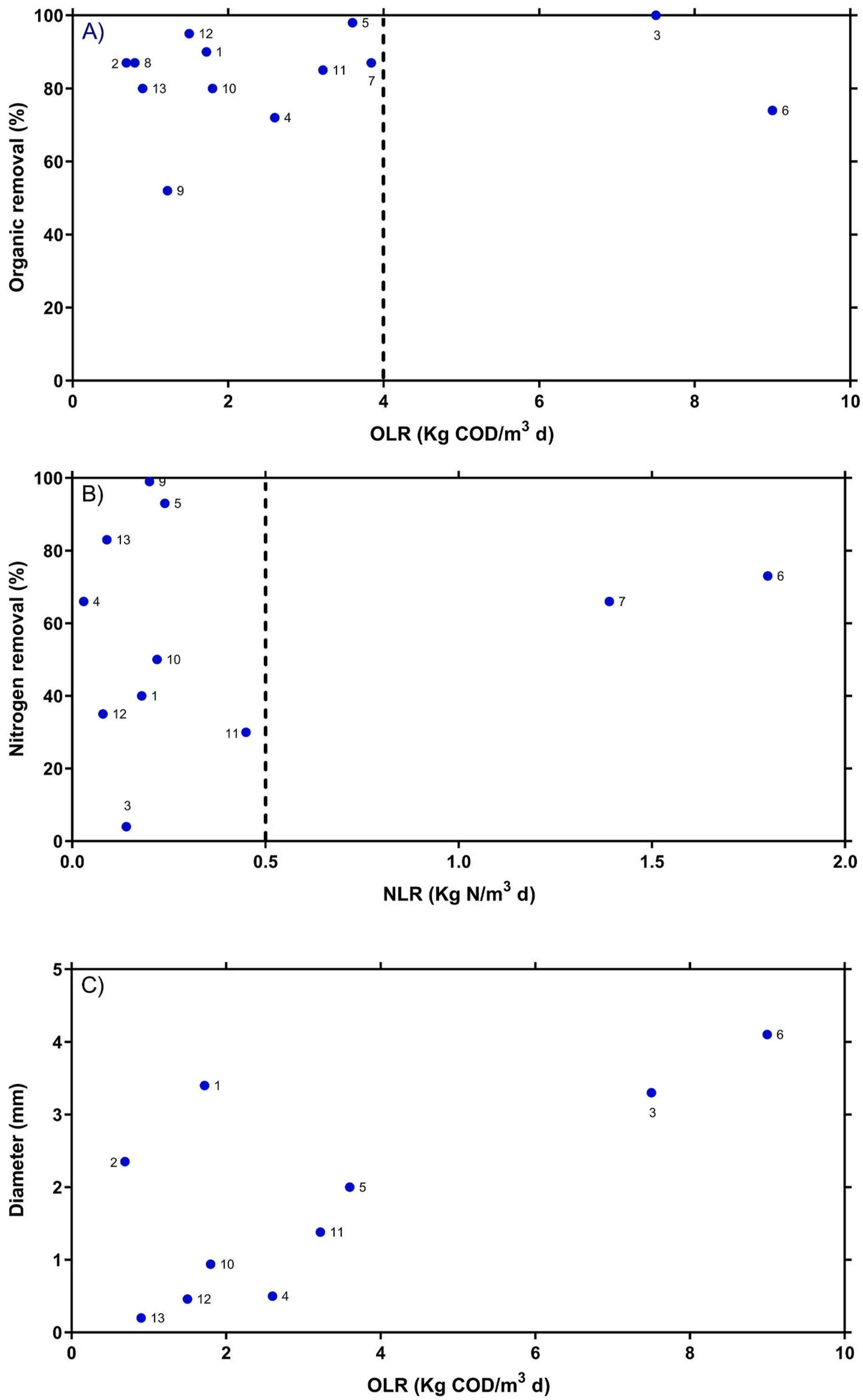

Fig. 4. Operational conditions and performance of different AGS systems reported in the literature. A) The plot of organic load rate (OLR) and organic removal. B) The plot of nitrogen load rate (NLR) and nitrogen removal. C) The plot of organic load rate (OLR) and granular diameter. In each plot, the number indicates the reference: (1) [21]; (2) [2]; (3) [3]; (4) [4]; (5) [17]; (6) [5]; (7) [18]; (8) [19]; (9) [8]; (10) [7] with anaerobic phase; (11) [7] without anaerobic phase; (12) [20]; (13) [22].

$$q_e = k_f C_e^{1/n_f} \quad (4)$$

Table 4
Cationic pollutant adsorption values with aerobic granular sludge.

Ref.	Sorbate	Carbon source	Granule diameter	T	pH	Incubation time	sorbate concentration	sludge concentration	Adsorption capacity
			(mm)	(°C)		(h)	(mg/L)	(g/L)	(mg/g)
[78]	Lead (II)	glucose	1.00–1.50	30.0	5.50	3.00	20.0–200	1.00	87.7
[66]	Lead (II)	acetate		25.0	5.00	4.00	10.0–150	0.50	102
[39]	Lead (II)		1.50	25.0	4.50	4.00	5.00–200	0.02	1590
[79]	Boron (III)	acetate	0.45–0.68			840	35.0	1.11	
[93]	Manganese (II)	acetate		20.0	6.00	2.00	20.0–500	1.00	311
[81]	Manganese (II)	yeast extract,			7.20	6.00 (days)	10.0–80.0		
[80]	Manganese (II)	glucose		28.0		75.0 (days)	10.0	11.0	
[100]	Chromium (III)	glucose	1.00	30.0	5.00	3.00	20.0–200	1.00	64.1
[74]	Chromium (VI)	glucose		20.0–30.0		80.0 (days)	10.0–30.0	3.20	175
[75]	Magnesium (II)	acetate	1.15		7.20	23.0 (days)	3.00	3.00	
[101]	Magnesium (II)	glucose	2.90	25.0		52.0 (days)	10.0	7.60	
[102]	Magnesium (II)	acetate		30.0; 40.0; 50.0		60.0 (days)	90.0	10.6	6.00–33.7
[102]	Calcium (II)	acetate		30.0; 40.0; 50.0		60.0 (days)	4460	10.6	3.00–32.4
[75]	Calcium (II)	acetate	1.15		7.20	23.0 (days)	5.00	3.00	
[103]	Calcium (II)	acetate		25.0	6.70–7.50	110 (days)	5.45	6.00	55.0
[75]	Iron (III)	acetate	1.15		7.20	23.0 (days)	4.20	3.00	
[93]	Iron (II)	acetate		20.0	3.00	2.00	20.0–500	1.00	232
[93]	Zinc (II)	acetate		20.0	6.00	2.00	20.0–500	1.00	350
[39]	Zinc (II)		1.50	25.0	4.50	4.00	5.00–200	0.02	1120
[94]	Zinc (II)	acetate	1.00	26.0	6.00	4.00	100	0.13	270
[93]	Copper (II)	acetate		20.0	6.00	2.00	20.0–500	1.00	198.2
[95]	Copper (II)	acetate	1.00	26.0	4.00	5.00	5.00–200	0.10	59.6

where C_e (mg/L) is the sorbate at equilibrium, q_e (mg/g) is the adsorption capacity, $q_{\max}$ (mg/g) is the maximum adsorption capacity, K_{eq} (L/mg) is the Langmuir constant related to the free energy of adsorption, k_f (mg/g $(\mathrm{L/mg})^{1/n}$) is the Freundlich constant representing the adsorption capacity of the sorbate, and n_f is the heterogeneity factor indicating the adsorption intensity of the adsorbent.

The sorption and adsorption are not the same. Both concepts are sometimes used as synonyms limiting the interpretation of the observed phenomena. Adsorption is often regarded as a molecular-scale process. Some authors have suggested that the term "sorption" should no longer be used, as some journals have outlined, since it describes the partitioning of dissolved species into the solid phase using an unspecified mechanism [37]. In particular, biosorption in general has the major problem that the mechanisms that dominated sorbent-sorbate interaction are unknown. Actually, a question without an answer has been the step control in the sorption process with AGS. Some works have wrongly claimed that biosorption can be studied using only kinetic empirical models such as pseudo-first-order kinetic model and a pseudo-second-order. Even more, the majority of works concluded that chemisorption process is the step controlling the sorption process [38,39]. However, these models lack physical information and supporting model. For this reason, they cannot be used to investigate the mass transfer mechanism [40,41]. In the same way, a version of granular sludge drying such biosorbent was used to determine that intraparticle diffusion is the main factor in the adsorption rate control [42]. However, in this study the step rate control was researched with an empirical model such Webber-Morris.

An alternative to solve this problem is to analyze the process through phenomenological models. As with any adsorbent material, biosorption has three stages that dominate the process [41]: (1) the transfer of sorbate between the boundary film and the surface of the sorbent (external mass transfer), (2) the transfer of sorbate from the sorbent surface to the active sites (internal mass transfer step), and (3) the adsorption onto active sites. Phenomenological models are described as differential equations which can be solved by the Runge-Kutta method [41]. The phenomenological models are external mass transfer (EMT) [43], internal mass transfer (IMT) [44] and adsorption onto active sites (AAS) [35,45], which are described mathematically in Equation (5), Equation (6) and Equation (7), respectively. This models allow to determine through analysis with experimental data which phenomenon dominates the sorption process.

$$\frac{dq_t}{dt} = k_{ext}(C_t - C_{et}) \tag{5}$$

where C_t (mg/L) is the adsorbate concentration at time t, C_{et} (mg/L) is the equilibrium concentration at the surface and k_{ext} (L/g h) universal external mass transfer coefficient, it is a constant that groups various terms such as efficiency factor, porosity and turbidities inside the granules [46].

$$\frac{dq_t}{dt} = k_{int}(q_{et} - q_t) \quad (6)$$

where k_{int} (1/h) is an internal mass transfer constant, q_{et} (mg/g) is the adsorption capacity at equilibrium in the pores of the adsorbent and q_t (mg/g) is the adsorption capacity at time t [46].

$$\frac{dq_t}{dt} = k_a C_t(q_{max} - q_t) - \frac{k_a}{K_L} q_t \quad (7)$$

where the isotherm representing adsorption must be represented by the Langmuir isotherm, and k_a (L/mg h) is the adsorption constant at the active sites, C_t is the concentration at a time t and K_L with q_{max} (mg/g) are constants determined by the Langmuir isotherm (L/mg) [46].

Likewise, using this type of models and experimentation at different temperatures allows to determine the type of interaction that occurs between the material and the sorbate. For example Monte Blanco et al. [47] the adsorption of reactive blue dye 5G by polymeric adsorbent Dowex Optipore SD-2 was analyzed with phenomenological models. The process was dominated by AAS; then different temperature adsorption experiments were performed using the Arrhenius expression to determine the type of interaction obtained. Thus with the activation energy ~11 kJ/mol the phenomena was produced by molecular interactions: electrostatic (6–80 kJ/mol), hydrogen bonds (4–13 kJ/mol). This same strategy with AGS and different sorbates could determine the type of interaction that occurs in the presence of the adsorption process: Physisorption or chemisorption.

Recent research on biosorption has revealed the complexity of the process, its dependence on physicochemical and biological factors, and the uncertainty about the dominant mechanisms. Biosorption, therefore, remains a developing technology; its commercial success will depend on a better understanding of the underlying processes.

2.3. Bioaccumulation mechanism

Bioaccumulation is a metabolic activity that requires energy expenditure by the sludge [1]. The main difference with biosorption is the active mechanism that participates in accumulating either atoms or molecules. Nutrient accumulation is important for AGS maintenance. Carbohydrates and proteins of the EPS are essential for developing the granular matrix [9]. In fact, the EPS are metabolic products accumulating on the surface of bacterial cells, which could alter the physicochemical characteristics of cellular surface such as its charge and hydrophobicity [10]. In addition, phosphorus-accumulating bacteria and glycogen-accumulating bacteria have been identified in aerobic granules developed under different conditions [10]. The polyphosphate-accumulating organisms (PAO) are essential to phosphorus removal. On the other hand, glycogen-accumulating organisms (GAO), which convert all acetate into glycogen or polyhydroxybutyrate (PHB) during the anoxic period, are essential for the granular structure [48].

The bioaccumulation of divalent cations such as calcium is essential to crosslink anionic polysaccharides such as alginate molecules present in the EPS, and they play a role in granule formation and stability [1]. Non-essential nutrients such as selenite are accumulated in aerobic granules [49]. In addition, the metal-tolerant mechanism exhibited by some bacteria is a type of bioaccumulation. Bacteria cell can limit the damage produced by high metal concentration with the sequestration of metals in storage proteins, making a cytosolic "buffer" [50]. If the metal concentration is too high, the efflux pump is activated and then with these mechanisms, the metal homeostasis is controlled [51].

In the literature the removal of polyvalent cations in AGS has been almost exclusively related to biosorption mechanisms, without accounting the contribution of bioaccumulation. Furthermore, the possibility of using granular sludge to adsorb cations and then desorb to recover, for example, metals, has not been extensively studied. One of the few studies that have evaluated this possibility, showed the adsorption and desorption of Pb(II), Cd (II) and Zn (II) in EPS from AGS. Afterwards, the metal ions were desorbed from EPS under acidic conditions (1–4 pH) with more than 90% recovery [39]. Since there are effluent treatment operations with granular sludge at low pH (pH 3) [52], the possibility of evaluating the biodegradation capacity of granules after desorption could make this technology suitable. The ability to recover polyvalent cations as metals and to maintain their nutrient removal capacities will be an environmentally friendly technological contribution since the technology could be used as a process and product.

Thus, the ubiquity of AGS in engineering treatment facilities offers an attractive technology to remove certain pollutants which have been traditionally subjected to physicochemical treatments. The carbon and nitrogen degradation removal and recovery of polyvalent cations must be further studied. Also, a better understanding of the relationship of the processes of biodegradation, bioaccumulation, and biosorption are challenges in AGS technology.

3. Fast start-up process: two new proposals

One of the main challenges of AGS processes is to reduce the start-up time. Some authors seed the reactor with anaerobic granules in order to enhance granule formation [53,54]; however, under aerobic conditions, granular sludge disintegration was observed. Other authors explored seeding the reactor with a mixture of crushed aerobic granules and floccular sludge [55]; however, this strategy needs a considerable amount of crushed aerobic granules, which is not frequently found in a well-operated reactor. In addition, this strategy depends on the presence of preexisting granules which for industrial-scale reactors is unrealistic in countries lacking this technology.

Two new alternatives are discussed in this work (1) the use of autoinducers (2) the generation of artificial granules.

3.1. Microbiological communication: the use of autoinducers for the fast start-up process

The cell to cell communication process in which bacteria synchronize their gene expression and physiological behavior using specific chemicals is called quorum sensing (QS). QS is considered to be the main molecular-level event responsible for aerobic granule formation [1]. Autoinducer molecules such as small lipids, oligopeptides or borate esters are the signal chemicals that mediate the microbial communication [1,15]. N-acyl homoserine lactones (AHLs) have been identified as the main autoinducer molecules responsible for quorum sensing in AGS [56,57]. During the initiation of granulation, specific AHL concentration increases 100-fold; on the other hand, during the granular disintegration phase the AHL concentration decreases [56]. Also, the addition of calcium ions benefits bacterial growth and promotes the production of cyclic diguanylate (c-di-GMP), as a second messenger. Both calcium ions and c-di-GMP have important roles in granulation [58]. AHLs are common in different processes that use granular sludge, including both Anammox and AGS processes, as summarized below.

(a) The impact of AHL on granular Anammox sludge has been among the most studied strategies. The release of three acyl-homoserine lactones (C6-HSL, C8-HSL, C12-HSL) by the Anammox sludge has been identified [59]. Also, a strategy for a fast start-up Anammox process by adding the reactor supernatant to a new reactor, reduced the start-up time from 80 to 66 days [60]. These results show the impact of AHL application; however, moving a large amount of effluent from one reactor to another implies high operating costs at an industrial scale.
(b) The same AHLs as those found in Anammox processes have been reported in AGS processes (Fig. 5). During the granulation phase in an AGS reactor C6-HSL concentration increases [56]. In addition, C10-HSL, C12-HSL and 3OC6-HSL have been reported to regulate the EPS production in AGS [57]. But, the concentration of C6-HSL, as well as if C8-HSL and C12-HSL decrease significantly during cell lysis and granular disintegration [56,61].

The interplay between AHLs and (1) a fast start-up AGS reactor and/or (2) performance improvement of an AGS reactor with low nutrient removal may provide a decisive advantage in the application of granular sludge in industrial wastewaters. However, these autoinducer molecules may be expensive to use in wastewater treatment [58,62]. Indeed, if an AHL extract or AHL concentrate from a well-performing aerobic granular reactor is used, such costs would be considerably reduced. The AHL addition in aerobic granular sludge has been studied with good results in biofilm production and nutrient removal performance. However, the AHL has been exclusively produced by bacteria grown on defined media [63]. Then, the potential of recovering AHL concentrated from aerobic granular sludge waste has not been researched, yet. Finally, the autoinducer molecules could be used such as a new fast start-up strategy.

3.2. Artificial granular sludge: alginate immobilization technique

The use of alginate as an immobilization technique has been proposed for any cell types with granular shape in many applications [14,64].

EPS, responsible for the formation and stability of the granules, are composed by polysaccharides and proteins [9]. The main hydrogel-forming polymer in their structure are alginate-like exopolysaccharides (ALE) [65]. The ALE are responsible for the stability

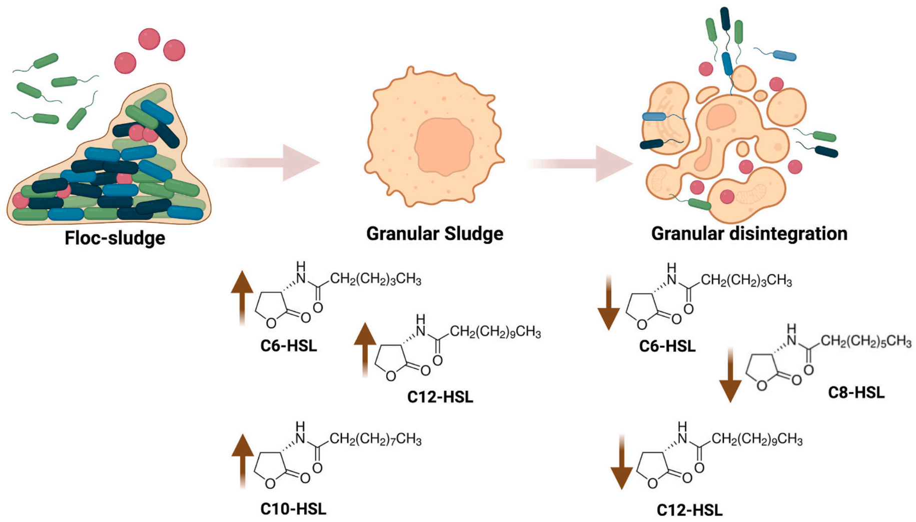

Fig. 5. Acyl homoserine lactones effect in the formation and disintegration of aerobic granular sludge adapted from Refs. [56,57,61]. C6-HSL: N-Hexanoyl-L-homoserine lactone; C8-HSL: N-Octanoyl-L-homoserine lactone; C10-HSL: N-Decanoyl-L-homoserine lactone; C12-HSL: N-Dodecanoyl-L-homoserine lactone. Created with BioRender.com.

and structure of granular sludge. The extraction of the EPS and the characterization of the type of polymers, such as polysaccharides and proteins, and potential industrial applications are the new topic of granular sludge research (Fig. 1). The ALE correspond to 35.1% of aerobic granular sludge composition and are mainly responsible for the biosorption capacity [66]. Indeed, biosorption experiments of EPS in AGS processes have determined sorption capacities greater than 10 times that of conventional biosorbents [39].

In addition, the long maturation times of AGS could be solved with common active sludge with high removal nutrients capacity immobilized in commercial alginate to produce artificial granules [14]. But it has not been successfully demonstrated for aerobic sludge granulation. For instance, bacterial cultivated cells from aerobic granular sludge were encapsulated in sodium alginate with a negative impact on the granulation [63]. However, the assay was carried out with cultured bacteria without taking into consideration that some bacteria of the aerobic sludge consortium may not be cultivated or are outperform by others under the fixed culture conditions. Notwithstanding, fast cultivation of Anammox sludge based on artificial granulation with alginate exhibited good short-term and rapid enrichment performance for the anammox bacteria [67]. In the same way, the optimal incubation time of this anammox granular sludge did not exceed 17 days with an increase of 4.3 times of anammox bacterial in 9 days of cultivation [67]. Therefore, more efforts are needed for extending the application of alginate artificial granules to AGS.

Probably, the high cost of compounds such as alginate and calcium chloride for the treatment of urban waters has not favored the use of this technique in aerobic granular sludge. However, with the ability of granular sludge to remove other non-biodegradable pollutants with a high operational cost, such xenobiotic, metal or polyvalent cationic pollutants, the production of "artificial granular sludge" starting with AS and using alginate immobilization technique would be a plausible way to operate. Besides, some industrial wastewater such as landfill leachate has calcium ion concentration of 10–7200 mg/L [68]; thus, the intrinsic characteristics of this substrate will benefit AGS granulation (Ca^{2+} is needed for cross-linking alginate polymers). Also, both mechanisms (1) the application of autoinducers and (2) immobilization with alginate can be used together, the former to ensure the metabolic activity of the granule and the latter to ensure its structure.

4. Cationic pollutant removal

The ability of the granular sludge to remove several pollutants contributes to the design of wastewater treatments with high load of pollutants. Landfill leachates are good example of complex wastewaters, as they contains organic matter, but also a high concentration of total ammonia nitrogen (TAN), dissolved solids, polyvalent cationic pollutants and xenobiotic organic compounds [69]. Leachates may be classified as young or old according to the stage of decomposition of landfilled wastes. Young leachates are characterized by the presence of easily biodegradable organic matter and acidic pH, whereas old leachates contain slow or non-biodegradable organic matter and high concentrations of TAN [8]. A biological process for leachate treatment must be robust since it should face high variations and high concentrations of polyvalent cationic pollutants such as boron, manganese, chromium, magnesium, calcium, iron, zinc, copper and lead [70–73]. Polyvalent cationic pollutants are typically removed by physicochemical processes (liquid-liquid extraction, coprecipitation, ion exchange, membrane filtration, and resin chelation). However, there are also disadvantages associated with those methods: (1) excessive use of chemicals, (2) toxic sludge production and (3) high operation time requirements [73].

The AGS technology has been reported to remove successfully polyvalent cationic pollutants. Table 4 shows examples polyvalent cationic pollutant removal with AGS. These works were conducted with synthetic substrates and aerobic granular sludge cultivated with different carbon sources. Cations composition of the synthetic substrates were the same as found in landfill leachates. Some works reported cationic bioaccumulation removal, with experiments carried out in SBR. Treatment times were of the order of days. For other works only biosorption was observed since experimental time was in the scale of hours.

The biosorption of chromium was proved through the reduction of Cr(VI) to Cr(III) during the bind to the AGS [74]. Kończak et al. (2014) [75] reported that combined divalent and trivalent cations, such as magnesium, calcium and iron play an important role in the formation of compact granules. In the same way, Yilmaz et al. [76] probed that iron ions (Fe^{2+}/Fe^{3+}) increase the diameter and

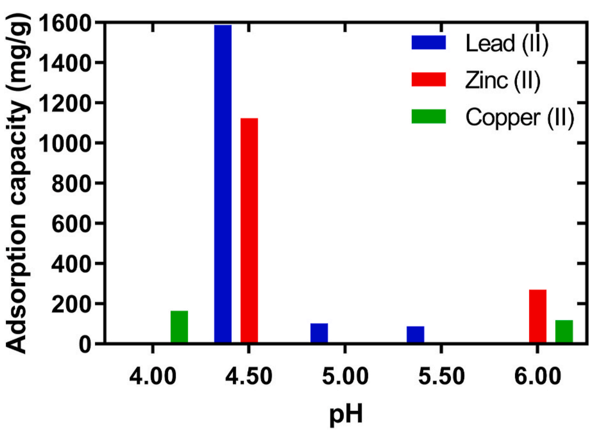

Fig. 6. The pH effect on the adsorption capacity of different metal cationic. (Data from literature in Table 4).

stabilization of AGS, however did not affect granulation time. Both studies consider polyvalent cations in the granulation process; however, they do not evaluate the biosorption capacity of AGS. Biosorption of zinc and copper was proved for the first time by Xu et al. [77] yielding an individual biosorption capacity of 180 mg/g and 246 mg/g, respectively. The carboxyl and hydroxyl groups on AGS have been related to excellent biosorption of lead [39]. Different values of lead biosorption capacity have been reported 44.3–1.59·10^3 mg/g; the wide range is due to (1) the degree of humidity and (2) the EPS content of the sludge [39,78].

Removed cations can exert a biological influence on the AGS consortium that are, in some cases, related to the bioaccumulation process. Zhang et al. [79] found that boron assimilation accelerated AGS growth, resulting in improved settlement performance and increased sludge concentration. Likewise, manganese has been reported as both stimulator and suppressor of the granulation process [80,81].

Most published works report adsorption capacities at pH between 3.0 and 7.5 (Table 4). The pH effect on the adsorption capacity of lead (II), zinc (II) and copper (II) are analyzed in Fig. 6. The highest adsorption capacity values were obtained at low pH (4.0–4.5), but this result is highly conditioned by the strategy used for the assays [39]. Liu et al. [39] studied the adsorption capacity of lead (II) and zinc (II) at pH 4.5 linking it directly the EPS adsorption capacity. In fact, they demonstrated at low pH that functional groups in EPS such as carboxyl (pKa ≈3.0), phosphoryl (pKa ≈6.5), amine (pKa ≈8.4) and hydroxyl (pKa ≈10.3) groups are responsible for the high metal adsorption capacity of AGS [39,78]. At extremely low pH the functional groups are protonated decreasing the adsorption of metal cations due to the electrostatic repulsion. For moderate pH, the functional groups are deprotonated with an increase in the adsorption capacity. On the other hand, very high pH produces metal precipitation [39,78]. This situation can be represented in Fig. 6, herein a pH of 4.5 the highest adsorption is produced. However, the highest values is produced by the use of EPS from AGS [39], then it is a need to normalize the experimental designs used by several authors. In fact, few works analyze the surface charge of AGS where the point of zero charge (PZC; isoelectric point). PZC is the pH value at which there is no surface charge [39]. PZC is an important parameter often used to characterize the adsorption properties of materials determined by potentiometric titration [82,83]. Besides, the specific surface of granules -that is linked to operating conditions-has not been precisely measured. For instance, reliable techniques for this like the Brunauer–Emmett–Teller (BET) method has not been reported so far, unless the AGS undergoes some pretreatment before being used as biosorbent material [42]. Probably, the extreme conditions of pressure and temperature in which this technique is performed have delayed its application [84].

Despite the existence of several reports of the removal of polyvalent cations by the granular sludge, there are several issues that limits it use for industrial applications: (1) cationic pollutant adsorptions have been reported almost exclusively in synthetic substrates; (2) There are only a few studies that evaluate the adsorption of several cations at same time by AGS; (3) Some works report qualitatively the sorption of the cationic pollutants, therefore the kinetics for some the cations are unknown; (4) Common techniques such as PZE and BET surface area must be studied to validate the quality of AGS such as adsorption material or other technique should be essayed and adapted.

When comparing the performance of chemical and sorption processes Table 5 and Fig. 7 shows different adsorbents with the ability to remove cationic metals. Different adsorption material such as chitosan, coal, and silica have been used. The silica has been the most used as solid support due to their important advantageous properties (fast metal sorption, good selectivity and mechanical stability, etc.) [85]. Chitosan is the only environment-friendly and biodegradable sorbent material that can be compared with AGS [86,87]. Chitosan, similarly to AGS, presents great adsorption potential by amino and hydroxyl functional groups [86]. However, in several studies, chitosan were physically and chemically modified [86,87]; unlike AGS which has been studied as granules without prior treatment, mainly. In addition, only Chromium (VI), Iron (III) and Boron (III) showed a higher adsorption capacity in other biosorbent materials than AGS (Fig. 7). Then, AGS is a biomaterial with a high potential to remove cationic compounds even compared to adsorbent of chemical and commercial origin. AGS to be used as sorbent can be obtained from wastewater treatment plants since sludge are continuously discarded.

Additionally, some studies evaluated the capacity of granular aerobic sludge to remove organic matter, ammonia and phosphorus (among others) from landfill leachate [8,88–90]. Carbon removal rate has been reported with very low values from landfill leachate [89]. Indeed, so far there are no scientific reports about the removal of polyvalent cations with AGS from leachate. However, this may be due to the fact that the granular sludge was used specifically for the removal of nutrients in landfill leachate, ignoring its ability to adsorb the cations typically present in this type of effluent (examples were shown in Tables 4 and 5). As was discussed above, this type of technology could not only be used to remove nutrients, necessary for the treatment of discharge, but also to recover cations, including valuable metals, for example from substrates as complex as landfill leachate.

5. AGS technology challenges: observations

Many studies of nutrient and cations removals using the AGS technology have been reported. On the other hand, the reduction of operational time of the start-up stage in AGS technology using (1) autoinducers and (2) the generation of artificial granules have been discussed. However, it has been detected that there are still some challenges in the application of AGS for fast start-up, nutrient and cationic pollutant removal for industrial wastewater treatment.

(1) AGS has been demonstrated to be an effective means for removing organic compounds through biodegradation mechanisms. A similar process can use to remove nitrogen, but only if the aerobic granular is PN-A. However, there are complex substrates with a high organic and nitrogen load that could remove by AGS with the correct selection of an operational strategy that allows the development and maintenance of nitrifying sludge in AGS.

Table 5
Different adsorbents with the ability to remove cationic metals.

adsorbent	Ref.	Adsorption capacity (mg/g)						
		Lead (II)	Zinc (II)	Copper (II)	Chromium (VI)	Manganese (II)	Iron (III)	Boron (III)
tetraphenylporphyrin in silica (SiNTPP)	[85]	55.2	34.6	19.1				
Dithiocarbamate	[92]	42.2	26.0	25.0				
Cetyltrimethylammonium bromide	[82]			32.2				
P (TA-TEPA)-PAM-RGO	[96]				394			
TGOCS	[97]				220			
Fe_3O_4@A/PDA	[83]				284			
modified coal gangue (MCG)	[98]					24.4		
DETA-MCM-41	[99]					88.9		
SiO_2-G2.0	[104]						21.2	
chitosan films	[86]						140	
Chitosan (CCTS)	[87]							35.1
Glycidyl methacrylate–methyl methacrylate–divinylbenzene (GMA–MMA–DVB)	[105]							23.2
Silica-polyallylamine composites (SPC)	[106]							16.8

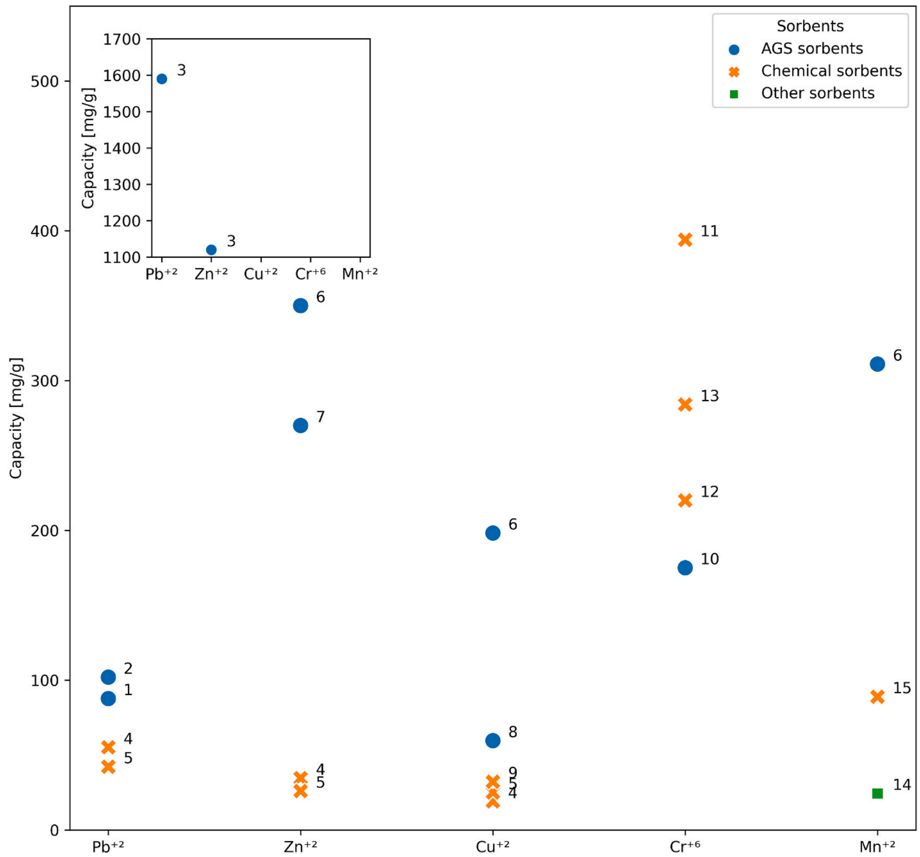

Fig. 7. The adsorption capacity of different adsorbent materials with the ability of recovery important metals. (1) [78]; (2) [66]; (3) [39]; (4) [85]; (5) [92]; (6) [93]; (7) [94]; (8) [95]; (9) [82]; (10) [74]; (11) [96]; (12) [97]; (13) [83]; (14) [98]; (15) [99].

(2) Higher organic load rate operation produced greater granular size. Therefore, operation strategies that ensure the diameter increase in the AGS should be studied. Thus the size of the reactors can reduce without losing removal efficiency.
(3) The elimination of cations by biosorption mechanisms has been studied without considering the relevance of bioaccumulation. Therefore, further studies on the inclusion of bioaccumulation and how it might influence cation assimilation processes are needed.
(4) The biosorption of metals by AGS shows higher capacity than some conventional sorbent materials. However, the application of AGS as a biosorbent material for valuable metals has been carried out exclusively on synthetic substrates. Adsorption and

desorption units must be investigated in the metal recovery. In addition, after the desorption process, the recoverability of biosorption and biodegradation properties should be evaluated. Also, the study of the phenomena that dominate mass transfer is inconclusive and has been based on exclusively empirical methods.

(5) The use of self-inductors such as AHL stimulates a fast start-up. However, they have a high commercial cost that conditions their use. The use of AHL recovered from discarded AGS could contribute to their industrial application.

(6) The use of alginate as an immobilization technique of conventional sludge can be used in granular sludge generation. It is necessary to study wastewater properties such as landfill leachate with a high calcium concentration that could contribute to cross-linking alginate polymers.

6. Conclusions

The biodegradation, biosorption and bioaccumulation properties of AGS could be used in the treatment of complex substrates such as they show wide ranges of concentration of organic matter, nitrogen, and polyvalent cations. The "product" (biosorption properties) and "process" (biodegradation properties) AGS can be used to complete the treatment in this type of streams. Production of AGS can be undertaken through wastewater facilities at low or zero cost, making it an attractive solution for complex substrates such as landfill leachates.

Notwithstanding, there are clearly several areas to deserve more attention. So far, cationic metal adsorption mechanisms have been accomplished almost exclusively in synthetic substrates; experiments with cationic metal in a complex matrix has not been reported and evaluation of cation selectivity has not been realized, yet. Another relevant issue is the start-up stage of AGS, whose biomass should be obtained from other existing reactor. To fast-up this stage, the use of autoinduction through quorum sensing or the use of artificial alginate granule are promising alternative strategies.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

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