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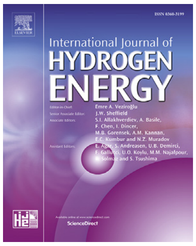

Biogranules applied in environmental engineering

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ABSTRACT

The efficiency of wastewater treatment with renewable energy generation has been greatly improved with the development of biogranules. In this review article, various types of biogranules (methanogenic, hydrogenic, aerobic, anaerobic ammonium oxidation, and oxygenic photogranules) applied in environmental engineering are introduced along with their history, theories on how they are formed, physico-chemical and morphological characteristics, and the effects on enhanced performance. Although each individual granule has its own characteristics, there might be something in common that the formation is related with high production of extracellular polymeric substances, and they all have high hydrophobicity, settling velocity, and density. To our knowledge, this is the first review article dealing with various types of biogranules. The information given herein will provide a chance for a deep understanding on biogranules in both fundamental research and engineering point of views.

Introduction

In the last decades, the generation of wastewaters (domestic and industrial) has been rapidly increasing with population growth, urbanization, and industrialization, and the amounts produced are already far beyond the self-cleaning limit of natural aquatic systems. Thus, it is essential to treat such waste streams before discharge to protect human populations and eco-systems, as well as to improve our environmental quality [1]. Carbon, nitrogen, and phosphorous are, in general, the main targets to be treated, and are removed through

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Abbreviations

AmGs	Anammox granules
Anammox	Anaerobic ammonium oxidation
ArGs	Aerobic granules
AUSB	Aerobic up-flow sludge blanket reactor
COD	Chemical oxygen demand
EGSB	Expanded granular sludge bed reactor
EPS	Extracellular polymeric substances
HGs	Hydrogenic granules
HPB	Hydrogen producing bacteria
HRT	Hydraulic retention time
LCA	Life cycle assessment
MGs	Methanogenic granules
OLR	Organic loading rate
OPGs	Oxygenic photogranules
SBR	Sequencing batch reactor
SRB	Sulfate reducing bacteria
UASB	Up-flow anaerobic sludge blanket reactor

physical, chemical, and biological units. In particular, biological treatments play an important role, being responsible for removal of more than half of all total organic pollutants in wastewater. They are also considered environmentally friendly technologies owing to less chemical use, and energy input, as well as renewable energy generation under certain conditions [2].

In biological treatment units, it is better to retain dense biomass to ensure the effectiveness of treatment and increase economic feasibility. Especially, it can directly determine the success of operation when slow-growing microbial species such as methanogenic and Anammox archaea perform main function [3,4]. The separation of cell retention from hydraulic retention has traditionally been carried out by settling of biomass and recycling them to the reactor. However, to reduce the footprint, advanced technologies such as use of immobilizing matrix, centrifugal systems, membrane filtration units, or other external sources of materials have been developed. Instead of using extra equipments, microbial granulation can be applied as an alternative method to attain high treatment efficiency [5].

Biogranules are discrete well-defined cell aggregates formed by cell-to-cell attraction that usually occurs in up-flow type reactors. Compared to conventional microbial flocs, biogranules have regular, dense, and strong structure with excellent settleability, enabling high cell retention, and the ability to withstand a high OLR [6]. The first biogranules discovered in the environmental field were used to treat industrial wastewater under anaerobic condition converting organics to CH_4 [3]. Until the end of 1990s, the main research on microbial granules was conducted for these MGs; however, this has expanded to various microbial processes, such as aerobic/anaerobic wastewater treatment, bio-H_2 production, Anammox, and photosynthesis. Color images with main reaction of each biogranules are shown in Fig. 1 [7–10]. It seems that each biogranules have different shapes, colors, and sizes, which might have resulted from different microbial species involved and formation mechanisms. There have been a few review articles of each individual biogranule type [11–13]; however, to our knowledge, no reports have investigated various biogranules applied in environmental engineering at the same time.

Therefore, this review was to introduce various biogranule types with their history, theories on how they are formed, physico-chemical and morphological characteristics, and their effects on enhanced performance by applying them. Moreover, future research required for each biogranule type is briefly addressed. The information here will enable a deeper understanding on biogranules from both fundamental research and engineering point of views.

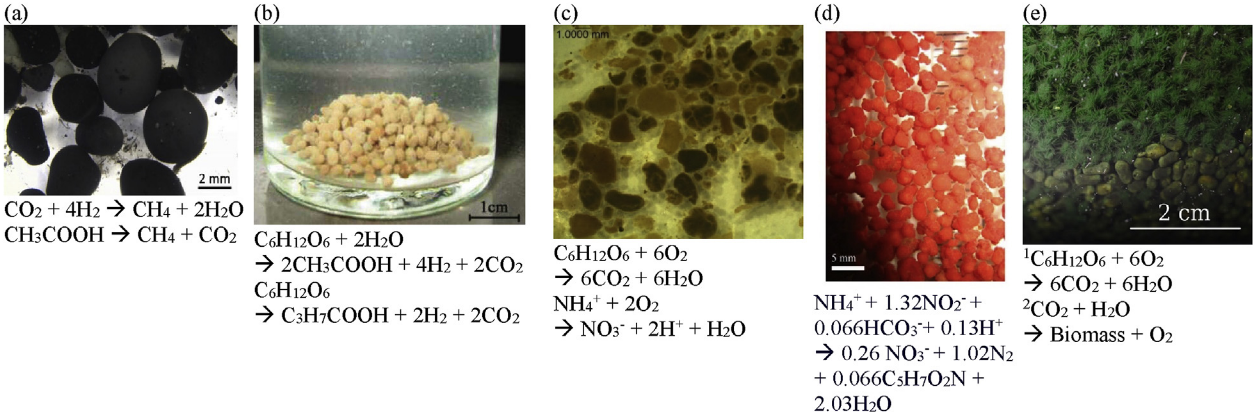
Various granule types

Methanogenic granules

History/theory

The first MGs were discovered in a novel high-rate reactor, known as an UASB, in 1976 by Gatze Lettinga's group at Wageningen University in the Netherland. This system was used to treat sugar beet wastewater with an OLR of 15–40 kg COD/m^3/d at HRT 3–8 h in a 6 m^3 pilot plant [3]. The advent of UASB with a core component of MGs has revolutionized the conventional wastewater treatment process and become the most popular high rate reactor configuration for anaerobic wastewater treatment.

The success of an UASB highly depends on the establishment of healthy and strong biogranules, and the long start-up period (2–8 months) for developing MGs is considered the major drawback. To enhance the understanding of granulation process, many mechanisms and models have been introduced based on various perspectives as summarized in Table 1 [3,14–31]. The first proposed MGs model was an Inert Nuclei Model developed by Lettinga et al. [3]. In the presence of microsize inert materials, anaerobic bacteria can attach onto inert particles to form an initial biofilm. Subsequently, granules mature through the growth of these attached bacteria. Hulshoff et al. [14] proposed the Selection Pressure Model, in which microbial granulation is the result of a continuous sludge selection through washing out light and dispersed particles and retaining heavier biomass in the UASB. As the surface of bacteria is negatively charged, reducing the electrostatic repulsion between negatively charged bacteria by introducing multi-valence positive ions, such as Al^{3+}, Ca^{2+}, Fe^{2+}, and Mg^{2+} was proposed as a granulation promotion method [16,17]. In addition, based on the microstructure of MGs under scanning electron microscope, Wiegant [23] proposed a Spaghetti model, in which the development of MGs is initiated by attachment of filamentous *Methanosaeta* on small flocs, followed by the formation of a three dimensional network through a branched-growth process. However, some models are known to be only applicable under specific conditions, and opposite observations have been widely reported. Furthermore, the validity of several suggested models is reportedly confined to only the initial stage of granulation, not the entire process. To accommodate the entire formation process of MGs, the following 4-step general model derived from the aforementioned previous models has been developed [11].



(a) $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
$CH_3COOH \rightarrow CH_4 + CO_2$

(b) $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$
$C_6H_{12}O_6 \rightarrow C_3H_7COOH + 2H_2 + 2CO_2$

(c) $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$
$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$

(d) $NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 0.26\ NO_3^- + 1.02N_2 + 0.066C_5H_7O_2N + 2.03H_2O$

(e) $^1C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$
$^2CO_2 + H_2O \rightarrow \text{Biomass} + O_2$

Fig. 1 – Biogranules applied in environmental engineering: (a) methanogenic granules [7], (b) hydrogenic granules [8], (c) aerobic granules [9], (d) Anammox granules [10], and (e) oxygenic photogranules (1: heterotrophic reaction, 2: photosynthesis).

Step 1 Initial contact among bacteria or attachment onto nuclei by physical movement.
Step 2 Maintenance of stable multi-cellular contacts by various attractive forces.
Step 3 Maturation of cell aggregation by various microbial activities.
Step 4 Shaping of aggregated cells (granules) by hydrodynamic shear force.

Characteristics/performance

The diameter of MGs varies from 0.14 to 5.0 mm depending on the type of wastewater and operating conditions. Similar to diameter, the shape of MGs widely varies, but they usually have a spherical form [32]. The color of MGs is normally black because they contain metal sulfides, and SRB. At 1990s, based on the microscopic observations, the microbial composition of MGs was believed to be different in each layer. The inner layer mainly consists of acetoclastic methanogens such as *Methanosaeta* sp., which may play an important role in initial granulation because of their filamentous shape. H_2 producing bacteria and utilizing methanogens (hydrogenotrophic methanogens) are dominant in the middle layer, while mixed microorganisms (HPB, SRB, hydrogenotrophic methanogens) predominate the outer layer [33]. However, several conflicting results (non-layered structure) have been also reported, which suggests that the structure of MGs is determined by dominant catabolic pathways [11].

It is not an exaggeration to state that the physico-chemical properties of MGs are directly related to process performance since they can directly influence not only substrate and nutrient transportation into MGs, but also maintenance of biomass concentration inside the reactor [34]. The typical settling velocity is between 18 and 50 m/h, which is much higher than those (0.6–15 m/h) of activated sludge [6]. According to Mu et al. [35], the porosity of MGs ranged from 0.64 to 0.90, indicating they were highly porous.

In the beginning, UASB was primarily applied to domestic wastewater treatment in tropical regions, and then its application has been broadened to various types of industrial wastewaters (such as breweries and beverages, distilleries, chemical, pulp and paper, food, landfill leachate, etc.) across the world [5]. Thereafter, numerous efforts have been made to maximize the process performance via modification of reactor configuration. Up to date, the best well known modified version of UASB is EGSB, developed in 1994. According to data base observation [36], the average OLR of 198 EGSB was over 20 kg COD/m^3/d, which was two times higher than the average OLR (10 kg COD/m^3 d) of 682 UASB. In addition, Yamada et al. [37] reported a multi-staged UASB, in which stable operation (>80% of COD removal rate) was maintained at 60 kg COD/m^3/d of OLR. The greater efforts to develop a novel reactor towards better process performance will be apparently given and MGs will be arguably main components in anaerobic wastewater treatment.

Hydrogenic granules

History/theory

There are several biological routes for H_2 production, but the formation of granules has exclusively been reported in dark fermentation [12]. It is the initial step of anaerobic digestion, in which organic polymers are converted to organic acids. Since COD removal is limited to less than 20% during the process, it has gained more attention in terms of clean energy generation rather than waste treatment. Unlike MGs, HGs were first observed in a completely-stirred-tank reactor operation, where high shear force is applied [38]. The flocculated-sludge turned into granules with a diameter and settling velocity of 1.6 mm and 50 m/h, respectively, within 80d under operating conditions of 26 °C, pH 5.5, and HRT 6 h. Later on, however, most of HGs were found in up-flow type reactors, in which higher cell retention, and thus higher cell-to-cell contact is expected.

Whatever the reactor might be, HPB can self-aggregate by the action of EPS. Under neutral and weakly acidic condition, the cell surface is generally negatively charged, creating repulsive force. However, it is known that functional groups associated with the EPS of one bacteria increase ionic interactions between oppositely charged functional groups in the EPS of other bacteria, leading to the formation of a bond

Table 1 – Summary of granulation models for methanogenic granules.

Category	Model	Brief mechanism	Reference
Physico-chemical approach	Inert nuclei model	The existence of nuclei or microsize biocarrier is necessary for granulation	[3]
	Selection Pressure model	Granulation is a protective microbial response against high selection pressures (light and heavy biomass)	[14]
	Attrition model	The origin of granules is fines formed by attrition and/or from colonization of suspended solids	[15]
	Multivalence Positive Ion-Bonding model	Granulation is the results of reduced electrostatic repulsion between negatively charged bacteria by addition of multivalence positive ion	[16,17]
	ECP Bonding model	Extracellular polymers not only mediate both cohesion and adhesion of cells but also change the surface negative charge	[18]
	Synthetic and Natural Polymer-Bonding model	Supplement of polymers promotes granulation	[19]
	Secondary Minimum Adhesion model	Granulation starts from the self-immobilization of bacteria through reversible adhesion and followed by irreversible microbial interaction	[20]
	Local Dehydration and Hydrophobic Interaction model	Local dehydration of bacterial surface and increased bacterial hydrophobicity promote the granulation	[21]
	Surface Tension model	Lower surface free energy of bacteria than that of liquid favors granulation	[20]
Structural approach	Capetown model	Excessive production of extracellular polymers is a key of initial granulation	[22]
	Spaghetti model	Granulation is initiated by attachment of Filamentous *Methanosaeta* on precursors and then followed by a formation of network in which other microbes are entrapped	[23]
	Syntrophic Microcolony model	The driving force for granulation is the needs for bacterial survival or functions	[24]
	Multilayer model	The microbial composition of methanogenic granules is different in each layer	[25]
	Ecological model	Granulation starts by covering filamentous *Methanosaeta by* other shape microbes	[26–28]
Other approaches	Proton Translocation-Dehydration model	Bacterial proton translocating activity at bacterial surface is a key of granulation	[29]
	Cellular Automation model	This model was suggested to describe the formation of microcolonies and biofilms	[30]
	Cell to Cell Communication model	Signal exchange (quorum sensing) among individual cells affects the spatial structure of methanogenic granules	[31]

between two cells. The specific production of EPS by HPB is 2–10 times higher than that of methanogens with a high content of carbohydrate [38,39]. Jung et al. [40] showed a gradual decrease of protein/carbohydrate ratio in EPS production from 2.0 to 0.2 with the increase of granule diameter. EPS also can determine the hydrophobicity properties of granule surface since it contains charged groups and apolar groups [41]. The water contact angle of sludge increased after the formation of mature HGs from 31° to 43°–54° [8,42]. The addition of divalent ions such as $Ca^{2+}/Mg^{2+}/Fe^{2+}$ is known to trigger the granulation process, neutralizing the negatively charged bacteria surface with an increase of hydrophobicity [43]. From a thermodynamic point of view, an increase in the hydrophobicity of sludge surface causes a decrease in the excess Gibbs energy of the surface [44].

Flocculated sludge has often been used as a seeding source for HGs formation, but there have been a few attempts to use MGs, to efficiently use the already-created rigid structure. However, pretreatments such as heat-shock or chemical (bromoethanesulfonate and chloroform) addition were crucial to suppress the activity hydrogenotrophic methanogens in MGs [45,46]. As operation went on, the rigid granule structure remained with high hydrophobicity, but the color was changed from black to white (or creamy), indicating a wash-out of sulfate reducing bacteria and methanogens.

Characteristics/performance

While MGs have normally dark color because of the sulfidogenic activity under alkaline condition, HGs normally have white or creamy-white color (Fig. 1(b)). The average size of HGs is dependent on environmental conditions such as reactor configuration and HRT or operation time, and mostly ranges from 0.4 to 3.5 mm [8,38,47,48]. Also, the size distribution and particle density are significantly relative to and vary across the different heights of up-flow reactor type [49]. HGs have a highly porous structure with multiple cracks on the surface that are likely to facilitate the transportation of nutrients and substrates as well as the release of H_2. Unlike MGs that usually formed as a multi-layered structure, HGs have a non-layered structure because of the simplicity of the acidification process [38,50]. A close examination of bacterial community analysis revealed that *Clostridium* sp., well known H_2-producing bacteria, were predominantly presented in HGs. Other H_2-producing bacteria such as *Klebsiella* and *Enterobacter* are also often detected, which can act as oxygen consumers to maintain an anaerobic condition within HGs [51,52]. The settleability of HGs is a critical factor to determine reactor performance and system stability under various operating conditions. Normally, HGs have higher settling velocity of up to 75 m/h than those of flocculated sludge, which can be attributed to their larger size and compact structure [8,38,53].

The main purpose of granulation is to treat high OLR with dense biomass. The performance in terms of volumetric H_2 production rate and H_2 yield with operating conditions is shown in Table 2 [8,38,42,54–60]. Various types of carbohydrates were used as feedstock to form HGs under a wide range of temperature (mesophilic, thermophilic, and even hyperthermophilic). Lee et al. [55] developed a carrier-induced granular sludge bed reactor that contained carrier matrices on the bottom to stimulate granule formation according to their previous finding that self-flocculated sludge formed when the bed porosity was high (>90%) and the HRT was low (<4 h), and achieved the maximum H_2 production rate of 9.3 L H_2/L/h at HRT 0.5 h. Similarly, Wu et al. [54] designed a reactor containing silicone-immobilized and self-flocculated sludge and obtained the highest H_2 production rate (15 L H_2/L/h) ever documented. The OLR in that condition was extremely high, reaching 1920 g COD/L/d. A high concentration of biomass up to 35.4 g/L was maintained even at HRT 0.5 h. Up to a certain point, it seemed that granule size was increasing under high OLR condition. This is generally accompanied by a high up-flow velocity, and creates an intensive hydro-dynamic force that results in an active mass transfer and stimulus of EPS production [47,48].

Aerobic granules

History/theory

ArGs were first reported in an AUSB where their formation was hypothesized to occur as a result of filamentous bacteria tangled with each other to form granules [61]. The schematic mechanism of aerobic granulation in SBR was also proposed based on the growth of filamentous fungi as a prerequisite step of granulation [62]. It has recently been widely accepted that aerobic granulation is a gradual process that occurs from seed sludge to ArGs [63].

ArGs have been successfully cultivated only in SBR, and it could be the key of aerobic granulation mechanism. SBR operation has several distinct features compared to conventional suspended activated sludge process, selection pressure and aerobic starvation period. The settling time is a major hydraulic selection pressure on the microbial community. A short settling time preferentially selects for the growth of fast settling bacteria, while the sludge with a poor settleability is washed out [64,65]. Studies have demonstrated that ArGs are formed with a short settling time, and the granules with a larger diameter developed as shorter settling times were applied [66,67]. Another distinct feature of SBR is aerobic starvation phase, under which bacteria secrete more EPS and became more hydrophobic [68]. However, the granules cultivated with extended starvation period showed poor settling ability [69,70]. Thus, reasonable starvation duration was necessary for the formation and stability of ArGs in SBR.

The stressful environment in SBR operation brought the positive impact on microbial granulation process by sophisticated cooperative behaviors and intricate communication capabilities [71]. Recently, the involvement of signaling molecules (autoinducer-2 for gram-positive & negative; AI-2, N-acylhomoserine lactones for gram-negative; AHLs) in aerobic granulation has been revealed [72].

The changes in cell surface properties have significant effects on microbial adhesion and granulation. The role of EPS in aerobic sludge granulation has been investigated and it acts as network structure through chemical bonding and physical composing to promote the microbial aggregates formation [73]. Generally, it was widely accepted that the content of EPS in ArGs was much higher than that in the flocculant sludge and biofilm [67,74]. The higher content of protein in EPS could strengthen the formation and stability of microbial aggregates by improvement of surface hydrophobicity and electronegativity [67,73], as well as polysaccharide in EPS contributes greatly to the strength and stability of granular sludge by formation of cross-network structure with cells [75,76].

Characteristics/performance

Compared to conventional activated sludge flocs, ArGs have a defined shape such as a spherical or elliptical shape. The color of granules was mainly yellow but was also black colored depending on the chemical composition, microbial population, and dissolved oxygen [77,78]. The average diameter of ArGs varied in the range of 0.2–16 mm (Fig. 1(c)) [65,79], and the average roundness in terms of aspect ratio is reported as higher than 0.6, even up to 0.8 [80]. Generally, ArGs have a higher roundness and a lower roughness than anaerobic granules due to intensive hydrodynamic shear force [6].

ArGs have a high density and compact structure, which helps in the biomass retention. The general specific gravity of ArGs typically ranges from 1.004 to 1.065, which was higher than that for flocculent sludge (1.002–1.006) (Table 3) [81–83]. Consequently, the water content of the granules (94–97%) was much lower than that of flocculent sludge (>99%) [84]. The high density of aerobic granule was closely related with settling behavior of granule. The settling velocity varied from 15 to 90 m/h, even up to 130 m/h and was significantly higher than that of sludge flocs (7–10 m/h) [82,85]. The sludge volume index, another parameter of sludge settling ability, of ArGs is generally below 80 mL/g, and even as low as 20 mL/g [82,85].

Microbial aggregation into compact aerobic granules offers many benefits, such as excellent settleability, high and stable rates of metabolism, protection against inhibition, resistance to chemical toxicity, and long biomass retention time [86]. ArGs have high resistance towards toxic compounds due to their compact structure, since the mass transfer barrier provides a lower concentration of toxic compounds [87]. Therefore, wastewater treatment system using ArGs have been widely applied for high strength organic wastewater to obtain high OLR. This includes toxic wastewater streams containing phenol, pyridine, dichlorophenol, and methyl *t*-butyl ether (MTBE) [86,88,89].

Anammox granules

History/theory

Anammox is considered one of the latest additions to the biogeochemical nitrogen cycle discovered in the 1990s [90]. The initial existence of Anammox was predicted based on nutrient profiles and thermodynamic calculations in a wastewater pilot plant at Delft University of Technology in the mid-1990s [91]. The process involves nitrogen removal as dinitrogen gas directly from ammonia under anaerobic condition with autotrophic oxidation. Anammox bacteria are quite amazing in their ecophysiology, cell structure, and

Table 2 – H_2 production performances of biogranules-applied reactors.

Reactor configuration	Performance		Operating condition (Temp (°C)./HRT(h)/substrate)	Pretreatment for inoculum preparation	Reference
	Volumetric H_2 production rate (L/L/h)	H_2 yield (mol H_2/mol $hexose_{added}$)			
CSTR[a]	0.54	2.14	26/6/sucrose	–	[38]
CSTR[a]	15.00	1.75	40/0.5/sucrose	Thermal	[54]
UASB[b]	0.05	1.45	38/18/sucrose	–	[8]
CIGSB[c]	9.30	2.00	35/0.5/sucrose	Acid	[55]
CSTR[a]	3.20	1.80	37/0.5/glucose	Acid	[42]
UASB[b]	0.14	1.61	39/13/glucose	–	[56]
UASB[b]	1.75	2.82	55/24/starch	–	[57]
UASB[b]	0.05	2.47	70/26.7/glucose	Thermal	[58]
EGSB[d]	0.18	0.92	30/10/glucose	Thermal	[59]
UASB[b]	2.37	2.25	35/2/galactose	–	[60]

[a] CSTR = Completely stirred tank reactor.
[b] UASB = Up-flow anaerobic sludge blanket reactor.
[c] CIGSB = Carrier induced granular sludge bed reactor.
[d] EGSB = Expanded granular sludge bed reactor.

Table 3 – General physico-chemical characteristics of aerobic granules and oxygenic photogranules with flocculated sludge.

	Size	Density (g/cm^3)	Settling velocity (m/h)	Sludge volume index (ml/g)	Porosity	Water content (%)
Aerobic granules	0.2–16 mm	1.004–1.065	18–130	Below 80	0.68–0.93	94–97
Oxygenic photogranules	0.1–5 mm	Highly variable	36–360	nd[a]	nd[a]	78–95
Activated sludge floc	0.5–1000 μm (mostly <100 μm)	1.002–1.006	0.6–15	100–150	>0.95	>99

[a] nd = not detected.

ability to oxidize ammonium anaerobically [4]. Compared to the conventional biological nitrogen removal processes, Anammox technology provides advantages including energy efficiency with less operational costs and higher nitrogen removal efficiency due to its low dependency on oxygen, none organic carbon consumption, and less sludge production [92]. To improve biomass retention, the formation of Anammox biofilm or granules is important, which is governed by the self-immobilization of hydrated EPS matrix produced from microbes [93,94]. Researches on startup characteristics of Anammox seeded with anaerobic granular sludge are still inadequate, and the variable properties of anaerobic granular sludge are currently unclear [95]. To enhance the treatment of ammonia-rich wastewater, successful active granules enriched with Anammox bacteria are essential; however, little is known about growth of Anammox bacteria and multiplication of inside granules [96]. The behavior and characteristics of Anammox granule is an emerging issue of further research.

Characteristics/performance

A record extremely high volumetric nitrogen removal rate of 45.24 kg-N/m^3/d was noted after operation for 230 d [97]. This could be achieved with the successful granulation of the Anammox microorganisms leading to stable and higher nitrogen removal performance [98]. However, a granule size bigger than 2.2 mm is not efficient for higher nitrogen removal and also causes granule flotation [99]. The underlying cause is excess EPS secretions in the AmGs that block gas tunnels obstructing gas release from deep inside the granules and increasing the granule's buoyant force [100]. As the volume of these types of gas pockets increases with the increasing diameter of the anammox granules, leading to decreasing density and settleability, and eventually causing the granules to floats [99]. This process the chance of washout of anammox bacteria. The optimum granule diameter ranges from 1.0 to 1.3 mm with an N-loading rate of 0.8 kg N/m^3/d, which provides maximum N-removal efficiency [101]. Based on a theoretical formula deduced by Lu et al. [99], the diameter threshold delimiting floating from settling granules is 1.35 mm. Granules ranging from 1.0 to 1.5 mm in diameter exhibited the highest activity [100]. In this process, the appropriate substrate concentration of ammonia and nitrate are also important to satisfy the demand of anammox bacteria. N-removal efficiency decreases with increasing loading rate of nitrogen [101]. Low substrate concentration (NO_2^--N, 240 mg-N/L) at high flow rate leads to a higher to higher nitrogen removal rate than high substrate concentration and low flow rate [97]. High salinity and low ambient temperature also limits the activity and growth of bacteria. The cell lysis weakens the specific Anammox activity and strength of anammox granules [102]. The demonstration of successful Anammox granulation was already processed through the DEMON-cyclone which plays a major role in establishing the anaerobic ammonia oxidation into commercial process. Though DEMON-cyclone application in the Strass wastewater treatment plant was limited with the granule size smaller than 1 mm, it provided a visible impact on sludge composition towards a more reddish and granular appearance. The demonstration showed 80% improvement in Anammox activity (28.6 mg N/g VSS/h) compared to the non-cyclone fed process [103].

Oxygenic photogranules

History/theory

OPGs are the most recent addition to the family of biogranules for biotechnological applications. The formation of these granules was first documented in unagitated, sealed vials exposed to natural light by Park and Dolan [104]. The vials were initially filled with activated sludge, which transformed over a period of several weeks into photogranules. These granules were then used as inoculum in SBRs where rapid growth of new granules occurred. OPGs in the SBRs can treat wastewater without external aeration [104,105]. Photogranule-like aggregates have recently been reported in the literature as mostly aerobic granules covered with an eukaryotic algae layer [106–110]. These types of granule differ in spatial organization and community composition from OPGs discussed here.

For biotechnological applications like wastewater treatment, OPGs possess a number of properties that make them potential candidates for alternatives to the costly activated sludge process. Specifically, OPGs produce in-situ O_2 through photosynthesis that is directly available for the conversion of organic matter to CO_2 and the oxidation of nitrogen species. At the same time, the autotrophic growth of cyanobacteria generates easily biodegradable biofeedstock that is immediately available as source of renewable energy, for example through anaerobic digestion of waste biomass [111]. As common to all granules, OPGs can be easily separated from the treated water.

The ongoing development of bioprocesses using OPGs need to be accompanied by eco-design recommendations through LCA. This allows the identification of key parameters that eventually determine the environmental feasibility of a future bioprocess as demonstrated for example for biodiesel from microalgae [112]. These parameters should then become priority objectives in OPG development. Similarly, the economic feasibility of OPG bioprocesses needs to be carefully assessed as costs for a new process design are difficult to up-scale from laboratory- and pilot-scale operations.

Characteristics/performance

In all laboratory-scale operations using OPGs, granules with different types of morphologies were present at the same time, notably bald granules resembling fluvial pebbles and filamentous granules, loosely reminiscent of dreadlocks (Fig. 1(e)). Similar morphologies were also observed by Arcila and Buitrón in a related system [106]. For both granule morphologies, microscopy and high-throughput sequencing identified cyanobacteria of the order *Oscillatoriales* as dominant organisms in the phototrophic part of the granules. *Oscillatoriales* are known for their filamentous morphology and gliding motility [113]. These properties may be a key element to the formation of the dense, cloth-like phototrophic layer of the OPGs.

The co-existence of the two morphotypes hints at a functional link between the two granule populations, possibly through a temporal succession. Preliminary data suggests that filamentous granules may be an early developmental state towards the formation of bald granules. During maturation, granules increase their net density via compaction, i.e., the loss of filamentous cells, and through the precipitation of calcite in the interior of granules, especially when OPGs are grown in environments with hard water. In these situations, bald OPGs can reach densities of up to 1.5 kg/L with a volatile solids content of around 30% (per total solids), whereas the density of filamentous granules is closer to that of water with volatile solid contents of more than 70%. Resulting is a range of settling velocities between 36 m/h for small, filamentous granules, to more than 360 m/h for bald granules (Table 3).

The generation of oxygen through photosynthesis from cyanobacterial growth is essential for the maintenance of COD and nitrogen removal capacities in OPGs. At the same time, the released CO_2 resulting from heterotrophic activity serves as a carbon source for autotrophic cyanobacteria. Consequently, successful wastewater treatment using OPGs requires good control of the activities of multiple interacting microbial populations.

Overall, the OPGs technology possesses the potential for energy-positive wastewater treatment. Since OPGs technology requires light, there might be a difficulty to apply in sunlight-limited region, and build a treatment tank with big depth. Process development guided through eco-design principles and ecological engineering of microbial interactions may turn the OpGs process into a stellar example of innovative process engineering.

Summary and future study

Over four decades, MGs in anaerobic wastewater treatment processes have shown their ability to achieve a higher organic removal rate, lower sludge yield, and lower energy consumption along with valuable CH_4 production. To meet stringent environmental regulations with increased of wastewater strength at affordable costs, anaerobic treatment of wastewater will be oriented towards to better valuable resource recovery and energy savings. To accomplish this, new promising technologies (membrane technology, nano technology etc.) should be applied and optimized. In addition, to achieve complete nutrient (N, P) removal in an anaerobic system, biological and physical-chemical processes should be considered to be integrated. The greater efforts to develop a novel reactor towards better process performance will be rewarded and MGs will arguably be the main components in anaerobic wastewater treatment.

The formation process of HGs takes less than a month, and is much faster than that of other biogranules. They are generally formed in up-flow type reactors, and the formation begins with a large production of EPS (in particular carbohydrate compounds), which is triggered by the increased hydrodynamic force, and the presence of divalent ions. HGs have a non-layered structure, consisting mainly of *Clostridium* sp. and a high settling velocity up to 75 m/h. An enormously high H_2 production rate of 15 L H_2/L/h was attained by applying

HGs. The methods to form HGs are quite well established and scientific research on morphological, biomolecular, and physico-chemical characteristics of HGs have been well documented. However, the long-term and pilot-scale experiences have rarely been reported. Since many gaseous products including H_2 and CO_2 are rapidly produced, they could affect the fluid dynamics condition inside the reactor, and foaming will occur. In addition, dark fermentation is accompanied by the production of various organic acids, which would decrease the pH, resulting in a low H_2 yield. The pH inside up-flow type reactors is non-uniform, and therefore, special care is required on how to control the pH [114].

The feasibility study in pilot scales showed that the ArGs technology is very promising compared to activated sludge process due to its distinguished advantages, such as excellent settleability and small footprint. ArGs have already been successfully applied in full scale wastewater treatment processes [115–117]. However, there are still some limitations for wide application that need to be studied further. These include long granule formation and maturation time, poorly understood molecular mechanisms, granule disintegration, unpredictable granule morphology, and inefficient nutrient removal.

Recently, the newly discovered anammox process is considered to be an innovative process that can replace the biological nitrogen removal process in the future because it can greatly reduce energy compared to existing technologies. It is possible to achieve a high nitrogen removal rate through the formation of AmGs and is actively applied to the treatment of wastewater containing nitrogen at a high concentration. However, it is necessary to study the physiological characteristics of AmGs to enable its application to sewage treatment, which shows low nitrogen concentration (40–60 mg N/L) and low temperature.

Innovation and targeted improvement of bioprocesses using granulated biomass is very likely over the next years as a vast basis of mechanistic understanding of the granulation process has been established. Even entirely novel processes can be imagined, for example using the recently described oxygenic photogranules (OPGs). Oxygenic photogranules tightly couple in-situ oxygen generation through photosynthesis with oxygen-consuming processes like organic matter conversion and nitrification, eliminating the need for external aeration. Formation mechanisms of OPGs may be different from other biogranules discussed in this review and merit further research towards successful ecological engineering of an OPG bioprocess.

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