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Review

Research progress on enhancing the performance of autotrophic nitrogen removal systems using microbial immobilization technology

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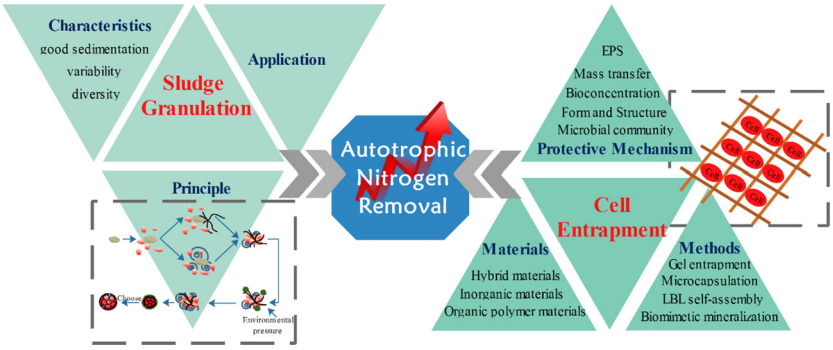
HIGHLIGHTS

- Research progress on granules in autotrophic nitrogen removal is reviewed.
- Options for entrapping materials and entrapping methods are discussed.
- The mechanism by which cell entrapment technology protects bacteria is analyzed.
- Further applications for cell entrapment in autotrophic N removal are proposed.

GRAPHICAL ABSTRACT



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ABSTRACT

The autotrophic nitrogen removal process has great potential to be applied to the biological removal of nitrogen from wastewater, but its application is hindered by its unstable operation under adverse environmental conditions, such as those presented by low temperatures, high organic matter concentrations, or the presence of toxic substances. Granules and microbial entrapment technology can effectively retain and enrich microbial assemblages in reactors to improve operating efficiency and reactor stability. The carriers can also protect the reactor's internal microorganisms from interference from the external environment. This article critically reviews the existing literature on autotrophic nitrogen removal systems using immobilization technology. We focus our discussion on the natural aggregation process (granulation) and entrapment technology. The selection of carrier materials and entrapment methods are identified and described in detail and the mechanisms through which entrapment technology protects microorganisms are analyzed. This review will provide a better understanding of the mechanisms through which immobilization operates and the prospects for immobilization technology to be applied in autotrophic nitrogen removal systems.

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Abbreviations: ANR, autotrophic nitrogen removal; AOB, ammonia-oxidizing bacteria; AAOB, anaerobic oxidizing bacteria; EPS, extracellular polymers; LBL, layer-by-layer; NOB, nitrite oxidizing bacteria; NRE, nitrogen removal efficiency; NRR, nitrogen removal rate; PVA, polyvinyl alcohol; SA, sodium alginate; WPU, waterborne polyurethane; MOFs, Metal-Organic Frameworks.

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1. Introduction

Autotrophic nitrogen removal (ANR) is an emerging biological nitrogen removal process developed in recent years that has the advantages of low energy consumption, no need for an organic carbon source, high nitrogen removal efficiency, and low greenhouse gas emissions (Li et al., 2019). The application of the ANR process (Eq. (1)) to wastewater treatment was first proposed by researchers at the Delft University of Technology in the Netherlands in 2002 (Sliekers et al., 2002). The ANR process includes two main biological processes. First, NH_4^+ is partially oxidized to NO_2^- (partial nitrification process, Eq. (2)) by ammonia-oxidizing bacteria (AOB), while NO_2^- is prevented from being oxidized to NO_3^- by nitrite oxidizing bacteria (NOB). Next, the remaining NH_4^+ and the produced NO_2^- are converted to N_2 (anammox process, Eq. (3)) by anaerobic ammonia-oxidizing bacteria (AAOB) (Chen et al., 2019b). In recent years, ANR has been widely used in the treatment of the wastewaters with high temperatures ($T \geq 30$ °C) and low C/N ratios, such as sludge digestion solution. To date, there have been hundreds of engineering cases across the world involving ANR, which have demonstrated its utility and potential for further application (Cao et al., 2017). However, engineering applications of this process under unfavorable environmental conditions, such as at low temperatures ($T = 10$–25 °C), in wastewaters with high C/N ratios, or in the presence of toxic substances, are still very limited (Cao et al., 2017). This is mainly because AAOB are sensitive to environmental conditions, and their growth rate is very slow (0.0026–0.0041 h^{-1}) (Ibrahim et al., 2016). Especially under adverse conditions, the metabolic activity, as well as growth and reproduction rates, of functional bacteria (AOB and AAOB) are inhibited, resulting in a decrease in the nitrogen removal efficiency of the reactors. In addition, microorganisms are easily washed out of the reactors (Ahmad et al., 2020), which leads to the loss of functional bacteria. Therefore, to expand the range of conditions under which ANR can be applied, it is necessary to improve the microbial growth rate and reduce loss of functional bacteria through biological washout, while simultaneously maintaining high microbial activity, even under adverse environmental conditions.

$$NH_3 + 0.85O_2 \rightarrow 0.11NO_3^- + 0.44\,N_2 + 0.14H^+ + 1.43H_2O \quad (1)$$

$$NH_3 + 1.5O_2 \rightarrow NO_2^- + H_2O + H^+ \quad (2)$$

$$NH_3 + 1.32NO_2^- + H^+ \rightarrow 1.02\,N_2 + 0.26NO_3^- + 2H_2O \quad (3)$$

Biomass retention is possible using immobilization technology, which can be divided into natural aggregation (granulation) and artificial immobilization (Ahmad et al., 2020). Granulation is a simple and natural carrier-free immobilization process (Ahmad et al., 2020). Studies have demonstrated that AAOB are able to autonomously select and fix cells, and then form granules (particle size ≥200 μm) (Ali et al., 2013). The granules formed by natural aggregation have good settling performance and can effectively be prevented from biologically washing out of the reactor (An et al., 2013). Moreover, in ANR reactors, the granules also have the characteristics of an aerobic outer layer and an anoxic inner layer. Through this feature, AOB, which require aerobic conditions, and AAOB, which require anoxic conditions, can be skillfully coupled together in a single granular sludge to achieve a balance (Cao et al., 2017), thus ensuring effective and stable reactor operation. At present, under suitable environmental conditions, the total nitrogen removal efficiency (NRE) can be stabilized at as high as 88%–95% using granules in ANR systems (Cheng et al., 2017; Lotti et al., 2014b; Winkler et al., 2012). However, under adverse conditions, the stability of granules is not always satisfactory. For examples, the granules may disintegrate at low temperature (Cao et al., 2017) or float up and thus cause sludge loss under high loading (Cao et al., 2017). Under dissolved oxygen shock, the activity of AAOB in the granules will be suppressed (Li et al., 2019). These problems may lead to a decrease in the nitrogen removal performance, or even instability and collapse of the reactors.

Artificial immobilization technology provides an effective way to solve these problems. Artificial immobilization fixes selected microorganisms on a limited carrier to maintain the high density and activity of the microorganisms (Partovinia and Rasekh, 2018). This artificial immobilization of microbes can be realized through adsorption, covalent bonding, cross-linking, entrapment, and encapsulation (Bouabidi et al., 2019). Among these, entrapment is widely used in the field of cell immobilization (Bouabidi et al., 2019). Cell entrapment aids in the separation of biomass from effluent and in the reduction of sludge flotation, leading to the complete retention of biomass (Isaka et al., 2017). Studies have shown that a porous network structure will be formed inside the carrier, which provides attachment sites for the growth and enrichment of AAOB (Wang et al., 2020c). By entrapping AAOB in a gel, reactor startup time was greatly shortened, and excellent nitrogen removal

performance was achieved (Tuyen et al., 2020). The total nitrogen removal rate (NRR) of an anammox reactor with entrapped biomass has been determined to reach 1.83 kg/m^3/d, which was 9.4 times that of a reactor with suspended sludge before entrapment (Wang et al., 2020a, 2020b, 2020c). Furthermore, the carrier can also alleviate inhibition of AAOB caused by salinity (Liu et al., 2020), oxygen (Benakova et al., 2018), loading shock (Chen et al., 2015), nitrite (Magri et al., 2012), and other adverse factors. For AOB, gel entrapment can also alleviate their inhibition by various inorganic salts (Yan et al., 2010), ameliorate the unfavorable influences of pH and temperature on AOB (Yan and Hu, 2009), and protect AOB from heat-shock treatment (Isaka et al., 2011), thus allowing AOB to outcompete NOB, realizing higher rates of nitritation, and providing sufficient substrate for the subsequent anammox process. Therefore, cell entrapment is expected to be one of the most effective means of improving ANR system performance.

Although cell entrapment technology has been studied for more than 20 years (Manonmani and Joseph, 2018b), cell entrapment research and applications in the field of sewage ANR are far from mature. Reviews focusing on the progress that has been made in research on cell entrapment for ANR are still lacking. Therefore, this review has the following objectives: (a) to review the impact of immobilization technologies (focusing on granulation and cell entrapment) on ANR reactors; (b) to compare, analyze, and summarize the effects of different materials and methods on entrapment microorganisms; (c) to distinguish and analyze the protective mechanisms of cell entrapment on functional bacteria in ANR systems.

2. Research progress on sludge granulation

2.1. Principle of granular sludge formation

There are different opinions on the mechanism underlying granular sludge formation, which include the selective pressure hypothesis (Tay et al., 2002), extracellular polymer hypothesis (Liu et al., 2004), crystal core hypothesis (Wan et al., 2015), biological self-aggregation hypothesis (Fang, 2000), and attrition hypothesis (Vlaeminck et al., 2010). These different hypotheses form the theoretical framework of granular sludge formation. All of these hypotheses involve the interactions between bacterial cells or those between bacterial cell surfaces and other substances, which involve repulsive electrostatic forces, attractive van der Waals forces, and repulsive hydration interactions. High cell hydrophobicity increases cell-cell interactions and forms dense granules (Manonmani and Joseph, 2018a, 2018b). In general, the formation of granular sludge is a complex process combining physical, chemical, and biological actions, and it could be believed that granular sludge is the product of the gradual self-immobilization of microbial cells.

First, microorganisms adhere, aggregate, and grow on the surfaces of substances in the reactor, such as proteins, small granular sludge, debris, or sediments (Chen et al., 2007; Manonmani and Joseph, 2018a, 2018b). During this process, microorganisms secrete viscous substances, such as extracellular polymers (EPS) and pectin (Liu and Tay, 2002), to promote mutual aggregation of bacteria. Simultaneously, the filamentous bacteria present in the reactor become entangled on the cell surfaces of functional bacteria, forming a network structure (Yamada et al., 2011), which further promotes the aggregation of microorganisms and the formation of a denser colony. Under external pressure, cells begin to secrete signal molecules. When bacterial density and the concentrations of signal molecules reach certain thresholds, quorum sensing is triggered to further promote the secretion of EPS (Zhang et al., 2019). EPS and microbial cells will form a network structure. In EPS, protein achieves adhesion between cells through chemical bonding, electrostatic attraction, hydrophobic interactions, and other interactions (Wang et al., 2020a, 2020b, 2020c), whereas polysaccharides in the matrix are cross-linked to form a three-dimensional network structure, fixing cells together (Seviour et al., 2012). The micelle forms a complex under the bridge between EPS and filamentous bacteria, and multiple complexes are finally integrated to form granular sludge (Xu et al., 2019). The large granules may decompose due to either internal decay or external wear resulting from shearing and collision, thereby forming new, smaller flocs or granules, and new large granules may be subsequently formed through the agglutination of these small flocs or granules. Similarly, a large granule extrusion could detach and grow as an individual granule (Vlaeminck et al., 2010; Manonmani and Joseph, 2018a, 2018b). When the wastewater contains positive ions (Ca^{2+}, Mg^{2+}, etc.), these positive ions can reduce the electrostatic repulsion between negatively charged bacteria, which is conducive to granule formation (Manonmani and Joseph, 2018a, 2018b). Finally, under the sheer force of the water flow in the reactor, the granular sludge will rotate slightly and entangle itself, making the granule structure more compact (Liu and Tay, 2002). The granular sludge formation process is depicted in Fig. 1, but the specific mechanism involved in granular sludge formation requires further exploration.

2.2. Characteristics of granular sludge

Sludge granules have the characteristics of regular morphology, good sedimentation, and size dependence. Anammox granules are mostly brick red, showing either spherical or ellipsoidal shape, which is related to the nitrogen load and shearing force (Li et al., 2013). The size of granules is generally 0.2–5.0 mm (Chen et al., 2014; Qian et al., 2017b; Xing et al., 2015), and the granule size affects the sedimentation performance and substrate utilization efficiency of the granules. According to their size, granules can be divided into floating zone (>4.5 mm), transition zone (2.5–4.5 mm), and non-floating zone (<2.5 mm) (Tan et al., 2020). However, other researchers believe that the non-floating granules are those with sizes less than 0.38 mm (Campos et al., 2017). The reason for this discrepancy may be that Tan et al. (2020) considered the influence of microbial cell lysis caused by insufficient substrate concentrations in their model. It is recognized that mass transfer resistance increases as granule size increases. Smaller granules have stronger mass transfer performance, whereas large granules accelerate bacterial autolysis in the core of the granule due to an absence of substrate, forming cavities (Tan et al., 2020) and resulting in reduced nitrogen removal performance. Therefore, controlling the granule size within a suitable range is essential for maintaining satisfactory sedimentation performance and substrate utilization by the granules.

Granules also have the characteristic of structural variability. During the process of self-immobilization, cavities and pores are formed inside and on the surfaces of the granules (Xu et al., 2019), through which the transfer of nutrients and metabolites between sludge and substrate can be achieved (An et al., 2013). Therefore, by physical state, granules can be divided into three parts: gas, liquid, and solid. The cavities and pores are occupied by gas and liquid, and the gas/liquid ratio changes periodically through the cycles of gas production and release (Xu et al., 2019). The solid part of granules is composed of both inorganic and organic matter, including bacterial cells, EPS, etc. The composition and proportion of these substances change during the self-immobilization process (Zhu et al., 2012). Therefore, during the process of granule formation and stabilization, the ratio of gas, liquid, and solid phases is dynamically changing, which affects granule size and sedimentation performance, thereby influencing the density and activity of functional bacteria.

Granules are also characterized by microbial diversity. In addition to AAOB-Planctomycetes, anammox granules contain other associated bacteria, such as Chloroflexi, Chlorobacteria, and Proteobacteria (Feng et al., 2018). Filamentous bacteria are entangled in the granules, bridging the glial mass and promoting granule formation (Yamada et al., 2011). In ANR systems, granules have aerobic outer layers and anaerobic inner layers. Their surface is often occupied by AOB, NOB, and denitrifying bacteria (Bhattacharjee et al., 2017). AOB and NOB consume oxygen on the surface of the granular sludge and thus prevent the internal AAOB from being exposed to oxygen. However, they will also compete with AAOB for substrates (Li et al., 2019). Therefore, the

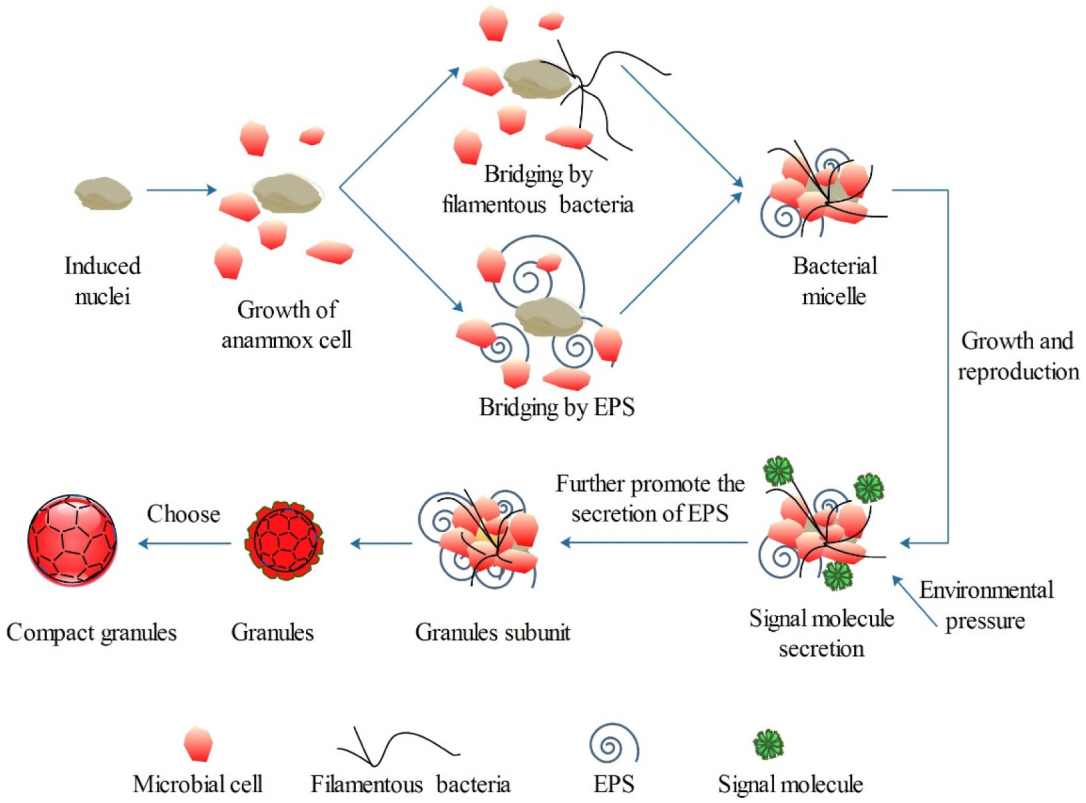

Fig. 1. Schematic diagram of the sludge granulation process.

microorganisms in granular sludge not only have symbiotic relationships but also competitive ones. Achieving the best balance and symbiosis between AAOB and other microorganisms in granules is key to maintaining the form of granules and ensuring efficient nitrogen removal in the reactors.

2.3. Application of granular sludge

Granular sludge in ANR processes has been widely used in laboratory studies (Chen et al., 2019a, 2019b), pilot-scale studies (Lotti et al., 2014a, 2014b), and even in full-scale operations (Cao et al., 2017). In studies conducted to date, high NRRs (0.2–0.9 kg N/m^3/d) and satisfactory NREs (70%–95%) with temperature variations between 17 and 23 °C have been achieved (Cao et al., 2017). This is because the granules have a good settling capacity and can reserve enough biomass for ANR systems to compensate for the slow growth of anammox bacteria (An et al., 2013). Additionally, granules can also buffer the negative impact of dissolved oxygen on AAOB (Qian et al., 2017a, 2017b) and improve the stability of functional bacteria during temperature shocks (Lotti et al., 2014a, 2014b). However, the inside of large granules (>2.5 mm) is susceptible to mass transfer restrictions (Tan et al., 2020). Due to the lack of substrate, the internal microorganisms are in an endogenous respiration state, which eventually leads to the formation of an internal cavity that causes the sludge to float. Studies have shown that the abundance of functional bacteria in the floating granules (~3.06 mm in diameter) is significantly lower than that of the sedimentation granules (~2.46 mm in diameter), and the cells are self-contained without a complete cell structure. The amount of microbial activity in floating granules is only half of that in the sedimentation granules (Tan et al., 2020). Therefore, granular size has a significant impact on microbial activity. Research has also found that the granular sludge structure becomes looser at low temperatures, resulting in deeper oxygen penetration (Cao et al., 2017). Therefore, controlling the form of sludge has also become a tedious task (Ahmad et al., 2020). Excessive removal of the flocs produced by the disintegration of granules will cause the loss of functional bacteria, and ultimately lead to system instability. However, experiments have found that in the presence of flocs, part of the habitat of AOB is changed from granular sludge to flocculent sludge (Li et al., 2020), and the mass transfer restrictions on it are reduced to a certain extent (Cao et al., 2017). The flocs can consume large amounts of dissolved oxygen in the reactor to avoid further oxygen poisoning of granules. Furthermore, experimental evidence has indicated that, when flocs-granules hybrid systems are used to treat wastewater containing high ammonia–nitrogen (655 mg L^{-1}) at 27 ± 2 °C, the NRE is as high as 92.1% (Luo et al., 2017); when treating wastewater containing low ammonia–nitrogen (40 mg L^{-1}) at 15 °C, an NRE of 83% can be achieved (Li et al., 2019). By using a cyclone separator, stable operation of a flocs-granules hybrid system also can be realized in a full-scale reactor (Shi et al., 2016). Therefore, the development of a flocs-granules hybrid system is one of the ways to solve the difficulties in controlling granule size and maintaining granule shape. Another measure used to maintain the sludge form is entrapment, in which the sludge is fixed inside the carrier.

3. Introduction of cell entrapment technology

Cell entrapment generally uses physical or chemical methods to wrap free cells either in a gel or in a semipermeable polymer film (Jen et al., 1996). Compared with free cells or granules, entrapped sludge has many advantages, such as providing high biomass, strong mechanical strength, and great protection of microorganisms from toxins (Bouabidi et al., 2019). Entrapped sludge generally does not float, which effectively prevents the loss of microorganisms (Manonmani and Joseph, 2018a, 2018b). At present, cell entrapment has been successfully applied to the treatment of a variety of wastewaters (Bouabidi et al., 2019), including wastewaters containing refractory organics (Banerjee and Ghoshal, 2016), heavy metals (Yu et al., 2020), and high amounts of nitrogen, phosphorus (Dong et al., 2017a; Zhu et al.,

2009), or industrial dyes (Park et al., 2006). It is an applicable and feasible option to improve wastewater treatment efficiency and maintain long-term, stable reactor operation.

The selection of the carrier material and the entrapping method are very important in the cell entrapment process, as it influences microbial activity, which in turn affects the efficiency of wastewater treatment. The ideal entrapping carrier should have the advantages of biocompatibility, low toxicity, stable structure, good mass transfer performance, long service life, and low cost (Leenen et al., 1996; Weiser et al., 2016). The type of carrier material also determines the method of entrapping. If organic polymer gel materials are chosen to entrap microorganisms, gel entrapping is often used (Ahmad et al., 2020). If a thin layer of membrane material is chosen, encapsulation is used for enclosing microorganisms (Tay et al., 2002). With further research and development, hybrid materials such as metal-organic frameworks (MOFs) have gradually emerged as carriers (Ji et al., 2018), and entrapping methods such as layer-by-layer (LBL) self-assembly (Liu et al., 2019) and biomimetic mineralization (Abdelhamid and Pack, 2020) have gradually evolved, which introduce functional groups according to the physical structure formed by the microorganisms (Amigoni et al., 2009; Jesionowski et al., 2014) and have good application prospects.

Whether it is self-immobilized granules or entrapped sludge, immobilization has an important contribution in maintaining reactor stability and improving reactor nitrogen removal performance. Fig. 2 shows the morphological characteristics of flocs, granules, and entrapped beads in ANR systems. Table 1 compares the performance of reactors with different sludge forms.

4. Entrapment materials

4.1. Inorganic materials

Inorganic carrier materials, such as activated carbon, phosphates, and SiO_2, generally immobilize microorganisms through adsorption or charge effects (Bouabidi et al., 2019). Among these inorganic carriers, SiO_2 has received the most attention (Hartmann and Kostrov, 2013; Jesionowski et al., 2014). According to requirements, SiO_2 can be included in materials with different pore diameters (e.g., small pores, mesopores, and macropores) (Hartmann and Kostrov, 2013; Thorn et al., 2011), adsorbing cells by its characteristics of large specific surface area and porosity. The interactions between inorganic materials and microorganisms, such as van der Waals forces, ionic forces, and hydrogen bonds, are weak, which leads to a high rate of leakage of cells during use (Bouabidi et al., 2019). Therefore, inorganic carriers are infrequently used in cell entrapment.

4.2. Organic polymer materials

Organic polymer materials are mainly divided into natural polymer materials and synthetic polymer materials. The former is represented by sodium alginate (SA), carrageenan, agar, etc., which have the characteristics of biocompatibility and good mass transfer performance but have the disadvantages of low mechanical strength and being easily degraded by microorganisms. The latter is represented by polyvinyl alcohol (PVA), waterborne polyurethane (WPU), etc., which have the advantages of high mechanical strength and durability but have the disadvantage of poor mass transfer performance (De-Bashan and Bashan, 2010; Zhang et al., 2007). Generally, the mass transfer performance of natural materials is better than that of synthetic materials (Manonmani and Joseph, 2018a, 2018b). However, the mechanical stability of synthetic materials is superior to that of natural materials (Wang and Hu, 2007). To obtain a high-efficiency entrapped sludge, the selection of materials should comprehensively consider many factors, such as mass transfer performance, mechanical properties, and structural stability. Therefore, chemical cross-linking or a combination of natural and synthetic polymer materials are often used to overcome the inherent limitations of a single material. For examples, adding polyethylene glycol and pentaethylene glycol as crosslinking agents can reduce the swelling and substantially improve the mechanical properties of hydrogels (Liu et al., 2020; Xu et al., 2017). Adding SA to PVA can improve the network structure of PVA, increase its specific surface area, and reduce its mass transfer resistance (Bae et al., 2015). Table 2 lists the operating efficiencies of several reactors using different entrapping materials.

4.3. Hybrid materials

Hybrid materials are obtained by combining organic and inorganic materials through physical or molecular means (Zhang et al., 2016). They show superior performance because they combine the high biocompatibility of organic materials with the superior stability of

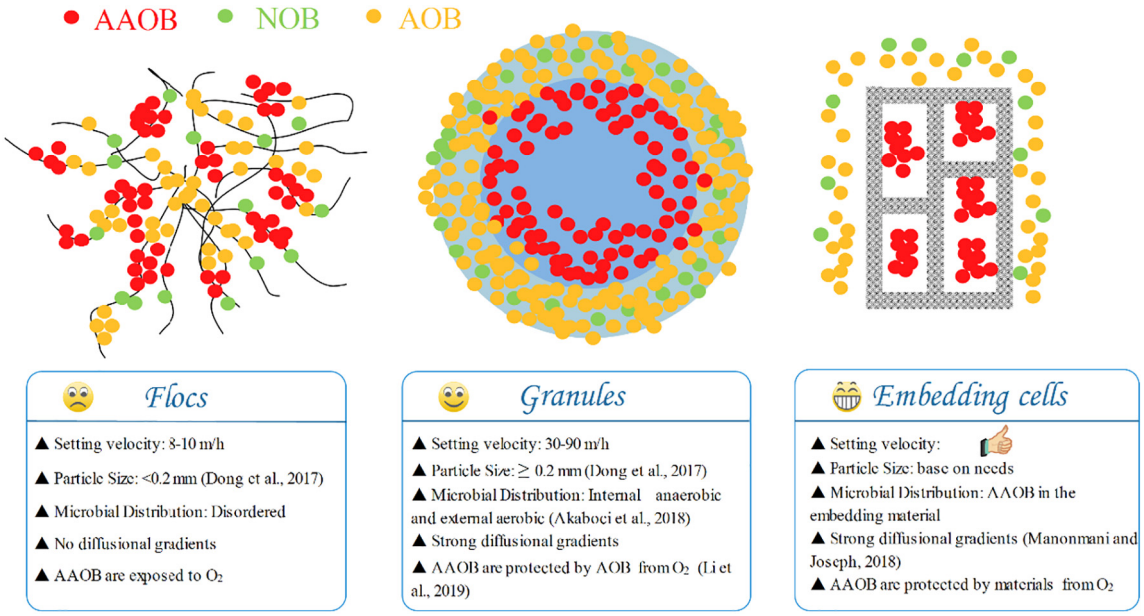

Fig. 2. Schematic diagram of the structure of flocs, granules, and entrapped beads.

Table 1
Comparisons of the nitrogen removal performances of different forms of sludge.

Reactors	Forms of sludge	influent NH_4^+-N (mg L^{-1})	Temperature (°C)	NRR (kg N m^{-3} d^{-1})	NRE (%)	Reference
Autotrophic Nitrogen Removal	Flocs	1100	15–35	0.3	86	Shi et al. (2016)
	Granules	150	30 ± 1	–	81.8	Qian et al. (2017a, 2017b)
	Granules	340	30 ± 1	2.52 ± 0.36	–	Wang et al. (2014)
	Granules	124	28 ± 1	2.83	85.7	Qian et al. (2017a, 2017b)
	Hybrid	100–220	35 ± 2	0.45	–	Vlaeminck et al. (2009)
	Hybrid	200	30 ± 2	0.31	93	Li et al. (2020)
	Hybrid	100	25	–	71.8 ± 9.9	Chen et al. (2019a, 2019b)
	Entrapped	100	30	–	81.3	Liu et al. (2020)
Anammox	Hybrid	150–300	–	0.6	72	Guo et al. (2016)
	Hybrid	25	15–17	0.5	80	Liu et al. (2017)
	Entrapped	186	32	0.505	80.7	Chen et al. (2016)
	Entrapped	230–600	30	4.0	>80	Furukawa et al. (2009)

inorganic materials. Hybrid materials are a hot research topic at present (Zhang et al., 2016). Studies have shown that adding porous materials such as peat, bentonite (Lee et al., 2012), and graphene oxide (Tang et al., 2020) to organic polymer materials can considerably improve the mass transfer performance of the entrapping carriers, which is conducive to the reproduction of microorganisms. The addition of magnetic materials can help stimulate bacterial growth and metabolism and increase the rate of pollutant degradation (Zhou et al., 2010). The addition of nanoparticles, such as nano-iron oxide (Yu et al., 2010) and nano-alumina (Kumar et al., 2011), can substantially improve the specific surface area and loading rate of the entrapping carriers, and form a structured microenvironment suitable for different bacterial groups, providing a reliable barrier for them (Ahmad et al., 2017).

In recent years, Metal-Organic Frameworks (MOFs), one of the hybrid materials, have received extensive attention from researchers. MOFs are organic-inorganic hybrid materials formed by self-assembly of metal ions or clusters and organic ligands through coordination bonds (Lu et al., 2020). They have the advantages of diverse components, high specific surface area, adjustable pore size, easy functionalization, and high biocompatibility (Slater and Cooper, 2015); as a result, they are widely used in enzyme entrapment (Nadar et al., 2020). Recently, researchers have also paid increasing attention to the application of MOFs in cell entrapment. Due to the affinity of the cell membrane to metal ions, MOFs materially nucleate and assemble at the interface of living cells, forming a protective outer layer, which allows the transfer of the matrix while protecting the cells from toxic compounds, radiation, and other adverse environmental factors, alleviating environmentally-induced reductions in microbial activity from fluctuating temperature, salinity, and nutritional levels (Liang et al., 2017). Ji et al. (2018) synthesized the MOF material and wrapped it around the microbial cell surface to form a strong shell, thereby increasing the survival rate of anaerobic bacteria in oxygenated environments from 50 ± 7% to 76 ± 8%. Furthermore, it was found that the coordination between bacteria and MOFs is dynamic and competitive, allowing newly proliferated cells to be entrapped through competition, which was also reflected in the research of Liang et al. (2017). Although research into the use of MOFs material in cells entrapment has only recently begun, it shows promising application prospects.

5. Entrapment method

5.1. Gel entrapment

The gel entrapment method uses polymers to trap cells inside the gel grid when forming gel beads. The loose structure of the gel beads enables effective substrate penetration and product diffusion, to achieve the purpose of maintaining cell viability (Li et al., 2011). The gel entrapment method not only easily separates the sludge from the solution and reduces the amount of biological outwash from the systems, but it also improves the mechanical properties of the sludge and facilitates the use of agitation to eliminate the air pockets formed in the sludge to prevent the sludge from floating (Ahmad et al., 2020). The diameter of the gel beads can be selected as required and is generally within 1–5 mm (Ali et al., 2015; Liu et al., 2020; Wang et al., 2020a, 2020b, 2020c). Presently, gel materials, such as PVA, SA, WPU, and carboxymethyl cellulose, have been widely used in the entrapment of the functional bacteria for ANR. Studies have shown that the use of gel beads can significantly shorten reactor startup time and significantly improve NRE (Ahmad et al., 2020; Ali et al., 2015; Chen et al., 2015).

Table 2
Reactor operating efficiency with different entrapping carriers.

Type	Material	Microorganisms	Reactor operation	Reference
Natural materials	SA	AAOB	Removal rate of NH_4^+-N and NO_2^--N was significantly improved.	Chen et al. (2015)
	SA	AOB	The negative influences of pH and temperature on microorganisms was obviously weakened	Yan and Hu (2009)
	calcium alginate	AOB	The performance of preservation, recycling, and ammonia oxidation ability were superior to those of non-immobilized AOB.	Dong et al. (2014)
Synthetic material	WPU	AAOB	The structure of the entrapped cells was stable, and the NRE of the reactor reached 80.98%.	Chen et al. (2015)
	Carboxymethyl cellulose (CMC)	AAOB	The removal efficiency of NH_4^+-N and NO_2^--N reached 100% and 95.3%, respectively.	Zhu et al. (2009)
	polyethylene glycol (PEG)	AOB	AOB were protected from heat-shock, and stable nitritation can be achieved.	Isaka et al. (2011)
Composite material	PVA-SA	AOB	The pH resistance, the number of reuses, material cost, heat resistance, and ammonia oxidation ability were superior to those of AOB immobilized by SA alone.	Dong et al. (2017a, 2017b)
Composite material	Polyvinyl alcohol-polypropylene (PVA-PP)	AAOB	The NRR was 9.4 times that of suspended sludge before entrapment.	Wang et al. (2020a, 2020b, 2020c)
	PVA-SA crosslinked with sodium sulfate	AAOB and AOB	The nitrogen loading rate reached 0.3 kg N m^{-3} d^{-1}, and NRE reached 70%.	Tuyen et al. (2020)
	PVA-SA	AAOB	NRR reached 10.8 kg N m^{-3} d^{-1} after 35 days of operation.	Ali et al. (2015)

Usually, after the gel and bacteria are mixed, freezing or chemical cross-linking is required to achieve fixation to the gel beads (Magri et al., 2012). Relatively speaking, the conditions of the chemical cross-linking method are milder, and the gel beads can be fixed just by dropping the gel-microbe mixture into the curing liquid to soak for several hours. The curing liquid is usually a dilute solution that has little effect on biological activity, such as iron chloride, calcium chloride, boric acid, or other similar solutions (Zhu et al., 2009). The type and concentration of the cross-linking solution as well as cross-linking time will affect the mechanical properties of the gel beads and ultimately affect the entrapment efficiency (Ahmad et al., 2020). For example, when using PVA as a carrier and saturated boric acid as the cross-linking solution, the acidity of the boric acid may inhibit AAOB and AOB, and the produced gel beads trend to agglomerate (Tuyen et al., 2020). Choosing sodium sulfate as the cross-linking solution can solve these problems (Tuyen et al., 2020).

5.2. Microcapsule entrapment

Microcapsule entrapment is a method in which a semi-permeable polymer membrane is used to encapsulate cells. Compared to gel entrapment beads, the diameter of microcapsules is generally smaller, between 1 μm and 1000 μm (Chavarri et al., 2010; Schrezenmeir et al., 1994). The cells fixed by the microcapsule are allowed to move freely in the core space and the mass transfer resistance of the microorganisms is smaller (Bouabidi et al., 2019). Both natural polymer and synthetic polymer materials can be selected as microcapsule shells. Usually, agar (Olguin, 2012), chitosan (Chavarri et al., 2010), polyacrylamide (Calvet et al., 2004), and fiber materials (Loh et al., 2000) are used.

The main purpose of microencapsulation is to enhance the resistance of bacteria to harsh environments. Because the core is isolated from the external environment, the internal microorganisms can be protected from changes in external temperature, oxygen, light, and other unfavorable factors (Rathore et al., 2013), and microencapsulation has achieved remarkable cell protection. Monir et al. used alginate-chitosan-alginate to encapsulate *Pseudomonas* sp. SA01 cells to prevent microorganisms from being inhibited by phenol and to improve phenol degradation (Mollaei et al., 2010). Fritzen-Freire et al. (2012) used reconstituted skim milk and the prebiotics inulin and oligofructose as the outer shell to encapsulate *Bifidobacterium* BB-12 cells to improve the cells survival rate when stored at low temperature (−18 °C). Pedroso et al. (2012) used interesterified fat with palm and palm kernel oils to encapsulate *Bifidobacterium lactis* and *Lactobacillus acidophilus* to enhance their resistance to simulated gastric fluid and simulated intestinal fluid. Although the microcapsule entrapment technology has achieved remarkable levels of cell protection, research on their application in ANR is still lacking and requires further exploration.

5.3. LBL self-assembly

LBL self-assembly is a method in which the materials alternately attach to the surface of the cell, layer by layer, through the interactions between the layers of molecules, such as electrostatic attraction, hydrogen bonding, and coordination bonding; the layers then spontaneously associate to form polymers with complete structure, stable performance, and specific functions (Cui et al., 2014). LBL self-assembly is often combined with microencapsulation for cell immobilization. Compared to traditional emulsion polymerization, interfacial polymerization, and other methods, LBL self-assembly can accurately control the composition, thickness, and surface morphology of the capsule wall at the nanometer scale (Amigoni et al., 2009; Cui et al., 2014). Furthermore, LBL self-assembly can also achieve cell surface functionalization by introducing functional groups to the cells (Liu et al., 2019; Tanner et al., 2011).

In recent years, LBL self-assembly technology has developed rapidly, and it has been widely used in the fields of biomedicine, biosensors, and molecular delivery. Researchers have found that the survival of probiotics under acidic conditions can be markedly improved by entrapping probiotics cells with chitosan and alginate using LBL self-assembly. This method provides a new option for delivering probiotics to the gastrointestinal microbiome (Anselmo et al., 2016). Wrapping a double-layer nanomembrane around cyanobacteria cells can protect the cells from excessive light and ultraviolet rays, thus forming an efficient photosynthesis bioreactor (Jiang et al., 2015). Moreover, through cell modification, the introduction of functional molecule dopamine in the LBL self-assembly process can substantially enhance the resistance of cells to external stimuli (Kim et al., 2014; Kim et al., 2015; Yang et al., 2011a). Additionally, LBL self-assembly technology has also been successfully applied to various cells entrapments, such as those for hematopoietic stem cells, pancreatic islet cells, and red blood cells (Ai et al., 2002; Balkundi et al., 2009; Mansouri et al., 2011), and has a protective performance on these different types of cells.

Although there are more and more studies on cell entrapment using LBL self-assembly technology, this approach is still in its infancy, and its application is still difficult to popularize. This is because biological cells are very sensitive to polyelectrolytes, nanoparticles, etc., and these materials may affect the stability of the charge on the cell surface during the LBL assembly process, which may negatively affect cellular activity. Additionally, the "shell" synthesized by LBL assembly technology is usually nanoscale (Jiang et al., 2015; Cui et al., 2014), which is much smaller than the cell diameter (AAOB diameter is ~0.8–1.1 μm). This size difference may cause local agglomeration of nanomaterials on the cell surface, and thus the cells cannot be completely entrapped. Most of the existing studies also focus on the entrapment of a single cell (Balkundi et al., 2009; Liu et al., 2019). As for flocculent or granular sludge in the ANR process, the difficulty of entrapping microorganisms with LBL assembly technology is increased dramatically. Therefore, the application of LBL assembly technology to the field of microbial entrapment of ANR sludge remains to be explored in depth.

5.4. Biomimetic mineralization

In the biomimetic mineralization method, inorganic nanomaterials are synthesized by cells, which use their own active substances, (e.g., proteins, polysaccharides, and enzymes) to regulate the production of exogenous substances in situ or extracellularly (Lin et al., 2019). This method mimics the chemical and structural relationships in biological systems, showing unparalleled flexibility and reliability (Ren et al., 2019). Additionally, the biomimetic mineralization process can be carried out under ambient conditions, such as room temperature and under neutral and normal pressure, and has structural controllability (Luckarift et al., 2004). Thus, it has been widely used in the field of bio-entrapment.

Biomimetic mineralization mainly includes bioinspired silicification (Abdelhamid and Pack, 2020), zirconization (Yang et al., 2011c), and titaniumization (Sumerel et al., 2003). Entrapment of cells by biomimetic mineralization has a few advantages: low leakage rate, high mechanical strength, and good stability and activity (Ardao et al., 2015; Jiang et al., 2014). Shi et al. (2011) synthesized a dual hard template with a silicon dioxide layer and a titanium dioxide layer and entrapped enzymes using a template through biomimetic mineralization and LBL self-assembly. Compared with microcapsule systems, the selectivity and stability of a multi-enzyme system prepared in this way is markedly improved (Shi et al., 2011). The biomimetic silicification process can also considerably increase the mechanical properties of the entrapped beads. Compared with the protein-silica hydrogel entrapped beads, the elasticity and stiffness of the entrapped beads formed via biomimetic silicification increases by two and three orders of magnitude, respectively (Cao et al., 2019). In cell entrapment, the cell surface helps in nucleation for biomimetic mineralization, and inorganic minerals are deposited on the cell surface to form a protective layer. Furthermore, researchers have introduced mineralized functional groups or molecules to the cell surface, leading to a structure of "shell–wall" (Wang et al., 2008). For example, Lee et al. (2015) deposited a thin silica layer on yeast cells. Compared with uncoated cells, the viability of silicified

yeast cells was three times higher. Additionally, by functionalizing the surface of the silicified yeast with sulfur during the biomimetic silicification process, the cells were further protected from the harsh environment (Yang et al., 2011b). Additionally, Lee et al. have found that compared to unmodified cells, living cells entrapped in silicified shells also showed higher viability and stability when exposed to a cytotoxic chemical (Lee et al., 2014). Unfortunately, although biomimetic mineralization shows great advantages and good application prospects in cell protection, research on biomimetic mineralization in the field of ANR is still just beginning, and more in-depth exploration remains to be done.

6. The protective mechanism of entrapment in autotrophic nitrogen removal systems

6.1. Protection provided by entrapment

The entrapment process provides a "shell" to cells, which can not only provide enough time for microorganisms to adapt to the environment, but also reduce the negative impacts of external unfavorable environmental factors on them. Therefore, the functional bacteria can react more efficiently in such systems, and the adaptability and capacity of these systems is thus improved (De-Bashan and Bashan, 2010). For example, entrapment can reduce the toxicity of metal ions in wastewater to bacteria (Yu et al., 2020), weaken the toxic effect of oxygen on anaerobic bacteria (Ji et al., 2018), and reduce the stress of low temperature on cell activity (Tang et al., 2020). Studies have also shown that, under environmental stress, ANR entrapment systems have better nitrogen removal performance than "naked" systems, as shown in Table 3.

The mechanism by which entrapment protects microorganisms can be explained by factors that describe in detail below.

6.2. Bioconcentration

The slow growth rate of AAOB (doubling time of 10–14 days) is the primary obstacle to the application of anaerobic ammonia oxidation in ANR processes (Cao et al., 2017). Due to the good sedimentation performance of entrapped beads, microorganisms can be completely retained in these systems, which makes up for the slow growth rate of AAOB (Manonmani and Joseph, 2018a, 2018b). Simultaneously, in the gel-entrapped beads, a larger specific growth rate of cells has also been observed, and AAOB doubling time was as low as 2.1–3.9 days (Zhang et al., 2017). This is because the microporous skeletons inside the gel carrier can provide space and favorable conditions for bacterial growth and reproduction (Bouabidi et al., 2019). The tunnel within the entrapped beads can also play a role in enriching microorganisms, thereby improving the nitrogen removal efficiency of the reactors. When high concentrations of microbes are present, the anammox aggregates in gel beads may improve chemical signaling and cooperation among cells, which also improves the nitrogen removal efficiency (Ali et al., 2015).

In addition to increasing the concentration of microorganisms, entrapment technology can also reduce the requirements for bioconcentration when the reactor is started. Research by Ali et al. (2015) showed that under the same conditions, the NRR achieved by an entrapment system with a biological concentration of 0.33 g VSS L^{-1} is about three times that of the granules-based system with a biological concentration of 2.5 g VSS L^{-1}. Other studies have also shown that the sludge concentration of the anammox granules-based systems is generally 3–25 g L^{-1} (Huang et al., 2016; Qian et al., 2017a, 2017b; Zhu et al., 2018), whereas the sludge concentration of the entrapped systems only needs to be maintained at 0.33–10 g L^{-1} (Ali et al., 2015; Chen et al., 2016; Tuyen et al., 2020). This is because the AAOB inside the granules barely contribute to nitrogen removal, while the gel beads can evenly distribute AAOB and the matrix can be used more effectively (Ali et al., 2015). Consequently, entrapment aids in the rapid startup and stable operation of reactors.

6.3. The form and structure of sludge

Although the granular sludge has the advantages of retaining microorganisms and shortening the reactor start-up time (Lotti et al., 2014a, 2014b), granular sludge fo is difficult to control. For example, during the reactor start-up process, natural granular sludge passes through the autolysis stage, and the particle size tends to decrease (Wang et al., 2020a, 2020b, 2020c). Under unfavorable conditions (e.g., low temperature), granular sludge is also prone to disintegration, leading

Table 3
Nitrogen removal performances of entrapped systems under adverse environmental conditions.

Sludge	Sources of inhibition	Nitrogen removal performance of Reactors	
		Entrapped systems	"Naked" systems
AAOB	Matrix deficiency	NRE increased from 36.7% to 87.1% within 5 days.	NRR was 1/8 of the entrapped system (Wang et al., 2020a, 2020b, 2020c.
AAOB	Nitrite	No inhibition when the nitrite concentration was lower than 400 mg N L^{-1} (Kimura et al., 2010).	Inhibition occurred at 40–280 mg N L^{-1} (Jin et al., 2012).
AAOB	Nitrite	Complete inhibition of activity occurred at 687–750 mg N L^{-1} (Magri et al., 2012).	
AAOB	Organic matter	When the COD was 400 mg L^{-1}, the contribution of anammox to total nitrogen removal remained at 58% (Wang et al., 2020a, 2020b, 2020c).	When the COD concentration was between 400 and 800 mg L^{-1}, the activity of AAOB was severely inhibited (Zhang et al., 2015).
AAOB	Organic matter	High concentration of BOD_5 (600–800 mg L^{-1}) and COD (400–4500 mg L^{-1}) did not inhibit AAOB (Furukawa et al., 2009).	
AAOB	SS	SS (200–3000 mg L^{-1}) did not affect microbial activity (Furukawa et al., 2009; Isaka et al., 2011).	SS would inhibit the efficiency of the reactors (Yamamoto et al., 2008; Sliekers et al., 2003).
AOB	Salinity	When the concentration of NaCl was 35 g L^{-1}, the NH_4^+-N removal rate reached 99% (Gao et al., 2020).	When the concentration of NaCl was 30.93 g L^{-1}, the NH_4^+-N removal rate was 56% (Zhao et al., 2016).
AOB	Salinity	AOB were inhibited when the concentrations of sulfate, chloride and phosphate were 500, 1000 and 700 mM, respectively.	AOB were inhibited when the concentrations of sulfate, chloride and phosphate were 300, 500 and 500 mM, respectively (Yan et al., 2010).
AOB	Temperature	The activation energy was 42.1 kJ/mol at 15–35 °C (Yan and Hu, 2009).	The activation energy was 73.5 kJ/mol at 10–35 °C (Benyahia and Polomarkaki, 2005).
AOB	Temperature	A high nitrification rate of 0.71 kg N m^{-3} d^{-1} was observed at 10 °C (Isaka et al., 2007).	Nitrification rate of 0.22 kg N m^{-3} d^{-1} was observed at 15 °C (Qi et al., 2020).
AOB and AAOB	Toxic compound	In the presence of quinoline, the removal rates of NH_4^+-N, NO_2^--N, TN, quinoline, and COD were as high as 98%, 99%, 97%, 100%, 98%, respectively (Ahmad et al., 2017).	Not mentioned

to an unbalanced microbial community structure (Cao et al., 2017). Compared with granular sludge, the structure of entrapped beads is more stable. PVA entrapped beads have a relatively dense "shell" and a compact internal grid structure (Zhu et al., 2009), and GO-modified PVA entrapped beads have a stable layered structure (Zhou et al., 2015). WPU entrapped beads have many channels, and bacteria are distributed on the surface and inside of the beads along the channels, which also have good stability (Chen et al., 2015). Under unfavorable conditions, the structure of the gel materials is not easily perturbed, and it continues to provide a suitable place for bacteria to attach and grow. Entrapment technology improves microbial stability by providing a "shell" for microbial cells, so that the sludge form and the microbial community structure can be maintained when the reactor is first started as well as under adverse conditions (Wang et al., 2020a, 2020b, 2020c).

6.4. Mass transfer

The carriers used for entrapment often have a porous structure, through which the matrix and metabolites can easily diffuse (Bouabidi et al., 2019). However, due to mass transfer resistance, the NRR of the entrapped beads may be lower than that of natural granules at the beginning of the reaction. In turn, in the later stage, the removal efficiency of ammonia–nitrogen and nitrite by the entrapped beads will exceed that of natural granules (Zhu et al., 2009). This is due to limited matrix transfer within 600 μm in the granular sludge (Zhu et al., 2018). The high biomass density and low effective matrix diffusion rate sharply reduce the concentration of ammonia and nitrite in the inner part of the granular sludge, thus decreasing AAOB activity and their contribution to total nitrogen removal (Ali et al., 2015). In contrast, the functional bacteria in entrapped beads are more evenly distributed, and the effective diffusion efficiency of the matrix in the gel beads is higher, so that AAOB activity inside the entrapped beads is maintained or even improved. It has been verified that the effective diffusion coefficient of PVA-SA gel beads is more than three times higher than that of granular sludge (Ali et al., 2015). Therefore, compared to natural granular sludge, the entrapped beads have a higher effective diffusion efficiency, which allows AAOB activity to be maintained. However, for flocculent activated sludge, the AOB in the gel beads seem to experience decreased activity due to mass transfer resistance, but the gel alleviates the adverse effect of increased osmotic pressure on the AOB, thus maintaining the activity of AOB in high salinity environments (Yan et al., 2010). Some studies have also found that the impact of mass transfer on microbial activity in gel beads can be divided into two stages. The first is the restriction stage, when the ammonia oxidation rate of the gel beads is lower than that of the suspended sludge. The next is the adaptation stage, when the ammonia oxidation rate of the gel beads is significantly increased, and the gel reduces the toxic effects on microorganisms (Morita et al., 2007).

6.5. EPS

It is well established that EPS released by bacteria play a decisive role in the process of sludge granulation, and they are an important part of maintaining the structure and function of microbial aggregates (Liu et al., 2010). In EPS, protein mainly functions to accumulate microbial cells through its hydrophobic effects, whereas polysaccharides play an important role in maintaining the stability of granular sludge (Hou et al., 2015).

The microenvironment formed by the entrapped beads is more conducive to the secretion of EPS than those of flocculent (Yu et al., 2020) and granular sludge (Wang et al., 2020a, 2020b, 2020c). EPS can combine with cells to form a huge network structure, thereby accelerating the aggregation of microorganisms and protecting cells from external pressure (Li and Pagilla, 2017). EPS secreted by AAOB in gel beads entangles the cells with the rough, porous gel, which makes the cells more densely clumped and, at the same time, makes the structure of the entrapped beads more stable. The microenvironment of the entrapped beads has a markedly higher promotional effect on protein secretion than on polysaccharide secretion (Wang et al., 2020a, 2020b, 2020c). High protein content greatly promotes hydrophobic interactions among the cells, so that the AAOB aggregate considerably (Ni et al., 2010), which can help improve the activity and growth rate of AAOB. Additionally, the secretion of EPS is also related to the nature of the entrapment materials. Studies have shown that gel beads with polyvinyl alcohol-chitosan-iron as carriers have better removal performance on organic matters than those with polyvinyl alcohol-chitosan as the carrier (Wang et al., 2019). EPS contains electronegative groups, such as carboxyl, hydroxyl, and azyl moieties, which can act as strong ligands for metal cation (Sheng et al., 2013; Wang et al., 2020a, 2020b, 2020c). Fe^{2+} forms a bond with the hydroxyl and carboxyl groups related to EPS, thereby promoting the immobilization of bacteria and greatly enhancing cell aggregation (Wang et al., 2020a, 2020b, 2020c).

6.6. Microbial community

During the operation of ANR systems, the diversity of bacteria in the entrapped beads is reduced, and a community with stable structure and specific functions is gradually assembled (Wang et al., 2020a, 2020b, 2020c), which is similar to granule-based systems, in which the biodiversity decreases after long-term operation (Sobotka et al., 2017). With the operation of the reactors, the presence of AOB and NOB will also be observed on the surface of the anammox entrapped beads, which is also similar to the process of ANR granular sludge formation. AOB and NOB on the bead surfaces consume oxygen and provide nitrite, whereas AAOB existing in the interior of the beads play a major role in total nitrogen removal (Tuyen et al., 2020).

In terms of microbial structure, the dominant anammox bacteria vary according to the operating conditions found in different entrapped anammox systems. The main observed taxa are *Candidatus Kuenenia* (Wang et al., 2020a, 2020b, 2020c) and Ca. *Brocadia* (Chen et al., 2015; Liu et al., 2020), which are consistent with those observed in "naked" systems (Bhattacharjee et al., 2017; Sobotka et al., 2017). By comparison, the dominant AAOB bacteria in entrapped beads have been found to be consistent with those found in the seed sludge (Chen et al., 2015); the other biological species observed in the entrapped beads are also consistent with those observed in the free biomass (Yan et al., 2010). Additionally, studies have shown that during the operation of the entrapment system, the relative abundances of *Thermomonas*, *Bacillus*, and *Pseudomonas* were reduced, and these have been shown to be heterotrophic nitrifying bacteria with simultaneous nitrification-denitrification abilities (Wang et al., 2020a, 2020b, 2020c). The relative abundance of the strictly anaerobic heterotrophic nitrifying genus *Igvanivibacterium* slightly increased, indicating that the entrapment carrier can provide a good anaerobic environment for internal anaerobic bacteria that protects them from oxygen poisoning (Wang et al., 2020a, 2020b, 2020c). The main AOB genus observed was *Nitrosomonas*, which is the primary AOB genus observed in most non-entrapment wastewater treatment systems (Benakova et al., 2018; Gao et al., 2020). No difference in AOB composition was observed between the entrapped beads and free sludge (Yan et al., 2010). Thus, the entrapping carriers do not change the bacterial taxa present, but they do have a positive effect on the enrichment of the dominant bacteria (Chen et al., 2015; Ibrahim et al., 2016; Wang et al., 2020a, 2020b, 2020c). Therefore, entrapped sludge shows stronger shock resistance and higher nitrogen removal performance than the natural sludge, which is mainly due to the protection provided by the entrapping carriers rather than the changes in microbial community composition (Chen et al., 2015).

7. Conclusions and outlook

This article reviews the progress that has been made in research on entrapment technology in the field of ANR, and mainly discusses the two methods of immobilization: natural aggregation (granulation)

and entrappment. The granulation of cells passes through four principle stages: free cells, micelles, micelle complexes, and granules. Among them, EPS plays an important role in the formation of granules. Granules can effectively retain microorganisms and significantly improve stability and nitrogen removal efficiency. However, under unfavorable conditions, the granule form is difficult to maintain, which easily leads to system performance degradation.

The beads formed by entrapment have good stability, and the external carrier material can alleviate the impact of some unfavorable factors on the sludge and improve the nitrogen removal performance of reactors in terms of increasing the biological concentration, maintaining the sludge form, promoting utilization of the substrate, and promoting microbial secretion of EPS. This article also summarizes the materials and methods used for entrapping microbial cells. With the in-depth study of immobilization technology by researchers, carrier materials have gradually transitioned from materials made from natural organic or synthetic polymers to modified materials and hybrid materials. The entrapment method has developed from traditional gel entrapment and microcapsule entrapment to LBL self-assembly technology and biomimetic mineralization, which can be "customized" according to cell characteristics; however, the application of cell entrapment to the field of ANR is still lacking.

Research on entrapment methods in the field of ANR should be further carried out to provide a basis for its engineering application. The author believes that further research should be conducted on the following aspects:

(1) The dynamic mechanism of interaction between materials and cells should be studied to develop more suitable entrapment materials.
(2) The metabolic mechanism of functional bacteria in the entrapment beads can be explored, and cell proliferation and death in the entrapment beads can be analyzed, thus providing a theoretical basis for the practical application of cell entrapment.
(3) The structural succession of the bacterial community in the entrapment beads can be explored, clarifying the mechanisms underlying interactions between the bacteria with nitrogen removal functions and other bacteria and providing guidance for effectively maintaining the balance of the bacteria in the entrapment beads.
(4) Hybrid materials represented by MOFs should be developed, and "customizing" the corresponding entrapment carrier according to the microbial characteristics is required to better adapt to microbial reproduction and to obtain entrapped beads with better stability and durability.
(5) LBL self-assembly, biomimetic mineralization, and other entrapment technologies may become the foci of future research. These methods can improve the biocompatibility and stability of the entrapped beads, as well as better protect cells from adverse environmental conditions.
(6) Specific analyses of the process through which the matrix and metabolites are transferred in and out of the carrier should be conducted. Based on these, an entrapment technology that accurately controls the pore size of the carrier material should be developed, which could prevent cell leakage while ensuring mass transfer, prolonging the service life of the entrapment beads.

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Declaration of competing interest

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