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Review

The Oxygenic Photogranules—Current Progress on the Technology and Perspectives in Wastewater Treatment: A Review

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Abstract: Wastewater generation is a worldwide problem, and its treatment is an important practice for maintaining public health and environmental protection. Oxygenic photogranules (OPGs) are a relatively novel type of biogranules that have the potential to substitute the conventional activated sludge (AS) process due to the production of in situ oxygen, better physical properties such as settling velocity and density, as well as carbon and nutrient removal efficiencies. The formation of the granules is attributed to many factors, among which the most influential are light intensity, ammonium nitrogen concentration, and the presence of filamentous cyanobacteria that, along with heterotrophic microorganisms situated in the granule's core, create a self-sustainable system that combines denitrification, carbon removal, and oxygen production. Hydrostatic and hydrodynamic cultivations are two ways that allow for obtaining OPGs. These two cultivation methods lead to the formation of various types of granules which differ in both structures as well as physical properties. This review article aims to aggregate the available literature information regarding the methods of cultivation of OPGs, their formation mechanisms, and factors that influence the cultivation as well as an overview of studies that were conducted thus far concerning this type of biogranules. Additionally, further research directions are proposed in the article.

Keywords: activated sludge; oxygenic photogranules; biogranules; wastewater treatment

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1. Introduction

Both wastewater treatment and environmental protection are crucial elements of today's public health. Despite the development of modern wastewater treatment approaches, there are still many people that do not have adequate sanitation. Therefore, additional new solutions and modification of the existing ones are required [1]. The activated sludge system (CAS) process is an established and the most popular biological method used for industrial and municipal wastewater treatment. Even though it is often employed around the world, it is still modified in aspects of its design and operational conditions. The CAS process requires a large area, complex design solutions, as well as aeration, which is associated with large operational costs that account for about 45–75% of energy costs for a wastewater treatment plant (WWTP) [2,3]. Moreover, it was estimated that the CAS consumes per m^3 treated wastewater usually between 0.30 kWh and 0.65 kWh energy, making it largely energy intensive [4–6]. Moreover, large area requirements are due to the poor settling property of AS flocs [7].

Depending on the type of wastewater, the efficiency of the CAS process varies, and, for example, the BOD removal efficiency of the process can be within the range of 39–86% [8]. In turn, for instance, COD, TSS, nitrate, and phosphate removal efficiencies make up about 50%, 20%, 75%, and 80%, respectively [9].

A large number of technologies have been developed over the last decade that may have a potential application in wastewater treatment in the near future, e.g., photocatalysis [10], microwave catalysis [11], catalysis with Perovskite materials [12], or application of nanoscale zero-valent iron (nFe^0) particles [13]. Photocatalysis is a new method that can be used to remove natural organic compounds before ultrafiltration [10]. Microwave catalysis can be used to convert biomass, e.g., sewage sludge, to valuable bioproducts (biooils and biochars) through pyrolysis [14]. In addition to the mentioned processes, new materials have also been developed for the existing conventional methods of water purification such as adsorption. For example, vanadium-SiO_2 composite (V/SiO_2), which has been recently synthesised, was used as a catalyst and showed good stability during the process [15]. The search for new solutions for wastewater treatment is one of the critical challenges, especially given the identification of new environmental and human health threats, such as emerging contaminants [16] or microplastics [17], as well the increasingly stringent legal standards for the quality of wastewater [18,19].

Recently, biogranules became an interesting solution for high efficiency wastewater treatment [20–22]. Biogranules have high specific gravity and settling velocity, which facilitated the separation of treated effluent from the sludge. There exist several types of biogranules that are applied for wastewater treatment, e.g., methanogenic granules (MGs), hydrogenic granules (HGs), anammox granules, and oxygenic photogranules (OPGs) [23,24].

OPGs are a relatively novel type of biogranules that could be applied for aeration free wastewater treatment due to their potential to produce in situ oxygen that aerobic heterotrophic microorganisms can use, thereby reducing wastewater treatment costs by about 25–60% [9,25,26]. OPGs were serendipitously discovered when AS was closed in glass scintillation vials and exposed to artificial light. Formation of the granules is associated with the presence of green algae and filamentous cyanobacteria, particularly the genus *Oscillatoria*, which play a significant role in the granules' development [25]. These microorganisms create a symbiosis with heterotrophic communities; i.e. oxygen produced by the phototrophic microorganisms is utilised for combined nitrification/denitrification and degradation of organic matter [1]. Moreover, OPGs have a 15–20% higher biological methane potential compared to AS, which makes them an attractive substrate for anaerobic digestion instead of waste AS [3].

This review article aims to aggregate available up-to-date information regarding the OPGs and their application for wastewater treatment. The review provides information on the structure and formation of OPGs depending on the type of cultivation as well as an overview of research that was conducted thus far in relation to this type of biogranules. Additionally, the review compares the existing kinds of biogranules with OPGs and reveals future prospects for the application of OPGs in environmental engineering. The efficiency and environmental impact of the CAS process and the ORPs system were also compared based on the available literature data.

2. Types of Biogranules

Wastewater treatment with the application of biogranules is not a new practice. A great amount of research shows that biogranules are more efficient in water and wastewater treatment than the CAS is [23,27]. The first reported application of biogranular technology dates back to the 1980s when anaerobic granular sludge was applied in an up-flow anaerobic sludge blanket (UASB) reactor [28]. The biogranules can be categorised into two major groups: anaerobic and aerobic granules. Anaerobic granules include methanogenic (MGs), hydrogenic (HGs), and anammox granules (Table 1) [23,29,30].

The mechanism of granulation of each granular type differs; for example, MGs are formed in the presence of attachment inert material which provides a surface for anaerobic bacteria to grow onto. Further growth of these attached bacteria is due to the sludge selection mechanism, i.e., washing out of the lighter sludge particles and leaving the dense biomass at the bottom of the reactor [28]. MGs have a layered structure in which the core accommodates methanogenic as well as hydrogen producing bacteria, whereas the outer layer supports filamentous and sulphate-reducing bacteria. Formation of MGs was first discovered in the UASB reactor during the treatment of sugar beet wastewater with an organic loading rate (OLR) of 15–40 kg-COD/m^3·d and hydraulic retention (HRT) of

3–8 h [31]. These types of granules have disadvantages associated with a long start-up period.

In turn, aerobic granules are formed from floccular AS without attachment material. The start-up for fully matured aerobic granules is usually high (several months), and they require optimal control of a set of operational parameters such as OLR, aeration time, mode of substrate feeding, etc. [32]. Aerobic granules are characterised by high density and, therefore, fast settling velocity (5–30 min are required for separation of the treated water from the biomass) [33]. HGs in turn are formed from flocculated AS (some studies also reported the use of methanogenic granules as seeding biomass) during dark fermentation, which is governed by a consortium of microorganisms, particularly, facultative anaerobic microorganisms [23,32]. A set of operational conditions, such as pH, HRT, OLR, substrate type, etc. need to be taken into account to achieve the successful granulation of HGs.

Table 1. Comparison of various types of anaerobic biogranules [23,34–40].

	Methanogenic Granules (MGs)	**Hydrogenic Granules (HGs)**	**Anammox Granules**
Date of discovery	1976 by Gatze Lettinga's group	-	1990s in a wastewater pilot plant at Delft University of Technology
Start-up period	2–8 months	A few months; the time of formation of granules may be shorter when acclimatised seed sludge will be acid incubated for 24 h after lowering the pH from 5.5 to 2.0 in an anaerobic CSTR; in this condition, the granules may be formed rapidly within 3–5 days.	14–800 days
Size /diameter, mm	0.14–5.0	0.4–3.5	1.75–4.0 Rare > 6.0 mm; not recommended because when granule size larger than 2.2 m may decrease nitrogen removal and cause granule flotation
Color	black	changed from black to white (or creamy),	carmine
Structure and microbiome	multi-layered structure The inner layer mainly consists of acetoclastic methanogens such as *Methanosaeta* sp., while the middle layer consists of hydrogenotrophic methanogens; in turn the outer layer consists of Hydrogen producing bacteria, hydrogenotrophic methanogens, Sulfate reducing bacteria	non-layered structure mainly: *Clostridium* sp., while *Klebsiella* and Enterobacter were also detected	Two- -layered structure consists outer aerobic layer containing ammonia oxidising bacteria (AOB) and an anoxic core of anammox micro-organisms (AMX bacteria) include members of the *Proteobacteria, Chlorobi, Bacteroidetes,* and *Chloroflexi phyla;* most commonly were isolated: *Candidatus* Brocadia *Candidatus,* Kuenenia, *Candidatus* Jettenia
settling velocity, m/h	18–50	Up to 75	35–160
the porosity	0.64–0.90	high	-
seeding source for biogranules formation		Flocculated sludge, rare: MGs, inoculum requires pretreatment	Anammox granules, Anaerobic granules, MGs, Activated Sludge, Nitrifying and anammox sludge, Inactive methanogenic granules
Potential role at WWTPs	decomposition of complex organic substances to methane mainly in the upflow anaerobic sludge blanket (UASB) and expanded granular sludge blanket (EGSB)	anaerobic hydrogen production from organic wastes; production of a new biofuel, namely the hythane gas (consists of 10%–30% v/v of hydrogen and 70%–90% v/v of methane)	For biological nitrogen removal (BNR), mainly for treatment of ammonium-rich wastewater (up to 2.5 kg N-NH$_4^+$/(m^3·d)

OPGs, a relatively novel serendipitous discovery of American scientists, have received much attention recently due to the advantages that their application can bring in relation to wastewater treatment [9,25,27,31]. The granules combine nitrification/denitrification processes with oxygen production, which is driven by photosynthesis, making the granules a promising solution to eliminate the mechanical aeration in CAS [41]. OPGs, when cultivated, can be utilised as easily recoverable biomass for anaerobic digestion or pyrolysis [23,41]. Compared to aerobic granules and AS floc, OPGs have a smaller diameter, lower water content, and large settling velocity, which make them a promising alternative to the existing CAS system [23].

3. Cultivation Methods, Structure, and Formation of OPGs

3.1. Cultivation Methods

OPGs are formed during static or hydrodynamic cultivations from AS illuminated with light during the cultivation period [31]. To promote granulation, AS is exposed to light (artificial) 24 h per day, or the combination of 12 h natural light/12 h artificial light can also induce OPGs formation [9]. Static cultivation includes the application of glass scintillation vials (Figure 1) into which AS is added (the filling volume is about 50–60% of the total volume of a vial) and the vials are held intact and closed during the whole granulation period to prevent disruption of OPGs formation [9,23,31]. Compared to other types of biogranules, e.g., aerobic granular sludge, the granulation of OPGs takes place without aeration [1,42].

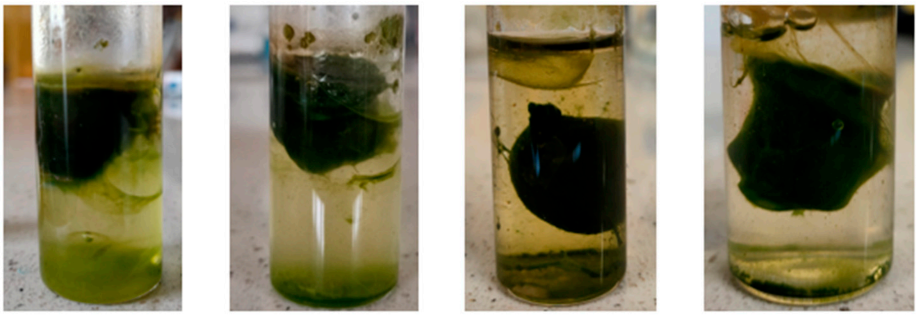


Figure 1. Maturated OPGs of sphere shape obtained during static cultivation (own study).

It takes approximately 34–42 days for the granules to mature in static cultivation and aggregate into a spherical or disk shaped structure [9,31]. The size of the granules is within the range of 10–20 mm [25]. To check whether cultivation has achieved success, a shake test is performed and if a granule maintains its aggregated state after the test and the liquid inside the vial has little to no cloud, it is deemed as successful [4,26].

A large number of studies were conducted in relation to the static cultivation of OPGs. For example, in the study in which AS was closed in vials up to 7 mL (15 mL total volume) and illuminated with artificial/natural light (cycle of 12/12 h), the effect of TSS was proved in relation to the cultivation success. Six types of AS with different characteristics were studied, and the study included light illumination at different power ratings. The granules with a density of 1250 kg/m^3 and a settling velocity of 36 m/h were obtained, which were used for SS treatment as inoculum. The efficiency of COD, TSS, Nitrates, phosphate, and BOD removals were higher at 50%, 29%, 80%, 84.56%, and 62%, respectively, compared to AS process [9].

In another study, the change in EPS concentration during OPG granulation was evaluated [43]. It was found that polysaccharide-based EPS extracted by sonication and just centrifugation play an important role in the promotion of the growth of filamentous cyanobacteria, which requires this EPS type for their motility, and a decrease in EPS concentration during the cultivation is an indication of the granulation success.

Hydrodynamic cultivation includes inoculation of an SBR reactor with statically formed granules that disintegrate into smaller particles after their addition into the reactor (diameter is less than 200 µm) [4,27,41]. Smaller OPGs provide less oxygen gradients and have lower settling velocity compared to the larger OPGs (size class of 0.5–1 mm) that are produced statically because of their less dense structure [4,25]. AS flocs have dimensions of 50–100 µm in diameter and have a large consortium of aerobic microorganisms that degrade organic matter. The reported cultivation period is typical during 148 d; however, long cultivations (250 d) were also conducted [44]. The next figure (Figure 2) presents the granules that were cultivated under hydrodynamic conditions.

A large number of studies were also conducted in relation to the hydrodynamic cultivation of OPGs. In one study it was shown that OPGs can be formed in less than 8 days and that only a combination of properly chosen magnitudes of operational conditions leads to granulation success. The study investigated the effect of the inoculum dilution with changes in light intensity and shear stress (mixing) applied. The best results were observed at 4x inoculum dilution with a light intensity of 6.4 klux and were mixed within the range of 20–50 rpm [45]. A similar conclusion was drawn from the study in which an SBR reactor was operated for 150 days, that the formation of OPGs under hydrodynamic conditions occurs along with filamentous cyanobacteria enrichment when low light intensity (expressed in PAR) is applied [27].

(**A**) (**B**)

Figure 2. OPGs formed during hydrodynamic cultivation: (**A**) in a vial, (**B**) an image obtained from a stereomicroscope (own study).

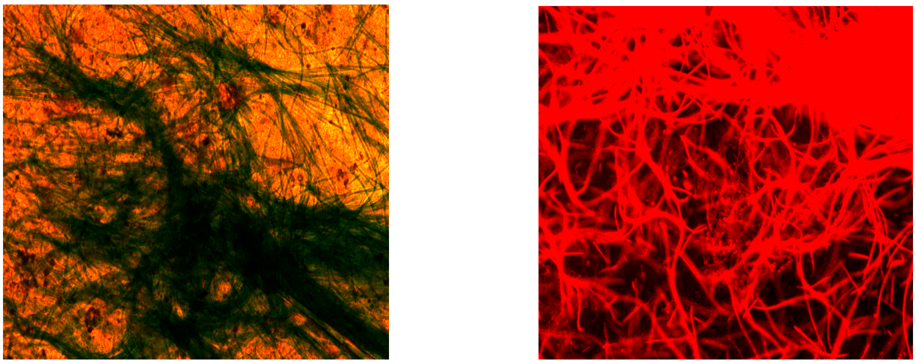
Another study, in which hydrostatically formed granules of different size classes were used as a seed inoculum in an SBR reactor of 4 L that was operated for 5 months, shows that an increase in the photogranule's size influences the change in the abundance of phototrophic microorganisms, which was proved through the analysis of the phycobilin levels of photosynthetic pigments [25].

Different types of bioreactors can be used for wastewater treatment and hydrodynamic cultivation of OPGs. Non-aerated photo-sequencing batch reactors (P-SBRs) are one of the most popular solutions. Recently, however, new developments in bioreactor design have been observed. Closed photo-sequencing batch reactors are one of these. Compared to conventional P-SBRs, these classes of reactor allow better control of operating parameters while increasing the residence time of the gases inside the reactor. Continuous-flow bioreactors, on the other hand, have advantages in terms of scalability, easy control of operating parameters, and high contaminant removal efficiency. Membrane reactors are also an interesting alternative. Unlike the previously mentioned solutions, they significantly reduce biomass washout from the reactors, which is particularly important in the start-up phase. They are also characterised by a longer retention time of microalgae inside the bioreactors. In contrast, sequencing batch suspended biofilm reactors are characterised by excellent mass transfer between the carriers [46].

3.2. Structure and Formation

The structure of OPGs share a similarity with cryoconite granules; i.e., there is a dense phototrophic layer (of 0.5–1 mm thickness in the case of static cultivation and 1.5 mm in the case of hydrodynamic cultivation) which accommodates motile and filamentous cyanobacteria that create a microbial mat [41]. The next figure (Figure 3) shows the inner structure of an OPG granule under both normal and fluorescent light.

The whole size of an OPG granule is within the range of 4–10 mm [9]. Such a dense structure of the phototrophic layer gives the granules exceptional settling properties (settling velocity of 36–360 m/h) due to the increase in their density (up to 1.5 kg/L) [23]. Cyanobacteria are known to perform phototaxis (movement towards the location where light conditions are more favourable), and that is the major mechanism that drives the granulation. Small changes in light intensity can affect granulation success [4].

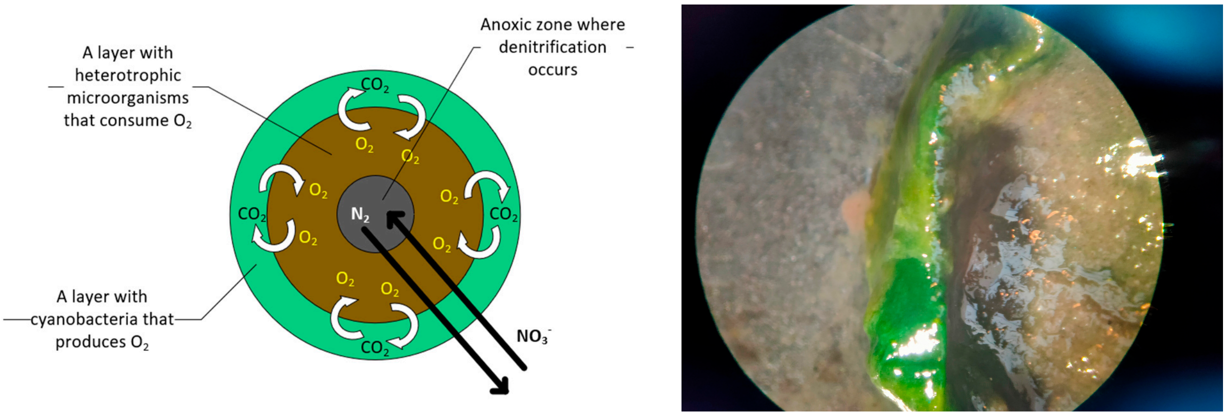


(**A**) (**B**)

Figure 3. OPGs under microscope: (**A**) 10× magnification, (**B**) fluorescent light image, 10× magnification (own study).

The phototrophic layer of cyanobacteria is maintained due to the release of extracellular polymeric substances (EPS) (mainly polysaccharides, proteins, lipids, nucleic acids, and phenols) by the microorganisms, and the layer is restricted only to the outer-most part of a granule because the light does not penetrate further to the depth. The light intensity is, therefore, the highest on the outer part of OPGs. Such granulation behaviour creates the oxygen gradient, which divides a granule into aerobic and anoxic regions, similar to a microbial mat [43,47]. Phototrophic microorganisms that accommodate this layer rely on photosystem II (PSII), which is a centre of oxygen production where oxidation of H_2O to O_2 occurs via the KOKs cycle [48]. As was reported in the studies [25,27], the amount of produced oxygen is about 12.6–21.9 mg-O_2/g-VSS·h, which is enough for the complete decomposition of organic matter.

The next figure (Figure 4) presents the approximate structure of an OPG. The inner core of an OPG granule has heterotrophic as well as nitrifying bacteria that perform aerobic oxidation of NH_3 and organic substances coupled with CO_2 utilization by microorganisms in the phototrophic layer. The denitrification process (reduction of NO_3^- to N_2 gas) also occurs in this region [48].



(**A**) (**B**)

Figure 4. A scheme of: (**A**) an OPG structure, based on [31,48] and (**B**) stereoscopic image of a granule obtained through static cultivation (own study).

The phototrophic layer is accommodated by cyanobacteria of genera *Microcoleus*, *Cyanobium*, *Geitlernema*, and predominantly *Oscillatoria*, which was proved through 23rRNA sequence inventory studies (up to 85 ± 18% of the whole cyanobacterial population in OPGs was accounted for by these mentioned genera). Wherein, unsuccessful cultivation was characterised by the high predominance of cyanobacteria of genus *Cuanobium* (about 8%). In another study, cyanobacteria of genera *Pseudoanabaena* and *Leptolyngbya* were identified in the OPGs structure [26]. Besides cyanobacteria, microalgae of genera Scenedesmus as well as bacteria genera *Lysobacter* and *Sediminibacterium* also predominate the OPG structure [1,23].

OPGs have two distinct morphologies; i.e., bald granules that resemble fluvial pebbles and filamentous granules that have a morphology of dreadlocks (Figure 5) [23].

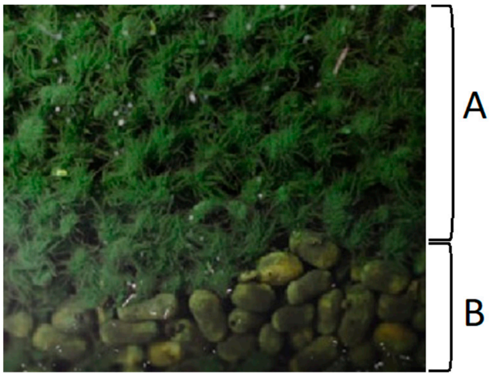


Figure 5. Two morphologies of OPGs: A—dreadlock-like and B—bald granules [23].

According to the granulation theory proposed by [28] the Inert Nuclei Model, MGs are formed due to the presence of inert material that serves as a place of attachment for bacteria. The same mechanism can also be applied in terms of OPGs. The theory suggests that there are four distinct stages of granulation, i.e., [31]:

- An initial contact between bacteria which is achieved by physical movement and further adhesion, which is the requirement for building the stable biofilm structure;
- Influence of the attractive physical and chemical forces that further maintain the contact between cells;
- Aggregation of cells as well as maturation (microbial forces);
- Influence of hydrodynamic shear force that shapes the structure of the final granule.

It is hypothesised that precipitated ions, fungal hyphae matrices, and dead bacterial and fungal cells serve as adhesion material for the first granulation stage. Attractive physical and chemical forces such as Van der Waals forces, opposite charge attraction, and hydrophobicity help to bring the bacterial cells together, which otherwise have repulsive electrostatic forces. Microbial forces, which are attributed to EPS production, serve as a critical element that help to maintain the cell-to-cell connections. For instance, high polysaccharide concentrations during the cultivation promote an increase in hydrophobicity which leads to better cell-to-cell interactions. An increase in hydrophobicity can also be attributed to the starvation conditions. The last step in OPGs formation is influenced by the hydrodynamic shear forces that play an important role in the granulation successes, especially in hydrodynamic cultivation [31].

As presented in the next figure (Figure 6), there are four distinct stages of the granulation: initial aggregation and compaction, followed by the initial granulation, main granulation, and then maturation stage at the end. Additionally, a mature OPG can have three distinct morphologies: a floating sphere, a settled sphere, as well as a disc-shaped granule [4].

Aggregation and compaction stages occur during the first three cultivation days, and in this period, AS settles to the bottom or floats (due to the denitrification process). Chlorophyll concentration is low at this stage. The second stage, which is the stage of initial granulation, occurs over 3–14 cultivation days, and it is accompanied by an increase in chlorophyll concentration, in particular, chlorophyll a (ratio of chlorophyll a to b is higher than 1 and can be as high as 7 to the end of the stage). Further granulation, which occurs between 14 and 21 days, is associated with the contraction of the sides of the biomass and formation of a spherical or disk shape. Maturation of the granules occurs at 21–28 cultivation days, and the motility of filamentous cyanobacteria decreases at this stage [43]. There are three strategies for breeding biogranules namely: (1) specific microalgae are inoculate aerobic granular sludge; the action allows a reduction in biogranule formation time of up to 18 days; (2) microalgae and AS are injected into the reactor simultaneously; the contents are mixed and aerated; (3) the AS is exposed to light under static conditions, resulting in the formation of bioaggregates after several months (Figure 7) [49]. In the formation of biogranules, the role of the inoculum can be taken up by AS, aerobic granular sludge (AGS), the microalgae and the AGS, biogranules (OPGs) and the AGS, and the OPGs alone. However, due to the presence of microalgae in the effluent, the addition of an inoculum is not required. [46,50].

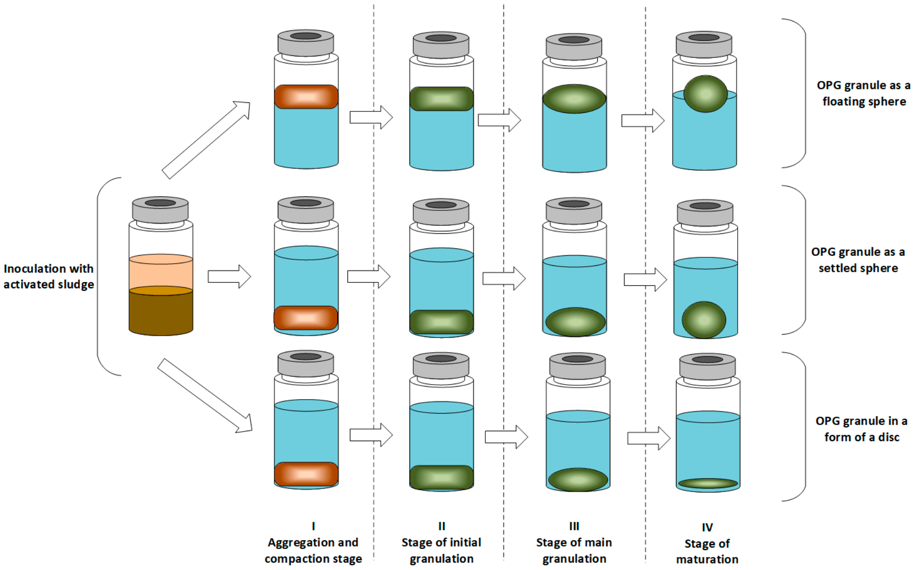


Figure 6. Stages of OPG granulation, based on [4,43].

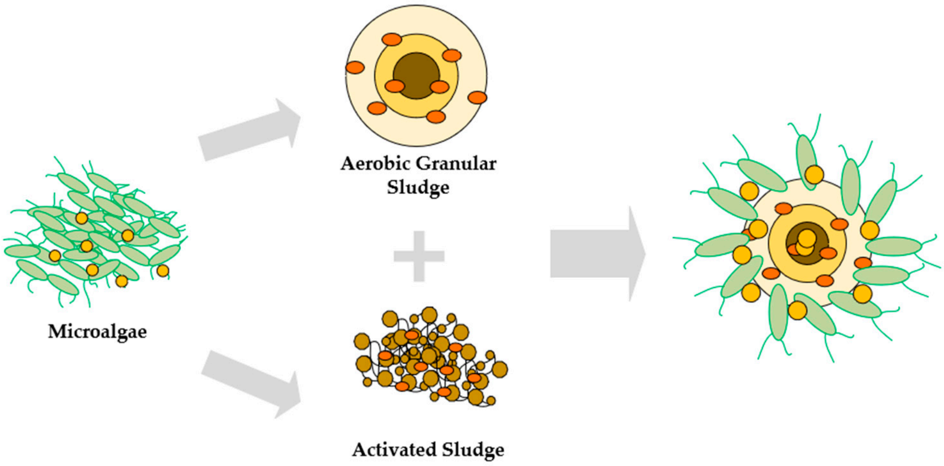


Figure 7. Strategies for breeding biogranules, based on [46,49].

4. Factors That Influence on Formation of OPGs

There are many factors that have an influence on the successes of the OPG granulation, and some of the most important ones are total suspended solids (TSS), the presence of cyanobacteria of Oscillatoria spp., N-NH_4^+ concentration, hydrodynamic shear (and related with this factor intensity of the mixing), temperature, and light intensity. Each of the factors is discussed further in the separate subsections. Changing the values of these parameters may promote the growth of OPGs with different structures and physicochemical properties, e.g., settling velocity, density, BOD, COD, and nutrient removal efficiencies [31,42].

4.1. TSS and Inoculum Concentration

As was reported in the study [9], TSS concentration of the initial AS material that is used for the cultivation plays an important role in its success. For example, AS with TSS of 12.5 g/L and fixed solids (FS) of 1 g/L had a successful cultivation compared to the AS that had TSS of 5.2 g/L and FS of 0.45 g/L. It was also noted that light intensity did not affect the results because both cultivations were conducted at 18 W, which meant that TSS was a decisive factor. On the other hand, [45] obtained the best results with a four-fold dilution of the inoculum (activated-sludge from WWTP) when the culture was conducted at low light intensity. Interestingly, sludge granulation occurred irrespective of the shear force tested. The use of high light intensity resulted in a failure of the culture. The quoted work demonstrates how important the determination of inoculation density is for the success of cultivation. If the value is too low, it may result in slow biomass growth, and if the value is too high, this may lead to inhibition of biomass production due to substrate shortages [49,51].

4.2. Presence of Cyanobacteria of Oscillatoria spp.

The presence of filamentous cyanobacteria is a crucial factor that determines the granulation success as was proved by the study. The cyanobacteria of the order *Oscillatoriales,* genus *Osccilatoria,* predominate the OPG cultivations and form a dense photoactive layer on the outer side of a granule [41].

It was reported that an increase in concentration of *Oscillatoria* spp. was associated with an increase in cultivation success as was proved by an addition of *Oscillatoria* to the synthetic media in ratio 3.6 to 14.4 (*Oscillatoria* to AS solids in mg wet mass). The granulation success was accounted to 90% when high ammonium concentration was also present [31]. The success rate can be linked to the specific properties of *Oscillatoriales*, specifically their gliding motility and the secretion of large amounts of extracellular polymeric substances (EPSs). The first property allows the cyanobacteria to move in relation to the light source. The second improves mineral binding and also attracts bacteria that utilise the cyanobacterial metabolites, including EPS and oxygen, promoting the agglomeration of bacteria and cyanobacteria into biogranules [52].

4.3. N-NH_4^+ Concentration

As was shown in the study, successful granulation is associated with availability of inorganic nitrogen, in particular, in the form of ammonium ion. At the initial stage of the granulation, N-NH_4^+ concentration was high, which then decreased dramatically after a few days accompanied with the increase in NO_3. On the other hand, an unsuccessful granulation did not have an increase in NO_3 concentration [26]. Furthermore, studies performed by Kuo-Dahab et al. [43] indicate that cyanobacterial mat and granular systems form along with nitrogen decrease through the process of denitrification, which can be observed within 3 days of static cultivation when the AS starts to float. In turn, Stauch-White et al. [26] observed that high concentration of ammonium nitrogen (20–30 mg-N/L) was associated with successful cultivation rather than low concentration (6–12 mg-N/L).

In another study it was found that the samples which had an increased ammonium nitrogen as well as *Oscillatoria* concentrations gave 90% successful granulation [31]. Initial ammonium concentration was observed to decrease throughout the cultivation period from day 0 to day 42, which signified that the granulation process took place.

4.4. Hydrodynamic Shear Force/Mixing Conditions

Hydrodynamic shear force is hypothesised to be an important factor in the formation of many types of granules, including OPGs. Some researchers suggest that the granulation is promoted when some shear stress is applied; however, OPGs are also formed in the absence of shear stress [31,52]. Tangential shear stress applied on microbial aggregates is known to promote the granulation due to enhanced aggregate–aggregate collision [52]. A reported value of the mixing speed which results in successful granulation lies within the range of 20–50 rpm; however, some studies report higher values such as 100 rpm [4,41,53].

Shear stress, if provided in the optimal magnitude, causes the granules to obtain their round shape and proper size, and the higher the magnitude is, the smaller and more spherical the granules are [45]. For instance, when the mixing speed was increased from 15 1/s to 140 1/s, the diameter of the granules decreased for 45%, i.e., from 5.5 mm to 2.5 mm, but a further increase in the mixing speed did not result in its decrease [52]. Shear stress causes the disintegration of the statically grown granules if they are used as an inoculum for SBR reactors. As was reported in the study [4], disintegration resulted in the dramatic increase in the number of smaller granules of smaller diameter (an average of 80 ± 18 more new granules).

4.5. Temperature

There is a lack of literature related to the impact of temperature on OPG cultivation. The majority of the studies were conducted at room temperature, i.e., 20–23 °C [4,26]. The cultivation was also conducted at a temperature higher than the mentioned range, i.e., 22–26 °C [41]. Filamentous cyanobacteria can grow at temperatures below 10 °C; however, the growth rate is lower, and at higher temperatures, i.e., higher than 25 °C, the growth is the highest [54]. The literature shows that the optimum temperature for granule formation is 30 °C. Interestingly, temperature significantly influences the symbiosis between cyanobacteria and bacteria and COD and phosphorus removal pathways. At low temperatures, bacteria dominate in OPGs, while the opposite trend is observed at higher temperatures. In the research of Ji et al. [55], the lowest COD removal was observed at 30 °C, while the highest phosphorus removal efficiency was recorded at 22 °C. The temperature did not affect ammonium nitrogen removal. Moreover, they observed that low temperatures could lead to the creation of "fluffy" granules (that have poor sedimentation properties) due to growth filamentous microalgae.

4.6. Light Intensity

Light intensity is a crucial parameter that directly influences OPG cultivation. Many studies report that optimal light intensity that promotes OPG formation is within the range of 8–10 klux. However, the effect of higher light intensity was also studied [4,26]. For example, a range of 6.4–25 klux was applied for the hydrodynamic cultivation of OPGs [45]. Too high light intensity promotes the growth of green algae rather than filamentous cyanobacteria [4].

Some studies report the values for the light intensity as photosynthetically active radiation (PAR). Some reported PAR values are 20–50, 160–200, 150, 101–115, and 117–450 $\mu\text{mol/m}^2\cdot\text{s}$ [4,27,45,48,56]. There are also publications claiming that it is possible for biogranules to be created at light levels of up to 2000 $\mu\text{mol/m}^2\cdot\text{s}$. However, in this case, one has to take into account the possibility of photoinhibition of cultivation at light intensities of 550 $\mu\text{mol/m}^2\cdot\text{s}$ [57,58].

The typical power rating of the lamps used for the studies is 15 W; however, higher values were also studied. For instance, in the study related to the static cultivation of OPGs [9], a wide range of light intensity was applied for static cultivation of OPGs, i.e., 9–27 W. It was found that too high or low light intensity leads to unsuccessful granulation and that an optimal value is required for OPG growth. From the point of view of biogranule cultivation, it is crucial to ensure a stable light–dark cycle and an adequate light intensity (see Table 2). Only such conditions are conducive to the development of cyanobacteria and the production of hydrophobic proteins, which are important for the formation of a stable biogranule structure with good sedimentation properties [49,59].

Table 2. Sedimentation properties and size of biogranules in relation to illumination [60].

Light Intensity ($mmol/m^2 \cdot s$)	Light/Dark Cycles	Size (mm)	SVI (mL/g)
2000	10 h/14 h	0.6	100
200	24 h/0 h	1.3	24
200	24 h/0 h	0.5	58
150	3.5 h/2.5 h	0.8	61
150	3.5 h/2.5 h	1.2	Nd
100	3.5 h/2.5 h	1.8	53
284	8 h/16 h	2.2	42

SVI—volume index; Nd—no data.

Besides light intensity, it was shown that wavelength also influences the cultivation of microalgae and cyanobacteria cultures [61,62]. For both microalgae and cyanobacteria, chlorophyll a is a primary photosynthetic pigment that has absorption peaks at 680 nm (red) and at 440 nm (blue). Besides chlorophyll a, phycobiliproteins act as accessory pigments that have absorption peaks at 620 and 650 nm [61]. Sunlight that has a broad absorption spectrum provides only 43% of energy for photosynthesis [63]. It was therefore proven that matching the wavelength of the light source with the pigments that depend on the microbial species provides improvement in the photosynthetic efficiency (six times as efficient as broad-spectrum light for the *Synechocystis* species) [61].

4.7. Condition of Cultivation

As mentioned in Section 3.1, the culture conditions influence the physical properties of the biogranules. For instance, Gikonyo et al. [64] compared the hydrodynamic properties of OPGs produced under dynamic (SBR reactors) and hydrostatic conditions. In the case of the first biogranulates, the average settling velocity was 0.0086 m/s (range: 0.0031–0.022 m/s) with a granule diameter ranging from 0.30 to 2.70 mm. For hydrostatically formed OPGs, on the other hand, settling velocities were 0.027 m/s (range: 0.01–0.05 m/s), while diameters of biogranules were in the field of 6.00–14.00 mm. In turn, the average density and porosity for reactor OPGs and hydrostatic OPGs were 1037 kg/m^3 and 86% and 1050 kg/m^3 and 81%, respectively. Moreover, the porosity of the reactor OPGs correlated with the size of the granules (decreased as their size increased), whereas this trend was not observed for hydrostatic granules. A decrease in settling velocities with increasing size of the hydrostatic granules was also observed. The authors link this phenomenon to the shape of the granules, which, unlike reactor OPGs, are rougher and thus experience higher frictional resistance.

5. Application of OPGs for Wastewater Treatment

Biological wastewater treatment is an essential part of any wastewater treatment that makes it possible to achieve the compliance with the environmental regulations related to the wastewater reduction and discharge or even in many cases surpass them. The widely applied AS process is a basis of biological wastewater treatment, which still provides decent treatment quality, despite outdatedness of the technology [31]. The next figure (Figure 8) provides a typical technological scheme of the conventional AS process [65].

The AS process relies on the large consortium of aerobic microorganisms that degrade organic matter in the form of wastewater. The microorganisms create flocs that exist in suspension and the air is supplied to maintain aerobic conditions during the treatment. Typical HRT for the conventional AS process is within the range of 5–14 h [66]. An interesting alternative to the CAS process is the possibility of using OPGs for wastewater treatment. This is supported not only by their physico-chemical properties (Table 3), but above all by their adaptability to widely different environmental conditions, the possibility of using both inorganic and organic nutrients in the effluent, the low energy requirement for treatment, low greenhouse gas emissions, and the production of many valuable products during treatment [46,49,59,67].

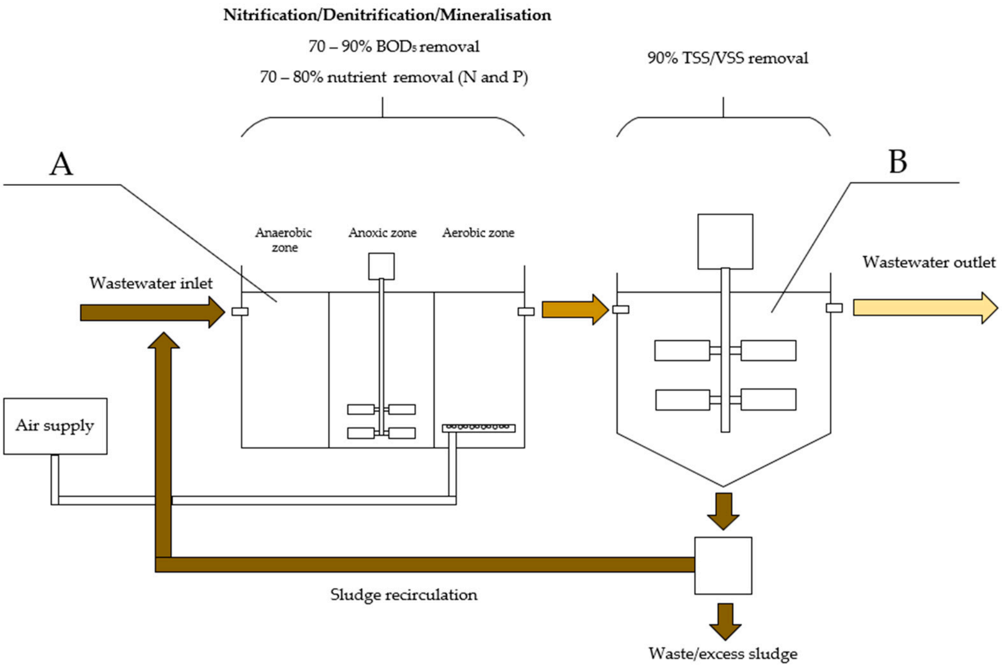


Figure 8. Conventional AS process scheme, based on [65]; where: A—reaction/aeration tank; B—secondary clarifier.

Table 3. Comparison of the physico-chemical properties of biogranules with AS flocks [23].

	Size	Density (g/mL)	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
OPGs	0.1–5 mm	Highly variable	36–360	Nd	Nd	78–95
AS flocs	0.5–1000 µm (mostly < 100 µm)	1.002–1.006	0.6–15	100–150	>0.95	>99

Nd = not detected.

So far, several successful studies were conducted in which the efficiency of OPGs in terms of wastewater treatment is evaluated [68,69]. OPGs were used for treatment of primary effluent (PE) as well as screened raw wastewater. The reactors were SBRs of 1.2–3 L that were inoculated with statically produced granules in order to reduce the start-up time (from 8 to 3 weeks). The stirring intensity was 100 rpm, and the reactors were illuminated with fluorescent light, delivering PAR of 90–150 μmol/m^2·s. The COD and nutrient removal efficiencies, however, were not mentioned in the paper [41].

A pilot scale study was also conducted in which PE was treated in 10 L and 30 L reactors illuminated with 10 klux lamps for 53 days (Pilot A). The average soluble chemical oxygen demand (sCOD) removal was 65% ± 8%. The successful operation allowed the researchers to initiate the second pilot reactor (Pilot B), in which the effect of higher OLR was studied. The average sCOD removal increased to 73% ± 6%. The third pilot (Pilot C) was conducted for 150 days, and the average sCOD removal was even higher 83–85%. The ratio of chlorophyl a to b was 6–7 as an average, which was associated with the intense growth of filamentous cyanobacteria [54].

The next table (Table 4) presents results of the studies that were conducted so far in relation to the application of OPGs for wastewater treatment.

Despite the small number of publications and the fact that most of the research was carried out at a very small laboratory scale, one publication evaluating the potential of the new technology in the context of wastewater treatment can be found in the literature. Based on experimental data from a laboratory-scale OPGs system and a reference CAS system, the publication's authors carried out a comparative LCA analysis for a hypothetical medium-sized wastewater treatment plant (equivalent to 10,000 to 50,000 people). The analysis clearly showed that the OPGs system had a lower environmental impact than the CAS system did. The disparity for impact categories ranged from 4% for freshwater eutrophication to 61% for ionising radiation. The only exceptions were noted for the impact categories of terrestrial eutrophication and acidification, respectively, two and three times higher for the OPGs system than the CAS system. The authors also showed a significant disparity in the electricity consumption of the two systems. The total electricity consumption for the OPGs system was 359 Wh/m^3 and was covered mainly by the combustion of the biogas produced, with only 90 Wh/m^3 coming from the electricity grid. The CAS system, on the other hand, required 263 Wh/m^3 from the electricity grid to cover its energy requirements, with the biogas produced covering only 137 Wh/m^3. The authors of the publication also identified the following key factors, the optimization of which will further reduce the environmental impact of OPGs technology: (1) reduction of electricity consumption associated with artificial lighting, (2) biomass concentrations, (3) nitrogen flows in the system, and (4) the fate of the biomass produced (transformation of the biomass into energy and organic fertiliser) [3].

Table 4. Operational conditions and results of COD as well as nutrient removals from some recent studies.

Type of Wastewater	Reactor Volume, (L)	PAR, (µmol/m^2·s)	Stirring Intensity, (rpm)	Time of Operation	COD Removal, (%)	Nutrient Removal (Nitrogen/ Phosphorus), (%)	HRT (d)	Reference
PE as well as screened raw wastewater	1.2–3	90–150	100	3 $^{(1)}$	Nd	Nd	0.75	[41]
PE	1.2	150	100	150 $^{(2)}$	82–86	90–96 $^{(3)}$ 52–57 $^{(4)}$ 21–44 $^{(5)}$	0.9, 0.75	[27]
Raw municipal wastewater	Nd	150	Nd	Nd	85	71 $^{(6)}$ 75 $^{(5)}$	0.5	[3]
Nd	1	Nd	Nd	Nd	59.68	87.50 $^{(6)}$ 85.37 $^{(5)}$	Nd	[9]
Synthetic wastewater (modified BG11 medium)	1.7	500	Nd	148 $^{(2)}$	Nd	Nd	0.33, 0.67, 1, 2	[44]
PE	1	101–115	100	150 $^{(2)}$	41–90	85–95 $^{(7)}$ 95–100 $^{(8)}$	1, 3	[4]
PE	2	150	100	Nd	50–98	14–65 $^{(4)}$	0.75	[53]
PE	8, 10–30	10 $^{(9)}$	100	53 $^{(2)}$	50–76	93	0.75, 1	[54]
High saline wastewater	3 $^{(9)}$	46	Nd	Nd	85.36 ± 2.84 $^{(10)}$	93.30 ± 2.07 $^{(3)}$ 77.68 ± 5.81% $^{(5)}$	1	[69]
Raw	60 (HRAP)	Natural sunlight	Nd	Nd	80	80 $^{(3)}$	Nd	[70]
Municipal	2 (P-SBR)	Artificial light	200	Nd	87	68 $^{(6)}$ 16 $^{(5)}$	2	[71]
Synthetic municipal	0.5	200	Nd	33 $^{(2)}$	47.6–59.9	56.5–78.1 $^{(3)}$ 61.5–74.25 $^{(5)}$	0.5	[72]

Nd—no data; $^{(1)}$—weeks; $^{(2)}$—days; $^{(3)}$—ammonia removal; $^{(4)}$ total dissolved nitrogen (TDN); $^{(5)}$—phosphorus removal; $^{(6)}$—total nitrogen (TN); $^{(7)}$—TN at 2-h light condition; $^{(8)}$—TN at 12 and 24-h light condition; $^{(9)}$—expressed in klux; $^{(10)}$—TOC removal; PAR—photosynthetically active radiation; HRAP—high-rate algal pond.

6. Conclusions and Potential Future Development Directions

The article's data clearly indicate the high potential inherent in OPGs. Benefits of the technology are:

- Excellent settling velocity which allows easy separation of biomass from treated water;
- Better COD and nutrient removal efficiencies compared to CAS;

- In situ oxygen production coupled with denitrification, so mechanical aeration, which characterises CAS is not required;
- Generation of autotrophically rich biomass that may be used as a source of renewable energy.

Notwithstanding the benefits of the technology, there is still a long way to go before they can be used in wastewater treatment at full scale. For this reason, future research in this area should focus on the following aspects:

- Scale-up, most research to date has been conducted at very small laboratory scale using synthetic wastewater under ideal or well controlled conditions;
- Impact of long-term process application on effluent quality;
- Adaptation of the OPGs production cycle to the natural diurnal cycle, weather conditions explore issues relating to symbiosis between bacteria and algae, particularly in the context of energy storage in the form of lipid, poly-P and glycogen;
- Developing new bioreactors and solutions for the cultivation of OPGs in order to ensure the transmission and penetration of light appropriate to their growth, also, include the stability of OPGs;
- The potential of OPGs for the removal of emerging contaminants;
- The reuse of the produced biomass of OPGs, including practical work on its conversion into biofuels and its thermal disposal, as well as the possibility of recovering energy from them through anaerobic digestion. The high methane potential of OPGs, estimated to be up to 20% higher than that of AS, argues in favor of directing research in this direction; to the best of the authors' knowledge, research in this direction has not yet been carried out;
- Research into technologies for converting biogranules into value-added products.

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