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# Microbial Granule Technology—Prospects for Wastewater Treatment and Energy Production

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**Abstract:** Recent years have brought significant evolution and changes in wastewater treatment systems. New solutions are sought to improve treatment efficiency, reduce investment/operational costs, and comply with the principles of circular economy and zero waste. Microbial granules can serve as an alternative to conventional technologies. Indeed, there has been fast-growing interest in methods harnessing aerobic (AGS) and anaerobic (AnGS) granular sludge as well as microbial-bacterial granules (MBGS), as evidenced by the number of studies on the subject and commercial installations developed. The present paper identifies the strengths and weaknesses of wastewater treatment systems based on granular sludge (GS) and their potential for energy production, with a particular focus on establishing the R&D activities required for further advance of these technologies. In particular, the impact of granules on bioenergy conversion, including bio-oil recovery efficiency and biomethane/biohydrogen yields, and bioelectrochemical systems must be assessed and optimized.

**Keywords:** aerobic granular sludge; anaerobic granular sludge; algal-bacterial granules; wastewater treatment; sewage sludge; waste to energy; energy recovery

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## 1. Introduction and Background

Recent years have brought significant evolution and changes in sewage treatment systems, both for domestic and industrial wastewater—a process driven by the ever-more stringent regulations regarding treated effluent quality and implementation of circular economies [1]. Other major drivers include global zero-waste policies, greenhouse gas (GHG) emission limits, as well as calls for reducing energy consumption and improving the share of renewables in the total energy mix [2]. Nowadays, wastewater is actually no longer treated as waste, nor are wastewater treatment plants (WWTPs) treated as systems designed primarily to degrade or neutralize pollutants for safe discharge into the environment. New WWTP installations and upgrades of existing ones are increasingly being designed in accordance with the integrated biorefinery approach, where energy and value-added substances/feedstocks are harvested from the wastewater and sludge [3,4]. Types of substances recoverable from wastewater are listed in Figure 1.

Despite the many reports supporting the practicability, competitive performance, and cost-effectiveness of energy/material recycling in WWTP systems, the vast majority of current plants still use the energy-intensive and waste-generating suspended growth (aerobic activated-sludge) process [5]. In this method, pollutants are removed by harnessing bacterial metabolism, activated by creating separate zones with different aerobic conditions [6]. Organic compounds are biodegraded over the entire WWTP process line in the aerobic zone, hypoxic zone, and anaerobic zone [7]. Nitrogen removal requires consecutive anaerobic ammonification steps, followed by nitrification in the aerobic chamber and denitrification, with nitrates converted to molecular nitrogen and released into the atmosphere [8]. Phosphorus is removed in two stages. Orthophosphates are first released into the wastewater under anaerobic conditions and then intensively accumulated by poly-P bacteria under aerobic conditions [9,10]. Phosphorus can also be removed via chemical precipitation with inorganic coagulants and polyelectrolytes [11]. A flowchart for a traditional suspended-activated-sludge WWTP is presented in Figure 2.

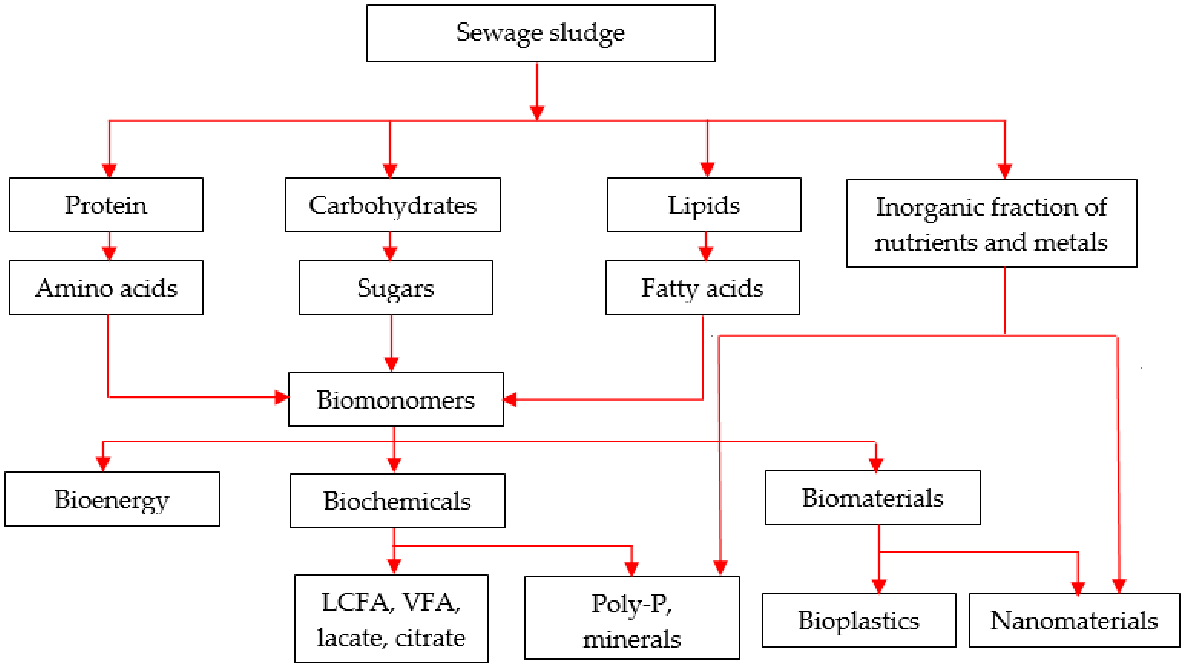


**Figure 1.** Energy carriers and added-value products recoverable under the 'WWTP as an integrated biorefinery' approach (inspired by [1,3,4]).

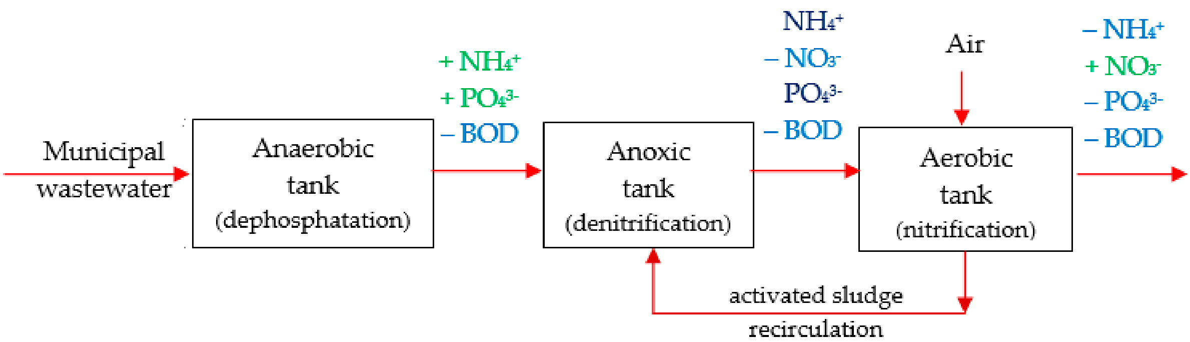


**Figure 2.** A process flowchart for a suspended-activated-sludge WWTP (inspired by [7–10]).

It should be noted that the activated-sludge method is not ineffective as such. However, it does require large-scale facilities, is very energy intensive due to the intensive aeration and stirring required, and generates environmentally-harmful sludge which is difficult to manage and recycle [12]. Digesters can serve as an alternative to the activated-sludge process, as they offer significant advantages and can be used in a wide range of applications in industrial and municipal sewage treatment systems [13]. Anaerobic WWTPs can efficiently biodegrade organic matter with relatively low investment and operating costs. Anaerobic plants and accompanying facilities do not take up much space and produce little in the way of anaerobic surplus sludge, further bolstering the attractiveness of the technology [14,15]. Finally, digestion technologies can also be used to produce and capture methane-rich biogas—a considerable advantage over the aerobic activated-sludge process [16]. Such reactors do not have to be aerated nor (at least in some cases) stirred, making them quite energy efficient [17]. A step-by-step conversion flowchart for the anaerobic wastewater treatment is given in Figure 3.

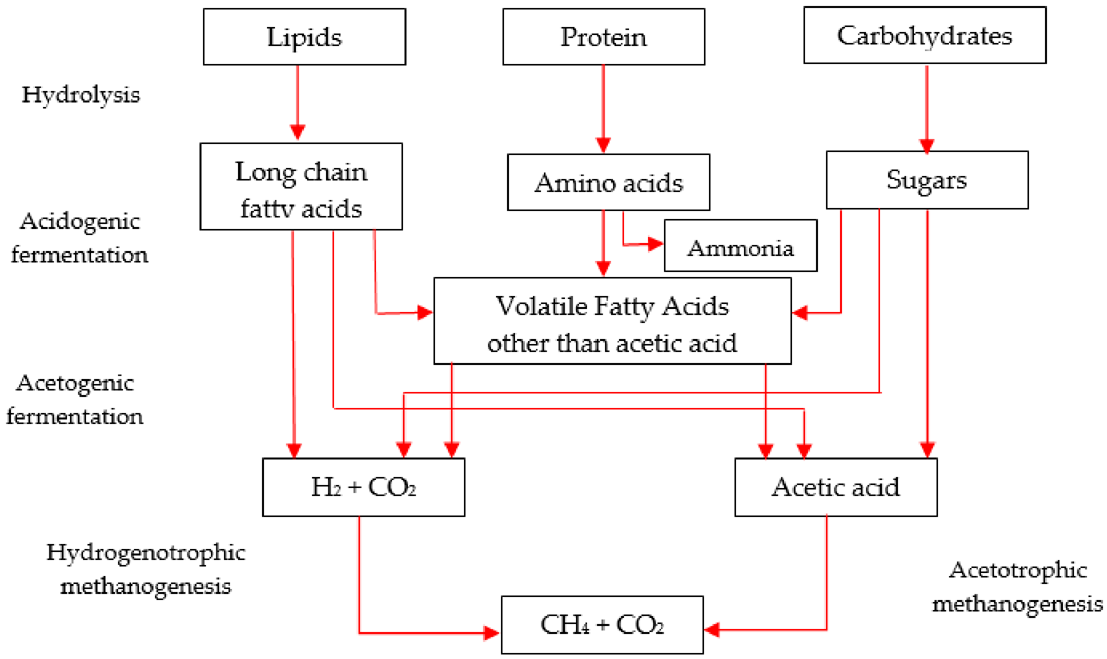


**Figure 3.** Methanogenic fermentation pathways for anaerobic wastewater treatment (inspired by [14–16]).

One often-cited weakness of anaerobic technologies is low nitrogen and phosphorus removal [18]. In the anaerobic processes, nutrients are only removed when incorporated into the microbial biomass [19]. This issue limits the versatility of the technology, which is why further advancement of anaerobic methods is predicated on eliminating this hurdle. For this reason, anaerobic technologies are very often treated as the first step in a more elaborate processing chain [20]. In order to achieve final effluent quality sufficient to discharge it to a receiving water body, digesters are usually integrated and coupled with other facilities and equipment. Several innovative solutions and methods can be used to overcome this deficiency of anaerobic reactors and improve their technological and commercial performance [21]. These include active filling, dissolution of metals, zeolites, adsorption/absorption methods, inorganic coagulants, alkaline substances, integration with microalgae cultivation systems, and many other physical and chemical treatments. Another problem with continuous-flow (fully-stirred) anaerobic reactors is the commonly-cited difficulty in separating the bacterial biomass from the treated wastewater [22]. Anaerobic bacteria are very small and of low-density, which complicates the processing due to their retention in the reactor [23]. Conventional settling tanks are not efficient enough, coagulation can lead to secondary wastewater contamination, and the highly advanced membrane filtration methods are costly and difficult to manage [24]. The anaerobic sludge discharged with the treated wastewater increases the pollutant levels in the final effluent, and in turn, its reduced levels in the digester lead to impaired biodegradation performance [25].

## 2. Microbial Granule Technology as an Alternative

The shortcomings of the above-presented methods necessitate the development and implementation of technologies that would provide high removal rates of organic compounds and biogenes while keeping investment and operational costs low. These criteria have been adopted as consistent with the current trends of circular economy and energy conservation, resulting in heightened interest in aerobic (AGS) and anaerobic (AnGS) granular sludge—which can, in many cases, be a feasible substitute for current processes [26,27]. Bibliometric analysis of the world's major academic research databases speaks to the popularity of granular sludge-based (GS) wastewater treatment methods. The 2010–2021 statistics for the search terms "aerobic granular sludge" and "anaerobic granular sludge", given in Figure 4, suggest that the topic is drawing ever-growing interest from research institutions worldwide.

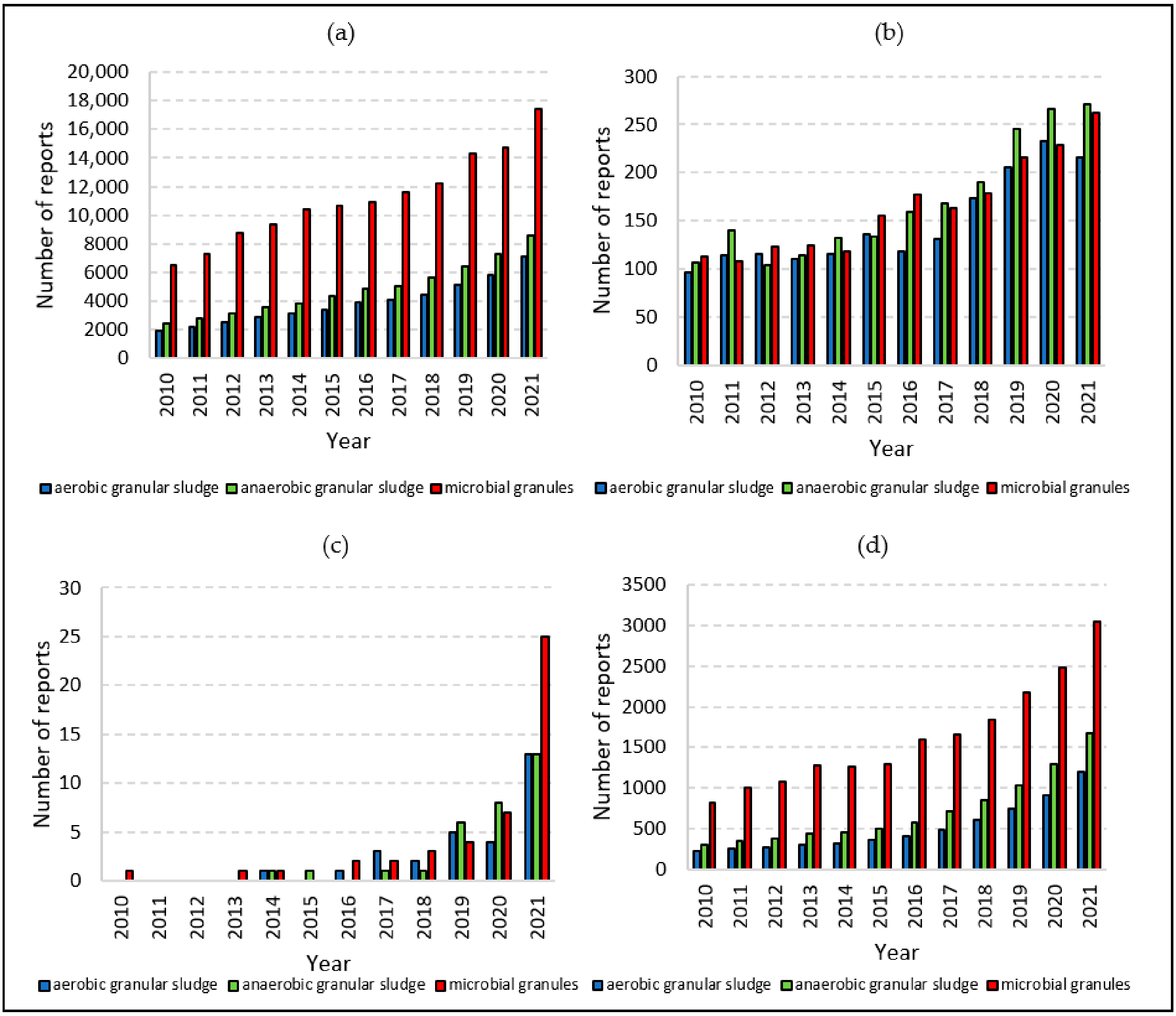


**Figure 4.** Number of articles found in (**a**) Google Scholar, (**b**) Scopus, (**c**) MDPI, and (**d**) Science Direct databases between 2010 and 2021 for the keywords "aerobic granular sludge", "anaerobic granular sludge", and "microbial granules". Accessed 30 October 2022.

Also of note is the newest approach that calls for harnessing the biological consortia of microalgal-bacterial granular sludge (MBGS) [28]. Through just the last few years, it has been cited as an alternative for the bacterial treatment of various sewages [29]. The microalgae in the MBGS generate oxygen for bacterial growth and take up carbon dioxide released in the course of bacterial metabolism, resulting in a strict symbiotic relationship between the microalgae and the bacteria [30]. The role of microalgae in this symbiotic system is threefold: they absorb mineral pollutants (mainly nitrogen and phosphorus compounds), generate the oxygen that feeds the metabolism of MBGS bacteria, and accumulate high-value-added products [31]. The innovative nature of this technology is demonstrated by bibliometric analysis of results for the search terms "microalgal-bacterial granular consortium" and "algal-bacterial granules" between 2010 and 2021 (Figure 5) as compared with the results for AGS and AnGS in Figure 4.

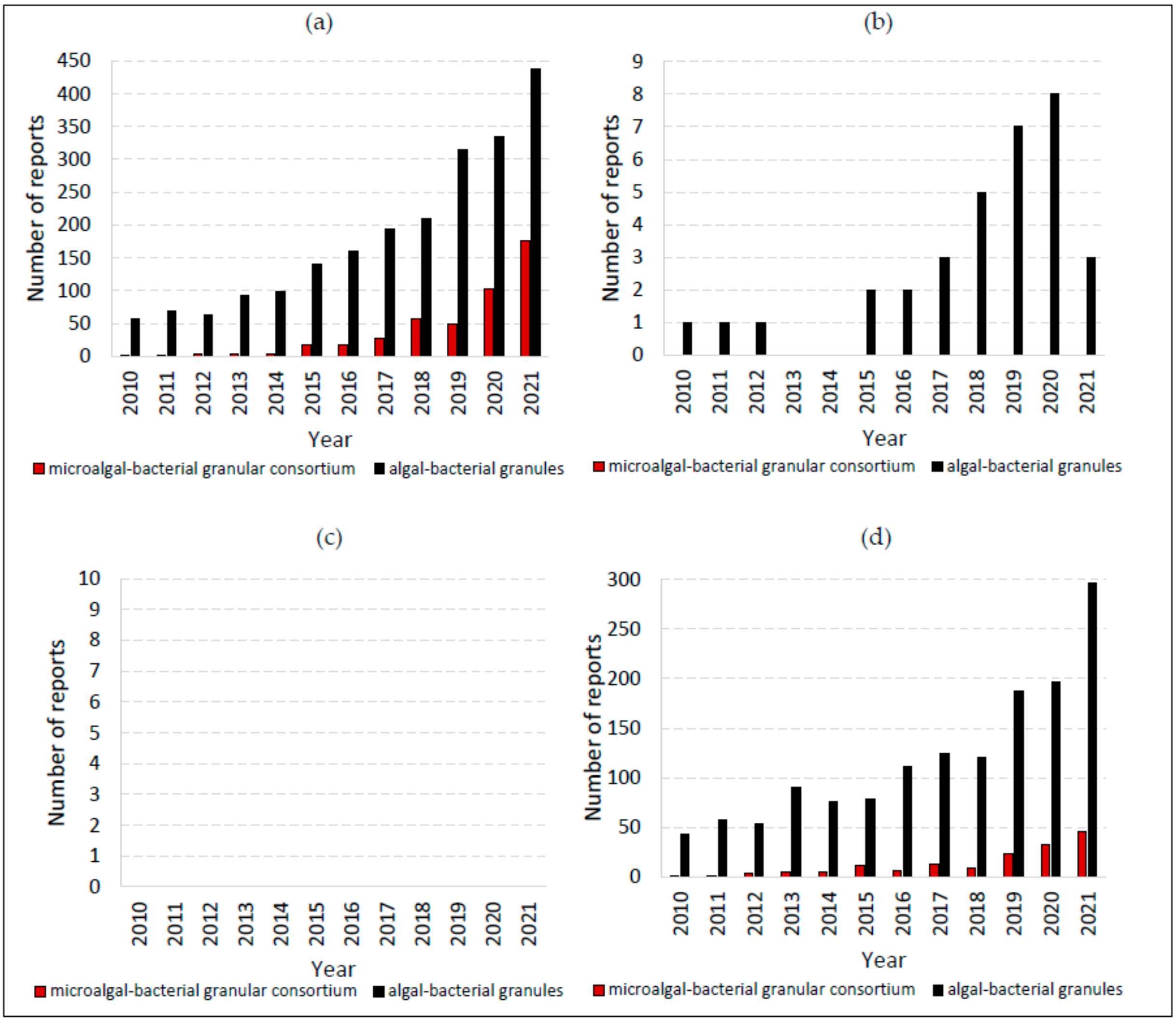


**Figure 5.** Number of articles found in (**a**) Google Scholar, (**b**) Scopus, (**c**) MDPI, and (**d**) Science Direct databases between 2010 and 2021 for the keywords "microalgal-bacterial granular consortium" and "algal-bacterial granules". Accessed 30 October 2022.

The aim of this paper is to present the strengths and weaknesses of GS-based wastewater treatment for aerobic and anaerobic pollutant removal. Its main focus was on delineating avenues of research, design, and deployment for the faster and broader development of AGS/AnGS. After all, the advantages of GS technologies have been extensively described in the literature, and it seems prudent to harness these strengths in commercial WWTPs. The paper also outlines the state of the art, and explores potential and promising applications of GS in energy production.

## 3. Why Granular Microbial Consortia?

Many technological parameters and aspects come into play when deciding which wastewater treatment system to employ [32]. An important step in the investment decision-making process is to assess the current "technological readiness level" (TRL) of the given process. TRL allows investors to assess the operational details and economic aspects of a product/technology, prospects for further development, and the associated innovative risk [33]. As such, it is a universal measure for assessing how far a technology has progressed and whether it is ready for commercial deployment. This is necessary to prepare materials and compile documentation, including environmental documentation necessary to obtain the relevant permits to launch the investment process [34]. The ultimate choice of technology is also dictated by its reliability, confirmed efficiency, performance, cost-effectiveness, space requirements, difficulty-to-use, availability of know-how, and other factors [35]. The main deciding factors in technology deployment are presented in Table 1 [36–38]. AGS and AnGS wastewater treatment seems to be competitive compared to popular conventional methods.

**Table 1.** Main deciding factors for choosing a wastewater treatment process.

| Parameter | Aim |
|---|---|
| Quality of treated effluent | Compliance with limits for discharged treated effluent |
| Variable loads in the wastewater | Tolerance to variations in hydraulic and organic loads |
| Toxic chemicals and/or metals | Tolerance to toxic pollutants |
| Reliability | Long-term stability and sustainable treatment |
| Operation and maintenance | Flexibility, simplicity, minimal complexity, and low cost |
| Capital costs | Minimum and optimal use |
| Operating costs | Lower energy consumption |
| Space requirements | Minimal space requirements |

AGS and AnGS technologies, alongside biofilm and fluidized-bed systems, fall under a relatively new paradigm of applying biomass-growing systems in WWTP reactors [39,40]. This is particularly relevant to the pollutant removal performance and the cost-effectiveness of the venture. Developing and implementing technologies that ensure the high density of the bacterial community is a vital aspect of the entire process, and one that calls for a re-evaluation of existing technical and technological assumptions for wastewater treatment systems.

AGS and AnGS granules are formed when microbial cells self-immobilize in response to certain specific environmental conditions in the reactor [41]. This means that these biological structures can be grown not only in newly designed plants, but in most existing wastewater treatment systems. An undeniable advantage of this technology is that it can be used to obtain a granular microbial biomass by controlling the technological parameters of the process or applying slight modifications to the bioreactor design. The primary triggers of granulation within a bacterial community are a high volumetric exchange ratio and high flow rate (usually generated via internal recirculation), as the resultant hydrodynamic forces act upon the biomass [42]. The scheme of microbial granule formation in the example of AGS is shown in Figure 6 [43].

The highly concentrated biomass obtained by microbial granulation allows bioreactors to handle much higher pollutant loads than conventional suspended-sludge systems. In fact, the specific pollutant load in the aerobic/anaerobic activated sludge and the resultant specific pollutant load rate for the reactor volume are the basic design parameters for WWTP [44,45]. The calculations usually draw on the organic load rate (OLR). The organic load in the reactors determines the ability of microorganisms to convert specific products. In aerobic systems, this mainly relates to effective nitrification for efficient nitrogen removal from the wastewater, which can only occur at low OLRs [46]. In anaerobic processes, the OLR mediates the balance between the acidogenic and methanogenic steps of digestion, which directly affects the quantity and composition of the resultant biogas, as well as the pollutant removal rate [47].

Increasing maximum OLRs through microbial granulation and incorporating higher OLRs into design and engineering practice would allow WWTPs to handle higher pollutant loads without changing facility size—a crucial feature when upgrading existing WWTPs. This can also be incorporated into the design and construction of new plants, i.e., building smaller bioreactors without loss of treatment efficiency, owing to the much lower pollutant load in the influent. Since AGS and AnGS wastewater biotreatment systems can run at relatively high pollutant loads, they have found widespread use in treating highly concentrated domestic and industrial sewage [48,49].

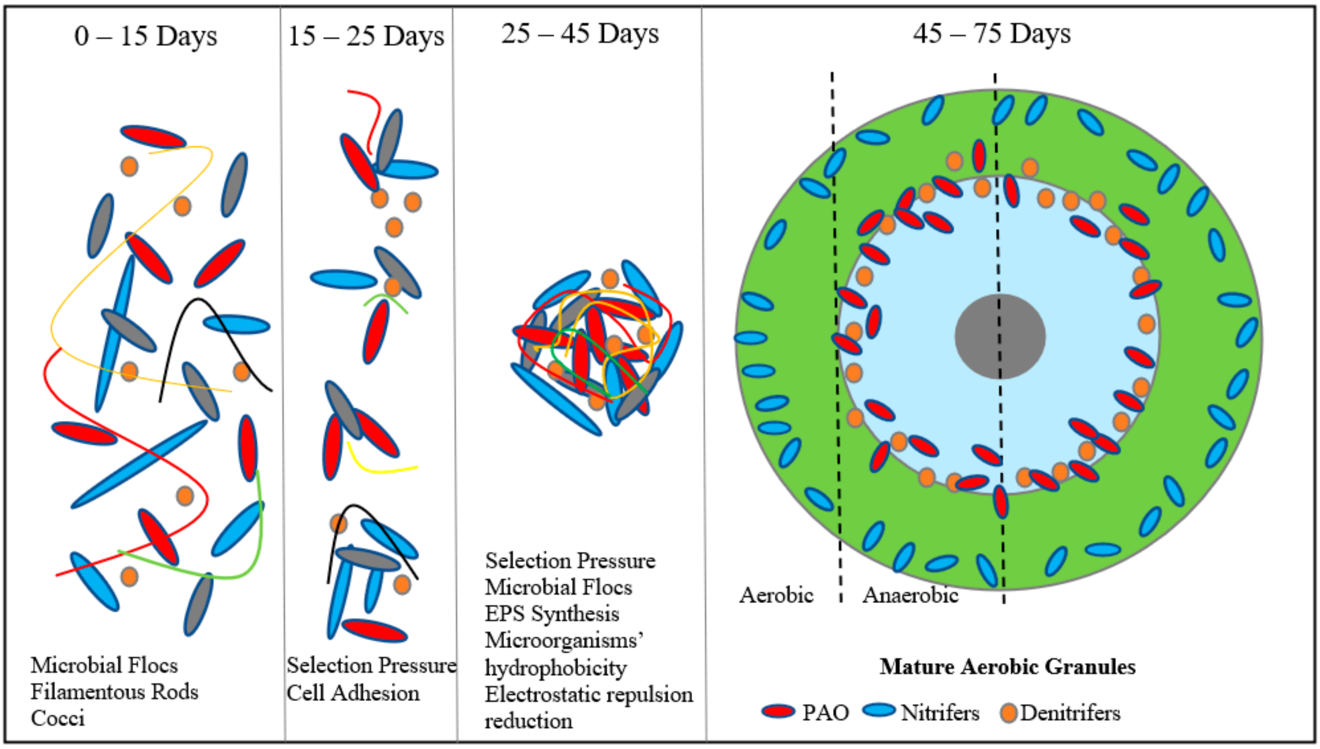


**Figure 6.** Illustrative chart of granulation process.

An important, positive aspect of the use of AnGS is the high and coordinated activity of anaerobic microorganisms involved in the subsequent stages of anaerobic digestion [50]. Ensuring conditions for efficient interspecies electron transfer (IET) between syntrophic symbionts is considered by many researchers to be the key to obtaining high technological efficiency of the process [51]. This phenomenon plays an important role in the biodegradation of complex organic compounds and the transformation of $CO_2$ to $CH_4$ under anaerobic conditions. It has been proven that in well-developed AnGSs, the balance of the syntrophic relationship is ensured, which provides thermodynamically favorable conditions for the biodegradation of carboxylic acids, and thus for the production of stable products of anaerobic processes [52]. Balance in the AnGS biocenosis is ensured by efficient indirect interspecies electron transfer (IIET) using electron carriers such as hydrogen and formate [53]. Disruption of balanced syntrophy can lead to the accumulation of intermediates such as volatile fatty acids, as well as high hydrogen partial pressure in the environment, which consequently causes a significant deterioration in the efficiency of anaerobic digestion [54]. An alternative to IIET in anaerobic conditions is direct interspecies electron transfer (DIET). DIET is judged to be energetically more favorable because it does not require the production of hydrogen for use as an electron carrier [55].

In addition to performance, the decision on whether to use AGS or AnGS is also dictated in practice by the economic aspect. It has been noted that the broader range of supported OLRs in the chamber can significantly reduce the investment costs of building the facility, including biological chambers and secondary settlement tanks [56]. The highly packed granules settle readily and quickly, while the high diversity of species within the spheres and the variety of conditions ensure high wastewater treatment performance [57]. The strengths and functional advantages of GS-based technologies are presented in Table 2 [58–60].

**Table 2.** Strengths and functional advantages of granular sludge technologies.

| **Functional capabilities** |
| --- |
| High retention of biomass for faster treatment |
| No problems with sludge-bulking |
| Combined COD, N, and P removal from wastewater |
| Simple process flow for N and P removal |
| P removal through increased biological removal of P |
| Removal of pollutants through biological redox reactions |
| Tolerance to shock loads, medium loads, and toxic pollutants |
| **Strengths** |
| Reduced sludge production and easy sludge dewatering |
| Lower energy cost due to minimal recirculation flows |
| Compact and fast-settling biomass, enabling reduced bioreactor volume |
| No secondary clarifiers |
| Lower space requirements and capital costs |

Given its morphology and diversity of aerobic conditions, each mature activated-sludge granule in an aerobic WWTP can be viewed as an individual mini bioreactor with integrated removal of carbon and biogenic compounds [61]. They allow concurrent organic removal, ammonification nitrification, denitrification, and orthophosphate fixation to be conducted in a single sequencing batch reactor (SBR), making it possible to resign from the construction of conventional process flows (three chambers with different aerobic medium conditions) requiring large areas [62]. The technological parameters required to ensure the effective removal of organic and biogenic pollutants during different biochemical conversion steps are presented in Table 3 [63–65].

**Table 3.** Pollutant removal steps in aerobic wastewater treatment systems and their required technological parameters.

| **Pollutant Removal Steps** | **Technological Parameters** |
| --- | --- |
| Dephosphatation | Oxygen content: about 0.2 mg $O_2/dm^3$<br>pH: 6.5–8.0<br>Temp.: optimum 18–20 °C<br>Availability of carbon source (raw sewage)<br>Holding time: about 1 h |
| Nitrification | Dissolved oxygen content > 2 mg $O_2/dm^3$ and theoretically 4 mg $O_2$/mg $NH_4^+$<br>pH: 5.5–9 (optimally 7.5)<br>Temp.: 20 °C<br>Microelements: Ca, Fe, Cu, Mg, P<br>Gaseous ammonia with a concentration below 1 mg/$dm^3$ (toxic to nitrifiers)<br>No other toxic compounds (phenols, antibiotics, etc.)<br>Neutralization of the formed nitrous acid, which inhibits both phases of nitrification<br>The presence of carbon dioxide or carbonates as a carbon source for autotrophs |
| Denitrification | Dissolved oxygen content < 0.5 mg $O_2/dm^3$<br>pH: 6.5–7.5<br>Temp.: 20 °C<br>$BZT_5$: $N_{og.}$ > 4–6 |

Other potential advantages of AGS include better take-up of dissolved oxygen, higher tolerance to non-uniform flow rate and wastewater composition (including the presence of toxic substances), and reduced generation of surplus sludge, which helps alleviate certain issues with sludge management in WWTPs [66].

In the case of AnGS, the reduced cost of wastewater treatment compared to aerobic processes stems from the lack of need for aeration. It is estimated that this can produce energy savings of up to 75% for anaerobic processes [67]. AnGS also eliminates reactor stirring, so the bulk of the operating costs is instead spent on keeping recirculation systems running so as to provide optimal hydraulic conditions for granule formation [68]. The granular form of sludge, with its higher density and significantly improved settleability, has much more of a positive impact on anaerobic wastewater treatment systems than aerobic ones. Aerobic activated-sludge bacteria can form a floc (even in suspended form), which can be easily removed while maintaining optimal hydraulic loads in secondary gravity clarifiers [69]. Unlike aerobic activated sludge, anaerobic bacteria in continuous-flow systems are highly dispersed and fragmented, which effectively translates to low density and settleability. Separating the bacteria from the treated effluent requires expensive and elaborate separation systems, including filtration and biofilm technologies [70]. Separation issues and release of anaerobic suspended sludge in continuous-flow reactors give rise to multiple technological issues, directly reducing the treated effluent quality due to higher levels of suspended solids, and, with them, biogenic substances and organics. They also complicate the maintenance of optimum anaerobic bacteria concentrations in the bioreactors [71]. As such, incorporating GS into anaerobic wastewater treatment systems should be a priority. Example applications of AGS and AnGS in wastewater treatment are presented in Table 4.

**Table 4.** Example applications of aerobic and anaerobic granular sludge in wastewater treatment.

| **Aerobic Granular Sludge** | | | |
|---|---|---|---|
| **Type of Wastewater** | **Operational Conditions** | **Results** | **Ref.** |
| Piggery wastewater | SBR:<br>Working volume: 30 L Air flow rate: 80–100 L/h<br>Cycle time: 3 h<br>pH: 7.0–7.5 | COD removal: 98%<br>Ammonia removal: 97%<br>TN removal: 92%<br>Antibiotics:<br>5.2% discharged 62.5% degraded<br>32.3% adsorbed | [72] |
| Pulp mill wastewater | SBR:<br>Working volume: 4.5 L Upflow air velocity: 2.2 cm/s | COD removal: 73%<br>TN removal: 74%<br>Phosphorus removal: 52%<br>Tannin/lignin removal: 54%<br>Phenols removal: 70% | [73] |
| Synthetic wastewater | SBR:<br>Working volume: 3.5 L Superficial air flow velocity:<br>1.3 cm/s | EE2: 16.09 µg/g, 77%<br>NP: 20.05 µg/g, 93%<br>CBZ: 10% | [74] |
| Synthetic wastewater | Lab-scale SBR: Working volume: 1.3 L Cycle time: 6<br>h<br>DO: 6–8 mg/L<br>SRT: 10 days<br>HRT: 12 h | Granular size:<br>Rb 1.18 mm and Rv 0.92 mm<br>SVI5: 32 mL/g(Rb) and 38 mL/g<br>(Rv)<br>Ammonia removal: > 99% Carbon<br>removal: > 95%<br>P removal: 70%–75% in Rb and 44%<br>in Rv | [75] |
| Domestic wastewater | SBR:<br>Cycle time: 3 h Working volume: 3 L 50 days with<br>addition of acetate as carbon source 125 days<br>without additional carbon source | Complete granulation after 51 days<br>Average diameter: 1.5–2.0 mm | [76] |

**Table 4.** *Cont.*

| Anaerobic granular sludge | | | |
|---|---|---|---|
| **Type of wastewater** | **Operational conditions** | **Results** | **Ref.** |
| Olive mill wastewater | Upflow anaerobic sludge blanket (UASB):<br>Working volume: 6 L<br>Total volume: 6.2 L<br>Continuous recirculation for gentle mixing of the bioreactor's content using an upflow velocity of 1 m/h.<br>The anaerobic granular sludge, consisting of uniformed granules (1–3 mm), was acquired from a full-scale UASB digester treating dairy wastewater.<br>Temperature: 37 ± 1 °C<br>HRT: 9 d<br>OLR: 4.21 g COD/($L_R$.d) | COD removal: 32 ± 12.7%<br>Phenols removal: 69 ± 14 % | [77] |
| Olive mill wastewater | HUASB (hybrid-UASB):<br>Working volume: 6 L<br>Total volume: 6.2 L<br>The plastic biomass carriers with an active area of 800 $m^2/m^3$ (actual size of 2.5 cm diameter and 0.3 cm height) were packed in the upper part of the bioreactor.<br>Continuous recirculation instead of agitation, for gentle mixing of the bioreactor's content using an upflow velocity of 1 m/h.<br>The anaerobic granular sludge, consisting of uniformed granules (1–3 mm), was acquired from a full-scale UASB digester treating dairy wastewater.<br>Temperature: 37 ± 1 °C<br>HRT: 9 d<br>OLR: 4.21 g COD/($L_R$.d) | COD removal: 32 ± 6.3%<br>Phenols removal: 46 ± 14% | [77] |
| Vinasse effluent | UASB reactor inoculated with granular sludge<br>Volume: 40.5 L<br>HRT: 2.8 d<br>OLR: 0.2–7.5 g COD/L·d<br>Upflow velocity: 0.019 m/h | The average COD removal: 49–82% | [78] |
| Vinasse effluent | UASB reactor inoculated with granular sludge<br>Volume: 21.5 L<br>HRT: 2.8 d for 219 days and then decreased to 1.8 d<br>OLR: 0.2–11.5 g COD/L·d<br>Upflow velocity: 0.018 m/h | The average COD removal: 49–82% | [78] |
| Synthetic wastewater | A lab-scale plexiglass UASB reactor with height of 71 cm, diameter of 6.8 cm, and total volume of 3.5 L. In stage I (days 1–65), the UASB reactor was started, and the sludge was domesticated with a hydraulic retention time (HRT) of 24 h. In stages II (days 66–91) and III (days 92–112), the HRT was gradually decreased to 6 h for increasing the upflow velocity. In stages IV (days 113–127) and V (days 128–143), 150% and 300% recycling were added to alleviate the antibacterial effect of allicin and increase the upflow velocity. The UASB reactor was fed by a peristaltic pump from the feed tank. The operational temperature was controlled at 30 ± 2 °C. | COD removal: 93.26%<br>EPS enhanced AnGS formation and allicin resistance under allicin stress. The bacterial community contained *Acinetobacter* and *Petrimonas* as dominant allicin-resistant genera in AnGS formation process cooperating with the EPS producers *Comamonas* and *Thauera*, which improved AnGS tolerance to allicin. | [49] |

## 4. Barriers and Limitations

Laboratory findings and operational data can easily be used to identify problems and weaknesses with bacterial-granule-based WWT processes. There have been many reported difficulties with obtaining the right granule structure, size, shape, and density, both for AGS and AnGS [79,80]. In both cases, growing fully-functional GS takes time—up to several months in extreme cases [81]. Granulation requires maintaining optimal wastewater treatment parameters, including a suitable flow rate and intensity, hydraulic load, pollutant load in the wastewater, specific organic load in the sludge, and, to a lesser extent, age of the sludge, intensity of aeration and stirring, and alternations to the design and process cycle of the biological reactor [82].

The time-consuming and difficult process of growing a GS inoculum translates to longer start-up periods and longer time to achieve the target/design treatment rate. These metrics need to be improved to bolster the performance and cost-effectiveness of the process. The low availability and steep market cost of the sludge provide another motivation to develop production protocols for aerobic and anaerobic GS. Optimizing the procedures for restoring the microbial community of sludge is especially important, as they have to be activated quickly in the event of a WWTP emergency (whether mechanical, technological, or energy-based). Failure to do so may lead to production stoppage and closure of the company—one of the most significant barriers to GS technology growth and dissemination.

There have been reported issues with granular biomass instability, even in well-operated WWTPs. In most cases, they emerge during long running times [83]. Granule disintegration leads directly to reduced pollutant removal, decreased sludge settleability, and secondary contamination of the final effluent with the dispersed bacterial suspension, resulting in higher levels of pollutants monitored during the treatment process. AnGS plants treating wastewater with high levels of lipids and suspended solids require an additional pre-treatment step [84]. In practice, this means that bioreactors usually have to be accompanied by a flotation tank, further complicating their exploitation and driving up the investment/running costs. Some researchers have also noted that high levels of extracellular polymeric substances (EPS) generated by granular sludge microbes can negatively impact pollutant removal rates [85–87].

Another barrier to the competitiveness of GS is the limited knowledge on how to manage and ultimately neutralize the resultant surplus sludge. Due to the different properties and characteristics of GS, the current sludge-to-energy processes need to be tested for suitability and effectiveness under the new conditions. The underpinnings and technological parameters of the process need to be validated and adapted to a substrate with a different chemical composition and properties. Relatively little research to date has focused on analyzing and optimizing GS use for energy, so there is a real need to review the existing findings and find prospective avenues for future research and practical efforts—ones that could further scientific understanding and the practical applicability of the process. The weaknesses of AGS and AnGS technologies are presented in Table 5 [77,88–90].

**Table 5.** Problems of granular-sludge technologies.

| Limitations |
|---|
| Slow granulation or start-up time |
| Poor long-term granule stability |
| AGS systems became less stable when high suspended (floccular) biomass fractions occur |
| The limitation of mass transfer is serious in granules with high density and big size, which affect the specific COD removal rate negatively |
| Various industrial wastewater characteristics negatively impact the sludge granulation process or even lead to de-granulation and loss of biomass |
| Although anaerobic granules were discovered first back in 1976, some definite limitations have been identified such as long start-up period, high operation temperature, and unsuitability for low-strength organic wastewater |
| In a UASB reactor, granule flotation and loss of structure can occur leading to biomass leaching |

**Table 5.** *Cont.*

| **Shortcomings** |
| --- |
| Novel technology requires more understanding and research |
| Technical bottlenecks such as long granulation period and long-term granule instability limit the rapid commercialization of this biotechnology |
| The lack of clear design and performance considerations for implementing AGS-based full-scale reactors which remain the major issues impeding the adoption of AGS in the municipal WWTPs |

## 5. New Directions of Biotechnological Granulation

Systems that harness microalgae-bacteria symbiosis represent a new direction in biotechnological microbial granulation [91]. Results so far have earned them a reputation as a very promising and versatile new technology which can be applied in the biological treatment of sewages of different types and origins [92,93]. Research is underway to identify the right conditions for the wastewater treatment process and the right technological parameters and medium conditions, with a view to developing a process that could provide repeatable and effective granulation of such consortia [94,95].

So far, microalgal-bacterial granules (MBGS) have been shown to form as cells self-aggregate in a sequential bioreactor under natural sunlight [96]. The resultant granules are highly stable, compact, and dense, performing well in terms of pollutant removal and gravity separation (settling) of the treated effluent [97]. It has been shown that filamentous bacteria in the activated-sludge bacterial community play an important role in the formation of stable and compact granules, forming the scaffold and structure of the granule, to which other bacterial and microalgal cells are then incorporated via external polymeric substances [98].

Compared with conventional AGS, as the microalgal biomass in the MBG grows, so do nitrogen and phosphorus removal rates [99]. It has also been demonstrated that granules formed through microalgae-bacteria symbiosis contain a high proportion of oil-producing microalgae [100]. This oil bestows the biomass grown during wastewater treatment with considerably higher calorific values and makes it a more universal substrate for energy production. MBGS can also accumulate other value-added substances, whose recovery from the surplus biomass may be technologically and commercially viable [101]. This is part of the reason why microalgal-bacterial granules are emerging as a promising and sustainable method for biotechnological wastewater treatment. Commonly cited advantages of this method include easy separation, excellent settleability, high pollutant removal rates, lower running (aeration) costs, and high-value-added biomass [102,103].

In microalgae-bacteria symbiosis systems, autotrophic organisms are responsible for boosting nitrogen and phosphorus removal—an essential feature from both a performance and commercial standpoint [104]. Removal of biogenic substances in traditional activated-sludge and bacterial-granular-sludge WWTPs requires complex and expensive processing and, in many cases, supplementary chemical treatment. For a purely bacterial community to remove nitrogen, the aerobic–anaerobic conditions have to be alternated in a sequence of ammonification, nitrification, and denitrification. The organic load rate in the sludge also has to be carefully controlled [8,105]. Similarly, phosphorus bioremoval requires sequential aerobic and anaerobic steps, very often supplemented with chemical precipitation. These treatments are expensive and elaborate, with considerable expenditure required to maintain aeration and recirculation within the plant [106].

In addition to absorbing nitrogen and phosphorus, MBG microalgae also produce molecular oxygen via photosynthesis [107]. The activated-sludge method is an aerobic WWT process that requires sufficient aeration. Maintaining this aeration is the primary cost driver for the process. Reducing the oxygen demand is the main obstacle to cutting emissions from activated-sludge WWTPs. In microalgae-bacteria symbiosis systems, the microalgae-produced oxygen feeds the metabolism of aerobic bacteria, improving the performance and cost-effectiveness of the process [108,109]. In turn, bacteria degrade pollutants into mineralized nitrogen and phosphorus, as well as carbon dioxide, which fuel the growth of microalgae in the granule [110]. As noted earlier, microalgae accumulate high-value-added products, such as astaxanthin, β-carotene, polyunsaturated fatty acids, and biodiesel, which facilitate the recovery and recycling of the algal-bacterial biomass [111]. It has also been demonstrated that microalgae can partially shift towards mixotrophic or even heterotrophic metabolism at high levels of biodegradable nutrients in the medium, boosting carbon removal from the wastewater [112]. The relationships between taxonomic groups present in MBGS are illustrated in Figure 7.

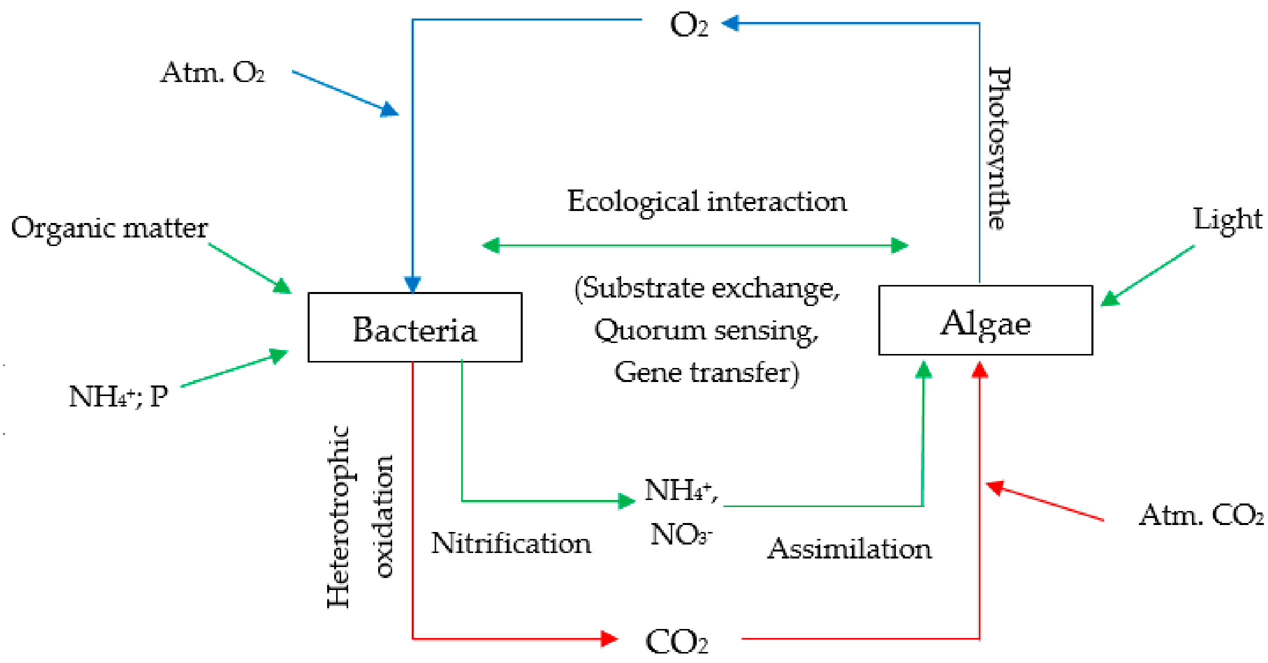


**Figure 7.** Symbiotic conversions occurring in microalgal-bacterial granules.

However, microalgae are primarily photosynthesizing autotrophs, whose growth and metabolic activity are predicated on adequate light conditions in the system. Prior studies have identified lighting intensity, light source, photoperiod, and light quality to be major determinants of microalgae growth, metabolic activity, and biomass composition [113]. Adequate illumination can significantly drive up the investment and running costs of a system, which is why researchers have mainly focused on finding ways to utilize sunlight and/or minimizing the use of artificial lighting [114]. This is all the more important as excessive light intensity can induce photoinhibition of microalgae, ultimately leading to the reduced metabolic capacity of the biomass and impaired performance of wastewater treatment [115].

Systems utilizing symbiotic microalgae-bacteria granules remain strictly in the realm of R&D, unlike purely bacterial AGS and AnGS [115]. In fact, some of the mechanisms governing microalgal-bacterial granulation have yet to be identified. Research is still underway to determine how various operational and environmental parameters affect the process, as well as to identify ways of accelerating the formation of granules and maintaining them for long-term bioreactor use [108,116]. These initial experiments bode well, both in terms of granule formation and the resultant wastewater treatment performance, giving reason to believe that we may see full-scale granule-based installations in the near future. Example applications of MBGS in wastewater treatment are given in Table 6.

**Table 6.** Example applications of microalgal-bacterial granular consortia in wastewater treatment.

| Type of Wastewater | Operational Conditions | Results | Ref. |
|---|---|---|---|
| Synthetic wastewater | Sequencing batch reactor:<br>Working volume of 0.92 L<br>Temp.: 20 °C<br>Illumination of 45 Lx (12 h/12 h)<br>Volume exchange of 50%<br>Operation time: 4 h<br>2 min feeding + 60 min non-aeration + 172 min aeration + 3 min settling + 2 min decanting + 1 min idle period. | COD removal: 98%<br>TN removal: 78%<br>TP removal: 71% | [117] |
| Synthetic wastewater | Stirring batch reactors:<br>Working volume: 250 mL<br>R1: no salinity<br>R2: 1% salinity<br>Temp.: 25 ± 2 °C<br>Operating cycle: 6 h (7 min feeding + 60 min of non-aeration + 282 min aeration + 2 min settling + 8 min decanting + 1 min of idling)<br>Volume exchange rate (VER): 50%<br>Aeration: 0.81 cm/s<br>Illumination: 6000 lx<br>Retention time: 30–40 days | COD removal: 96.5%<br>TN removal: 78–85%<br>TP removal: 80.8% | [118] |
| Synthetic wastewater | Glass bioreactor:<br>Working volume: 50 mL<br>Temp.: 26 °C<br>Illumination intensity:<br>210 µ mol/$m^2$s | COD removal: 70.5%<br>TN removal: 80.7%<br>TP removal: 73% | [30] |
| Synthetic wastewater | Sequencing batch reactor:<br>Working volume: 500 mL<br>Temp.: 25 °C<br>4 h operational time<br>Volume exchange ratio of 50%<br>Aeration of 0.87 cm/s<br>Illumination: 3600 lx | COD removal: NM<br>TN removal: 66%<br>TP removal: 70% | [119] |
| Simulated wastewater | Sequencing batch reactor:<br>Working volume: 2.0 L<br>4 h cycle (2 min feeding + 232 min aeration + 4 min settling + 2 min decanting)<br>Volume exchange ratio: 50.0%<br>HRT: 8.0 h<br>DO: 7.0–9.0 mg/L<br>Aeration: 1.2 cm/s (2.0 L/min)<br>Temp.: 18.0–23.0 °C<br>Illumination: 1531 mmol/$m^2$s<br>COD/$NH_4$-N: 309.4 /213.6 mg/L | COD removal: NM<br>TN removal: 96.5%<br>TP removal: NM | [120] |
| Simulated wastewater | Glass bottles:<br>Working volume: 50 mL<br>In open environment 12 h/12 h | COD removal: during day 59.9 ± 6.8%, during dark 47.6%<br>N removal: during day $NH_4^+$-N: 78%, during dark 56%<br>P removal: during day 61%, during dark 74% | [91] |

## 6. Energy Aspects

Extracting energy and materials from sludge, including granular sludge, has great commercial and environmental value. This feature is a key justification for including wastewater treatment systems in circular economies. Depending on its process of origin, granular sludge can contain between 50 and 70% organic matter by dry mass, 30–50% nitrogen, and trace phosphorus, as well as a wide variety of other nutrients with potential energy, commercial, and/or agricultural uses [121]. On the other hand, GS may also contain high levels of hazardous substances and toxic pollutants, as well as pathogenic bacteria, parasites, and their spores [122]. Neutralization, management, and recycling of sewage sludge is energy-intensive, very expensive, and harmful to the environment. It is estimated that sludge management is responsible for around 40% of WWTP greenhouse gas emissions and accounts for around 50% of annual running costs [123].

The characteristics of GS suggest that—just like regular surplus sludge—it should be treated not as waste, but rather as a by-product with a wide variety of potential uses, including energy production. This approach opens the door to harnessing the many value-added substances contained in GS and recovering energy from it. It is also fully in line with the principles of circular economy, bioeconomy, and energy and material recycling. The calorific value of GS is estimated to be between 10 kJ/kg DM and 16 kJ/kg DM, similar to that of conventional fuels such as coal and other types of biomass used in the wider energy industry [124]. In this light, surplus GS can be viewed as a solid fuel or as starting feedstock to produce liquid or second-generation biofuel gas. The recovered energy can be utilized in-house to heat bioreactors or dewater/dry sludge—in fact, there are already many examples of such facilities attaining positive energy balance [125,126]. Excess heat and power can also be fed into the grid. Applications of GS in energy generation are given in Figure 8.

Anaerobic digestion is one of the most common sludge-to-energy technologies [127,128]. This bioconversion method is widely used, owing to our excellent understanding of the technology, its relatively straightforward nature, low cost, and ability to convert high-moisture organic waste. The resultant biogas—a mixture of carbon dioxide and methane—can be enriched and upgraded into biomethane [129]. Biomethane, a substitute for natural gas, can be fed into the pipeline, used to fuel compression-ignition engines, and/or converted into heat and power via cogeneration [130]. Sludge can also be processed through dark fermentation to obtain hydrogen gas [131]. For this particular process, the inoculum must be purified by expunging methane-producing bacteria from the medium, leaving only the hydrolyzing and acid-producing species [132]. This is usually done through thermal conditioning of the anaerobic sludge [133]. Another important variable is the OLR, which is significantly higher than in the case of methane fermentation [134]. This parameter can play various roles in anaerobic digestion and fermentative hydrogen production, depending on the type of granule used (AGS, MBGS, or AnGS). For AGS and MBGS, a typical organic feedstock is used, which is converted into gaseous metabolites by anaerobic bacteria [135]. In turn, AnGS processes employ digester inoculum (primarily of continuous-flow reactors) which mediates the successive anaerobic conversions such as hydrolysis, acidogenesis, acetogenesis, methanogenesis, and hydrogen production [136].

Some applicable waste-to-energy technologies are also based on thermochemical conversion. These include combustion, pyrolysis, and gasification [137]. However, these processes require lower levels of moisture in the biomass, and sludge dewatering processes are very expensive both in terms of the initial investment and the ongoing costs (energy consumption, repair, maintenance, and operation). At present, the standard operating procedure is to incorporate a heat recovery process into the thermal treatment of sludge in incineration plants [138]. This enables heat recovery from flue gas. The recovered heat is used to heat a liquid (usually water), which can be used directly for heating or to generate electricity using a steam turbine. Pyrolysis is conducted in an oxygen-free atmosphere at a temperature range of 300 to 900 °C [139]. This thermal treatment results in a mixture of pyrolysis oil, biochar, and assorted gases (CO, $H_2$, $CO_2$, $CH_4$, and other hydrocarbons) [140]. Operating temperature, heating rate, and residence time in the high-temperature zone are parameters critical to the quality of the end product [141]. The bio-oil can be upgraded and used as a liquid fuel, or reformed into syngas. Biochar, gases, and bio-oil can also be used as fuel and combusted to generate electricity and heat [142]. Gasification is another well-known sludge-to-energy technology. This method converts organic compounds by partial oxidization (at oxygen levels lower than the stoichiometric demand) at high temperatures (650–1000 °C) to maximize the production of gas (CO, $H_2$, $CO_2$, and low-weight hydrocarbons), especially syngas, which is composed mainly of CO and $H_2$, and has a calorific value of 4 to 28 MJ/Nm (depending on the gasification agent and temperature) [143]. Syngas can be combusted as-is in gas turbines, or subjected to upgrading and further processing [144].

Bioelectrochemical systems are a less popular and attractive (in terms of performance and cost-effectiveness) sludge-to-energy process, used to convert organic compounds to $H_2$, $CH_4$, and value-added chemical products (e.g., acetates, alcohols, and fatty acids) [145]. This process utilizes microbial fuel cells and electrochemical reduction and serves as an example of a promising and sustainable sludge-to-energy technology that has yet to be deployed on a commercial scale [146]. Due to the various technological limitations, such as low power density, low conversion factors, and low performance (which determine scalability), these systems are considered expensive and require further development [147].

MBGS can additionally provide extractable bio-oil, which can be transesterified into biodiesel [148]. Another concept, still considered innovative, is the integrated biorefinery approach, which provides for extracting value-added products and nutrients from sludge, with a focus on amino acids, proteins, biopesticides, fatty acids, phosphorus, bioflocculants, enzymes, bioplastics, and biofuels [115]. Though still nascent, the technology is ultimately intended to produce liquid transport fuels (biodiesel and bioethanol), high-added-value chemicals, syngas, heat, and power. Accordingly, it has potential uses for optimal recovery and reuse of resources from waste streams. Example GS applications in energy generation and their efficiencies are presented in Table 7.

**Table 7.** Example applications of granular sludge in energy generation and their efficiencies.

| GS | Type of Wastewater | Operational Conditions | Results | Ref. |
|---|---|---|---|---|
| AGS | Municipal wastewater | Glass bottles (OxiTop system):<br>Temp.: 36 ± 1 °C<br>HRT: 21 days<br>OLR: 2, 4, 6 kg VS/$m^3$·d | $CH_4$: 272.5–357 L/kg VS | [149] |
| | Municipal wastewater | Flasks with a volume of 2 L<br>Temp.: 35 ± 1 °C<br>Time: 44 days<br>120 rpm | $CH_4$: 197 ± 11 L/kg VS | [150] |
| | Synthetic wastewater | Flasks with a volume of 525 mL:<br>Temp.: 37 °C<br>OLR: 0.7–0.9 gCOD/ L·d<br>SRT: 15–>40 d | $CH_4$: 245–285 L/kg VS | [135] |
| | Municipal wastewater | Quartz cylindrical reactor time Pyrolysis time: 4 h<br>Temp.: 500–800 °C with a heating rate of 3 °C/min | Maximum yield of bio-oil:<br>43.6% of weight-lost percent during GSS pyrolysis | [151] |

**Table 7.** *Cont.*

| GS | Type of Wastewater | Operational Conditions | Results | Ref. |
| --- | --- | --- | --- | --- |
| AnGS | Olive mill wastewater | Upflow anaerobic sludge blanket (UASB):<br>Working volume: 6 L<br>Total volume: 6.2 L<br>Continuous recirculation for gentle mixing of the bioreactor's content using an upflow velocity of 1 m/h.<br>The anaerobic granular sludge, consisting of uniformed granules (1–3 mm), was acquired from a full-scale UASB digester treating dairy wastewater.<br>Temp.: 37 ± 1 °C<br>HRT: 9<br>OLR: 4.21 g COD/($L_R$.d) | Biogas Production Rate: 0.91 ± 0.25 $L_B$/($L_R$.d)<br>$CH_4$ content: 34.07 ± 8.20%<br>Yield: 0.21 ± 0.07 L $CH_4$/g COD converted | [77] |
| | Olive mill wastewater | HUASB (hybrid-UASB):<br>Working volume: 6 L<br>Total volume: 6.2 L<br>The plastic biomass carriers with an active area of 800 $m^2/m^3$ (actual size of 2.5 cm diameter and 0.3 cm height) were packed in the upper part of the bioreactor.<br>Continuous recirculation instead of agitation for gentle mixing of the bioreactor's content using an upflow velocity of 1 m/h.<br>The anaerobic granular sludge, consisting of uniformed granules (1–3 mm), was acquired from a full-scale UASB digester treating dairy wastewater.<br>Temperature: 37 ± 1 °C<br>HRT: 9<br>OLR: 4.21 g COD/($L_R$.d) | Biogas Production Rate: 1.01 ± 0.23 $L_B$/($L_R$.d)<br>$CH_4$ content: 35.93 ± 5.53%<br>Yield: 0.27 ± 0.08 L $CH_4$/g COD converted | [77] |
| | Vinasse effluent | UASB reactor inoculated with granular sludge<br>Volume: 40.5 L<br>HRT: 2.8 d<br>OLR: 0.2–7.5 g COD/L·d<br>Upflow velocity: 0.019 m/h | Average conversion efficiencies of the removed COD into methane: 48–58%. The largest methane yield values: 0.181 L $CH_4/gCOD_{removed}$. These values were attained after 140 days of operation with an OLR of 5.0–7.5 g COD/L·d. | [78] |
| | Vinasse effluent | UASB reactor inoculated with granular sludge<br>Volume: 21.5 L<br>HRT: 2.8 d for 219 days and then decreased to 1.8 d<br>OLR: 0.2–11.5 g COD/L·d<br>Upflow velocity: 0.018 m/h | Average conversion efficiencies of the removed COD into methane: 39–65%. The largest methane yield values: 0.185 L $CH_4/gCOD_{removed}$. These values were attained after 140 days of operation with an OLR of 5.0–7.5 g COD/L·d. | [78] |
| | Synthetic wastewater | Bottles<br>Volume: 125 mL<br>Time: 21 d<br>Temp.: 30 °C<br>Gas pressure: 0.5 bar | Ethanol production: 17.1 mM<br>Propanol: 8.08 ± 0.85 mM<br>*n*-butanol: 3.66 ± 0.05 mM | [152] |

**Table 7.** *Cont.*

| GS | Type of Wastewater | Operational Conditions | Results | Ref. |
| --- | --- | --- | --- | --- |
| | Dairy wastewater | Bottles<br>Volume: 125 mL<br>Time: 408 h<br>Temp.: 25 °C<br>Gas pressure: 1.8 bar | Ethanol production 17.1 mM | [153] |
| | Food industry wastewater | Batch bioreactors<br>Temp.: 35 °C<br>pH: 5.5 ± 0.3 | Biohydrogen: 72.9 ± 5.7 mL $H_2$/g $COD_{rem}$ | [154] |
| | Municipal wastewater | AnGS was successfully demonstrated as a novel and efficient biocatalyst in METs such as microbial fuel cells. Three different strategies were explored to shift the microbial composition of AGS from methanogenic to exoelectrogenic microbes, including varying the external resistance and organic loading and manipulating the anode potential | The significantly high current response: 10.32 A/$m^2$ and 100% removal of organic carbon from wastewater. | [155] |
| M-BGs | Synthetic wastewater | Photo sequencing batch reactors (PSBRs)<br>Illumination incubator (12 h light/12 h dark, 6000 ± 200 lux)<br>Influent feeding 1 min, aeration 356 min, sedimentation 2 min, effluent withdrawal 1 min<br>Aeration intensity: 4 L/min Temp.: 26 ± 1 °C<br>pH: 7.5 ± 0.1 | Biodiesel production: 66.21 ± 1.08 mg/g·SS | [156] |
| | Synthetic wastewater | Fixed bed reactor:<br>Temp.: 673–1073 K<br>Time: 1 h | Biooil: 39.5–45.4 wt%<br>Biochar with a nitrogen content of 3.7–7.0 wt% | [157] |
| | Municipal wastewater | Lab-scale identical SBR reactors made of acrylic plastic<br>Working volume: 2.0 L<br>Dark/light cycle: 12 h/12 h<br>Light intensity: 0–225 µmol/ $m^2 \cdot s^1$<br>Temp.: 23 ± 2 °C<br>HRT: 8 h | Lipid content: 31.2–59.6 mg/g-SS | [158] |
| | Synthetic wastewater (Ammonium-rich wastewater) | Lab-scale sequencing batch reactors (SBRs) made of transparent acrylic plastic<br>Effective working volume: 2.0 L Average light illuminance: 190 µmol/$m^2$/s with a constant dark/light (12 h/12 h) cycle<br>3 h cycle: 2 min of feeding, 20 min of non-aeration, 152 min of aeration, 4 min of settling, and 2 min of decanting<br>Temp.: 20–23 °C<br>HRT: 6 h | Lipid content: 57.4 mg/g-SS | [159] |

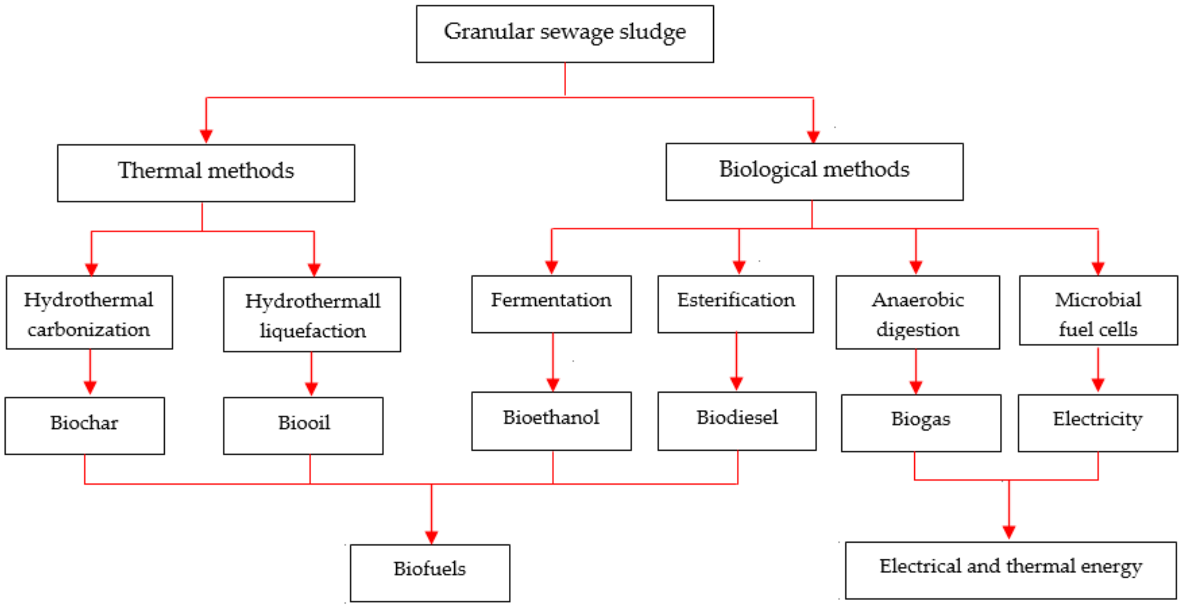


**Figure 8.** Applications of granular sludge in energy generation (inspired by [119–124]).

## 7. Conclusions

There is fast-growing interest in WWT processes based on microbial granules, as evidenced by the number of studies on the subject and by the rising number of full-scale installations. These trends speak to the promising nature of processes utilizing aerobic and anaerobic granular sludge, especially as they have been shown to be effective in practice. Multiple improvements to this technology have been proposed to further enhance pollutant removal and cost-effectiveness. Mechanisms are still being sought to accelerate the processes of granule formation, and research is underway to determine the optimal technological conditions for long-term sustainable activity—a prerequisite for maintaining a consistently high wastewater biotreatment performance.

These efforts are exemplified by attempts to harness granules that harbor symbiotic communities of microalgae and aerobic bacteria. This technology, though still innovative, can help produce stable microbial granules, improve pollutant removal, limit energy consumption by reducing the oxygen demand, and expand potential uses of granular surplus sludge in energy production. Of course, this approach is an emerging technology, and much work still needs to be completed: precisely characterizing the microalgal-bacterial granules, exploring granulation pathways and mechanisms, identifying granulation catalysts and process parameters that boost granule formation, as well as finding ways to facilitate granulation and to ensure long-term granule stability.

It is essential to pursue effective and sustainable management of surplus granular sludge with a strategy that provides not only for neutralization, but also for recovery of value-added substances and energy. Any such strategy must draw on a comprehensive and sustainable biorefinery approach that is fully in line with the principles of circular economy as well as emission and waste-mitigation policy. The unusual characteristics and properties of the granules will also be a factor in determining the potential and development of granule-to-energy processes. In particular, the impact of granules on bioenergy conversion (including bio-oil recovery efficiency, and biomethane/biohydrogen yields) and bioelectrochemical systems must be assessed and optimized. Harnessing microbial granules for environmental technologies is a very forward-looking approach that offers a feasible and practical substitute for current processes.

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