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# New Advances in Aerobic Granular Sludge Technology Using Continuous Flow Reactors: Engineering and Microbiological Aspects

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**Citation:** Rosa-Masegosa, A.; Muñoz-Palazon, B.; Gonzalez-Martinez, A.; Fenice, M.; Gorrasi, S.; Gonzalez-Lopez, J. New Advances in Aerobic Granular Sludge Technology Using Continuous Flow Reactors: Engineering and Microbiological Aspects. *Water* **2021**, *13*, 1792. https://doi.org/10.3390/w13131792

Academic Editor: Hongyu Ren

Received: 26 May 2021
Accepted: 25 June 2021
Published: 29 June 2021

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**Abstract:** Aerobic granular sludge (AGS) comprises an aggregation of microbial cells in a tridimensional matrix, which is able to remove carbon, nitrogen and phosphorous as well as other pollutants in a single bioreactor under the same operational conditions. During the past decades, the feasibility of implementing AGS in wastewater treatment plants (WWTPs) for treating sewage using fundamentally sequential batch reactors (SBRs) has been studied. However, granular sludge technology using SBRs has several disadvantages. For instance, it can present certain drawbacks for the treatment of high flow rates; furthermore, the quantity of retained biomass is limited by volume exchange. Therefore, the development of continuous flow reactors (CFRs) has come to be regarded as a more competitive option. This is why numerous investigations have been undertaken in recent years in search of different designs of CFR systems that would enable the effective treatment of urban and industrial wastewater, keeping the stability of granular biomass. However, despite these efforts, satisfactory results have yet to be achieved. Consequently, it remains necessary to carry out new technical approaches that would provide more effective and efficient AGS-CFR systems. In particular, it is imperative to develop continuous flow granular systems that can both retain granular biomass and efficiently treat wastewater, obviously with low construction, maintenance and exploitation cost. In this review, we collect the most recent information on different technological approaches aimed at establishing AGS-CFR systems, making possible their upscaling to real plant conditions. We discuss the advantages and disadvantages of these proposals and suggest future trends in the application of aerobic granular systems. Accordingly, we analyze the most significant technical and biological implications of this innovative technology.

**Keywords:** aerobic granular sludge; continuous flow reactor; granular stability; microbial community; reactor design

## 1. Introduction

Aerobic granular sludge (AGS) technology is today often regarded as a promising option for biological wastewater treatment because it has demonstrated excellent physico-chemical performance at the pilot scale; even the AGS full-scale has gained an optimum position in the area of treatment of urban and industrial sewage [1]. Granular sludge was first discovered in upflow sludge blanket anaerobic systems in the 1980s [2]. Later, at the end of the 1990s, it achieved the growth of AGS without any supporting carrier operated in sequential batch reactors (SBR) [3,4]. Currently, aerobic granular sludge technology remains challenging to implement for treating sewage water, several authors reporting the instability of granular biomass for long-term operations [5,6]. Nevertheless, several mainstream full-scale systems are being substituted by AGS technology worldwide by Nereda® for treating urban and even industrial wastewaters [7–10]. Undoubtedly, AGS technology has numerous advantages in comparison with the most common technology implemented: conventional activated sludge (CAS). Conventional technology needs longer hydraulic and sludge retention times, more time to treat and recycle sludge and a larger surface area for implementation. For these reasons, AGS has become increasingly popular among the scientific and engineering communities.

AGS technology has been employed for treating highly toxic and recalcitrant compounds in wastewater at full scale, including particulate matter, nitrogen, phosphorous, pharmaceutical compounds, phenols, nuclear waste and heavy metals [11–14]. However, it is important to mention that most recently applications are being focused on the denitrification of groundwater contaminated with pesticides and nitrates, implemented at full scale and thereby providing biosafety and high-quality drinking water [15]. Thus, nowadays the technology continues innovate in the water biological treatment area [16].

Granules are defined as compact and dense spherical biofilms with a solid structure, conformed by aggregates of prokaryotic and eukaryotic microorganisms embedded in extracellular polymeric substances, constituting a tridimensional matrix without any supporting material and encouraged by self-immobilization [17]. The excellent properties of granular biomass encouraged the high settleability, and consequently, the technology is able to remove organic matter and nutrients in a compact design reactor. Moreover, the microbial relationships found in the granular sludge encourage several metabolic pathways to degrade pollutants in syntrophic association, such as reported by Kasina et al. [18] These factors promote a technology with a low footprint, a small surface in need of implementation and low energy costs [16].

## 2. Characterization of Aerobic Granular Sludge Biomass

### *2.1. Physicochemical Characterization*

(a) Regular shape

Granular sludge is defined as a biofilm with a high degree of sphericity and a smooth surface, owing to the hydrodynamic pressure exerted by the circular and continuous motion within the bioreactor. Viruses, bacteria, archaea, protozoa, fungi and metazoan microorganisms, among others, are joined through the excretion of extracellular polymeric substances, which act as glue among cells. The spherical conformation is produced without the presence of any supporting material for promoting growth, as seen in other biofilm technologies [19,20].

(b) Excellent settleability

Settleability is a significant advantage of AGS relative to CAS. In wastewater treatment plants (WWTPs) with CAS systems, it is essential to separate the liquid phase from the solid fraction. In this sense, CAS systems require a larger land surface for building a WWTP due to floccules' lack of settleability. However, the incomparable granular decanting ability allows for easy and fast solid-liquid separation because the granules are in different phases. This fact is driven by the negative cell surface charge of granules and the hydrophobic compounds they produce, which are mainly constituted by proteins [21]; the feast–famine periods produced changes on cell surface charges that intensified the better settleability.

This settling capacity allows granules to reach a decanting velocity of up to 138 m $h^{-1}$, as reported by Muñoz-Palazon et al. [17], whereas floccules of CAS show velocities below 10 m $h^{-1}$ [11]. Such impressive settleability allows operations to be conducted in shorter time periods, ranging from 2 to 10 min. The recurrence of this action enables the selection of the most compact granules, while fluffy and filamentous flocs are washed out [22] in sequential batch reactors. The separation of solid–liquid phases takes place in the same reactor; hence, secondary clarification after biological treatment is not required. All these characteristics reduce both the surface required for implementation and the investment for the build, as also given by the vertical design of the technology.

(c) Dense structure

Dense and compact granules are selected due to their excellent settleability, as mentioned in the previous paragraph. However, this structure is also important for conferring one of the most important characteristics and advantages of granular sludge: the dense layers promote the existence of several microniches, driven by mass transfer behaviors from the outer layers to the internal layers. This mass transfer encourages the limitation of oxygen in the environment as well as the nutrients consumed in the external layers; their metabolites are thus transported to the interlayer and the nuclei. These physical properties bestow the coexistence of aerobic, anoxic and anaerobic organisms in a single granule (Figure 1). Therefore, the outermost layer is in contact with a higher dissolved oxygen concentration in the medium, which is where the heterotrophic and nitrifying organisms that carry out the oxidation process of raw water are located. Subsequently, polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) compete in the interlayers for the substrate, where oxygen is presented in small concentrations or is even absent. Finally, denitrifying microorganisms, which need anaerobic conditions, are located in the core of the granules in order to carry out the denitrification process in the absence of oxygen. In this way, the granular structure enables the removal of nutrients and organic matter within the same reactor, whereas modifications of CAS are required to remove phosphate and nitrogen [12].

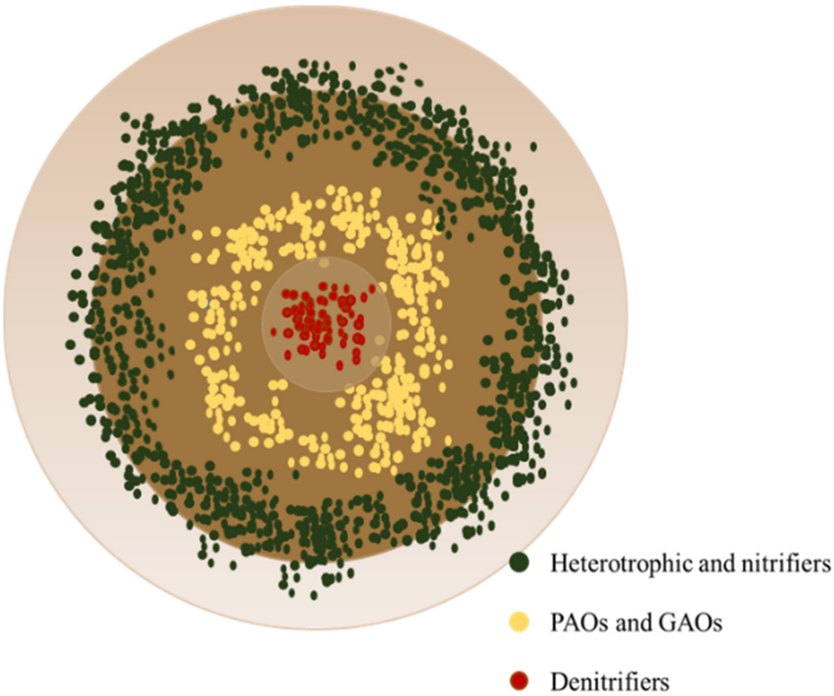


**Figure 1.** Schematic diagram of niches located in aerobic granular sludge biomass.

(d) Removal of organic matter and nutrients within the reactor

As mentioned in Subsection c, granules are able to remove organic matter and nutrients such as phosphorous and nitrogen under the same operational conditions. Nitrogen is removed via several steps. First, ammonium is oxidized to nitrite and subsequently to nitrate under aerobic redox conditions. Then, nitrite and nitrate are reduced by denitrifying microorganisms, which transforms them into nitric oxide, nitrous oxide and finally dinitrogen gas [12,16,23]. The degradation product of one microorganism can serve as the substrate for another microorganism, facilitating the complete degradation of pollutants.

In the enhanced biological phosphorus removal (EBPR) process, PAOs can store polyphosphate through the release of phosphorous in the anaerobic phase and then the 'feast uptake' of phosphorus in the aerobic stage. Both PAOs and extracellular polymeric substances can accumulate phosphorous and act as a reservoir in the biomass [24,25]. Moreover, granules can eliminate organic matter, nitrogen, phosphorous and more; they are even able to biotransform, biodegrade, bioadsorb and remove priority and emerging pollutants as highly recalcitrant substances from the wastewater of industries such as tanneries, textiles, pharmaceuticals and the chemical and mining sectors [1].

(e) Tolerance to a high organic loading ratio and high biomass retention

The high biomass retention and concentration reached in the system allow AGS to withstand high organic loads, treating more than 15 kg $m^{-3}d^{-1}$ [26,27]. AGS has been reported to be highly resistant to a wide range of organic loading rates (OLRs). The organic loading ratio and the substrate determine the kinetic behavior and morphology of granules; likewise, the granulation process is dependent on the load [26,27]. Due to the high specific surface and biomass retention capacity of granules, the technology can handle high organic loading ratios in comparison with conventional activated sludge [27]. A significant substrate concentration can promote increased microbial activity and a greater mean diameter [13]. Some studies have defined OLR as a key operational parameter for the design of WWTPs [28,29].

In addition, AGS has a high biomass retention capacity, making it possible to treat large amounts of inflow in a smaller bioreactor relative to other technologies such as CAS because, as mentioned before, the surface in contact with raw water is greater [11]. Some research has reported a concentration of mixed liquor suspended solids (MLSS) close to 10 g $L^{-1}$ [6], while in CAS technology this usually ranges from 2.5 to 4.5 g $L^{-1}$ [30].

(f) High toxicity resistance

Biological processes are poorly effective for biodegraded toxic substances due to their recalcitrant nature. However, several studies have demonstrated AGS's resistance to the toxicity of several chemicals due to the microbial activities and high levels of biomass retention provided by the layered structure [1,11,14]. Bioaggregation is a means of protecting single cells from environmental stresses and toxic compounds. Furthermore, significant levels of microbial diversity can affect various organisms differently, causing syntrophic relationships of cohabitants within granules. Redox conditions and diffusional resistance towards external molecules and then high tolerance to toxicity make granules different from the flocs found in CAS or membrane bioreactors (MBRs) [31].

### *2.2. Advantages over Conventional Activated Sludge*

The use of AGS has emerged as a feasible alternative technology to traditional floc-based CAS for the second step of wastewater treatment. It has been demonstrated at the laboratory, pilot and full scale that granular sludge has remarkable advantages relative to CAS systems (Table 1). All of the characteristics of granular biomass described previously render this technology a promising system for biological wastewater treatment, whereas activated sludge is typified by slow-settling flocculent biomass, demanding a large secondary clarifier, a lower MLSS concentration and large amounts of system energy [32]. Furthermore, AGS systems reduce the footprint due to the lower hydraulic retention time. Given these advantages, CAS infrastructure may be replaced through the implementation of AGS. Indeed, AGS requires a 25–50% smaller area for implementation, 23–40% lower electricity costs and 20–25% lower operational costs.

CAS systems are able to remove pathogen microorganisms. Floc biomass is an ecosystem where predation and biomass adsorption promote inactivation or destroy pathogens. The main way to predation is carried out by protozoan microorganisms, which have a high capacity to consume particulate matter; in this sense, pathogen bacteria can be ingested by eukaryotic organisms [13]. Alternatively, viral removal can be promoted by physical procedures, mainly by biomass adsorption, because viruses are adhered to the surface and subsequently microbial inactivation occurs [33]. Despite efforts to increase knowledge about the removal of pathogens, few studies have concluded that AGS is able to remove F-specific RNA bacteriophages such as *E. coli*, Enterococci and thermotolerant coliforms as efficiently as CAS [34]. However, there is a clear need for further studies to determine if the removal mechanisms in the two systems are comparable. This issue is promoted by the complex ecosystem found in the granular sludge able to establish several relationships between organisms. Moreover, the high abundance of extracellular polymeric substances (EPS) could act with success mechanisms to bioadsorb viral particles, which have an effect on the microbial dynamics.

**Table 1.** Comparison of AGS and CAS.

| Parameters | CAS | AGS |
|---|---|---|
| Settling ability | <10 m h$^{-1}$ | 15 to 140 m h$^{-1}$ |
| Size | <0.2 mm | 0.2 to 50 mm |
| Redox | Aerobic | Aerobic, anoxic and anaerobic |
| Compactness | Absence | High |
| EPS production | Low | High |
| Resistance to toxicity | Low | High |

Another important aspect of AGS is its resistance against toxic compounds, meanwhile AGS could treat high load shocks; in terms of organic matter, nutrients or chemical compounds, biomass concentration of CAS is deeply affected by these kinds of influents. Actually, CAS technology is a treatment not specifically designed for the removal of emergent contaminants. Then, the discharge of effluent from this kind of WWTP is a focus point for the release of emergent and priority contaminants such as pharmaceutical compounds, which reach natural water bodies [35].

## 3. Granular Formation Mechanisms and Technological Characterization

Most of the studies pointed out that the ideal conditions for the aggregation, compactness and stability of the granules are mainly given by the pressure exerted in SBR systems due to the hydrodynamic shear force and slow-sludge discharge. [11–13,36,37]. Nevertheless, this batch operation is almost incompatible with most WWTPs worldwide due to the nature of continuous flow. Some essential parameters to obtain granules are hydrodynamic shear force, the feast–famine period, circular and continuous motion and the segregation of extracellular polymeric substances.

In fact, granular formation is usually described in terms of the four stages involved. First is cell-to-cell attachment, stimulated by the physical motion caused by the contact among cells, which encourages movement within the reactor. Some of these physical forces are hydrodynamics, diffusion, gravity, cell mobility and thermodynamic forces such as Brownian movement, as reported by Sarma et al. [38]. The second stage is based on the attachment of cells and the formation of microaggregates. The forces acting on granulation can be physical, chemical and biochemical. The consensus indicates that cellular surface dehydration, cellular membrane fusion, the formation of ion pairs or an interparticle bridge, Van der Waals forces or opposite charge attraction are the strongest. In this sense, it is important to note that hydrophobicity is indirectly related to the excess Gibbs energy of the cell surface; when this is low, cell-to-cell contact is encouraged. The third stage is enhanced attachment by EPS production, whereby the essential mechanism involved is quorum sensing (QS), whose signals contribute to increased polysaccharide content, promoting strong aggregates due to their role as glue. Higher QS activity during the previous granulation stages is linked with the greater production of gel-forming EPS [39]. By contrast, the opposite process of quorum quenching (QQ) has a relevant influence on granulation because with higher QQ activity, granules trend to be fluffier and floccular [40]. The last stage comprises granular conformation and maturation by hydrodynamic shear forces, allowing mass transfer behavior in order to facilitate diverse metabolic pathways to obtain energy.

### 3.1. Extracellular Polymeric Substance Segregation

The role of EPS is to assist cell adhesion, which promotes the initial stages of granulation. Usually, the EPS are constituted by lipids, carbohydrates, proteins and polysaccharides. Some authors have noticed that they can act as a bridged union between cell and particulate matter [11] (Figure 2). Proteins and humic compounds are the most representative substances, followed by polysaccharides. These substances can be degraded during the starvation phase, which is one of the two periods of the feast–famine regime, in which there is a shortage of carbon source. That degradation during starvation stage causes an increase of the cell surface hydrophobicity and the promotion of the stability of granules. Although the enzymatic hydrolysis of proteins, lipids and α-polysaccharides has little effect on the structure of granules, the hydrolysis of β-polysaccharides causes granules to break and disintegrate [41]. Actually, some minerals could be bonded to the internal layers through EPS or fatty acids [18]. The efficient capacity of bioadsorption of AGS can be given by the EPS content [42]. It also protects single microorganism cells from shock loads and toxic compounds such as antibiotics or phenolic compounds. Reino et al. [43] have reported that EPS support the survival of a microbial consortium under adverse environmental conditions.

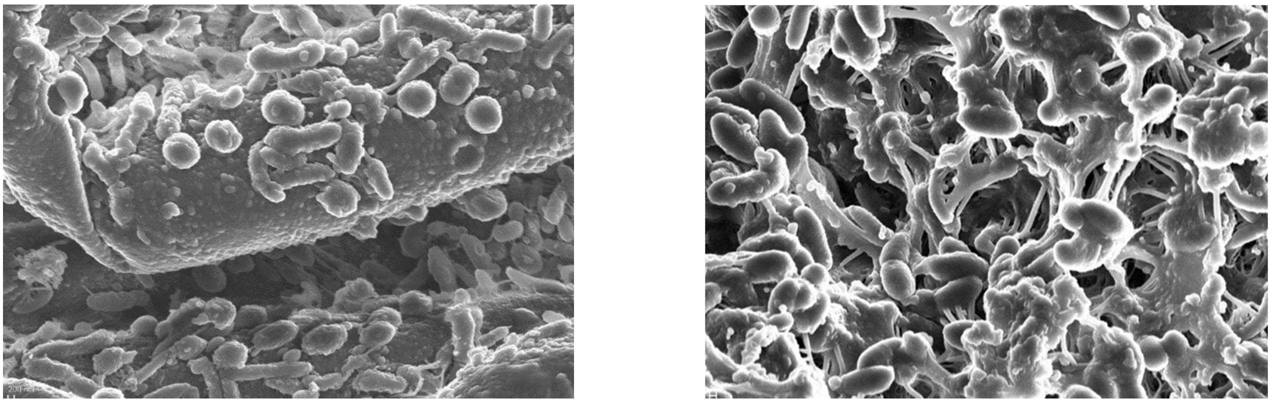

**Figure 2.** Segregation of EPS found in the core of the granules treating wastewater.

Many efforts have been made to determine EPS in AGS due to the difficulties reported by the extraction of substrates from the complex matrix in comparison with activated sludge [41]. It has been discovered that the high content of alginate polysaccharides provides the hydrophobicity and elastic assembly of granules, generating great interest in recovering the alginate contained in granular waste for food industries [44] because it acts as a stabilizing and gelling agent used in bakeries, canneries and drink industries Moreover, alginate extends shelf life and minimizes changes during the storage and handling of food [45]. Another interesting substance is named Granulan, an EPS exclusively found in granules that treat high concentrations of N and P from wastewaters, although it is unclear whether it can be found in other systems [46]. Both Granulan and alginate are soluble under mild alkaline conditions, attributed to their easy isolation and characterization, although completely opposite to the extraction of other EPS from granules [46].

### 3.2. Technological Operation

AGS technology is operated in sequencial batch reactors (SBRs), in four stages: feeding, aeration, settling and draining. Granular cultivation and operation in a steady state is thoroughly understood for achieving dense and compact biomass as well as impressive physicochemical performance in sequential batch cycles. For example, it is well-known that a longer reaction time promotes feast–famine phases, and consequently, the starvation stage encourages the density of granular sludge. Furthermore, the pressure exerted by shorter settling times makes a strong selection of rapidly decanting granules, while slow-decanting flocs are washed out. Both of the previously described examples are essential for obtaining a stable granule structure for long-term operations. However, the lack of knowledge about aerobic granular sludge rectors operated in continuous flow reactors (AGS-CFR) makes diffuse the reactor design and the mechanisms to filamentous microorganisms washed-out. In general, it is referred to as the optimum operation condition for getting stable granules using activated sludge as inoculum or to maintain the compact structure of mature granules. The loss of stability for long-term operations has been reported in SBR [47] to a greater extent than in CFRs [48]. In recent years, many efforts have been made to develop AGS technology in CFRs because control and operations in WWTPs at the full scale are easier, and the current infrastructure could be re-used [49]. In this respect, interesting advances have been achieved. Several authors have designed engineering models for the successful cultivation of granules using a range of strategies, and the number of scientific papers about AGS operated in continuous flow is continuing to increase. Perhaps one of the biggest problems facing the development of a continuous flow design for AGS is the impact of the outgrowth of filamentous bacteria in the granules because an uncontrolled proliferation of such slow-decanting microorganisms could instigate the granules' loss of stability and breakage.

## 4. Aspects of AGS in Continuous-Flow Reactors

### *4.1. Design Reactors and Engineering Aspects*

In general terms, AGS systems are built on a cylindrical column with a variable height/diameter (H/D) ratio and are operated in sequential batch cycles. The cycles in batch mode firstly consist in an aeration stage, which is used for the degradation of organic matter, nutrients and pollutants. Then, the aeration is stopped, and the granular biomass is allowed to settle at the bottom of the reactor. This parameter is essential because biomass selection pressure depends on settle time. The wash-out of biomass is subsequently linked with the discarded effluent. Finally, the reactor is re-filled with raw water. However, this operational mode presents certain disadvantages, such as the relatively low volume of treated water compared with other technologies that operate continuously. Therefore, from a technical point of view, the development of AGS working in continuous flow may represent an advantage over sequential systems.

In recent years, different designs of potentially useful granular CFR systems have been tested, in each case in an attempt to attain an optimal system that would enable a reactor to keep the granules in a steady-state, preventing the proliferation of slow-growing microorganisms. These microorganisms are responsible for the instability of granular biomass as a consequence of the outgrowth of filamentous organisms. Despite the different approaches carried out, we can affirm that for the moment, none of the experiences reported at the laboratory scale have been able to be transferred to the real scale, sometimes due to the complexity of the design or the lack of requirements for implementation. In this context, a large number of CFRs are being tested at the laboratory scale using different strategies, for example, bubble columns, serial multiple chambers, clarifiers after or within the bioreactors, or submerged membranes. Some designs are even hybrid reactors, combining aspects of SBR and CFR. A brief description of these designs is presented below.

(a) Bubble columns with baffles

Chen et al. [48] reported an upflow sludge bed reactor (USB) connected to an aeration column as a continuous flow granular system (Figure 3). The effluent from the USB was flown to the aeration tank and water and granular biomass returned to the USB to ensure a high level of biomass retention. In order to avoid the loss of biomass, a solid–liquid–gas separator was set to the top of the USB reactor. A similar separator has been employed by other authors [50,51]. Thus, Zhou et al. [50] operated a continuous flow airlift fluidized bed reactor (CAFB) that consisted in two columns, one inside the other, with a bubble burst at the top (Figure 3). The granules flew up through the internal tube and fell down by the annular tube that rounds the internal cylinder. To acclimate the sludge, the reactor operated in batch mode for 2 days and was then configured in continuous mode. In the same way, Wan et al. [51] designed a continuous-flow aerobic granular reactor (CFAGR) composed of a cylindrical reactor containing a three-phase separator and a two-decker stabilizer to avoid the loss of biomass. Kishida et al. [52,53] also designed a reactor with a similar baffle that operated in an aerobic upflow fluidized bed (AUFB) in continuous flow. This reactor consisted of a column with a gas–solid separator (located in the upper part of the reactor) and a lamella dividing the aeration zone from the effluent output zone (Figure 3).

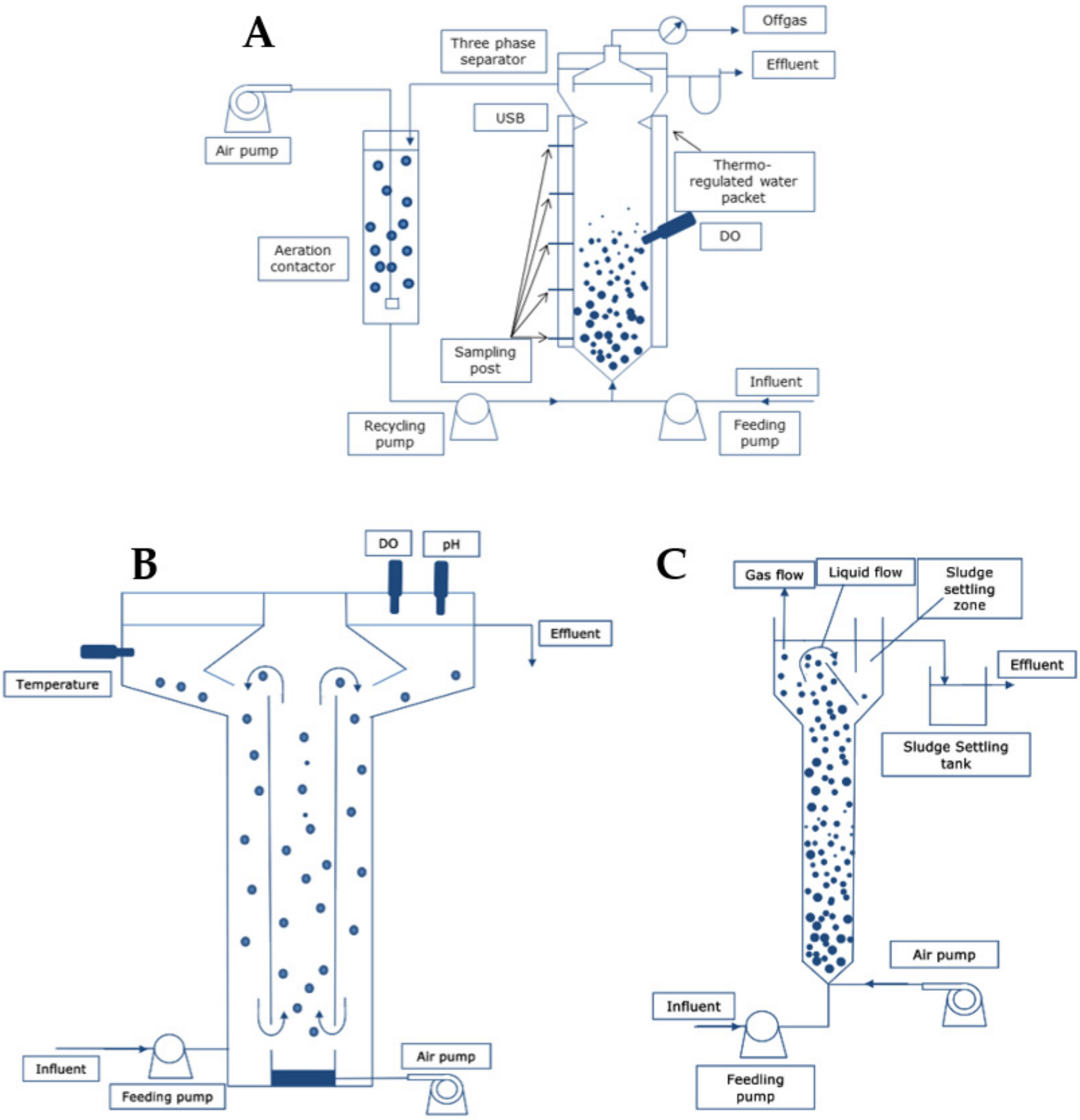


**Figure 3.** Diagram of AGS operated in a continuous flow reactor using bubble columns with baffles adapted from (**A**) Chen et al. [48], (**B**) Zhou et al. [50] and (**C**) Kishida et al. [53].

(b) Serial multiple chambers

A different mechanism to avoid the wash-out of granular biomass was operated using serial multiple chambers to retain the granules, such as the bioreactor designed by Li et al. [54]. The length of the reactor was divided by baffles to create 10 chambers in order to increase the H/D of the full reactor. Each chamber was connected with the chambers placed next to it. There was an aerator in all the cells. The direction of the influent flow changed every 2 h. On the opposite sections of the extremes, there was an agitator, but only one of them was working at the same time: the agitator located in the chamber of the influent input. Following this idea, the aerator of the last section was disconnected to create a settling zone at the bottom, where the granules were accumulated to avoid their wash-out. This configuration improved the feast–famine mechanism (Figure 4).

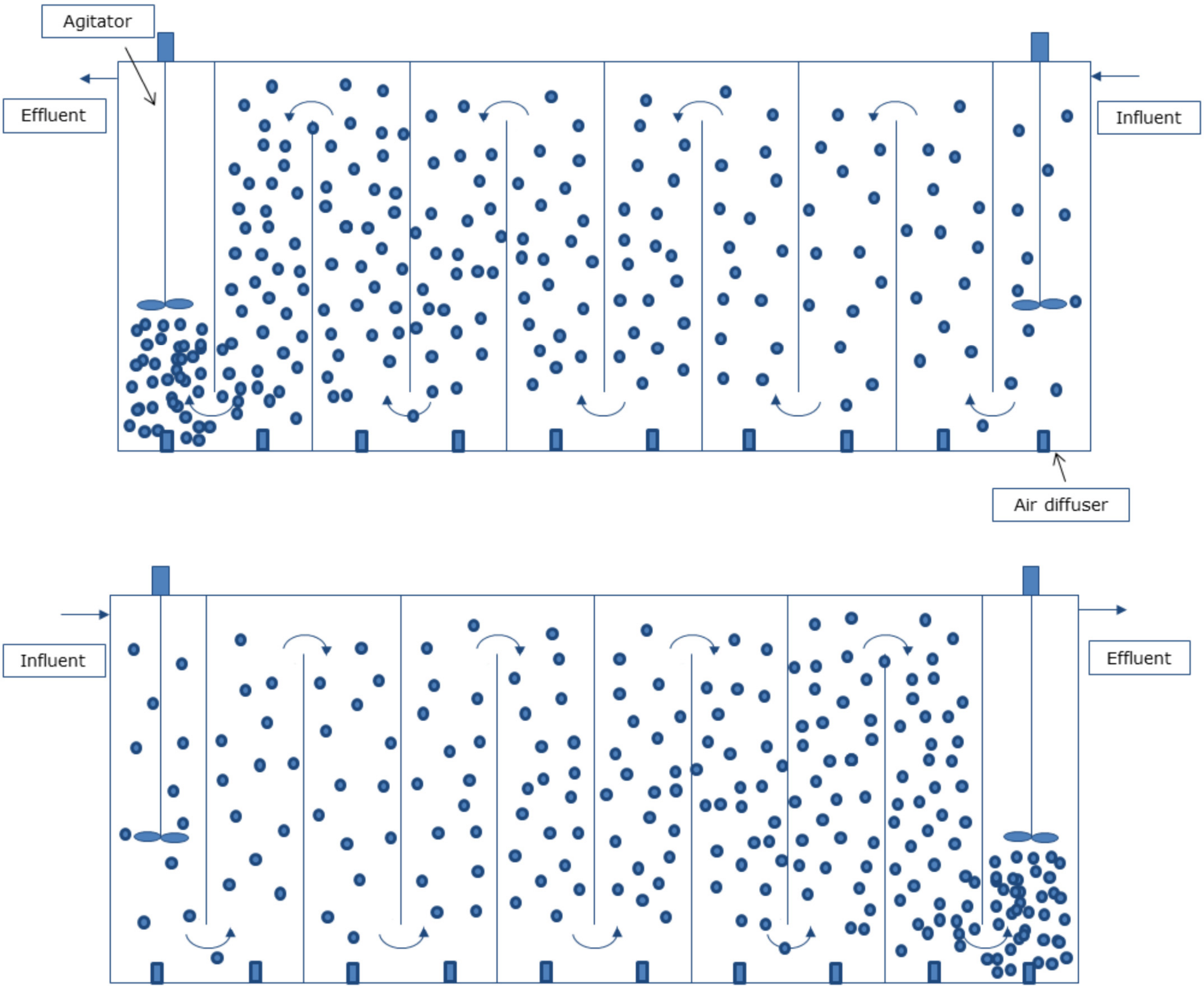


**Figure 4.** Diagram of a continuous flow reactor using a serial multiple chamber, adapted from Li et al. [54].

(c) Use of clarifiers

Several studies have presented CFR designs connected with clarifiers, where granules could be retained. Long et al. [55] reported a CFR system with a double column cyclic aerobic granular reactor (DCCAGR), which consisted in two equal bioreactors composed by a column and a settling tank connected by an inclined tube (Figure 5). Both reactors were able to separate the solid, liquid and gas phases. The settling tank had a removable baffle, allowing the selection pressure to be modified. Moreover, both reactors functioned alternatively as the first and the second reactor, using the effluent of the first as the influent of the second one. Another example of a CFR system combined with clarifiers was reported by Chen et al. [56]. In this case, the CFR system was built with a cylindrical column with an H/D ratio of 1, composed by a mixer and an aerator. Moreover, the system incorporated a clarifier tank, where granules were accumulated and returned to the aeration tank

(Figure 5). Continuous flow AGS has also been developed to remove P from wastewater. For instance, Li et al. [57,58] reported a CFR system composed of two chambers (anaerobic and aerobic) followed by one settling tank, named the 'Tube Seller', which recirculated 50% of the granules' volume to the anaerobic zone (Figure 5). According to these authors, this continuous flow granular technology allowed granular stability to be maintained and very high C, N and P removal yields to be obtained.

Recently, Sun et al. [59,60] described a very innovative CFR system incorporating a plug-flow aerobic granulation reactor, formed by multiple completely stirred tank reactors (CSTRs). The main objective of this design was to create different feast–famine conditions and evaluate the influence of this parameter on granular stability. In this sense, a value of 0.33 was determined to be the optimum feast–famine ratio. Thus, granules were very stable for a feast/famine ratio of 0.33, while for ratios of 0.5 and 1, their stability was greatly affected, and their breakdown was frequently observed.

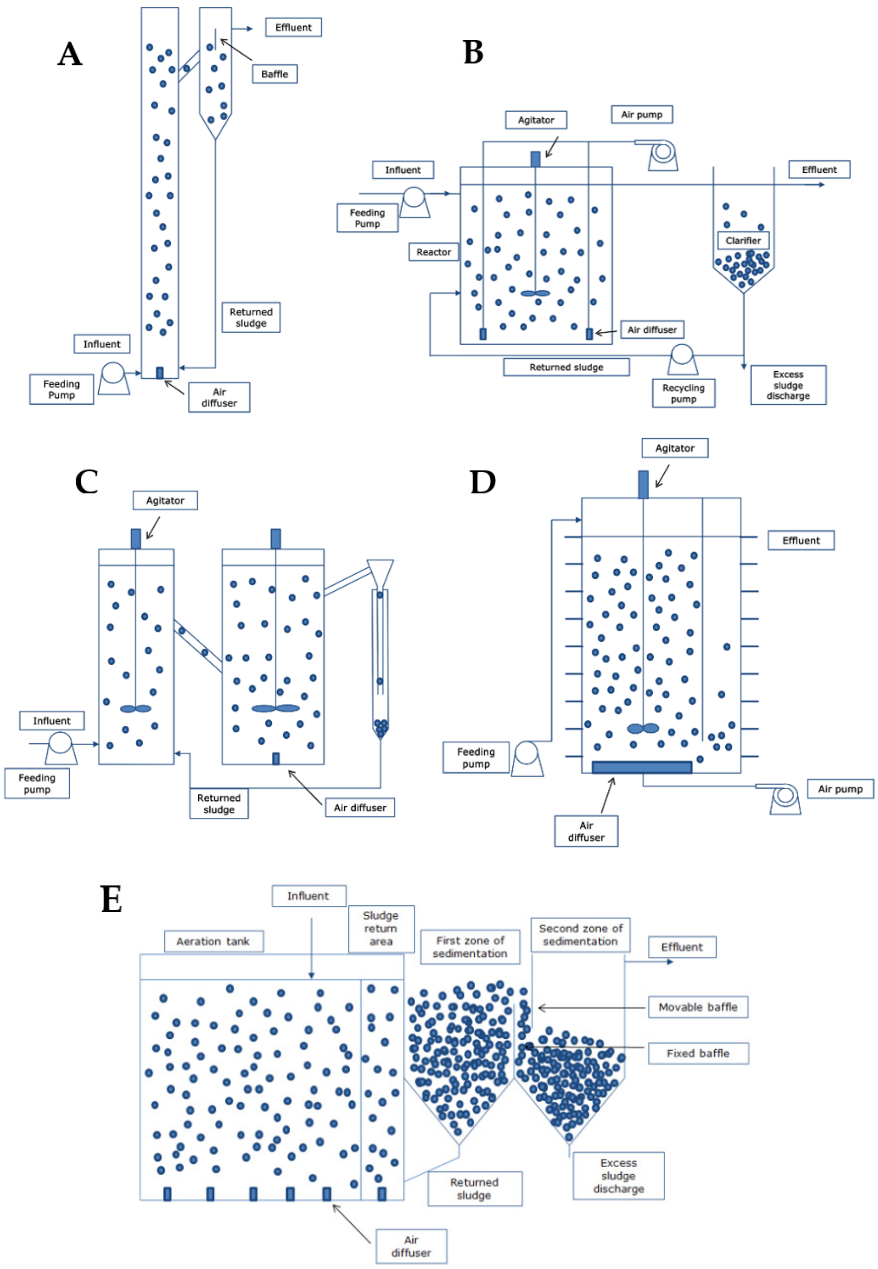


**Figure 5.** Diagram of a continuous flow reactor with clarifiers to avoid the wash-out of biomass, adapted from (**A**) Long et al. [55], (**B**) Chen et al. [56], (**C**) Li et al. [57,58], (**D**) Li et al. [61] and (**E**) Xu et al. [62].

Li et al. [61] reported an innovative system that conceptually integrated an SBR system and an advanced continuous flow reactor (ACFR). Basically, this technology consisted of an aerobic zone with a specific area for granular selection (Figure 5). The granules with good settleability went back to the aeration zone, while poorly decanting fluffy granules were accumulated and discharged every 24 h for effluent clarification purposes. Another example of a CFR system that incorporates clarifiers was described by Xu et al. [62]. These authors reported a continuous-flow reactor with a two-zone sedimentation tank (CFR-TST). The reactor was composed by an airlift system followed by an aeration tank. After the aeration zone, a double sedimentation tank was implemented, where well-settling granules from the first clarifier were returned to the airlift system. At the same time, the floccular biomass from the second clarifier was discharged (Figure 5).

(d) CFR with submerged membranes

A truly innovative technological alternative is the design of granular systems coupled with submerged MBRs. In such cases, the application of continuous AGS-MBR systems enjoys the advantages of both granular and membrane technologies, enabling the production of treated water with tertiary quality. Moreover, AGS-MBR technology significantly reduces membrane-fouling processes, probably a very important problem in membrane systems [63,64].

When AGS-MBR systems are implemented in continuous mode, the main problem pertains to keeping the granular biomass stable for long periods of time, due to the lack of a starvation phase, which avoids the collapse of granules. However, this problem can be solved by setting a famine phase. In this sense, the design of reactors is constituted by two serial reaction chambers (Figure 6), one of them working at a high OLR and the other at a low OLR. With this plant configuration, feast and famine conditions can be respectively promoted [63,65].

Liu et al. [66] reported a continuous-flow granular self-forming dynamic membrane bioreactor (CGSFDMBR) that consisted in a sequencing batch airlift reactor (SBAR), a settling tank, a dynamic membrane bioreactor (DMBR) and a sludge selection tank (Figure 6). The granular biomass was produced in the SBAR tank, while the separation of granular and filamentous biomass was produced in the settling tank. Then, the granular sludge was recirculated to the SBAR tank, and the flocculent sludge was driven to the DMBR. Subsequently, the treated water went out as effluent, while the mixed liquor of DMBR passed to the sludge selection tank once a day, where the granules that did not cross a sieve were returned to the DMBR tank, and the floccules that passed the sieve were discharged as excess sludge.

(e) Hybrid SBR-CFR system

Li et al. [67] developed a hybrid SBR-CFR system comprising four identical columns (Figure 7). One of the reactors acted as an anaerobic column and was also responsible for feeding two aerobic reactors. These two reactors then fed a fourth reactor that acted as a clarifier. Therefore, the bioreactors worked like clarifiers or anaerobic or aerobic chambers, depending on the direction of the inflow (Figure 7).

### 4.2. Physicochemical Parameters and Granular Formation in CFR Systems

There is a broad consensus that for the formation of a stable granular biomass, it is essential that the bioreactor generates a hydrodynamic shear force and that filamentous microorganisms can be washed out. These two processes can easily be performed in SBR systems by settling the velocity or particle size selection [49]. However, in CFR systems, achieving these processes is more complicated and challenging.

Granular biomass selection based on particle size can be promoted through the implementation of different types of membranes [66,68], although it has been concluded that the enrichment of granular biomass in continuous flow aerobic granular systems using size as the selection procedure is not the most efficient way of keeping the granules in the reactor [49]. Thus, granular selection based on the settling velocity can be regarded as the most appropriate procedure for developing stable and efficient CFR systems. In this context, CFR bioreactors use both internal baffles that provide settling zones [69] and external separators that produce different reactions and settling zones that take place in separate spaces. They are considered without doubt the most suitable for future implementation on a full scale. However, it is evident that the settling velocity of granules formed in CFR is notably slower than in SBR [17,68].

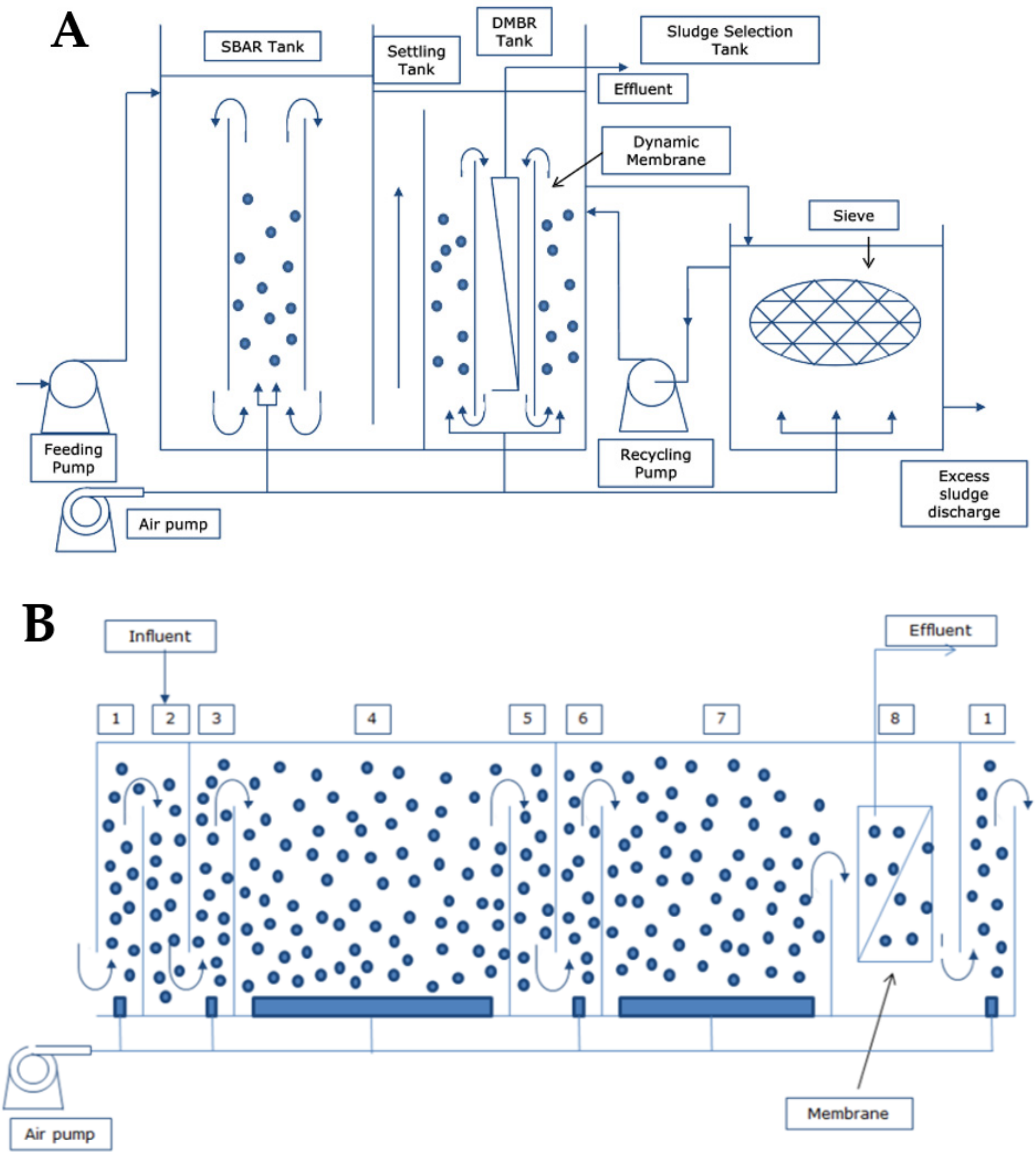


**Figure 6.** Diagram of continuous flow reactors with submerged membranes, adapted from (**A**) Liu et al. [66] and (**B**) Corsino et al. [63].

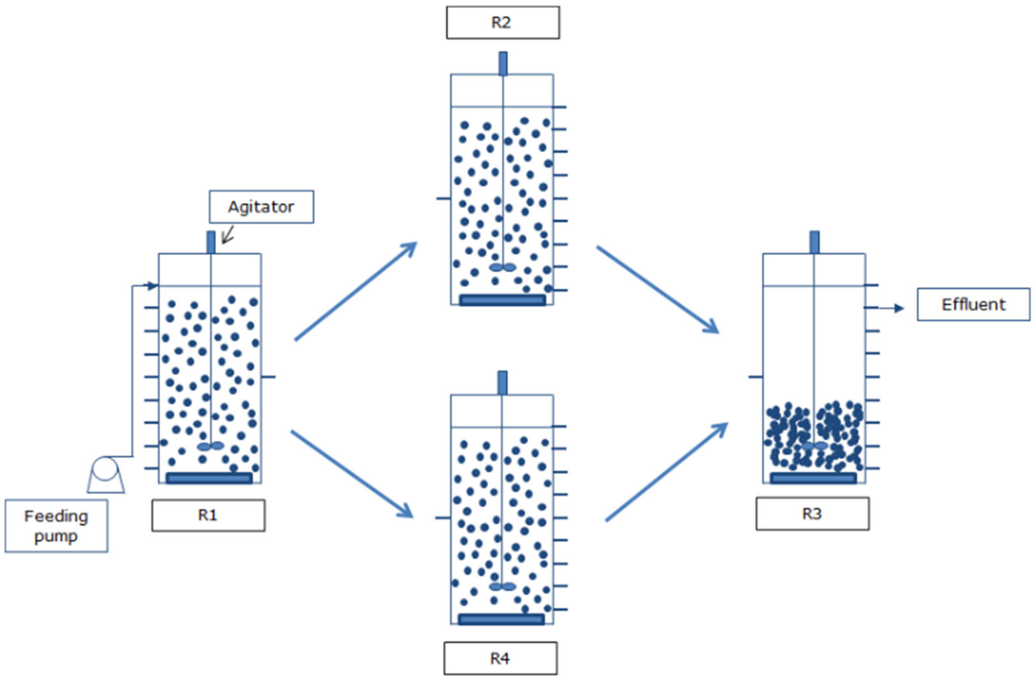


**Figure 7.** Diagram of a hybrid sequential batch reactor and a continuous flow reactor, adapted from Li et al. [67].

The granular size in CFR systems is highly variable and is clearly affected by the design of the bioreactor in question. Thus, granule sizes between 0.135 mm and 6.1 mm have been reported [54,70]. Chen et al. [70] described the formation of granules larger than 5 mm using aerobic granular as inoculums, a high C/N ratio and OLR values between 6 and 30 kg $m^{-3}$ $d^{-1}$. However, it is important to mention that under the highest OLR values the performance of the CFR system was unpredictable, presenting significant operational stability problems in the bioreactor. In general terms, it could be concluded that the size of the granules in CFR systems is certainly smaller than in conventional SBR systems. In this sense, most studies indicate that aerobic granules in CFR systems have an average size of about 2 mm [49], while in conventional SBR systems they are larger with values of 14 mm [17]. The granules found in digestion processes achieved mean sizes close to 7 mm [71]. Table 2 shows some general characteristics of the aerobic granules produced in SBR and CFR systems. The influence that the design of the system exerts on granular biomass is evident.

**Table 2.** Characteristics of aerobic granular biomass formed in SBR and CFR systems.

| Type of Reactor | Settling Velocity (max)($mh^{-1}$) | Mean Size (mm) | SVI (mL $g^{-1}$) | Nucleus Core Formation (d) | Temperature (°C) | HRT (h) | Reference |
|---|---|---|---|---|---|---|---|
| CFR system with baffled bubble column | - | 0.2–2 | 33.5 | - | 20 ± 2 | 4.08–24 | [53] |
| CFR system with serial multiple chambers | - | 0.135 | 43 | 21 | - | 5.5–16.4 | [54] |
| CFR system with clarifiers/sieve | 35.4 | 0.34–0.42 | 25–56 | 14 | 25 ± 5 | 16 | [62] |
| CFR system with MBR | 15–25 | 1.0–6.0 | 25–40 | Inoculated with granules | 22.5 ± 2.5 | 13 | [66] |
| Hybrid CFR/SBR system | - | - | 56.2–101.7 | Inoculated with granules | - | 6–12 | [67] |
| Conventional SBR system | 138 | 14 | - | 35 | 8–26 | 6 | [17] |
| Conventional SBR system | - | 1.2 | 15 (SVI8) * | Inoculated with granules | 8–20 | 5.6 | [22] |

* Sludge volumetric index at 8 min.

The concentration of organic matter in CFR systems is also a very important parameter to consider. However, continuous-flow AGS systems can operate at a wide range of OLR values, highlighting their versatility. For instance, Hou et al. [72] reported excellent performance in an airlift reactor MBR reactor operating with chemical oxygen demand (COD) values close to 9 kg $m^{-3}d^{-1}$, while Wan et al. [51,73] demonstrated that CFR systems could operate with OLR values lower than 0.1 kg COD $m^{-3}d^{-1}$. Certainly, Wan et al. [51,74] suggested that to treat effluents with low OLR values, the C/N ratio must also be low in order to increase EPS production in the granular biomass and consequently increase the stability of the granular structure.

Dissolved oxygen (DO) and temperature are key parameters for the successful performance of biological wastewater treatment, which are directly correlated with microbial metabolisms. In this sense, temperature tested in AGS-CFR varying from 30 °C to 6 °C, but the most of studies are referred to mild temperatures [53,70]. Lee and Chen [75] reported a drastic decrease in COD removal performance in CFR systems when the temperature dropped from 28 to 10 °C. However, this fact has not been exclusively reported in AGS-CFR: it has been corroborated in several biological technologies, even in AGS-SBR [12,17].

Stable granules and excellent performance have been reported in CFR systems at very low DO concentrations (0.1 mg $O_2$ $L^{-1}$) but also at 8 mg $O_2$ $L^{-1}$ [72,73]. Nevertheless, the control of DO is a requirement for the selection of some bacteria, such as ammonia oxidizing-bacteria (AOB), which had competitive advantages over nitrite-oxidizing bacteria (NOB) at low DO [49]. In summary, it can be established that CFR systems behave in general terms like all other biological wastewater treatment systems.

### *4.3. Granular Biomass in CFR Systems: Microbial Aspects*

Due to the diversity of microbial populations, reactor design, operational conditions and environmental parameters play a key role when selecting the desired species to achieve successful performance. Although lower diversity has been reported in granules operated with continuous flow, little information in this regard is currently available [61]. For instance, Li et al. [54] reported that *Nitrospira* and a phylotype belonging to *Gammaproteobacteria* were dominant organisms in the granular biomass formed in CFR systems, while under the same conditions except when operating in batch cycles, the dominant bacteria in SBR systems were *Adhaeribacter.*

With the aim of researching the optimal continuous operation with granular sludge in CFR, different inoculation methods have been described by several authors in order to achieve successful and long-term granular stability. Long et al. [55] reported successful inoculations using activated sludge, mature granules or biofilms. However, it has recently been shown that the system that determines the best results in inoculation is with mature granules previously cultivated in a conventional SBR system [51,57,58,61,63,66,76].

The inoculation of CFR systems with activated sludge for inoculation was employed by the pioneers in testing AGS systems in continuous flow [48], and its use has since been extended [50,53,54]. Long et al. [27] used a mixture of activated sludge at 70% and mature granules at 30%, but the instability of granules was observed during the first days of operation and ultimately the biomass disintegrated into smaller granules or flocs. These findings were corroborated by Corsino et al. [63], who suggested that the inoculation of CFR systems with activated sludge increases the amount of flocculent sludge and decreases EPS segregation, especially with regard to protein molecules. Moreover, the growth of filamentous bacteria was found to cause the breakage of spherical biomass. The researchers suggested that this may be due to the absence of a feast–famine period. In parallel, Corsino et al. [63] described how intermittent feeding with short periods of starvation could encourage and improve the granular structure and stability.

Filamentous and slow-growing microorganisms have been reported as a positive influence in the initial granulation process and in the structural skeleton of granules [17,36], influencing their compactness, stability and porosity. Some of these microorganisms are nitrifying and denitrifying bacteria, which proliferate slowly even under optimal environmental and substrate conditions [77]. One important factor for promoting the selection of nitrifying and denitrifying bacteria is the COD/N ratio. Luo et al. [78] analyzed the effect of this parameter on the abundance of ammonia and NOB, observing that AOB were abundant even with a low COD/N ratio. Certainly, in AGS-CFR systems, *Nitrosomonas* and *Azoarcus* genera have been reported as the most representative bacteria involved in ammonia oxidation [79]. With regard to NOB, Li et al. [54] found that *Nitrospira* remain stable from inoculum to mature granules, suggesting that a low COD/N ratio promotes the dominance of this phylotype.

Several authors [36,80] described in AGS systems operated in extreme environments some genera belonging to *Xanthomonadaceae* involved in the nitrogen cycle, while the presence and abundance of the well-known nitrifying bacteria such as *Nitrosomonas* were very low. However, this fact has not been confirmed in CFR systems, considering that nitrifying bacteria could contribute to high settleability, a smooth surface and long-term stability [22,53].

The COD/N ratio affects the compactness of granules, disturbing their porosity. Compactness and density affect the anaerobic core of granules, where denitrifying bacteria are usually found. Among the denitrifying genera found in granules grown in CFR systems, the *Denitromonas* genus has been described as having a total relative abundance of 15% [79]. According to Juang et al. [76], the dominant phylotypes in CFR systems are *Bacteroidetes* (56%), *Proteobacteria* (30%) and *Actinobacteria* (14%), whose abundance and dominance are in accordance with the findings of Wan et al. [74]. Most of these microorganisms were carefully identified as denitrifying bacteria by the latter [74]. Furthermore, Chen et al. [56] have corroborated the presence and high abundance of *Proteobacteria* and *Bacteroidetes*, but they also noticed the dominance of *Firmicutes* in terms of microbial diversity. Essentially, Chen et al. [56] detected phylotypes that commonly inhabit contaminated soils and aquatic environments with hydrocarbons and suggested that these phylotypes could involve the degradation of hydrocarbons hydrolyzed from milk powder.

Li et al. [57,67] described members of the *Rhodocyclaceae* family as being dominant in CFR systems, highlighting the role played by this group in terms of nitrogen, glycogen and COD removal. Moreover, these authors reported that an increase in the loading rate promoted the proliferation of *Thiothrix* described as filamentous bacteria, as reported in granules cultivated in SBRs [36], while the abundance of PAO was compromised. Li et al. [58] observed that the abundance of PAOs increased over operational time from inoculation to 100 days of operation, when the operational conditions were modified from SBR to CFR and the '*Candidatus* Accumulibacter' genus was kept in the system.

## 5. Technical Application of Aerobic Granular Continuous Flow Systems: Future Prospects

Most of the research carried out so far in CFR systems has had as its objective the treatment of industrial or urban wastewater. Moreover, the results obtained have generally been at the laboratory scale. However, its potential application at the real scale is undoubtedly a future reality. It is evident that granular systems have great operating advantages compared with CAS systems; furthermore, CFR systems bring additional advantages. In this sense, aerobic granular technology can be regarded as undergoing a clear expansion and its incorporation as an emerging technology is a consolidated fact in the integral water cycle. For instance, a full-scale plant (Nereda Technology) provides a very successful and stable operation with AGS in a conventional SBR system located in numerous countries, such as Portugal, the Netherlands, the United States, South Africa, Brazil, Australia, the Philippines, France and Germany. It is indisputable that in the near future, aerobic granular sludge in CFR systems must be considered a potential system for degrading organic matter and nutrients, especially nitrogen and phosphorous [81], which are common compounds in real urban wastewater.

Recent studies [82] have reported that AGS-CFR systems represent a useful technology for the bioremediation of industrial wastewater with high ethylene glycol concentrations. Moreover, Meng et al. [79] have demonstrated that AGS-CFR systems can be applied for the treatment of saline wastewater, suggesting that this technology is especially useful for the treatment of industrial effluents with extreme characteristics that are difficult to treat by other conventional biological systems. However, a lack of knowledge about the capacity and efficiency to remove recalcitrant compounds by granular sludge in continuous flow remains an issue. Many efforts should be made to develop a deeper understanding of the initial granulation process, stability in the absence of pressure selection, the chemical behavior of EPS and the relationship among cells from layered niches. AGS-CFR systems are especially beneficial for treating chemicals, as they are maintained at lower concentrations than in SBRs [83].

It could be argued that CFR technology is experimentally mature enough to make a leap to the real scale in the near future and should be applied to mainstream wastewater treatment, given by the continuous flow nature of the WWTP already implemented worldwide. Furthermore, interest in the comprehensive operation of this mode should be increased. Some knowledge previously well described in conventional SBR systems could be used for the improvement of CFR systems.

**Author Contributions:** A.R.-M.—conceptualization, investigation, writing—original draft preparation; B.M.-P.—conceptualization, investigation, writing—original draft preparation; A.G.-M.—supervision, writing—review and editing; S.G.—validation, writing—review and editing; M.F.—supervision, writing—review and editing; J.G.-L.—funding acquisition, project administration, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data sharing not applicable.

**Acknowledgments:** The authors would like to acknowledge the support given by the Institute of Water Research of the University of Granada, Spain and Universitá degli Studi della Tuscia, Italy.

**Conflicts of Interest:** The authors declare that there are no conflict of interest in this work.

## Abbreviations

| | |
|---|---|
| ACFR | advanced continuous flow reactor |
| AGR | aerobic granular sludge |
| AOB | ammonia oxidizing-bacteria |
| AUFB | aerobic upflow fluidized bed |
| CAFB | continuous flow airlift fluidized bed |
| CAS | conventional activated sludge |
| CFAGR | continuous-flow aerobic granular reactor |
| CFR | continuous flow reactor |
| CFR-TST | continuous-flow reactor with two-zone sedimentation tank |
| CGSFDMBR | continuous-flow granular self-forming dynamic membrane bioreactor |
| COD | chemical oxygen demand |
| CSTR | completely stirred tank reactor |
| D | diameter |
| DCCAGR | double column cyclic aerobic granular reactor |
| DMBR | dynamic membrane bioreactor |
| DO | dissolved oxygen |
| EBPR | enhanced biological phosphorus removal |
| EPS | extracellular polymeric substances |
| GAO | glycogen-accumulating organism |
| H | height |
| HRT | hydraulic retention time |

| | |
|---|---|
| MBR | membrane bioreactor |
| MLSS | mixed liquor suspended solids |
| NOB | nitrite oxidizing bacteria |
| OLR | organic loading rate |
| PAO | polyphosphate-accumulating organism |
| QS | quorum sensing |
| QQ | quorum quenching |
| RNA | ribonucleic acid |
| SBAR | sequencing batch airlift reactor |
| SBR | sequential batch reactor |
| USB | upflow sludge bed |
| WWTP | wastewater treatment plant |

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