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Continuous-Flow Aerobic Granular Sludge Treatment of Dairy Wastewater

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Abstract: The authors conducted a study on treating synthetic dairy wastewater using aerobic granular sludge (AGS) in a laboratory-scale continuous flow reactor (CFR) system. The system consisted of an anaerobic reactor, an aerobic reactor, and a settling sedimentation tank, with different hydraulic retention times tested over a 90-day period. The study monitored sludge characteristics and effluent treatment performance and found that the system achieved excellent removal rates for chemical oxygen demand and total carbon, exceeding 90%. As a result, the effluent met Portuguese laws for direct release into the water environment. Moreover, the study found that the AGS system improved the sludge sedimentation capacity from 272 to 80 mL/g, demonstrating its effectiveness as a viable treatment alternative for this type of effluent.

Keywords: aerobic granular sludge; biological wastewater treatment; continuous flow reactor; dairy industry

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1. Introduction

Wastewater from the dairy industry arises from the cleaning and washing processes during the production of milk and dairy products, such as cheese, butter, whey, and cream [1]. According to the Food and Agriculture Organization of the United Nations (FAO), around 928 million tons of milk were produced globally in 2021, an increase of 1.3% compared to 2020 [2]. For every liter of processed milk, between 6 and 10 L of wastewater are generated, and between 4 and 11 million tons of that wastewater are released into the environment worldwide [3]. This effluent is characterized by high chemical oxygen demand (COD) concentrations due to the presence of lipids, sugars, oil and grease, fatty acids, and proteins [4,5]. In addition, some amounts of detergent used during washing also appear. COD values can range from 650 to 68,000 $mgO_2 \cdot L^{-1}$ [6,7], depending on the type of dairy produced. Phosphorus and nitrogen, the main nutrients to be removed from dairy wastewater [8,9], can also vary widely (P 5–640 mg/L and N 10–1120 mg/L) [6,10].

The activated sludge (AS) process is one of the most widely used biological aerobic methods for wastewater treatment. By forming dense cultures of microorganisms, suspended AS can effectively biodegrade organic matter in water and is a competent method for removing carbon and nitrogen [11]. However, this technology has some shortcomings in separating the solids in suspension from the liquid phase due to the development of weak flocs and filamentous bacteria [12,13]. This way, the study and knowledge of aerobic granular sludge (AGS) technology are gaining relevance. Granules have properties that can overcome some of the problems presented by AS, such as improved liquid-solid separation and simultaneous nutrient removal, acting as an upgrade of AS [14–16]. While the aggregate size of AS varies between 50 and 300 µm, under specific conditions, it is possible to form granules between 0.3 and 5 mm [17].

Granules are compact, dense, and spherical biofilms that form solid structures. Aggregates of microorganisms incorporated in extracellular polymeric substances (EPS) are developed, creating a three-dimensional matrix stimulated by self-immobilization without material support [18]. By having an anaerobic layer (in the granule core), an anoxic layer, and an aerobic layer (on the granule surface), granules ensure the simultaneous removal of carbon, nitrogen, and phosphorus [19,20]. That is, aerobic granulation compacts the agglomeration of nitrifying bacteria, denitrifying bacteria, aerobic heterotrophs, and acidifying bacteria [18,21,22]. The implementation of feast/famine periods and settling velocity-based selection pressure, dissolved oxygen (DO) concentration, organic loading rate (OLR), influent composition, the amount of filamentous bacteria and EPS, solids retention time (SRT), hydraulic retention time (HRT), hydrodynamic shear force, metal ions concentration, temperature, and pH are some of the factors that can influence AGS formation, development, and stability [23–25].

The first successful granulation was reported in 1997, when the authors applied an exclusively aerobic feast/famine period [26]. However, more recent studies have shown that granulation can be achieved by promoting periods of anaerobic feast and aerobic famine [27,28].

The enhanced biological phosphorus removal (EBPR) process helps the development of granules as it promotes the selection of slow-growing microorganisms such as polyphosphate-accumulating organisms (PAO) and glycogen-accumulating organisms (GAO), and consequently denser and stronger granules are formed than granules formed by other heterotrophs [29,30]. PAO and GAO are fundamental in forming dense and stable granules, and anaerobic feeding encourages their proliferation [31,32]. Thus, when the aim is to develop strong and compact aerobic granules, anaerobic feeding is the most common strategy [18,33–38].

AGS is a technology mostly used in sequencing batch reactors (SBR) due to the ease of controlling the conditions for developing aerobic granules. So far, it has only been possible to implement full-scale AGS technology in SBR due to the limitations of granulation in continuous flow reactors (CFRs), such as the application of settling or size-based selection pressures, feast/famine conditions, and sludge recirculation [23]. However, most common wastewater treatment plants have continuous installations, making it difficult to implement SBR [39,40]. That is due to the benefits of continuous processes, such as low construction and operating costs, as well as the ease of maintenance and control. Granular systems allow for high biomass retention, leading to improved performance of CFR [23,24].

For this reason, it is necessary to study the formation and stability of AGS in CFR [39,41–44]. One of the solutions is to transfer mature aerobic granules, produced in SBR, to CFR. However, the long-term stability of the granules in CFR should be further investigated [24,45]. In continuous systems, it is fundamental to guarantee a good mixture of reactional contents to avoid the sedimentation of sludge portions at the base of the tanks. Additionally, an extra tank is required for the clarification stage, creating difficulties in controlling sludge separation because of the poor settling characteristics of the activated sludge flocs [46,47]. It has already been reported that the instability associated with CFR is also linked to difficulty controlling factors fundamental to the granulation process, such as changing feast/famine periods and hydraulic selection pressure [48]. Table 1 summarizes some AGS technology approaches applied in CFR systems.

Table 1. Summarized AGS approaches in continuous flow systems.

Setup	Characteristics	Results	Ref
Column-type (120 × 6 cm)	• Synthetic wastewater • Inoculated AGS developed in SBR • OLR 7 $kgCOD/m^3 \cdot d$ • HRT 24 h	• Granular stability for 216 days • COD removal 83–84% • High levels of ammonium salts enhance the granule structure stability • Mean diameter 1.9 mm	[41]
Airlift bioreactor with a settling tank and a membrane	• Synthetic and real wastewater • Inoculated AGS developed in SBR • OLR < 1 $kgCOD/m^3 \cdot d$ • HRT 13 h	• More than 2 months with stable granules • COD removal 83% • Max. diameter 200 µm	[49]
Double column cyclic reactor (188 × 8.4 cm)	• Synthetic wastewater • Inoculated 70% of AS and 30% of AGS • OLR 6 $kgCOD/m^3 \cdot d$ • HRT 4 h	• Inoculated AGS helped the granulation of AS • COD removal 97.6% • The large granules were not stable • The granules formed had an irregular shape • Max. diameter 157 µm	[42]
5 CSTRs in serie	• Synthetic wastewater • Inoculated AS • OLR < 2 $kgCOD/m^3 \cdot d$ • HRT 6 h	• Selection pressure influences the washout of flocculent sludge • sCOD removal 75–90% • Granule characteristics changed with selective wasting • Diameter max. 200 µm	[50]
Reverse flow baffled reactor	• Real wastewater • Inoculated sludge with small granules • OLR 0.54, 1.2 and 1.6 $kgCOD/m^3 \cdot d$ • HRT 16.4, 7.3 and 5.5 h	• 3.3 H/D promoted shearing forces • Sludge with excellent settling capacity (SVI 33 mL/g) • Max. diameter 135 µm • BOD5 removal 90–94% • Results from SBR were better	[51]
Continuous flow airlift fluidized bed reactor	• Real wastewater • Inoculated AS • OLR 5 $kgCOD/m^3 \cdot d$ • HRT 2–3 h	• Max. diameter 800 µm • Granulation successfully achieved • COD removal 90%	[52]
Aerobic upflow fluidized bed	• Synthetic wastewater • AS inoculated • HRT 5 days to 7.6 h • High H/D	• Spherical granules with a diameter of 346 µm on day 300 • Good nitrification performance	[53]
Anaerobic and aerobic reactors, and a settling tank	• Synthetic wastewater • AGS inoculated • OLR 1.2 $kgCOD/m^3 \cdot d$ • HRT 6 h	• Max. diameter 900 µm • COD removal 95–98% • Granules developed in 16 days • PAOs promote improved sedimentation	[54]
Continuous flow membrane bioreactor	• Synthetic wastewater • Inoculated AGS developed in SBR • HRT 7.5 h • Intermittent feeding applied as an alternative	• Mean diameter 1.5 mm • AGS lost the initial structure • Intermittent feeding favoured the AGS stability	[55]

The difficulties of granulation in continuous flow are well known. Rosa-Masegosa et al. [18] compared the data between aerobic granules developed in SBR and CFR systems, and the continuous reactors have (to date) failed to reach SBR values. In SBR, granules with a sedimentation velocity of 138 m/h and a size of 14 mm were developed [56], whereas in continuous reactors, the speed does not exceed 40 m/h and the size is 6 mm [18,49,57]. Nevertheless, Sun et al. [58] presented an interesting alternative to CSTR and tested the feasibility of pelleting in a plug flow reactor. After 90 days of operation, the characteristic differences between biomass initially inoculated into the reactor (AS) and biomass obtained (AGS) were evident. These authors noticed that the morphology of the granular sludge developed in the study was similar to the morphology of the granular sludge formed in SBRs, namely the fluffy surface of the granules. The system could produce granules with a mean diameter of 3.4 mm. In addition, parameters such as particle size, SVI, and EPS content were comparable to the parameters achieved by SBR.

In overview, most studies are conducted at the lab scale, and many strategies may not be practical when transposed to the full scale. Concerning industry, the transition from AS plants to AGS plants requires further research. Kent et al. [23] state that after granule formation in continuous mode, the transport of granules through the tubes may block due to the amount and size of solids. The complexity of the design or the unavailability of conditions for its implementation are also some of the adversities. However, the implementation of AGS is expected to increase solids retention in the reactors and thus increase the maximum treatment capacity [47].

This study focuses on developing aerobic granular sludge and its removal performance in a continuous flow reactor system composed of an anaerobic tank and an aeration tank for treating dairy industry wastewater. Keeping in mind the possibility of AGS development under HRT of 24 h or less [41,42,58,59] and high shear force [14,41,60], this investigation aimed to study the feasibility of using long HRT (2–8 days) by promoting low OLR and low upflow air velocity. Granular development and nutrient removal performance were followed under different substrate flow rates.

2. Materials and Methods

2.1. Influent

For simulating dairy wastewater, the influent was prepared manually by diluting low-fat commercial milk 80 times with tap water and stored in a reservoir with a maximum capacity of 20 L. The reservoir was covered with ice to keep the wastewater temperature low to avoid acidification and the formation of milk coagulum. The pH was controlled daily so the CFR system would not be fed with acidic wastewater. The reservoir was washed and replaced every day (except at weekends) to prevent significant changes in the wastewater composition. The COD, sCOD, TC, TN, and TP concentrations of the synthetic influent were determined before the start of the activity (Table 2). The ratio COD:N:P (144:15:1) obtained by diluting commercial milk differs from the conventionally used ratio of 100:5:1 [61].

Table 2. Characterization of the synthetic influent and similar real dairy wastewater.

COD (mg/L)	sCOD (mg/L)	TC (mg/L)	TN (mg/L)	TP (mg/L)	pH	Ref.
1031.3 ± 50.1	839 ± 30	516.9 ± 2.9	109.6 ± 13.5	7.14 ± 0.41	6.03 ± 1.48	Used in this study
662–1293	-	-	8.1–38.8	0.79–6.84 *	5.3–7.0	[62]

Note: * Concentration of $P\text{-}PO_4$.

2.2. Inoculum

The seed sludge used in this experiment was collected from a municipal WWTP in Coimbra and implemented in the CFR system. At start-up, the AS placed in both reactors had similar total suspended solids concentrations (2.39 and 2.44 gMLSS/L). The initial SVI_{30} was 272 ± 37 mL/g.

2.3. Image Analysis

Sludge development was monitored weekly using microscopic images provided by a Leica DM2000 microscope. The microscope was coupled to a camera (Leica), allowing monochrome images to be collected at 400× magnification onto the software to follow the sludge's morphology.

2.4. Solids and SVI Measurements

The evolution of solid concentration in the reaction contents was followed by the determination of Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS). Both parameters were carried out according to the Suspended Solids 2540 D and Volatile Suspended Solids 2540 E methods from Standard Methods for the Examination of Water and Wastewater [63], respectively. The settleability of the sludge was evaluated weekly by calculating the Sludge Volume Index (SVI) using Method 2710 D of the Standard Methods for the Examination of Water and Wastewater [63] after 10 min (SVI_{10}) and after 30 min (SVI_{30}) of settlement.

2.5. Analytical Methods

The wastewater treatment involved analyses of the amounts of COD, sCOD, total carbon (TC), total nitrogen (TN), and total phosphorus (TP) in the synthetic influent, the reaction contents of the two reactors, and the treated effluent. COD and sCOD were determined by the Closed Reflux, Colorimetric Method 5220D of the Standard Methods for the Examination of Water and Wastewater [63]. TC was evaluated by NDIR using the Shimadzu Total Organic Carbon Analyzer (TOC-V CPH/TOC-V CPN), and TN was measured by chemiluminescence using a Shimadzu Total N Measuring Unit TNM-1. TP was determined according to Method 4500PD of the Standard Methods for the Examination of Water and Wastewater [63]. The pH was measured using a WTW Multi 9269 IDS analyzer equipped with the pH-Electrode Sentix 980 and dissolved oxygen (DO) with a WTW FDO 925 probe.

2.6. Setup

A setup based on Li et al.'s [54] continuous-flow EBPR granule reactor was used. The system comprised a feed reservoir (20 L), two cylindrical reactors, a clarifier, and a tank for the treated effluent (Figure 1). The first reactor (d = 28 cm; h = 38 cm) represented the anaerobic zone and contained only one stirrer (Heidolph® RZR 1) to ensure homogenization of the reaction contents (23.4 L). The aerobic zone was performed in the second reactor (d = 28 cm; h = 31 cm) using an air diffuser (Envicon®) at the bottom of the reactor, which provided oxygen and ensured the mixing of the reaction contents (19.1 L). The clarifier (3 L) allowed the sedimentation of the sludge and the withdrawal of the treated effluent. A peristaltic pump was used (Ismatec® Reglo Digital MS-2/8) for feeding the synthetic flux, and a peristaltic pump was used to recycle sludge from the clarifier to the anaerobic reactor (Gilson® Minipuls Evolution).

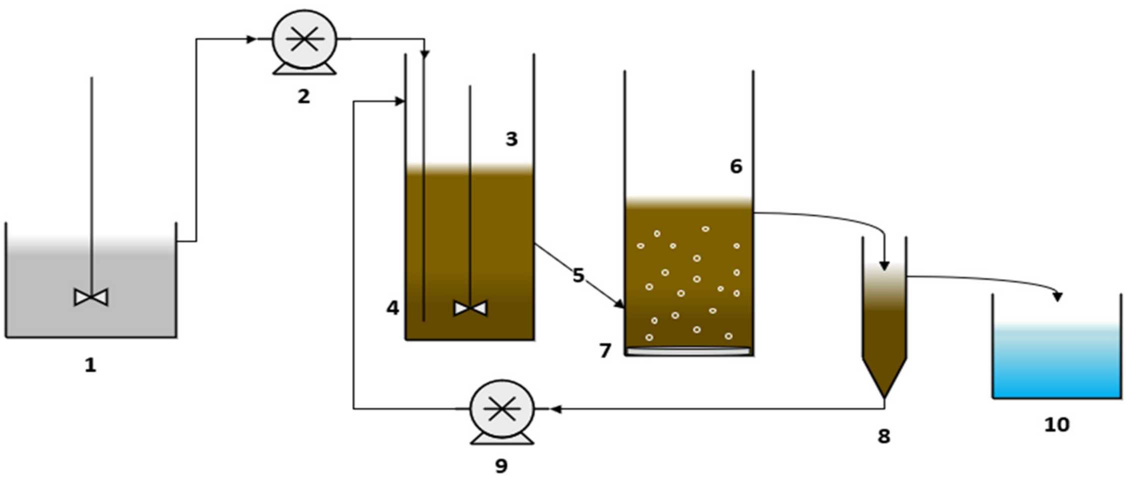


Figure 1. Schematic figure of the lab-sale CFR system. 1—Feed reservoir; 2—Feeding pump; 3—Anaerobic reactor; 4—Bottom feeding; 5—Connecting pipe between reactors; 6—Aerobic reactor; 7—Air diffusor; 8—Clarifier; 9—Recycling pump; and 10—Treated effluent tank.

The reactors were positioned to allow the sludge to flow between the anaerobic and aerobic reactors by gravitational effect. A pipe allowed sludge to flow to the clarifier from the surface of the reaction contents of the aerated reactor. The sludge settled in the clarifier, which separated the sludge with better sedimentation (granular sludge) from the sludge with low sedimentation velocity (floccular sludge). The treated effluent and the low sedimentation velocity sludge were continuously removed from the clarifier by the surface into a tank. Sludge from both reactors was acclimated to the dairy wastewater for 14 days.

2.7. Operating Strategy

For 90 days, the system was fed continuously with synthetic milk influent. In the same way, recycling sludge from the clarifier to the anaerobic reactor (100% recycled) also worked continuously. The CFR system operated at a room temperature of 24.3 ± 2.5 °C. Additionally, the pH of the influent and reactant contents was controlled manually and daily to be maintained above 6.2. Four different approaches were tested during the operation, changing the feed and sludge recycle flow rates (Table 3). The total HRT depends on the feed flow rate and the reactors' usable volumes; therefore, the HRT was a variable parameter during operation. The aerobic reactor was aerated with an airflow of 225 L/h throughout the operation, promoting a low upflow air velocity of 0.1 cm/s and a constant DO concentration of approximately 8 mgO_2/L.

Table 3. Conditions of operation performed during the operation of the CFR system.

Phase	Operating Time (Days)	Feeding Flow (L/d)	Recycle Flow (L/d)	OLR (kg COD/(m^3·d))	Total HRT (d)
1	23	5.3	7.2	0.234	8.01
2	27	10.1	7.2	0.445	4.20
3	25	15.1	20.2	0.666	2.81
4	15	15.1	36.2	0.666	2.81

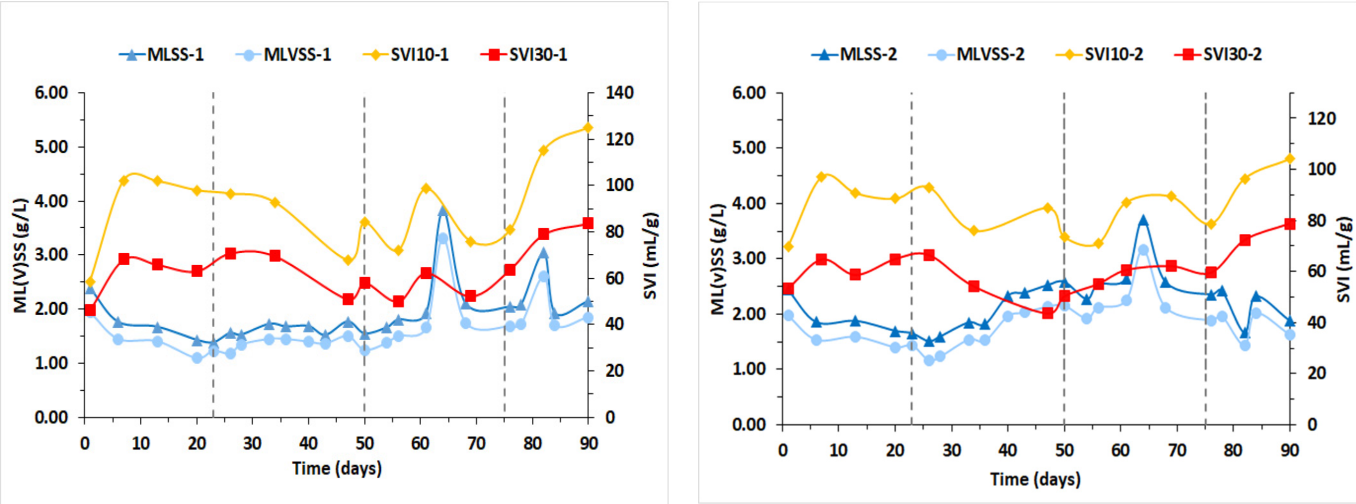
3. Results and Discussion

3.1. Sludge Characteristics and Development of AGS

In this study, MLSS, MLVSS, and SVI were measured to evaluate the growth of sludge and its conversion into granules. The sludge's initial biomass concentration was 2.39 gMLSS/L and 2.44 gMLSS/L in the anaerobic and aerobic reactors, respectively. The settling capacity of the floccular seeded sludge from the WWTP (272 ± 37 mL/g) was close to the values of other studies: 250 mL/g [13], 107 mL/g [64], 70–211 mL/g [65], and 60–190 mL/g [66]. It was possible to observe that the concentration of microorganisms throughout the operation represented more than 80% of MLSS in the two reactors.

During phase 1, it was noticed that the biomass concentration in both reactors gradually decreased. The flocculated sludge was removed in the clarifier, but the reactors' biomass growth did not compensate for this removal. The OLR used seems to be insufficient to guarantee the growth of microorganisms, a situation already reported in another study [67], in which a lack of carbon source was observed when an OLR of 1.3 kg/(m^3·d) was used. Notwithstanding, in phase 2, the OLR was sufficient for the growth and maintenance of microorganisms, as seen in Figure 2.

The values of SVI_{10} and SVI_{30} oscillated, but the best results were obtained during phase 2. Moreover, on day 47, the closest proximity between SVI_{10} and SVI_{30} values was recorded. The proximity of these two values indicates the quality of sludge sedimentation. In fact, regarding AGS, SVI_5 and SVI_{10} should be considered, as these are better parameters for quantifying sedimentation than SVI_{30} [68].



(**a**) (**b**)

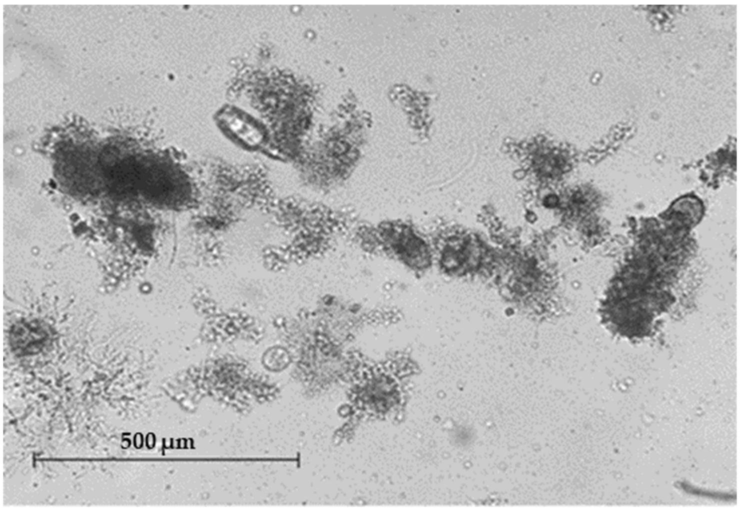
Figure 2. Evolution of the sludge parameters during the operational period in the anaerobic reactor (MLSS-1, MLVSS-1, SVI_{10}-1, and SVI_{30}-1) (**a**); and the aerobic reactor (MLSS-2, MLVSS-2, SVI_{10}-2, and SVI_{30}-2) (**b**).

The system used allowed for an improvement in the sedimentation capacity for SVI_{30} values below 80 mL/g. This value is similar to some SVI values achieved for aerobic granulation in SBR [69–71] and continuous flow systems [72–74]. This result is a positive indicator, as it demonstrates that the system configuration under high hydraulic retention time and low upflow air velocity achieved good sedimentation characteristics, comparable to previous studies that used low HRT and high upflow air velocities as operating conditions.

The sludge in the anaerobic reactor formed aggregates, but they were not as densely packed as those in the aerobic reactor, which contained more compact and spherical aggregates and fewer loose ones (Figure 3). The use of a peristaltic pump for sludge recirculation between the clarifier and anaerobic reactor may have hindered biofilm compaction. The presence of the protozoan Vorticella in the aerobic reactor indicated a lack of oxygen, as noted in a previous study by Arregui et al. [67]. However, since there was enough oxygen in the aerobic reactor, this could be an indication that the microorganisms were able to move through both reactors, as hypothesized.

It was clear that a few small, rounded structures were formed (≥200 μm), surrounded by loose sludge and microorganisms that are characteristic of AS [67]. During all phases, the presence of rotifers was verified, which contributes to reducing filamentous bulking, preventing the growth of filamentous bacteria, and improving the sludge settling ability [75,76].

The sludge was passed through two sieves (1 mm and 0.6 mm). The aerobic reactor contained small granules, which, although reduced in size and number, were retained on the 1 mm sieve as well as smaller granules that were retained on the 0.6 mm sieve. The dimensions of the observed granules are similar to those obtained in studies that successfully performed aerobic granulation in continuous flow [52–54]. In fact, there were no significant amounts of granules larger than 1 mm. As demonstrated before, the recirculation of biomass by a peristaltic pump may have compromised this parameter [58].



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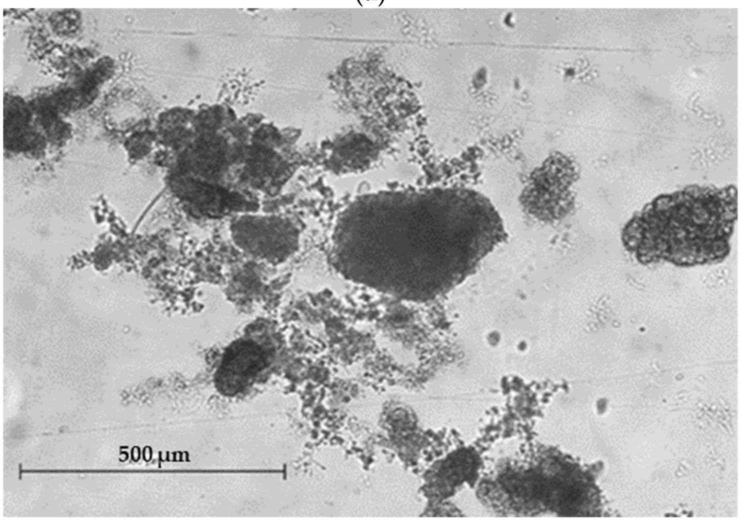


(**b**)

Figure 3. Typical microscopic image of the sludge from the anaerobic reactor (**a**) and the aerobic reactor (**b**). Scale bar: 500 μm.

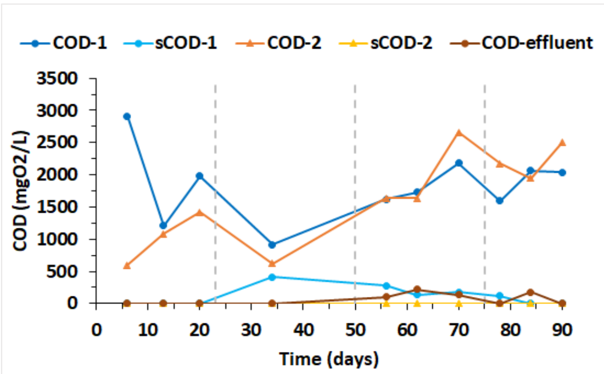
Although aeration provides the sludge mixture, the output from the aerobic reactor was located near the surface of the reactor contents. Bearing in mind that the mini-granules formed are denser than the loose sludge, it is most likely that they could not easily leave the reactor to circulate through the whole system (clarifier and anaerobic reactor), which is a relevant aspect to be improved in this type of system. This situation occurs because the sedimentation velocity may be higher than the upflow air velocity, hampering the recirculation of the granules [77]. Chen et al. [78] concluded that granules might deteriorate under low upflow air velocities (0.8–1.6 cm/s). Therefore, the low upflow air velocity during all phases of operation (0.1 cm/s) may have impaired the development of the granules. The reactor diameter directly influences the upflow air velocity. This study used an H/D ratio close to 1, which did not increase the hydraulic shear forces. Most studies use an H/D ratio greater than 5 [20,55,79–82]. On the contrary, Henriet et al. [83] used a H/D ratio of 1.8 combined with a purge of selected fractions, resulting in dense granules. Furthermore, the authors also used low upflow air velocity (0.42 cm/s), proving that it is possible to form granules with a low H/D ratio and low air velocity. Ji et al. [84] showed that despite not obtaining granules at a reduced air velocity of 0.07 cm/s, they already managed to form stable granules at speeds of 0.3 cm/s, with good performance at 0.56 cm/s [85].

Concerning the HRT and the recirculation flow, the study verifies that increasing the recirculation flow rate (from 7.2 to 36.2 L/d) does not seem to affect the granulation process significantly. At the same time, the high HRT (2–8 days) used seems not to favor granulation. Different HRT led to distinct aerobic bead morphologies. Rosman et al. [86] found that the mean size of granules increased when the HRT was reduced from 24 h to 6 h, although they used a different type of wastewater. The authors further suggest that this increase may be due to the decrease in aeration time. Although it is reported that low HRT can lead to the wash-out of reactor solids, Morales et al. [77] were able to form granules (mean diameter 6.8 mm) using 1 h HRT in a continuous reactor. On the contrary, with an HRT of 6 h, filamentous-shaped bacteria appeared.

The feast/famine ratio has an impact on aerobic granulation. In this experiment, a feast/famine ratio slightly higher than 0.5 was used, which does not seem to favor granulation, in line with studies that suggest that this is the limit value for obtaining good granulation results [87–90].

3.2. Performance of the CFR System

The evolution of COD, TC, and TN concentrations is presented in Figure 4. It can be seen that most of the COD was removed aerobically (Figure 4a). The system guaranteed a COD removal of more than 90% on most days, reaching COD values of the treated effluent below 100 mgO_2/L complying with the requirements for discharges in the water environment in Portugal (150 mgO_2/L) [91] and being in line with the results observed in other continuous AGS systems [42,50,60].



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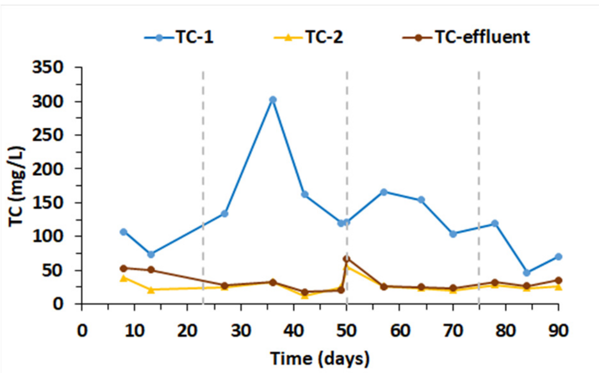

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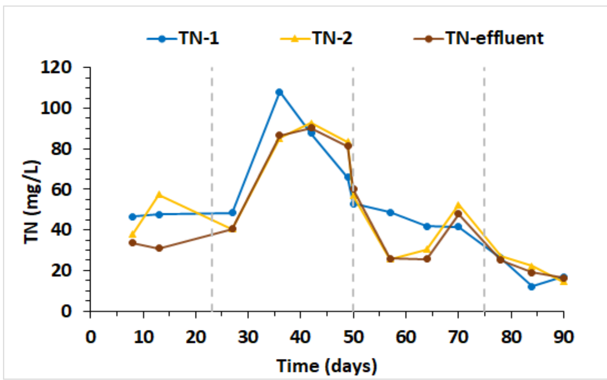

(**c**)

Figure 4. (**a**) Evolution of the COD and sCOD concentrations in the anaerobic reactor (1), the aerobic reactor (2), and the treated effluent. The values presented as 0 mgO_2/L correspond to values <100 mgO_2/L (below the limit of quantification). (**b**) Evolution of the TC concentrations in the anaerobic reactor (1), the aerobic reactor (2), and the treated effluent. (**c**) Evolution of the TN concentrations in the anaerobic reactor (1), the aerobic reactor (2), and the treated effluent.

The removal of TC remained efficient in all phases (Figure 4b). The influent inlet concentration of 516.9 ± 2.9 mg/L was reduced to values below 70 mg/L, setting the removal rate mostly above 90%.

The biological nitrogen removal processes were less effective than those for COD and carbon (Figure 4c). In phase 2, the removal of TN was the lowest at 15.1%. The TN concentrations in the aerated reactor were always close to those in the treated effluent. In the last two phases, nitrogen removal improved, and on the last day, the highest removal efficiency of 86.7% was achieved. The synthetic influent was characterized by a low C:N ratio (4.7:1), implying low nitrogen removal, which could be minimized by adding carbon sources (increasing the C:N ratio) [92,93].

Furthermore, when small granules are developed in CFR, denitrification is compromised. DO can penetrate the granule and prevent the formation of the anoxic core, where the denitrification process occurs [23,94]. The influence of low OLR on the nutrient removal performance has already been demonstrated by Li et al. [95] when using a loading of 0.43 kg COD/m^3·d, the nitrogen and phosphorus treatment decreased significantly. In addition, the sedimentation capacity and the mean diameter also worsened. In a different study, under a relatively higher OLR (0.8 kg COD/m^3), the previously developed and stable granules disintegrated, demonstrating that the granules' size is defined by the OLR [96,97].

The TP values determined during the experiment demonstrated phosphorus accumulation at some moments (Table 4). In phase 1, when the influent flow rate was the lowest, the TP concentrations in both reactors were lower than the initial influent concentration (TP = 7.14 ± 0.41 mg/L). A higher phosphorus concentration would be expected in the anaerobic reactor (phosphorus release) and a lower concentration in the aerated reactor after phosphorus uptake, considering the EBPR process. The former was only confirmed in phases 2 and 4. Notwithstanding, it was possible to achieve phosphorus removal of 85%, as in several studies already published on the nutrients removal performance of AGS [83,98], although it is possible to achieve values higher than 95% in some cases [54,99,100].

Table 4. Results of TP concentrations in the anaerobic reactor (1), the aerobic reactor (2), and the treated effluent during the experiment.

	TP (mg/L)		
Phase	**Anaerobic Reactor-1**	**Aerobic Reactor-2**	**Effluent**
1	0.53	0.53	1.15
2	2.84	1.34	1.34
3	2.38	3.72	3.16
4	3.58	1.23	1.10

It has been previously investigated that HRT influences phosphorus removal efficiency [101]. Decreasing the HRT increases the food-to-microorganism ratio and induces denitrification and phosphorus release simultaneously. Thus, the phosphorus uptake in the aerobic step increases as well.

Phosphorus accumulation by PAO is also a very important factor for granule development, and these results do not show strong PAO activity. Under low DO concentrations, these organisms growth promotes granule stability [33]. In phase 4, the greatest TP difference between the two reactors demonstrated a great phosphorus uptake in the aerated reactor. Nicholls [102] found that aerobic microorganisms accumulated phosphorus, concluding that polyphosphate is converted to ATP for energy or orthophosphate for the growth process in the absence of oxygen, resulting in more phosphorus diffusing to the exterior of the cell than into the cells. Activated sludge can store up to 10% of phosphorus internally in the form of polyphosphate when submitted to alternating anaerobic/aerobic conditions. However, successful phosphorus removal depends ultimately on the ability of the biomass to store carbon in the form of polyhydroxybutyrate and phosphate as polyphosphate [103]. In anaerobic conditions, organic carbons accumulate inside the cells mostly as polyhydroxyalkanoates. When the sludge moves to the aerobic tank, without a sufficient amount of organic carbon, stored polyhydroxyalkanoates are used as an energy source, and orthophosphates are absorbed from the wastewater and subsequently accumulate within the cell as polyphosphates [104]. The recovery of phosphorus accumulated in granular sludge could increase the value of this waste for agricultural purposes, further promoting the circular economy and highlighting the value of AGS technology. This is an area that warrants further study in future research [105–107].

4. Conclusions

The continuous experiment used a lab-scale CFR system to achieve AGS at low feeding rates from synthetic dairy wastewater for 90 days. Generally, good removal of TC was achieved, reaching a reduction of more than 93% on most periods, and a maximum TN removal of 86.7% was achieved. The TP has been removed up to 85%. The CFR system proved to be very efficient in reducing the COD values (>90%), making it possible to reduce COD to values lower than 100 mgO_2/L, making the effluent capable of being released directly into the water environment.

Small granules were developed (>1 mm) and the system improved the sludge sedimentation capacity from 272 to 80 mL/g, attesting that the aerobic granular sludge system constitutes a good treatment alternative for this type of effluent.

The obtained results also demonstrate that the granulation process strongly depends on some operating variables, namely OLR, HRT, and rising air velocity, which can be optimized to obtain larger granules and greater stability, improving the system's performance.

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