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Development of aerobic granular sludge for real industrial/municipal wastewater treatment

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ABSTRACT

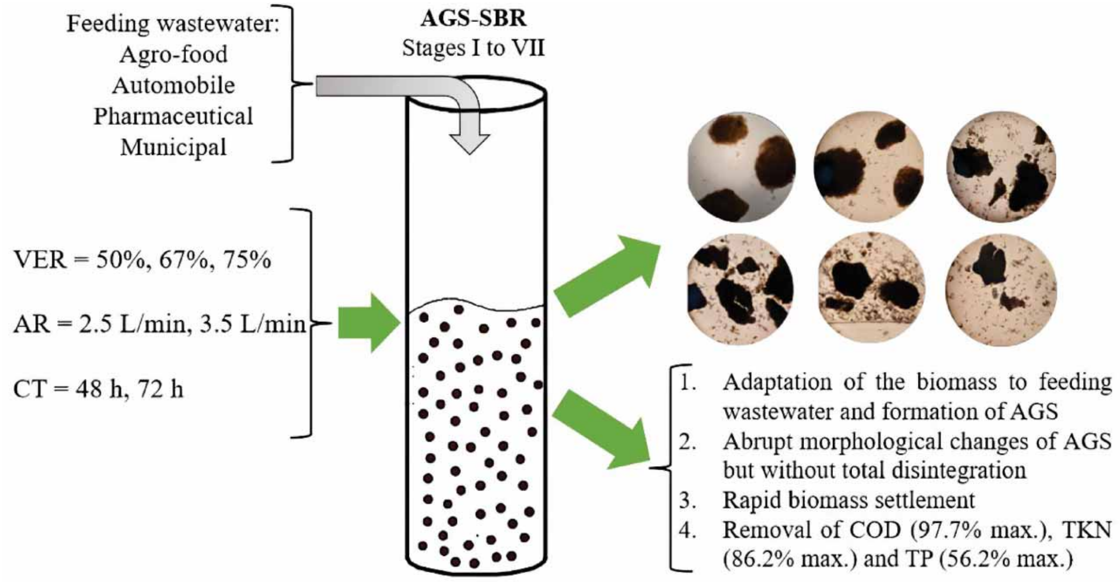
The formation and evolution of aerobic granular sludge (AGS) developed in a sequential batch reactor (SBR) were evaluated to understand the effect of influential operating parameters on its morphology, stability, and removal performance while treating industrial/municipal wastewater. After 18 days of operation (stage I), mature granules were identified in the reactor, and in 25 days, the AGS system reached a stable operation. The chemical oxygen demand (COD) and total Kjeldahl nitrogen (TKN) were affected by the applied operating variations (from stages II to VII). Until day 48 (stage III), the aerobic granules did not show relevant changes in shape and stability. During this stage, the AGS system achieved high removal efficiencies of COD (97.7%) and TKN (86.2%) and a sludge volume index (SVI) of 65 $\pm$ 6.7 mL/g-total suspended solids. From stage IV until the end of the reactor operation, partial disintegration and rupture occurred in the system, but granules did not completely disintegrate. Specifically, a volumetric exchange ratio (VER) of $>$67% and an aeration rate (AR) of $<$2.5 L/min promoted the compactness and the structural integrity of AGS. The principal component analysis corroborated that the rise in the VER is an effective strategy for improving AGS stability and organic pollutant removal.

Key words: aerobic granular sludge, influential operating parameters, principal component analysis, real wastewater, removal performance, stability

HIGHLIGHTS

- The VER was the factor most influential on the settling capacity of aerobic granules.
- The AR and VER significantly changed the morphological characteristics of AGS.
- Wastewater with the origin mainly from automobile and agro-food sectors has a similar behavior under a VER of $\geq$67 and cycle time of 48 h.
- Aerobic granules presented partial disintegration and rupture from stage IV, which affected their integrity, stability, and removal performance.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Aerobic granular sludge (AGS) technology has been extensively studied due to its various advantages for wastewater treatment, such as excellent settleability, high retention of biomass, simultaneous nutrient removal, lower area requirement, strong resistance to organic loading rate, and lower energy expenditure during the process (Chen *et al.* 2008; Alves *et al.* 2022). AGS technology is commonly applied in sequential batch reactors (SBRs) under operating factors and conditions strictly controlled to assist granule formation (Rollemberg *et al.* 2020). The popularity of SBRs is further cemented by an operating principle that provides appropriate feeding/starvation conditions, alternating aeration for sludge granulation, and nutrient removal, thanks to its particular hydraulic pattern (Li *et al.* 2019). AGS technology was first developed on a large scale in the Netherlands and has been applied in countries such as France, Australia, and Brazil (Xavier *et al.* 2021; Royal HaskoningDHV 2022).

Despite AGS systems having been used to treat wastewater on large scales, there are still challenges and limitations that the constrain application of the technology. The main difficulty is directly related to the instability of granules and the loss of biological activity caused by their size and structural properties, which depend on a complex interaction of different environmental and operational parameters (Wilén *et al.* 2018; Xavier *et al.* 2021). Specifically, aeration rate (AR) is one essential operating parameter since it provides the dissolved oxygen (DO) required for SBR and generates the hydrodynamic shear strength needed for the formation of AGS and its optimum performance (Gao *et al.* 2013).

Cycle time (CT) is also an important parameter since it is closely linked to hydraulic retention time (HRT) and operating regime type (condition and distribution in reaction-phase time), which affect the duration of contact between wastewater and AGS and the morphological and bacterial properties given to AGS during the reaction phase. The volumetric exchange ratio (VER) is another influential parameter since it influences the organic load rate (OLR) and thus affects the characteristics of the biomass. Many studies have addressed the AR, CT, and VER parameters in isolation in order to evaluate the effect of each one on the development and removal performance of AGS (Gao *et al.* 2013; Dobbeleers *et al.* 2017; Rollemberg *et al.* 2020; Wang *et al.* 2020b; Xavier *et al.* 2021; Silva *et al.* 2022). However, much of this research was carried out in lab-scale SBRs fed with simulated domestic wastewater. These study conditions have not contributed to overcoming the issues which arise in AGS systems, such as the stable development over a long period of time, with the appropriate granular properties for high nutrient removal when using real effluents on a large scale. Therefore, the innovation of the present study is to evaluate the effect of simultaneous variation of VER, CT, and AR as influential AGS–SBR operating parameters on the formation, stability, and removal performance of an AGS system for the treatment of mixed industrial/municipal wastewater with a complex composition. It is expected that this study will provide not only new insights into the morphological behavior and removal performance of AGS systems but also a basis for proposing suitable operating ranges and ensuring their stability when treating mixed wastewater with a complex composition.

2. MATERIALS AND METHODS

2.1. Reactor setup and experimental design

A bench-scale column-type SBR was constructed using transparent acrylic plastic. The 12 L reactor (internal diameter – 14 cm, height – 90 cm) had a working volume of 4.5 L. Effluent withdrawal was conducted at different flows through a sampling port located at a predetermined elevation on the reactor to achieve volumetric exchanges of 50, 67, and 75%. Airflow rates were provided using Elite-801 and Elite-802 air pumps, which were installed according to the applied operating configuration (Figure 1).

The total operating period was divided into seven consecutive stages: a start-up period (stage I) and six periods with different operating conditions depending on the level assigned to each influential operating parameter (stages II to VII). In stage I, all the parameters were fixed at constant conditions until biomass adaptation and the formation and stabilization of granules were achieved. For stages II–VII, 12 combinations (two per stage) were applied over periods of 6 or 9 days (Table 1). These combinations were set according to the proposed parametric levels for AR (2.5 and 3.5 L/min), CT (48 and 72 h), and VER (50, 67, and 75%). The 48 and 72 h CT consisted of 5 min of feeding, 10 min of settling, and 10 min of effluent withdrawal, resulting in aeration times of 2,855 and 4,295 min, respectively. Therefore, HRT varied as a function of the CT and the VER applied at each stage.

2.2. Wastewater source and seed sludges

The SBR was continuously fed using samples of mixed industrial/municipal wastewater collected from a wastewater treatment plant (WWTP) with an installed capacity of 210 L/s, which treats effluent from the valley of Cuernavaca and the

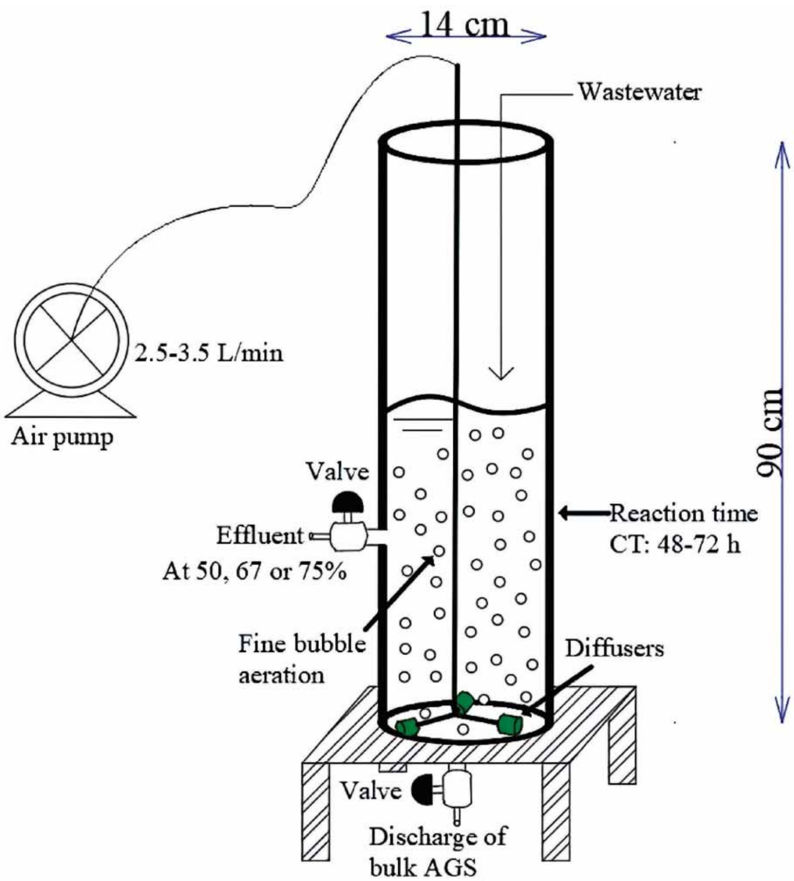


Figure 1 | Schematic of a bench-scale sequencing batch reactor for the formation and evolution of AGS.

Table 1 | Operating strategies applied to the SBR during the different stages

Stage	Operation period (days)	Cycles	Aeration rate (L/min)	CT (h)	VER (%)	HRT (h)
I	1–27	9	2.5	72	67	108
II	28–39	4	2.5–3.5	72	50	144
III	40–57	6	2.5–3.5	72	75	100
IV	58–69	6	3.5–2.5	48	67	72
V	70–81	6	2.5–3.5	48	75	64
VI	82–93	4	3.5–2.5	48	50	96
VII	94–111	6	3.5–2.5	72	67	108

area of Jiutepec, Morelos, Mexico (Table 2). The average values were obtained from the data obtained for the periods under the study. The samples were taken after pre-treatment (coarse and fine screening, sand, grit, and grease removal and primary sedimentation). The seed sludge was collected from a facility with extended aeration located in the Instituto Mexicano de Tecnología del Agua (IMTA) in Morelos, Mexico. This seed sample had previously been adapted to diverse synthetic substrates to simulate wastewater from the dairy and beef industry in earlier studies. The initial mixed liquor suspended solids (MLSS) concentration was ~4.8 g/L. The average age of sludge was ~6.75 days.

2.3. Analytical procedures

Total suspended solids (TSS), MLSS, and the sludge volume index (SVI) were analyzed in accordance with standard methods (APHA 2005). Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were determined in accredited laboratories in accordance with NMX-AA-030/2-SCFI-2011 and NMX-AA-028-SCF1-2001, respectively. For total Kjeldahl nitrogen (TKN) measurements, effluent samples were taken, preserved with H_2SO_4, and stored at 4 °C to be transferred to the Engineering Research Laboratory at the Universidad Politecnica del Estado de Morelos (UPEMOR). The samples were analyzed in a Büchi Kjelmaster K-375 instrument. Nitrites (NO_2-N) and nitrates (NO_3-N) were measured following the 8,153 and 8,039 HACH colorimetric methods using a DR-6000™ spectrophotometer. Likewise, total phosphorus (TP) was determined by a colorimetric method (10,127 HACH). DO was monitored every 24 h with a YSI model 58. Temperature, pH, and conductivity were measured *in situ* in a WWTP with an OAKlon PC540 potentiometer. The morphology of AGS was observed using an achromatic microscope (MOTIC™ model B1 digital) through a stereoscopic 40× lens (4/0.1). At the end of each cycle, the granules were collected using different bacteriological loop sizes (1.5, 1, and 0.5 mm) based on the observed size of the granules.

2.4. Statistical analysis

A multivariate principal component analysis (PCA) was carried out to identify the relationships between the most significant configurations which affect the performance of the system. Also, the statistical significance of the differences in pollutant removal was analyzed using two-way variance analysis (ANOVA) without interaction. All these analyses were performed

Table 2 | Average composition of mixed industrial/municipal wastewater used in this study

Parameter (mg/L)	Average	Maximum	Minimum
COD	2,316 ± 1,287	4,938	839
TSS	569 ± 1,146	1,160	167
BOD	709 ± 122.7	972	356
TP	24.4 ± 7.2	31	10
TN	85.1 ± 37.02	179	44
pH*	7.32 ± 0.37	6.88	7.93

*pH units; $n = 45$.

with Minitab® 19 software, where the relative probability of error of rejecting the hypothesis of equal means was set at 5% ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Adaptation and development process of AGS

The adaptation of the seed sludge to the characteristics of feed wastewater and the formation of the granules occurred simultaneously in stage I of the operation. Due to the high complexity in the composition of used wastewater, feeding into the SBR was carried out gradually. Feed wastewater was diluted with tap water, starting with (1:3), then (1:2), next (1:1) and finally feeding undiluted wastewater into the reactor. These ratios changed every two operating cycles. As the adaptation was successful, the color gradually changed from tan to dark brown, which might be due to pigment absorption from wastewater of industrial origin.

Mature granules were identified in the AGS system on day 18 as compact aggregates with a well-defined morphology. On day 24, the AGS had a dense, compact, and spherical shape, with an average size of $\approx 1.2 \pm 0.4$ mm, due to the previous adaptation of the biomass to the wastewater variability used in this study. Some recent studies have reported that the suggested minimum concentration of COD and the OLR in feed wastewater to maintain the integrity of aerobic granules is 200 mg/L and 0.6 kg/m^3-d, respectively (Peyong *et al.* 2012; Long *et al.* 2015). However, they seldom represent the complex composition of real municipal or industrial wastewater and its load variations. In this study, the AGS system maintained COD and OLR concentrations above 800 mg/L and 2.0 kg/m^3-d throughout the operation. These concentrations prevented the total disintegration of the AGS despite the high organic loading shocks and abrupt changes in the configurations applied. The adaptation and formation of aerobic granules in steady-state conditions occurred in approximately 25 days.

3.2. Morphological changes to aerobic granules

During the application of operating strategies, morphological changes in the AGS were observed (Figure 2). From stage II to stage VII, granules with different characteristics were studied to evaluate the behavior of the AGS system. Size changes in the aerobic granules were linked to the operating conditions. In fact, the AR and the VER were the main operating factors that directly influenced morphological AGS characteristics. The first parameter affected mixing, biomass diffusion, and applied

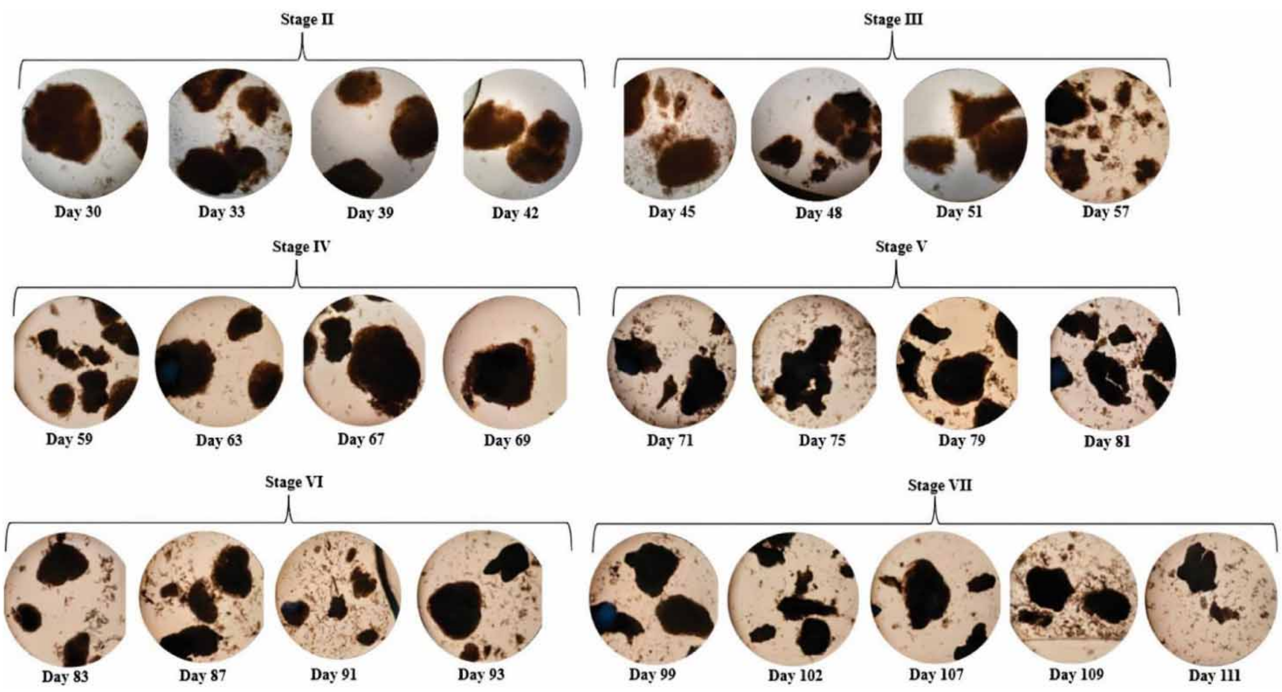


Figure 2 | Changes occurring in the granular morphology of the AGS system during operating combinations.

hydrodynamic strengths, which directly impact the density, stability, and structure of granules (Hamza *et al.* 2022). The second influenced their size and sedimentation properties (Wang *et al.* 2006).

Up to day 48, no relevant changes were identified in the shape and stability of the aerobic granules. However, the granule fragmentation increased considerably (~day 51) when the AR was modified from 2.5 to 3.5 L/min in stage III. Later, granule stability recovered when the AR changed to 2.5 L/min in stage IV (~day 63). Partially, broken or disintegrated granules tended to appear within 3 days of the changes of configuration that initiated each 6 or 9 days, and these promoted a flocculent medium, smaller size, and modifications in the granular structure (days 33, 45, 67, 71, 87, and 109). However, the system returned to stable operation about 4 or 5 days after the operational change in each stage, which promoted the recovery of granule structure, agglomerated the biomass again, and improved compactness and size, although with different morphologies (days 39, 48, 63, 79, 83, and 107). These effects on AGS characteristics mainly occurred when the VER changed from 50 to 75% in the system. It was found that operating conditions with a VER of 50% meant that the AR supplied to the system (2.5 and 3.5 L/min) caused an excessively high hydrodynamic shear strength, promoting granule instability and generating filaments around the aggregate. However, with VER values of 75%, granule instability decreased, allowing the AGS system to reach stable operation faster than lower VER values. This latter is due to the fact that hydrodynamic shear strength applied to the granules is lower under a high VER and an AR of 2.5 L/min. Some authors have shown that a lower AR reduces the fragmentation and disintegration of sludge particles during the early stage of operation, due to the low hydrodynamic shear strength and rapid growth of filamentous bacteria, which is beneficial for aerobic granulation and the amount of biomass retained in the reactor (Wang *et al.* 2020a). However, the excessive reduction of AR causes increased growth of filamentous bacteria and a lower amount of extracellular polymeric substances (EPS), which leads to the disintegration of granules (Hamza *et al.* 2022).

Other authors have employed morphological parameters for granules, such as aspect ratio and shape factor, for the purpose of monitoring AGS characterization under diverse conditions. Silva *et al.* (2022) showed that a progressively stronger granular structure was developed under high solidity and eccentricity values and low convexity values. On the other hand, lower solidity and convexity values and higher eccentricity reflected disturbances in the granulation process, possibly due to a higher presence of flocs, resulting in more suspended solids in the effluent. In this study, as the operating combinations were applied to the AGS system, the regeneration capacity of the granules decreased until broken granules completely changed their properties (from day 71). The latter might have been due to a high solidity that prevented the total disintegration of the granules and a low convexity that caused a higher release of suspended solids (days 79, 87, 91, and 99). However, despite the morphological alterations and possible microbial inhibitions that occurred in the AGS system under the different operating conditions, adequate granule integrity was maintained, which allowed an average settling capacity below 85 mL/g-TSS until stage VI.

3.3. Effect of the SVI on settling time under the operating conditions

The settling behavior of AGS was evaluated according to the SVI measured. Various authors have reported SVI values in AGS–SBR systems from 20.8 to 74 mL/g-TSS (Aqeel *et al.* 2016; Krishnen *et al.* 2017; Yin *et al.* 2019; Leal *et al.* 2020; Xavier *et al.* 2021; Alves *et al.* 2022). These SVI values were the highest achieved depending on the different structural properties given to granules based on the operating strategies applied. The SVI in this study ranged from 56 to 110 mL/g-TSS from day 39 (beginning of stage 3) until the end of the operation. Table 3 shows the highest average settlement depending on the best operating combinations applied at each stage. In the stages where the VER value applied to the system setup was $\geq$67%, an increase occurred in biomass sedimentation through high solid/liquid separation. It has been reported in the literature that AGS systems developed in the SBR and operated at constant volume with a high VER (75%) produce a significant fraction (>80%) of granules with very good settling properties (SVI <70 mL/g-TSS) (Xavier *et al.* 2021). Moreover, multivariate analysis supported the finding that an increment in the VER from 50 to 75% is an important strategy for AGS stability (Xavier *et al.* 2021). In this study, when the VER value applied to the system was $\geq$67%, the density and stability of the sludge improved, which increased sedimentation of the granules (stages III and VII). The PCA biplot of Figure 5(a) shows that the operating combinations that applied a VER equal to 75% (close to PC1) had a positive influence on SVI and TSS values. However, when the VER decreased to 50%, the SVI was directly affected. Moreover, the ANOVA analysis showed a significant effect of VER (75%) on SVI and TSS, where $p = 0.04$ and $p = 0.045$, respectively. Therefore, VER values can directly influence the settling capacity of granules.

Table 3 | Average AGS settling capacities depending on the operating conditions applied during each stage

	Operational configurations			
Stages	AR (L/min)	CT (h)	VER (%)	SVI (mL/g-TSS)
II	2.5	72	50	71 $\pm$ 3.1
III	3.5	72	75	65 $\pm$ 6.7
IV	2.5	48	67	75 $\pm$ 0.8
V	3.5	48	75	87 $\pm$ 11.2
VI	3.5	48	50	89 $\pm$ 6.3
VII	3.5	72	67	70 $\pm$ 5.5

$n = 3$.

In this regard, when a VER value equal to 50% was applied to the AGS system, high suspended biomass fractions appeared due to the partial disintegration of granules. The above produced high concentrations of suspended solids, which caused a decrease in the settlement, less stable granules, and, consequently possible modifications in microbial characteristics (stage VI). Each change in operating conditions placed stress on the presence of microorganisms, resulting in the generation of these biomass fractions. In addition, the AGS system required more time to become stabilized at operating conditions where the VER was equal to 50%, which affected the removal performance and biomass settling capacity. However, when the VER increased to 67%, the system improved sedimentation in stages III and VII. During these periods, an AR equal to 3.5 L/min significantly influenced the improvement of the settling capacity under these operating conditions. In fact, it has been shown that sludge settling capacity is significantly improved by increasing superficial air velocities (Zhu *et al.* 2015), reaching an average AGS settling capacity of up to 38.7 $\pm$ 6 mL/g-TSS under an AR of 4.79 L/min (Leal *et al.* 2020). In this study, the maximum settlement capacity obtained with the AGS system was 56 mL/g-TSS, which was produced from days 48 to 57 (stage III) under the highest operating configuration, with CT, VER, and AR being equal to 72 h, 75%, and 3.5 L/min, respectively. Also, the granules maintained a compact structure and a larger size ($\geq$1 mm), which significantly reduced the concentration of suspended solids in the outflow.

SVI behavior was strongly linked to the increase or decrease in biomass productivity during the operating period. Figure 3 shows the variability in SVI performance as a function of the MLSS concentration present in the AGS system. Recent studies have established that when biomass productivity increases, the SVI value improves and *vice versa*. Therefore, it is advisable to maintain an MLSS concentration between 3,500 and 5,000 mg/L (Ferrara-Giner & Ramírez 2013) to improve the AGS settling properties and thus avoid sludge settlement in the reactor during the reaction phase. The average MLSS concentration during the operating combinations was 4,625 mg-TSS/L. However, on day 61, the MLSS decreased to 3,240 mg-TSS/L, probably because the type of feed wastewater (automobile) affected the SVI, which resulted in 90 mL/g-TSS.

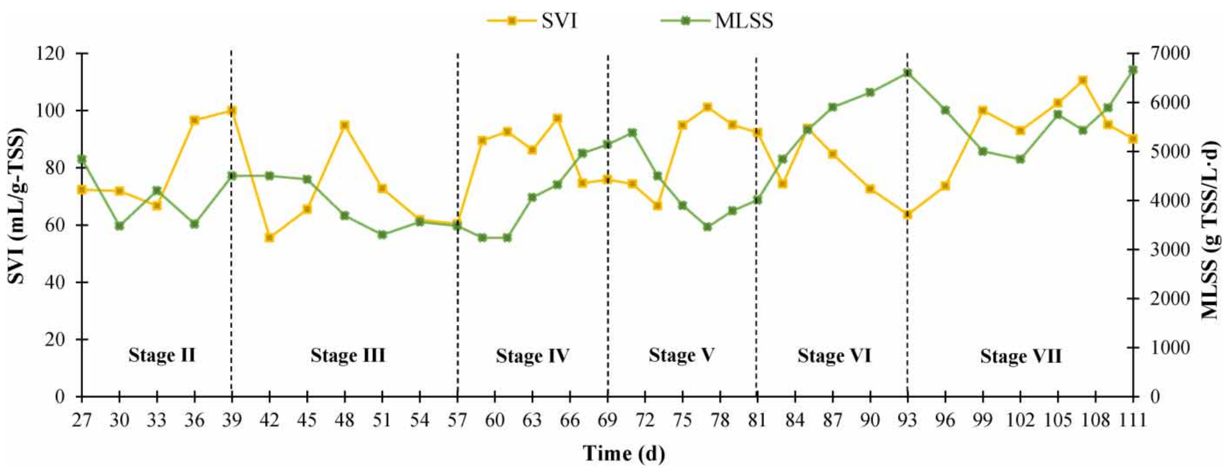


Figure 3 | SVI and MLSS performance under the operating conditions applied.

Later, on day 71, the MLSS concentration increased to 4,320 mg-TSS/L, and the SVI decreased to 75 mL/g-TSS. This biomass recovery was due to the change in feed wastewater characteristics (from automobile to agro-food/municipal) and the increase in the VER from 67 to 75% at the beginning of stage V.

In this regard, the range of MLSS concentration in the AGS system was between 3,200 and 6,600 mg-TSS/L. However, over the first 87 days, the MLSS measured was below 5,200 mg-TSS/L, which promoted good stability and high settling capacities in the AGS system, despite the composition of feed wastewater.

3.4. Classification of feed wastewater and organic matter removal

The feed wastewater was categorized according to color and odor detected during sampling. The predominant industrial sources were the automobile, pharmaceutical, and agro-food sectors. The ratio of industrial to municipal wastewater contained in the samples was around 60/40. This ratio of the feed volume also affected the morphological characteristics and the structural stability of aerobic granules. The severest morphological changes to the granules occurred when the AGS system was fed with wastewater from the automobile and pharmaceutical industries during stages V and VI. Figure 4 shows the PCA score plot on the relationship and trend of feed wastewater from the predominant origin used in the study. It is possible to observe three main clusters, corresponding to removal performance based on the morphological changes undergone by aerobic granules due to feed wastewater throughout the operating conditions applied from stages II to VII. These clusters show that most wastewater which is mainly municipal had a positive correlation, which indicates that operating configurations applied to wastewater samples with these characteristics produced similar performance (cluster 1 with high scores in PC2). On the other hand, wastewater which was mainly of automobile and agro-food origin showed a similar positive trend when operating conditions included a VER of ≥67% and a CT of 48 h (cluster 2 with high scores in PC1). This latter was supported by ANOVA analysis, in which no significant differences ($p > 0.36$ and $p > 0.92$, respectively) were found between wastewater mainly from automobile and agro-food origin for COD removal efficiency under the influence of VER and CT parameters.

However, when operating conditions changed to the VER of 50% and the CT of 72 h in mainly automobile wastewater, a negative correlation was observed (cluster 3 with higher values on PC1 and PC2). Likewise, wastewater which was mainly of

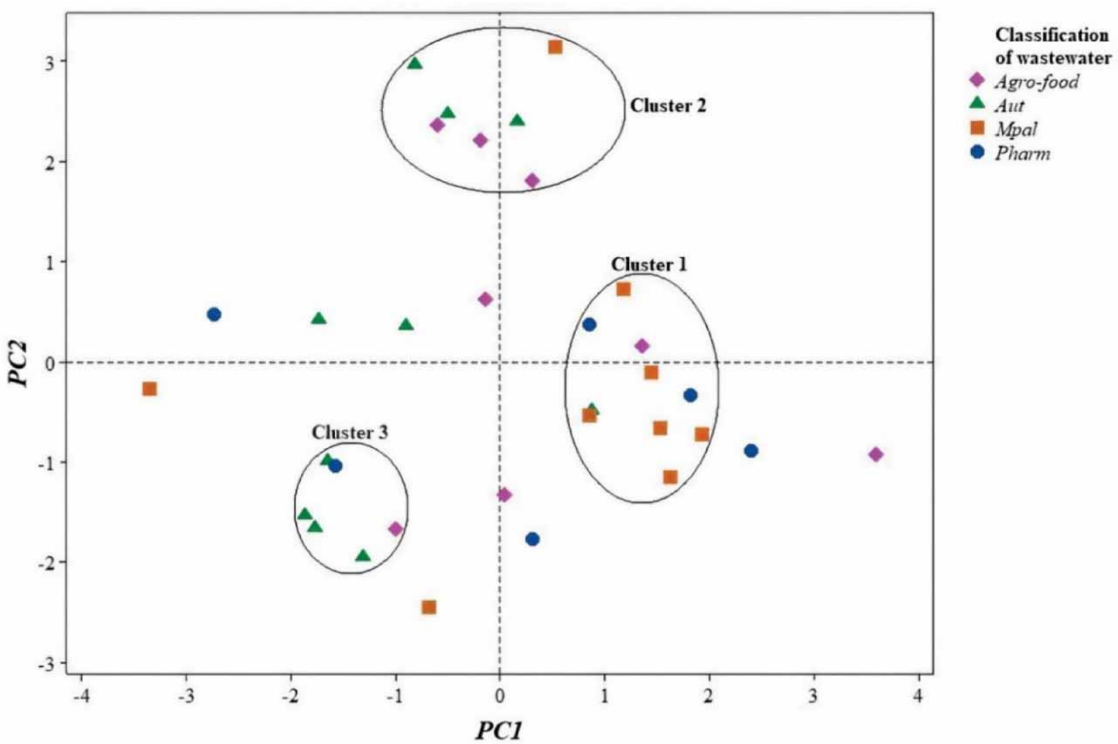


Figure 4 | PCA score plot for feed wastewater.

pharmaceutical origin did not show similar behavior under any specific operating conditions. Even some variables from the various wastewater types showed inversely correlative behavior among themselves. This last shows the substantial effect of operating conditions applied to the system operation. In this regard, AGS performance is related to the concentration of compounds present in feed wastewater and to the specific operating parameter set, since these factors affect the properties of the granules throughout the operating period, which in turn strongly influences microbial selection, carbon availability, and organic compound removal.

The AGS system performance is also supported by Figure 5, which shows the PCA biplot for all VER, AR, and CT levels applied to the AGS system. In Figure 5(a) and 5(c), operating conditions that included a VER of 67% and a CT of 48 h (variables close to PC1) positively influenced BOD removal. In addition, operational combinations where the VER was equal to 75% (variables close to PC1) had a positive effect on COD and TSS degradation but hampered TKN and TP removal. In addition, both AR levels positively and significantly influenced COD, BOD, and TSS removal efficiency under the VER and the CT of ≥67 and ≥48 h, respectively. Indeed, the COD variable in the variance analysis showed a significant difference ($p = 0.031$) for the VER, which supports the results found in the PCA biplot. Also, it was noted that the AGS system was fed with wastewater of mainly municipal and agro-food origin under these operating conditions.

The abundance of wastewater types in each sample implied the presence of organic and xenobiotic compounds that determined the microbial properties of the granules and thus modified the AGS system performance. The COD concentration of feed wastewater ranged from 800 to 7,000 mg/L throughout the operating period. This wide variability was linked to the volume of municipal and industrial effluent discharged on any given day. The automobile industry was predominant in 31% of the feed wastewater, which produced the highest COD concentrations, ranging from 1,420 to 4,938 mg/L (Table 4). Although wastewater mainly from the automobile industry had the lowest BOD/COD ratio (0.31 ± 0.12), the AGS system achieved an average COD and TSS removal efficiency of 94.5 and 90.7%, respectively (COD concentration to the outflow of 100–185 mg/L). This was the highest removal efficiency achieved among the wastewater types identified.

This predominantly automobile wastewater was mixed with effluents mainly of municipal and agro-food origin. By contrast, predominantly pharmaceutical wastewater had the lowest concentration (from 839 to 2,630 mg COD/L) and accounted for 17% of the total effluent treated. This feed wastewater type produced the average COD and TSS removal of 90.4 and 85.7% (COD to outflow from 95 to 170 mg/L). In this regard, the low carbon availability in pharmaceutical wastewater and the possible presence of active pharmaceutical compounds affected AGS properties, and consequently, the removal performance of the biological process was negatively affected. Similarly, 25% of feed wastewater was mainly of agro-food origin, mixed with effluents mainly from the municipal origin, and registered average COD and TSS removal efficiencies of 93.8 and 91.0% (COD to outflow from 89 to 170 mg/L). Some studies have reported high COD removal rates of up to 92.4% during simulated pharmaceutical wastewater treatment using the AGS under stable conditions (Wang *et al.* 2016). However, research on real pharmaceutical wastewater using AGS systems is limited. Furthermore, the present authors did not find any reports on the performance of AGS systems in the treatment of wastewater from the automobile industry during an exhaustive search of the literature.

Even though the BOD/COD ratio of feed wastewater oscillated between 0.3 and 0.5, nearly all operating cycles of the AGS system showed COD and BOD removal efficiencies above 90 and 98%, respectively (Figure 6). The latter was because the DO concentration in the AGS system oscillated between 3.5 and 6.1 mg/L during the whole operation. This high oxygen concentration allowed for organic matter degradation due to the development of heterotrophic microorganisms as dominant populations. Although there was no significant difference in the removal efficiency of organic matter under the different operating combinations applied, a decrease from 89 to 81% in the efficiency of the AGS system was observed between days 90 and 99, with CT, VER, and AR values of 72 h, 50%, and 3.5 L/min, respectively. This reduced performance was due to the modification in operating conditions and wastewater fed to the system (from municipal to pharmaceutical). This greatly affected carbon availability in the AGS system, which could modify the COD/N ratio present and affect structure, stability, and AGS operation (Wang *et al.* 2020a).

Amorim *et al.* (2016) assessed the performance of AGS–SBR systems for simulated wastewater treatment. They observed that when a load of 100% municipal wastewater was changed for wastewater with pharmaceutical compounds, COD removal decreased by up to 57%. These results were obtained under operating conditions where the VER, AR, and CT were 40%, 4 L/min, and 6 h, respectively (COD to outflow from 38 to 84 mg/L). However, before the addition of the chiral pharmaceuticals, the COD efficiency was ~90%. Likewise, the COD values obtained in this study can be compared to several references found in the literature regarding the expected COD removal of stable and mature AGS (Table 5). Moreover, it was corroborated that

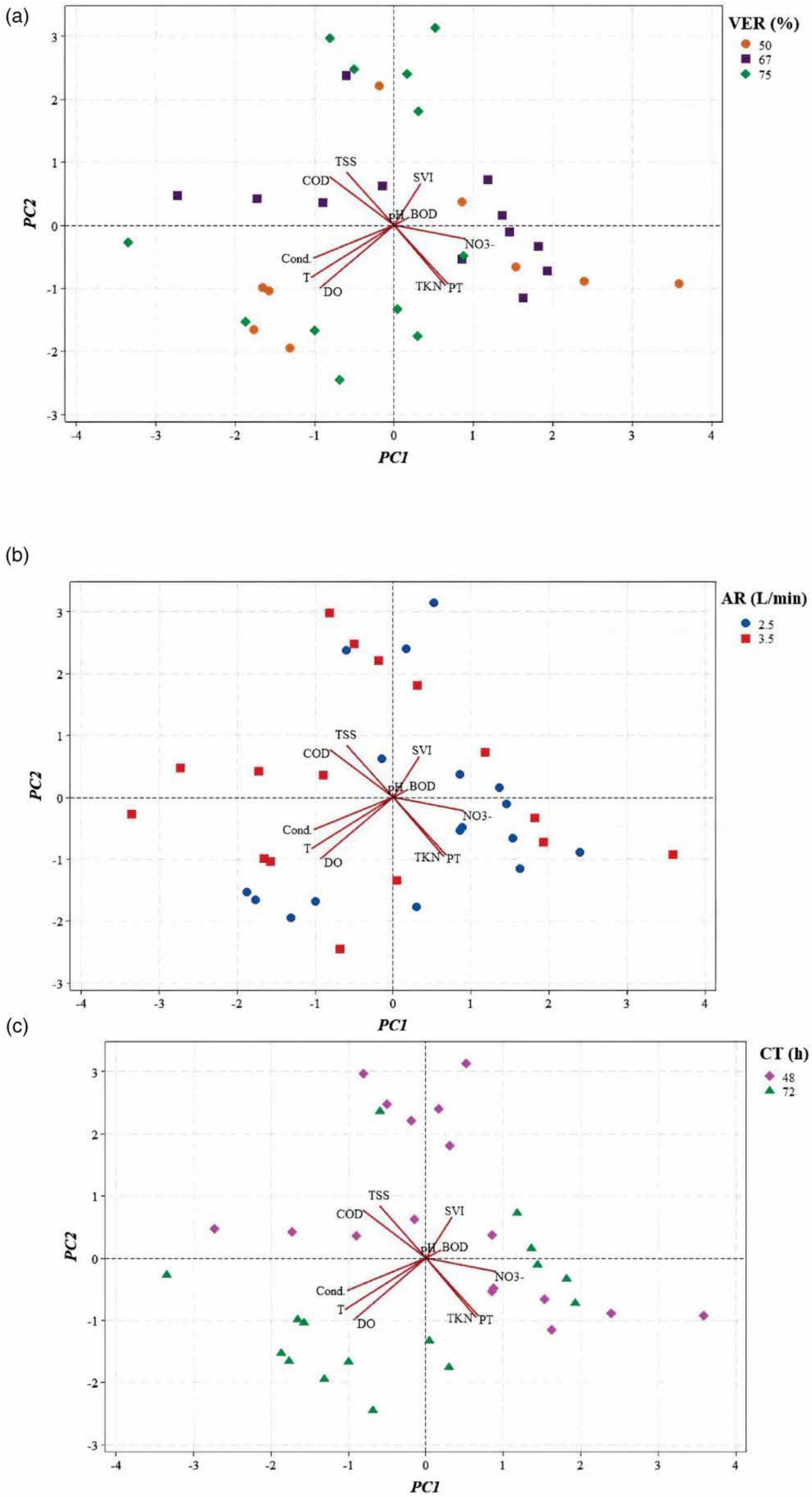


Figure 5 | PCA biplot for AGS system performance: (a) VER (%); (b) AR (L/min); (c) CT (h). Scale for loadings: [PC1 and PC2 from −0.5 to 0.4].

Table 4 | Average COD and BOD removal from the AGS system depending on the type of feed wastewater

Type of wastewater	Initial concentration (mg/L) COD	BOD	BOD/COD	F:M ratio	Removals COD mg/L	%	BOD mg/L	%	TSS mg/L	%
Municipal	1,750 ± 543.9	743.6 ± 133.6	0.47 ± 0.10	0.155	124.8 ± 31.1	92.1	13.3 ± 2.9	98.2	36.5 ± 14.4	87.4
Pharmaceutical	1,586 ± 615.5	679.8 ± 103.76	0.40 ± 0.25	0.142	130.8 ± 31.9	90.4	11.2 ± 5.0	98.3	32.2 ± 20.4	85.7
Automobile industry	2,459 ± 1,043.1	671.1 ± 69.93	0.31 ± 0.12	0.140	119.5 ± 24.7	94.5	12.4 ± 5.2	98.04	27.9 ± 23.6	90.7
Agro-food industry	2,073 ± 656.7	717.2 ± 150.82	0.36 ± 0.09	0.149	122.2 ± 23.3	93.8	11.5 ± 4.5	98.2	23.3 ± 10.4	91.0

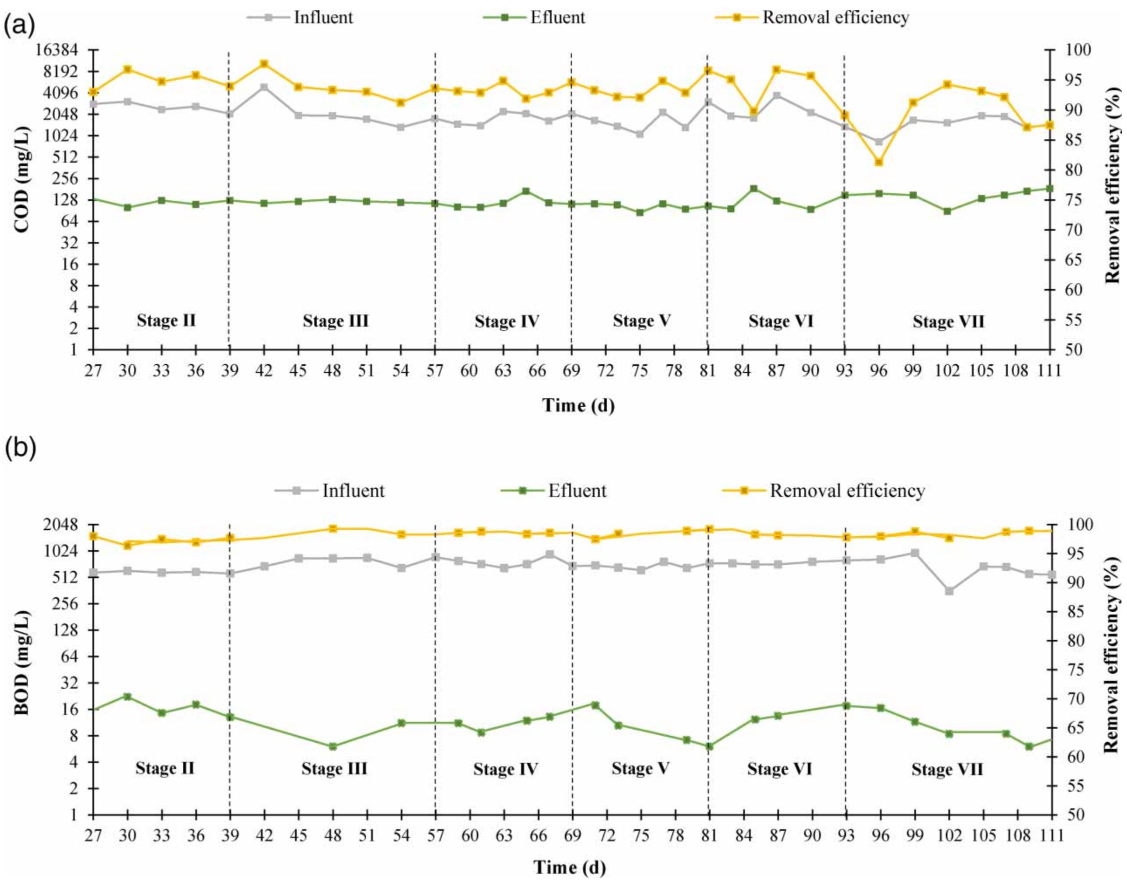


Figure 6 | Organic matter removal properties of AGS: (a) COD removal and (b) BOD removal. *Y*-axis scale: binary logarithm ($\log_2 n$).

when the DO concentration is ≥2 mg/L, the AGS system can obtain COD efficiencies higher than 85% in both municipal and industrial wastewater, with an excellent solid/liquid separation ratio.

In this study, the highest COD removal efficiency obtained with the AGS system was 97.7% after 42 days of operation. This removal efficiency was achieved with wastewater mainly of automobile origin and with CT, VER, and AR figures of 48 h, 75%, and 2.5 L/min, respectively. Furthermore, the wide variety of types of wastewater used in this study affected the

Table 5 | Removal efficiencies achieved in previous works using AGS–SBR systems

			Operating conditions				Removal efficiency (%)			
WW type	WV (L)	Influent concentration (mg/L)	CT h	VER %	DO mg/L	AR or SAV	COD	TN	TP	References
Synthetic	2	1,000 mg COD/L; 200 mg NH_4-N/L; 16 mg P/L	6.5–7	17–29	0.5–2	n.d.	93.8 ± 3.2	94.6 ± 5.6	83.7 ± 8.6	Dobbeleers *et al.* (2017)
Chemical industry	2	1,000 mg COD/L	6	25–75	n.d.	2–4 L/min	97	n.d.	n.d.	Rafiee *et al.* (2018)
Synthetic	1.4	600 mg COD/L; 100–200 mg NH_4-N/L; 10 mg P/L	4	54	7–9	2.0 cm/s	92	99	10	Huang *et al.* (2015)
Petrochemical	3	340–610 mg COD/L	4	0.5	n.d.	2–3 cm/s	>85	n.d.	n.d.	Milia *et al.* (2016)
Synthetic	2	500 mg COD/L; 50 mg NH_4-N/L; 5 mg P/L	6	50	n.d.	2–1 L/min	90–96	>80	n.d.	Wang *et al.* (2020b)
Synthetic	2	2,000 mg COD/L; 100 mg NH_4-N/L; 10 mg P/L	12	50	2	1 L/min	98.8	81.9	n.d.	Yin *et al.* (2019)
Dyeing	4.6	569 mg COD/L; 0.524 mg NT/L	6	50	7.75	4 L/min	87	n.d.	n.d.	Bashiri *et al.* (2018)
Synthetic	115.5	206.4–222.5 mg COD/L; 28.1–33.7 mg NH_4-N/L; 3.1–3.7 mg P/L	4	71	8	27 L/min	80 ± 11	83 ± 20	55 ± 24	Alves *et al.* (2022)
Agro-based	3	25,000 mg COD/L; 45 mg NH_3-N/L	3	50	n.d.	2.5 cm/s	91.1	97.6	n.d.	Abdullah *et al.* (2011)
Synthetic	18	2,000 mg COD/L; 100 mg NH_4-N/L; 20–25 mg P/L	4	60	8–10	2.7 cm/s	98 ± 1	75 ± 17	59 ± 11	Pishgar *et al.* (2019)
Rubber	1.8	1,820 mg COD/L; 250 mg TN/L	3, 6, 12	50	2.4–7.6	1.7 cm/s	98.4	89.5	n.d.	Rosman *et al.* (2014)
Textile synthetic	1.5	1,000 mg COD/L	6	50	n.d.	n.d.	80.0	n.d.	n.d.	Lourenço *et al.* (2015)
Mixed industrial/ municipal	3–6	2,316 mg COD/L; 85.1 mg NH_4-N/L; 24.4 mg P/L	24–72	50–75	3.5–6.6	2.5–3.5 L/min	92.1–94.5	68	41	This study

WW, wastewater; SAV, superficial air velocity; WV, working volume; n.d., no data.

performance of the AGS system, since they contain high concentrations of organic pollutants and complex industrial substances that may modify the selection and properties of the bacteria responsible for removing the organic matter (Ouyang *et al.* 2019).

3.5. Nutrient removal performance

TKN and TP removal performance was also measured during the application of the operating combinations (Figure 7). A TKN decrease was mainly due to cell synthesis and nitrification. However, simultaneous nitrification–denitrification reactions possibly also contributed to TKN removal, as previous research analysis of microbial communities in granular sludge has indicated the presence of both nitrifying and denitrifying bacteria (Gao *et al.* 2011). Phosphorus (P) removal was mainly due to cell synthesis and biomass adsorption. The TKN concentration of feed wastewater during operating combinations ranged between 43.8 and 179.1 mg/L, whereas the TP concentration was between 9.5 and 31.4 mg/L. The average removal concentrations achieved at the outflow were TKN – 24.4 $\pm$ 7.2 mg/L and TP – 14.0 $\pm$ 3.3 mg/L (removal efficiencies of 67.5 and 41.4%, respectively). However, TKN concentration in the effluent oscillated between 9.1 and 37.7 mg/L, whereas TP ranged between 4.9 and 18.2 mg/L (removal efficiencies of 53.4 – 86.2% for TKN and 25.5 – 56.2% for TP). This variability in nutrient removal was strongly influenced by DO levels in the system (up to 6.1 mg/L), the COD/N ratio found in feed wastewater, and the AGS properties which evolved, such as the distribution of aerobic, anoxic, and anaerobic zones within granules and the microbial growth rate in each zone, which altered during the operating configurations.

Besides the feed wastewater characteristics and the frequent morphological AGS changes occurring throughout the operation, those strategies that included a CT of 72 h and an AR of 3.5 L/min promoted a medium with high DO concentrations ($>$5.5 mg/L), which negatively affected the performance of TKN and TP removal in stages II, III and VII. The foregoing is

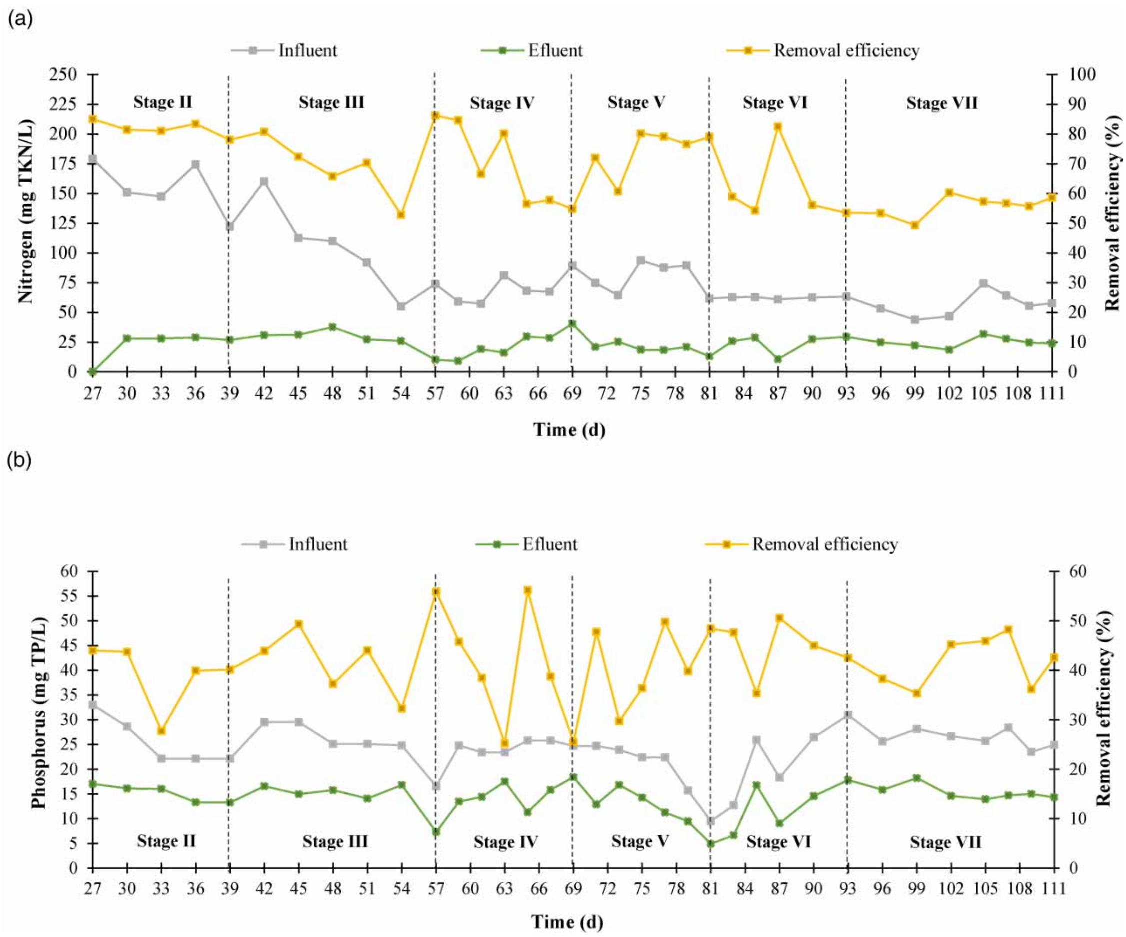


Figure 7 | Performance of the AGS–SBR in terms of (a) TKN removal and (b) TP removal.

illustrated in Figure 5(b) and Figure 5(c), which shows that operating combinations that included an AR of 3.5 L/min and a CT of 72 h (variables close to PC1) were directly linked to high DO concentrations. These conditions in the system had a direct effect on nutrient removal (TKN, $NO_3^- - N$, and TP). It is well known that when an AGS system is exposed to excessively high DO, it generates a broader aerobic zone inside the granule, which limits the suitable development of the anoxic/anaerobic zones (Liu *et al.* 2017). Such properties of an AGS prevent the growth of the denitrifying bacteria responsible for nitrate removal and of the phosphorus-accumulating organisms (PAOs) responsible for phosphorus degradation. The growth of these organisms is inhibited since they need relatively high residence times and low DO concentrations in order to develop (Li *et al.* 2016; Wilén *et al.* 2018). Also, it has been reported that in larger AGS and with low DO concentrations, a greater more anoxic zone may form inside the AGS, providing a larger available area for denitrifying bacteria (Wang *et al.* 2020b).

In this study, the granules formed were small to medium size with a mainly aerobic surface, which possibly promoted the nitrification of part of nitrogen (N), thanks to the abundant development of nitrifying bacteria (such as ammonia-oxidizing bacteria and nitrite-oxidizing bacteria), which was in turn stimulated by a broader aerobic zone toward the inside of the AGS. Consequently, although large CT values were applied, the high AR levels supplied to the AGS system prevented the generation of suitable anoxic/anaerobic conditions for the microbial growth responsible for removing nitrates and P. On the other hand, it has also been pointed out that monitoring the COD/N ratio is essential to ensure enough N content for heterotrophic microorganisms' metabolism. Yin *et al.* (2019) reported that when the COD/N ratio was 100/5, part of the $NH_4^+–N$ was oxidized to $NO_2^-–N$ and then to $NO_3^-–N$ by autotrophic nitrifying bacteria. However, $NH_4^+–N$, $NO_2^-–N$, and $NO_3^-–N$ coexisted in the effluent, which may have been due to the washout of autotrophic nitrifying bacteria caused by the competition for space between heterotrophic and nitrifying bacteria. This latter possibly resulted in the limited growth of autotrophic nitrifying bacteria in the aerobic zone of the AGS, which prevented the nitrification process completely during the reaction stage. In practice, in this study, the TKN concentration measured at the outflow of the AGS process was in the form of $NO_3^-–N$ and $NH_4^+–N$, which made up more than 97% of the total concentration. In this regard, the competition between heterotrophic and nitrifying bacteria resulted in a sharp decrease in nitrifying bacteria from the aerobic zone and hence lower TKN removal efficiency.

Nearly all operating combinations during stages II, III, and V produced cycles where TKN removal ranged between 60 and 85%, whereas, in stages VI and VII, the removal efficiency of most operating cycles decreased to 50 and 66%. This behavior also occurred during P removal, where some isolated cycles from phases II and IV had removal efficiencies of just 27.7 and 25.2%, whereas, in stages III, V, VI, and VII, the efficiencies achieved ranged between 32.3 and 56.4%. In fact, the highest TP removal efficiencies occurred when the VER and the CT were 75% and 72 h, which is applied from days 42 to 54 (stage III). Stages II and V used wastewater mainly of automobile origin, while stages III, VI, and VII involved agro-food and pharmaceutical wastewater. Stage IV used mainly pharmaceutical wastewater. All wastewater from these stages was combined with municipal wastewater. So, the operating conditions were determining factors in AGS for nutrient removal performance in each of them.

Some authors have made significant efforts to study AGS – SBR systems for N and P removal from different types of wastewater (Table 5). Most of these studies have evaluated the organic loads as well as COD/N and food/microorganism ratios using different operating strategies and configuration mechanisms. The results indicate that AR and load rates are essential for effective N removal. At least 34% of the studies with a superficial air velocity between 1.7 and 2.5 cm/s and a VER equal to 50% reported a TN removal efficiency higher than 80%, despite the differing types of wastewater used, such as synthetic municipal, agro-based, and rubber. On the other hand, the P removal efficiencies reported were limited, since only 42% of the total studies reported TP removal values, which were less than 60% for synthetic municipal wastewater treatment. The above values support the relevance of the results of this study, since up to 85% TKN and 56% TP removal values were achieved during the treatment of real industrial/municipal wastewater with a complex composition.

4. CONCLUSIONS

The main findings of this study are listed below:

1. Wastewater of industrial origin was mainly from the automobile (31%), agro-food (25%), and pharmaceutical (17%) sectors.
2. Adaptation and stable formation of aerobic granules with a diameter of $\approx 1.2 \pm 0.4$ mm were achieved in industrial/municipal wastewater with a complex composition after 25 days of operation through an SBR at the bench scale.

3. AR and VER were the main operating factors that directly influenced the morphological characteristics of the AGS. The greatest morphological changes occurred when the AGS system was fed with wastewater mainly from the automobile and pharmaceutical sectors.
4. The AGS process achieved an SVI up to 56 mL/g-TSS during stage III, which was the maximum settling capacity obtained in the AGS system.
5. The optimum removal efficiency of COD (97.7%) was achieved in phase III with VER, AR, and CT of 75%, 2.5 L/min, and 72 h. Also, optimum removal efficiencies of TKN (86.2%) and TP (56.2%) were achieved in phases III and VI.
6. The multivariate analysis corroborated that aerobic granule performance was mainly correlated to DO concentrations and morphological changes which occurred due to the operating conditions applied.
7. The results of the study suggest that ranges applied to influential operating parameters are significant for improving the treatment of industrial/municipal wastewater with a complex composition. Therefore, future research should focus on evaluating the best configurations for mixed wastewater treatment, considering the possible variations at larger scales.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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