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Scientific Paper

Aerobic granulation process applied to landfill leachate treatment

Processo de granulação aeróbia aplicado ao tratamento de lixiviado de aterro sanitário

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ABSTRACT

The performance of aerobic granular sludge (AGS) process was evaluated for real leachate treatment, assessing the system capacity to form granules, biomass characteristics, and other engineering and microbiological aspects. Two sequencing batch reactors were operated with a leachate concentration of 25% (R1) and 50% (R2), with an 8-h cycle. The time required for granulation was greater than 80 days in both reactors, and solids loss was significant. The sedimentation rate was also outside the typical values for AGS reactors, with a sludge volumetric index in 30 min (SVI30) greater than 70 mL/g. Although the granules produced in R2 were more compact (200 µm), they were more resistant. Proteobacteria and Rhodobacteraceae were the phyla and the most abundant family in R2. The phylum Planctomycetota and the family Pirellulaceae were the most abundant in R1. Settling time reduction, feeding phase increase, and increased dissolved oxygen (DO) levels were fundamental strategies to improve reactors' performance and stability.

Keywords: aerobic granular sludge; complex effluents; microbial community composition; sequencing batch reactor; simultaneous nitrification and denitrification.

RESUMO

O desempenho do processo de lodo granular aeróbio (LGA) foi avaliado no tratamento de lixiviado real, considerando a capacidade do sistema de formar grânulos, as características da biomassa e outros aspectos de engenharia e microbiológicos. Dois reatores em bateladas sequenciais (RBS) foram operados com concentração de lixiviado de 25% (R1) e 50% (R2), com ciclos de 8 horas. O tempo necessário para a granulação foi superior a 80 dias em ambos os reatores, e a perda de sólidos foi significativa. A velocidade de sedimentação também ultrapassou os valores típicos para reatores LGA, com o índice volumétrico de lodo em 30 minutos (SVI30) superior a 70 mL/g. Embora os grânulos produzidos no R2 fossem mais compactos (200 µm), eles apresentaram maior resistência. Proteobacteria e Rhodobacteraceae foram o filo e a família mais abundantes em R2, enquanto o filo Planctomycetota e a família Pirellulaceae foram os mais abundantes em R1. A redução do tempo de sedimentação, o aumento da fase de alimentação e o incremento dos níveis de oxigênio dissolvido (OD) foram estratégias fundamentais para a melhoria do desempenho e da estabilidade dos reatores.

Palavras-chave: lodo granular aeróbio; efluentes complexos; composição da comunidade microbiana; reator em batelada sequencial; nitrificação e desnitrificação simultâneas.

INTRODUCTION

Aerobic granular sludge (AGS) system in treating leachate has been reported as very promising. However, biomass loss, granule size, great time demand for granulation, and low denitrification efficiency are some of the characteristics that limit the direct technology application for this type of effluent (REN *et al.*, 2017a; BUENO *et al.*, 2020; SAXENA *et al.*, 2022). As a strategy to minimize these problems, several authors have started the AGS systems by adopting synthetic effluents or real low-load wastewater, with a gradual increase so that there is no toxicity and hydraulic shock.

Different zones are created with oxygen gradients that decrease toward the aerobic biomass nucleus and are correlated with granule size (NANCHARAIAH; REDDY, 2018; ROLLEMBERG *et al.*, 2018). These zones are important for the simultaneous nitrification and denitrification (SND) process, as the dissolved oxygen (DO) and the soluble substrate available outside the granule diffuse into the aerobic zone, depending on DO, biodegradable organic matter, and ammonia concentrations. Moreover, the nitrite and nitrate produced in the aerobic zone would diffuse to the inner granule layer, being electron donors for denitrification. Thus, nitrogen removal efficiency tends to be better in larger granules.

For granulation from real leachate, two strategies were investigated: (1) physical-chemical treatment with polyaluminum chloride (PAC), in which granules with sizes between 360 and 600 µm were obtained (WEI *et al.*, 2012), and (2) a mixture with domestic sewage (10% raw leachate), in which the

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biomass was considered granular after 70 days, with an average diameter of 480 µm (REN; FERRAZ; YUAN, 2017a). As the granules obtained were not large, some processes were harmed, and NO_x accumulation occurred. Only investigations with low-load synthetic effluent, with greater influent constituents' control, could obtain larger granule dimensions, reaching up to 900 µm (BELLA; TORREGROSSA, 2014) or even 1100 µm (REN *et al.*, 2017a).

Leachate treatment in AGS systems is still incipient and needs to be thoroughly studied. Therefore, it is necessary to understand leachate constituents' interference in the granulation process without pre-treatment and dilution with domestic effluent (synthetic or real). Thus, this investigation sought to evaluate the AGS process performance for real leachate treatment, assessing the system capacity to form granules, biomass characteristics, and other engineering and microbiological aspects.

MATERIALS AND METHODS

Reactor operation

Initially, the biomass was acclimatized for 2 days with the stipulated following leachate dilution to minimize the hydraulic shock toxicity. After this period, two sequencing batch reactors were operated under the same conditions, changing only the influent leachate dilution factor, 25% in R1 and 50%, both raw and diluted with tap water.

The experiments were carried out in two identical RBSs (Figure 1) with a working volume of 7.6 L, an internal diameter of 10 cm, and a height of 100 cm at a height-to-diameter ratio of 10, with an exchange volume of 50%. Each cycle lasted 8 h and consisted of feeding (20 – 40 min), aerobic reaction (429 – 439 min), sedimentation (20 – 10 min), withdrawal (1 min), and discard (1 min). Air was injected into the lower part (air compressor Yuting SUN, China) of the reactors through fine-bubble porous diffusers, satisfying a DO concentration between 3 and 6 mg/L during the aerobic phase. The operation of the systems was automated through synchronized timers. Both reactors were operated at room temperature, and the wastewater inside the reactors was around 28 ± 2°C. During the operation, there was no manual removal of sludge, and the sludge retention time in the reactors was determined due to biomass washing, which was not measured due to constant biomass washouts.

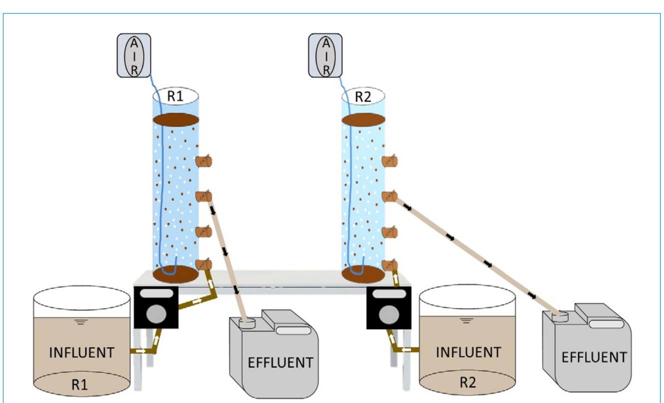

Figure 1 – Scheme of the sequencing batch reactor-aerobic granular sludge experimental system in this study in which the effluent from R1 contained 25% leachate, while, in R2, it was 50%.

The reactors operated for 95 days which was divided into three periods. In the first part of the experiment (30 days), feeding time (T_f) and settling time (T_s) were 20 min (Period I). Subsequently, T_f was increased to 40 min, and the T_s was reduced to 10 min (Period II). Finally, the DO rate was changed from 3 to 6 mg/L (Period III). According to previous research, these strategies favor the development and performance of granular biomass (ROLLEMBERG *et al.*, 2018). Furthermore, feeding mode, especially prolonged anaerobic feeding, is also one of the key factors in the granulation process (DE KREUK; VAN LOOSDRECHT, 2005).

Seed sludge and leachate collection

Aerobic seed sludge had approximately 4 g/L mixed liquor suspended solids (MLSS) with 89% volatile suspended solids (VSS)/total suspended solids (TSS) and sludge volumetric index in 30 min (SVI_{30}) of 189.5 mL/g. Feeding was carried out from the reactor bottom. The leachate was collected from the Municipal Sanitary Landfill (ASMOC, Ceará, Brazil). The composition was previously described by Silva, Rollemberg, and Santos (2022).

Analysis of biomass development and systems' performance

The collection of raw and treated samples was performed weekly. System influent and effluent COD, pH, NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P, MLSS, mixed liquor VSS (MLVSS), and sludge volumetric index (SVI) in 10 – 30 min (SVI_{10} and SVI_{30}) were analyzed 2 – 3 times a week and determined according to APHA (2012). DO was measured by a YSI 5000 probe (YSI Incorporated, USA). Total inorganic nitrogen (TIN) was considered the sum of NH_4^+-N, NO_2^--N, and NO_3^--N. The method of determining the extracellular polymeric substances (EPS) content was previously described (SILVA; ROLLEMBERG; SANTOS, 2022). Diameters of 0.2, 0.6, and 1.0 mm sieves were used to determine the granulometric distribution of the granules produced. From microscopic images, it was possible to measure the average granule diameter. The structure of the mature granules was analyzed as established elsewhere (SILVA; ROLLEMBERG; SANTOS, 2022). The reactor reaches the aerobic granulation stage only when more than 80% of the biomass has a diameter greater than 0.2 mm.

Microbial community analysis

Analyses to determine the composition of the microbiota were performed in the inoculum and at the end of each period in both reactors. All samples were collected during the aerobic phase (0.5 g fresh), in order to guarantee that all the biomass was homogenized in each reactor. Initially, DNA extraction was performed. For this, the V4 region of the 16S rRNA gene was amplified by polymerase chain reaction (PCR) with primers (515 F-Y and 806R) (PARADA *et al.*, 2015; CAPORASO *et al.*, 2011). Each PCR reaction was performed in a volume of 30 µL where 2 µL of genomic DNA (5 ng/µL), 0.75 µL of each primer (10 µM), 6.0 µL Go taq G2 HotStart Promega (5X), 3.6 µL of MgCl2 Promega (25 mM), 0.6 µL of Dntps Promega (10 mM), 0.2 µL of Promega Taq polymerase (5 U/µL), and 16.1 µL of nuclease-free ultrapure water (Promega, Madison, WI, USA) were added.

The reactions were incubated in the Eppendorf Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany) through DNA denaturation using the following temperatures and times: 95°C, 3 min; 98°C, 30 s (35 cycles); 55°C, 30 s; 72°C, 45 s; and 72°C, 5 min. Amplicons were checked on a 2% (w/v) agarose gel and purified with the Ampure XP beads (Beckman Coulter, Inc., Brea, CA, USA). After purification, the amplicons were subjected to a new PCR for insertion of the Illumina sequencing adapters using 25.0 μL of 2X KAPA HiFi Ready Start Mix (Roche), 5 μL of each Nextera XT index (Illumina, San Diego, CA, USA), 5 μL of library, and 10 μL of water. After indexing, libraries were purified using the Ampure XP beads.

The concentration of each library was determined using a Qubit® 2.0 fluorimeter (Invitrogen, Carlsbad, CA, USA) and then normalized according to the protocol for preparing the sequencing library (Illumina, San Diego, CA, USA). Equimolar concentrations of each library were pooled in a single tube forming a pool that was diluted and denatured. Sequencing was performed using the Miseq V2-300 cycles reagent kit, according to the manufacturer's instructions (ILLUMINA, 2013).

Sequencing reads were trimmed, assembled, and denoised using the DADA2 package v1.20.0 (CALLAHAN *et al.*, 2016). The resulting amplicon sequencing variants (ASVs) were taxonomically classified using the IDTAXA classifier (MURALI; BHARGAVA; WRIGHT, 2016), from the DECIPHER package v2.20.0, based on release 138 of the SILVA rRNA database (QUAST *et al.*, 2012).

The absolute abundances of the target enzymatic functions were predicted from taxonomic data with PICRUST2 (DOUGLAS *et al.*, 2020). It is important to mention that PICRUST2 has limited credibility because it extracts functional information from metataxonomic data. For this reason, PICRUST2 results were considered only as an indication of the overall metabolic activity found within the samples and not as a detailed description. KEGG Orthology codes for the target functions were obtained by keyword-searching with the KEEGREST engine v1.32.0 (TENENBAUM, 2021), resulting in the following codes: K10944, K10945, K10946, K10535, K05601, and K15864 for ammonia-oxidizing bacteria (AOB); K00370 and K00371 for nitrite-oxidizing bacteria (NOB); K20932, K20933, K20934, and K20935 for anaerobic ammonium oxidation (ANAMMOX); K20812, K00975, K00688, and K02438 for glycogen-accumulating organisms (GAOs); K00937 and K22468 for phosphorus-accumulating organisms (PAOs); and K00372, K00360, K00367, K00370, K00371, K00373, K00374, and K10534 for denitrifying bacteria (DNB). The Bioconductor (HUBER *et al.*, 2015) packages DADA2, DECIPHER, and KEGGREST were run in R, version 4.1.2.

The abundance-based coverage estimator (ACE), Chao1 index, Shannon index, and Simpson index were calculated using the Phyloseq package. After sequencing, the results obtained were stored in the National Center for Biotechnology Information (NCBI) BioProject database, ID PRJNA836880.

Statistical methods

Statistical analyses were performed with the Origin 2018 computer software applying the Mann-Whitney rank-sum test to compare the performance of the reactors at a 95% confidence level, where the data groups were statistically different when $p < 0.05$.

RESULTS AND DISCUSSION

Granular biomass development

The biomass concentration dropped significantly in both reactors after contact with leachate (Figure 2). During Period I, solids loss between reactors was statistically similar, suggesting that leachate toxicity reduces biomass retention.

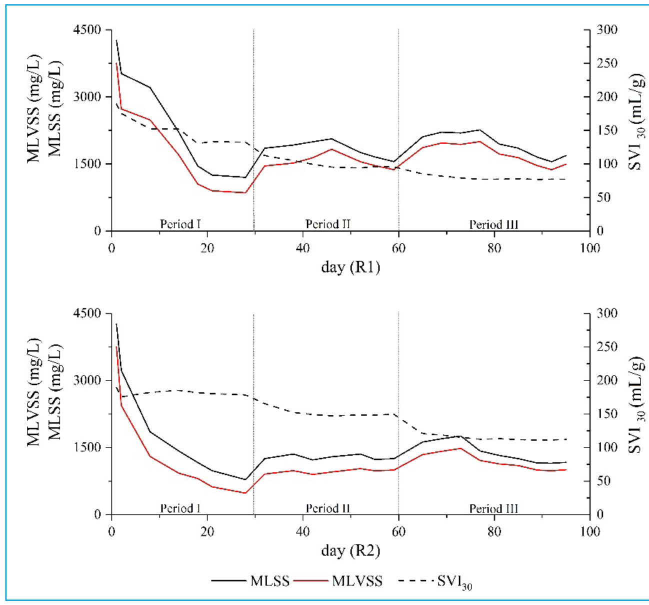

Figure 2 – Stability in terms of solids and SVI_{30} of aerobic granular sludge systems with 25% (R1) and 50% (R2) leachate with feeding and settling time of 20 min (Period I), feeding time of 40 min, and settling time of 10 min (Period II) and with increased dissolved oxygen concentration (Period III).

Throughout the strategies adopted, the two reactors showed significant differences. T_s reduction and the feeding time increase (Period II) and DO increase rate (Period III) favored biomass retention, reducing the effluent solids concentration. In the system with the highest leachate load (R2), the biomass retention was significantly lower in both Period II ($p < 0.001$) and Period III ($p = 0.001$).

It was also possible to verify that the final proportion of VSS in relation to TSS was 88% in R1 and 84% in R2. It was to be expected that R2 would have the lowest solids concentration as several studies indicate that higher leachate loads generate more filamentous biomass, which is easily washed and consequently worsens sedimentation. Despite this, solids loss in R2 with 50% leachate was still lower than the values reported by Bueno *et al.* (2020) when using much lower concentrations of leachate (5%) and by Ren *et al.* (2017a) by increasing the influent ammonia concentration.

Still, a pattern behavior identified was that, after the optimization stipulated in each period, there was an improvement in biomass development that worsened again until the beginning of the next optimization. Thus, this improvement and worsening peaks suggest that the raw leachate's toxicity does not support biomass growth, which always returns to the decay stage.

As for settling, the reactors had similar behavior, but the R2 presented higher SVI ($p < 0.001$). As with solids, the change in settling and feeding times and DO concentration favored settling. However, these optimizations were not enough to achieve the desirable SVI levels for AGS reactors (< 50 mL/g) as, at the experiment completion, R1 had an SVI_{30} of 79.1 mL/g and R2 of 114.1 mL/g. It was already expected that R2 would present this sedimentation outside the acceptable range, but R1 should have better sedimentation rates since it had less toxicity and less tendency to develop filaments.

The settling results with 50% real leachate alone (R2, tap water dilution) were similar to or better than those with lower concentrations of leachate diluted in synthetic wastewater (BUENO *et al.*, 2020; SAXENA *et al.*, 2022). For instance, after the granulation phase, SVI_{30} of 210 and 155 mL/g were achieved for 5% and

10% leachate dilutions, respectively (BUENO *et al.*, 2020). Influent suspended solids (SS) impair granulation and produce poor-quality sludge (PRONK *et al.*, 2015). Settling time reduction contributed to this poor-quality sludge being washed. However, both systems showed considerably high SVI values for aerobic granulation reactors, implying that leachate made the granulation process difficult and impaired granule settling.

Granules' characteristics

Among the main problems reported in the literature when treating landfill leachate in AGS systems are the recurrent disintegration and the long time required for granulation (REN; FERRAZ; YUAN, 2017b; BUENO *et al.*, 2020). This investigation was no different (Figure 3). Although the SVI in 10 and 30 min (SVI_{10}/SVI_{30}) ratio remained between 1.0 and 1.5, which is referred to as a granulation stage (KOCATURK; ERGUDER, 2015; HAMZA *et al.*, 2018), it took 81 and 89 days for the R1 and R2 systems, respectively, to meet the requirements to be considered granular.

Concerning average granule diameters, although it is possible to find R1 values larger than R2, there were no statistically significant differences between them in Periods I and II (p = 0.67) and III (p = 0.67). In Periods I and II, both reactors presented granules with average diameters of 100 ± 100 µm, while, in Period III, the average diameters were 300 ± 100 µm (R1) and 200 ± 100 µm (R2).

As the reactors' granules were not large and the smallest sieve opening in the granulometry was 200 µm, it was not possible to verify the occurrence of disintegration episodes. In addition, it is important to emphasize once again that verifying complete biomass washouts in both systems was common, as it was necessary to return the biomass to the system manually. These washes were more frequent during the first two periods. However, they did not stop happening during the last period.

Microscopic analyses with a precision of 3000× were used to evaluate the granules' surface (Figure 4). Their structure was very similar, presenting an irregular surface with the development of an ascending coccus. Despite this, it was possible to verify a strong microbial aggregation without the presence of exposed communities. In R2, the reactor with the highest leachate concentration, the presence of small internal structures that suggest fragmentation is evident. In this reactor, channel presence in the surface structure was also observed, contributing to the substrate permeability in the innermost layers of the granules.

Thus, this microbial aggregation is directly influenced by EPS production, which plays a key role in aggregation (ROLLEMBERG *et al.*, 2018). In this regard, to complete the granular biomass profile, the protein (PN) and polysaccharide (PS) fractions were measured (Figure 5). Protein production was

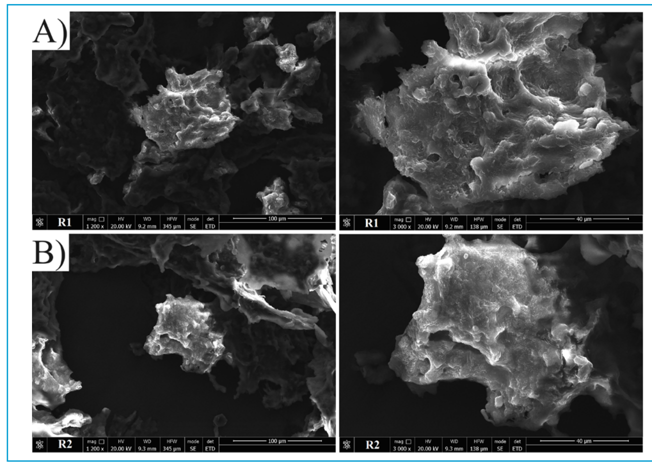

Figure 4 – Granule scanning electron micrograph of the reactors R1 (A) and R2 (B) at the end of Period III accurate to 1200× (left side) and 3000× (right side).

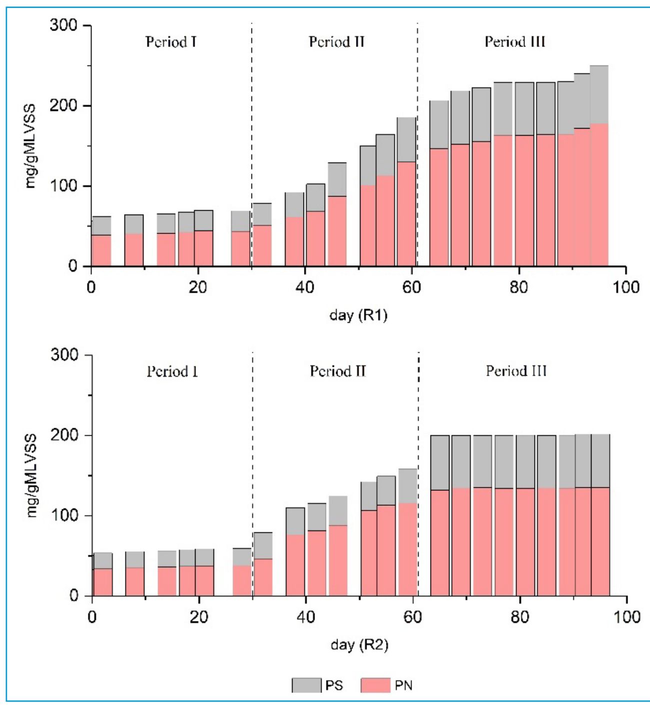

Figure 5 – Average quantification of extracellular polymeric substances.

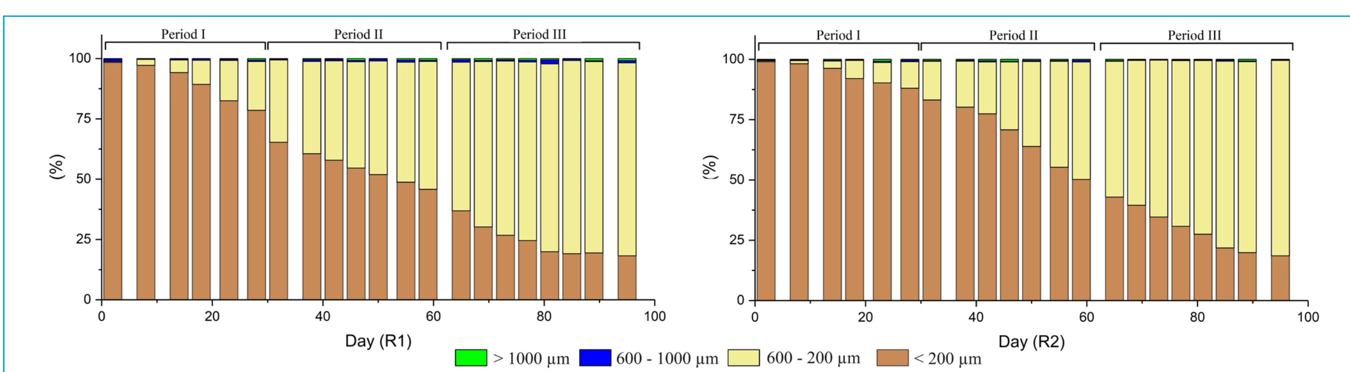

Figure 3 – Granule size distribution (% mass) in aerobic granular sludge systems.

greater than that of polysaccharides in both reactors and remained in the same proportions. This condition is AGS systems, as PN is responsible for granule aggregation, forming organic molecular bonds that are responsible for catalysis and degradation processes, while PS forms carbonaceous bonds that favor sedimentation, generating granule mechanical stabilization (SHI *et al.*, 2017; ROLLEMBERG *et al.*, 2018).

During Periods I and II, there were no significant differences between the reactors regarding total EPS production ($p = 0.76$ and $p = 0.94$, respectively), with the mean values in Period I being 64 ± 5 mg/gMLVSS (R1) and 56 ± 9 mg/gMLVSS (R2) and in Period II being 129 ± 39 mg/gMLVSS (R1) and 129 ± 32 mg/g MLVSS (R2). In Period III, when the DO concentration increased, R1 continued to produce EPS, and R2 remained stable. In R1, the mean production was 238 ± 25 mg/gMLVSS, which was significantly higher than the mean production of 198 ± 3 mg/gMLVSS in R2 ($p = 0.032$).

DO concentration and control are essential so that microbial communities can secrete EPS at satisfactory levels, favoring cell adhesion and generating the necessary stability for granule activity (BELLA; TORREGROSSA, 2014; ADAV; LEE, 2010). As the EPS production was similar to the usual high production when simpler wastewaters were used, microscopic images revealed this strong aggregation on the granules' surface. Furthermore, despite their reduced diameters, the EPS production was expected to be high due to leachate toxicity as, in environments with toxic substances, heavy metals, and severe conditions, microorganisms produce more EPS as a defense mechanism. Based on this principle, as in the last operation stages, R2 produced less EPS, it can be inferred that the granules produced were more resistant to leachate toxicity, not feeling the need to produce more EPS as a defense strategy.

Removal of carbon, nitrogen, and phosphorus in reactors

During Periods I and III, organic matter removal was significantly higher in reactor R1 (Table 1), which had the lowest leachate concentration ($p < 0.001$ and $p = 0.001$, respectively). During Period II, there were no statistical differences between the two reactors. In this study, chemical oxygen demand (COD) removal was very low, similar to experiments with 90% leachate (REN; FERRAZ; YUAN, 2017a; 2017b). That is, the readily available organic matter presented a recalcitrant character, hindering the assimilation conversions necessary for the biomass. A COD removal decrease by increasing the leachate influent organic matter load is also reported (BELLA; TORREGROSSA, 2014).

As part of the organic matter in AGS systems is used for biomass growth and another part to sustain the denitrification process, the granules did not grow, and the nitrogen removal efficiency was impaired because the organic constituents are difficult to degrade. Thus, all nitrogen conversion processes were unsatisfactory, generating nitrite accumulation at concentrations above 60 mg/L in the last period.

As R2 had the highest leachate concentration, it would consequently have a higher non-biodegradable organic matter concentration and lower COD removal. On the contrary, this remaining COD could sustain the denitrification process. Thus, R2 showed the highest removal of total nitrogen. Nitrification in the two reactors only showed a significant difference in Period III ($p = 0.037$), in which the R2 efficiency was higher than R1. However, nitrite production was statistically similar between the reactors, except in Period I. In R2, verifying a slight nitrate accumulation was also possible, greater than in R1 in all periods ($p < 0.001$). NO_x accumulation has been commonly reported as one of the main limitations of nitrogen removal upon aerobic granulation technology (SAXENA *et al.*, 2022; SILVA *et al.*, 2022). This accumulation may result from the high load of free ammonia and free nitrous acid available in the leachate, which can favor the nitritation route and negatively interfere with the microorganisms responsible for nitration and denitrification via nitrite or nitrate (CHUNG *et al.*, 2015). In any case, the nitrogen removal results of this study were similar to previous research (BELLA; TORREGROSSA, 2014; REN; FERRAZ; YUAN, 2017a), in which TN removal was not greater than 30%, even at low leachate concentrations.

Phosphorus removal only showed significant differences in Period III ($p = 0.02$), in which the DO concentration was higher. The lower leachate concentration (R1) was fundamental for the higher phosphorus removal, despite being much lower than those found elsewhere (REN *et al.*, 2017b; 2017b; REN; FERRAZ; YUAN, 2017b; BUENO *et al.*, 2020), even with high leachate concentrations.

As the granules found in the above-mentioned literature were larger, and the granulation process was not exclusively with leachate, it was expected that

Table 1 – Performance of aerobic granular sludge systems in terms of chemical oxygen demand, nitrogen, and phosphorus.

Characteristics	Period I		Period II		Period III	
	R1	R2	R1	R2	R1	R2
COD_{inf} (mg/L)	935 ± 254	1892 ± 305	969 ± 196	1938 ± 375	996 ± 236	1905 ± 433
COD_{eff} (mg/L)	748 ± 52	1625 ± 53	755 ± 59	1542 ± 51	629 ± 36	1415 ± 42
COD removal (%)	19 ± 3	13 ± 2	21 ± 3	18 ± 5	36 ± 3	24 ± 4
NH_4^+-N_{inf} (mg/L)	166 ± 38	374 ± 49	183 ± 42	367 ± 35	165 ± 31	384 ± 49
NH_4^+-N_{eff} (mg/L)	125 ± 8	215 ± 8	109 ± 7	200 ± 4	81 ± 7	165 ± 5
NO_2^--N_{eff} (mg/L)	36 ± 15	75 ± 19	57 ± 13	65 ± 20	62 ± 9	72 ± 12
NO_3^--N_{eff} (mg/L)	6 ± 3	25 ± 8	12 ± 3	39 ± 7	7 ± 2	30 ± 9
NH_4^+-N removal (%)	35 ± 6	41 ± 9	37 ± 5	42 ± 4	48 ± 5	56 ± 6
TN removal (%)	9 ± 6	27 ± 4	20 ± 8	30 ± 6	21 ± 10	33 ± 9
PO_4^{3-}-P_{inf} (mg/L)	6 ± 2	12 ± 3	6 ± 1	13 ± 4	5 ± 3	10 ± 4
PO_4^{3-}-P_{eff} (mg/L)	5 ± 1	11 ± 2	5 ± 2	11 ± 1	4 ± 2	9 ± 2
P removal (%)	2 ± 1	3 ± 2	8 ± 3	6 ± 2	11 ± 1	6 ± 3

organic matter, nitrogen, and phosphorus removals would be lower. Despite this, nitrogen removal was similar to the previous rates reported, even at influent leachate loads greater than those used in this work. In addition, the low removal of organic matter and phosphorus in R2 is a consequence of the toxicity conferred by the higher leachate influent load. In R1, it was expected that the removal of the contaminants would be greater as the influent leachate load was lower. This suggests that it is necessary to create a granulation optimization protocol that favors granule growth and reduces leachate toxicity so that, consequently, there is an improvement in the removal of other organic and phosphorous-based constituents.

Molecular biology

Taxonomic composition

From gene taxonomic analyses, it was possible to determine and analyze microbial communities at the phylum and class levels (Figure 6). The most abundant phyla in both reactors and in the inoculum were Planctomycetota and Proteobacteria. This result was consistent with previous experiments with leachate treatment in AGS systems, in which more than 80% of microbiota belonged to these two phyla (SAXENA *et al.*, 2022), being reported as the main microorganisms involved in COD, NH_4^+-N, and PO_4^{3-}-P removals (WANG *et al.*, 2020).

In the inoculum, these two phyla were in the same proportions. However, the leachate load significantly influenced the microbial ecology over time. In R1, the Proteobacteria abundance was much higher than that of Planctomycetota, while, in R2, the opposite occurred. Thus, it is possible to infer that Proteobacteria were more sensitive to leachate toxicity as, when increasing the influent leachate load (R2), this phylum was reduced, consequently favoring Planctomycetota and Verrucomicrobiota. The latter is reported as essential in organic components removal, so its development was necessary to increase organic removal in the systems (LI *et al.*, 2020). Furthermore, the presence of unclassified phyla was greater, suggesting that the leachate eliminated most of these phyla, which were probably more sensitive and did not resist the imposed selection pressure. The same happened with Firmicutes and Bacteroidota. The presence of Bacteroidota favors SND in aerobic granules (LI *et al.*, 2020), and its small abundance is consistent with the low TN removals in both reactors.

At this level, it is possible to verify that microorganisms of the phylum Proteobacteria (Alphaproteobacteria and Gammaproteobacteria) were present, and Gammaproteobacteria were more sensitive to the leachate. This type of effluent also favored the development of Planctomycetes and Verrucomicrobiae, which were more abundant at higher leachate concentrations. Furthermore, the presence of Acidimicrobiia indicates a slightly acidic environment and favors a stronger structuring, improving granules' stability (LI *et al.*, 2020).

Key functional groups

In the last stage of the microbiological analyses, the families found in the systems were categorized according to their functional type (Figure 7) and separated into AOB, NOB, DNB, GAOs, and PAOs.

The lowest abundances found were AOB and NOB. As the granules formed were small, these organisms that develop in the outermost layer were inhibited and possibly washed away. Slowly growing nitrifying bacteria improve biomass stability (ROLLEMBERG *et al.*, 2018), reflecting this low abundance in instabilities throughout the operation. The increase in leachate concentration also influenced the microbial distribution, inhibiting NOB-type microorganisms, in which no families belonging to this group were found in R2.

The two reactors showed similar abundances of DNB, with R1 represented mainly by Rhodobacteraceae and R2 by Pirellulaceae. Thus, the increase in leachate concentration favored Pirellulaceae and inhibited Rhodobacteraceae. This was observed in DNBs, as well as in PAOs and GAOs. Comamonadaceae was the most dominant DNB family in the inoculum and is essential in the nitrogen removal cycle (FAN *et al.*, 2018). However, they were inhibited in both reactors in the presence of leachate for requiring specific conditions. In general, great abundances and diversities of AOB, NOB, and DNB microorganisms were not found, which explains the low TN removals and NO_x accumulations.

Under conditions where the oxygen concentration is not zero, phosphate-accumulating microorganisms use polyhydroxyalkanoates (PHAs) to accumulate phosphorus. As organic matter is the energy and carbon source for many organisms in the microbial community, including PAOs, competition is inevitable. At the end of the experiment, it was possible to verify that the competition between PAOs and DNBs was not at considerable rates as the microorganisms of

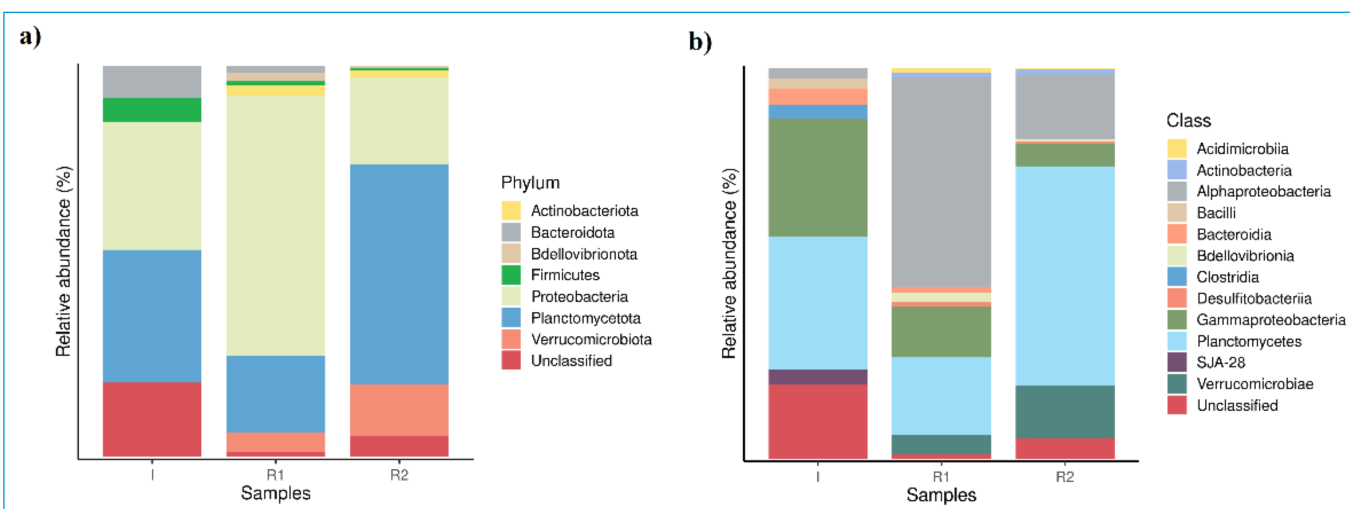

Figure 6 – Bacterial community structure at the phylum (a) and class (b) level of the two reactors and the inoculum (I).

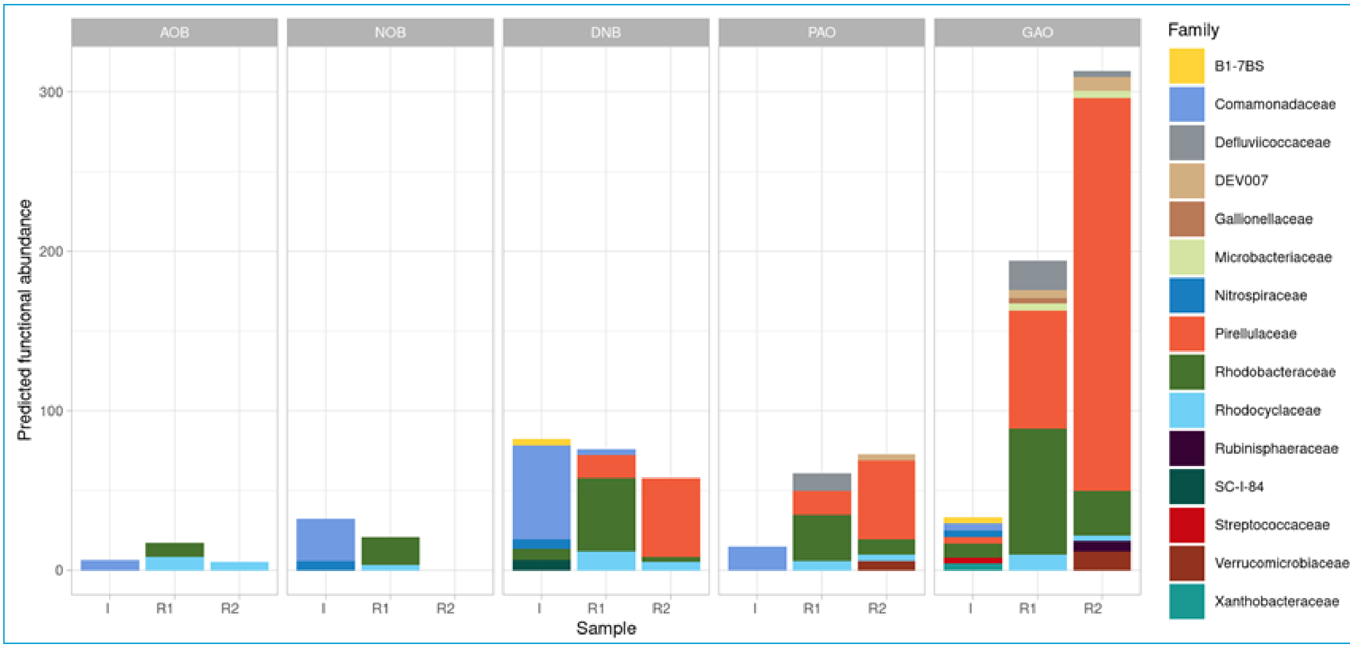

Figure 7 – Functional taxonomic classification distribution at the experiment's end.

these two functional groups were practically at the same concentrations in both reactors. On the contrary, GAOs predominated in relation to PAOs. This fact may indicate that their competition was more intense and that the available organic matter benefited GAOs.

Microorganisms from the Pirellulaceae and Rhodobacteraceae families were the main PAOs and GAOs, respectively, found in the two reactors. In R1, they were practically in the same proportion. In R2, Pirellulaceae was much more abundant. These microorganisms prevent filamentous bacteria from developing in periods with excess oxygen by immediately consuming part of the available substrate (ROLLEMBERG *et al.*, 2018). Therefore, the greater abundance of these families was not a cause for concern in this study, as they tended to be stable.

In general, the leachate load increase in R2 inhibited the development of important microorganisms for complete nitrogen removal, such as AOB and NOB, which was reflected in the low TN removal and incomplete denitrification values. The leachate load also influenced the profile of DNB, PAOs, and GAOs as Pirellulaceae was the most abundant family with higher leachate concentrations, suggesting that these microorganisms are less sensitive and more tolerant to the toxicity imposed by the leachate. In the reactor with the lowest leachate load, the Rhodobacteraceae family was the most abundant, both DNB and PAO and GAO types.

Strategic discussions

Considering that this investigation joins the small group of works that used the AGS technology to treat real leachate, some conceptions could be established and paradigms broken.

Apparently, an anaerobic phase becomes unnecessary concerning cycle distribution, unlike AGS systems for the treatment of simpler effluents, such as municipal wastewater, which is essential. For more complex effluents such as leachate, fermentation during this phase is insignificant and can select microorganisms of low energy value, such as PAOs. The cultivation of PAOs has been a widely used strategy in most granulation works, as they remove P and favor the formation and stability of aerobic granules by accumulating PHAs. However, as leachate has a low phosphorus concentration, other constituents' removals, such as nitrogen, COD, and toxic compounds, among others, are more challenging.

GAOs have a higher growth rate than PAOs, being dominant at temperatures greater than 20°C, whose increased metabolism favors the stability and development of granules (ROLLEMBERG *et al.*, 2018). That is, the temperature is a limiting factor for the growth of PAOs. In this sense, it is more advantageous to cultivate GAOs, which are similar to PAOs and favor mature granules' formation and stability but do not accumulate phosphorus. Like PAOs, GAOs also store volatile fatty acids in the form of PHAs and use glycogen as an energy source. Furthermore, as both groups present denitrifying microorganisms (DPAOs and DGAOs), nitrogen removal would also be favored by the greater kinetics of GAOs.

The available organic matter in the leachate is insufficient to sustain complete nitrogen removal and to cultivate dense granules, requiring COD supplementation. This approach, in addition to favoring denitrification and reducing the accumulation of nitrite and nitrate in the effluent, also contributes to biomass growth. However, depending on the landfill age, the concentration required may be different, being higher in older leachates, which have a lower COD load. Therefore, organic matter supplementation is also the key to reducing the time required for granulation because a fraction will be used for granule development.

Special attention must be given to the granule size, as one of the problems frequently reported in investigations dealing with leachate in AGS systems is granule disintegration. This happens because the developed granules use only the organic matter available in the leachate, which is divided with the other processes, producing weaker granules. Furthermore, leachate toxicity plays a major role in this disintegration, which is minimized when dilutions and organic matter supplementation are used. The source of organic matter selected is expected to favor the development of more resistant granules, reducing the disintegration episodes, and also to favor specific microbial groups, such as GAOs and DGAOs.

It is important to note that the most common on a full scale is the simultaneous treatment of leachate and domestic effluents (co-treatment). As mentioned, this dilution also reduces leachate toxicity. In addition, this co-treatment is advantageous, as the daily sewage flow is much greater than the leachate flow. Thus, large leachate dilutions are acceptable (up to 20% leachate), eliminating the need to deal with the impacts of high leachate concentrations.

However, in sewage treatment plants, there will be an increase in the concentration of phosphorus, slowly biodegradable organic compounds, ammoniacal nitrogen, heavy metals, and other substances that inhibit biological processes, making it more challenging to comply with the release limits. Thus, this dilution must be carried out most appropriately toward the ideal granule formation protocols, which must consider the mixture percentages, the cycle times, and the frequent alternation of aerobic and anoxic phases in the cycle.

Compared with the most used biological treatment system in the world, the conventional activated sludge (CAS) system, granule disintegration can be a determining factor. In general, the resistance of the aerobic granules may be lower at high leachate concentrations than the activated sludge flocs resistance, but the recovery time is also shorter. Ren *et al.* (2017a) found that, when free ammonia (FA) inhibited biomass, AGS systems could recover faster than CAS systems. Furthermore, FA concentration that inhibits microorganisms in AGS systems is twice as high as in CAS systems (REN; FERRAZ; YUAN, 2017a). Thus, when performing adequate dilutions in AGS systems, resistance to toxicity will be amplified, and recovery will occur in shorter times.

Therefore, given the above, the AGS technology for leachate treatment, whether in the sanitary landfill or co-treatment with sewage, is feasible as long as all the aspects summarized here are considered.

CONCLUSION

The granulation process from real leachate required a high temporal demand and produced very small and unstable granules. By presenting a higher influent leachate load, the biomass in R2 was more susceptible to toxicity, which could have impaired granule development and reactor performance. On the contrary, R1 contradicted the initial hypotheses, presenting unsatisfactory results, being inferior and/or similar to R2 and those reported in the literature.

The two reactors showed settleability out of ideal and low solids retention. However, in R2, this retention was similar to works with lower leachate loads. This loss of solids in R2 was not enough to impair nitrogen removal, which, linked to the available organic load during the cycle due to the low COD removal, favored SND, which was higher than in R1 (with lower leachate load). Furthermore, EPS and microbiology results suggest that granules produced in R2 were more resistant to leachate toxicity.

Thus, the influent leachate concentration was also fundamental to differentiate the removal of C, N, and P and to shape the microbial profile. In both reactors, there were accumulations of NO_x and reflections of the inhibition of AOB, NOB, and DNB microorganisms by the increase in the leachate load.

Although the results were not desirable and the influent leachate concentration was high, they were similar to those obtained in studies with lower leachate loads diluted in domestic sewage and with previous granulation in the absence of raw leachate. In addition, settling time reduction and DO concentration increase were fundamental to increasing solids retention, favoring microbial aggregation, and improving reactor performance. Thus, new optimization strategies must be investigated for applying real leachate, focusing on protocols for granulation and cycle phases with oxygenation gradients to favor the development and stratification of oxygen inside the granules. A previous physical-chemical pre-treatment to reduce leachate toxicity and a COD supplementation by using a readily available substrate are also recommended to be investigated.

AUTHORS' CONTRIBUTIONS

Silva, V.: Writing – Original Draft ; Writing – Review & Editing. Rollemberg, S.: Writing – Original Draft ; Writing – Review & Editing. Santos, A.: Writing – Review & Editing; Funding acquisition.

REFERENCES

ADAV, S.S.; LEE, D.J. Potential cause of aerobic granular sludge breakdown at high organic loading rates. *Applied Microbiology and Biotechnology*, v. 85, n. 5, p. 1601-1610, 2010. https://doi.org/10.1007/s00253-009-2317-9

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). *Standard Methods for the Examination of Water and Wastewater*. 22. ed. Washington, DC: American Public Health Association, American Water Works Association, Water Environment Federation, 2012.

BELLA, G.; TORREGROSSA, M. Aerobic granular sludge for leachate treatment. *Chemical Engineering Transactions*, v. 38, p. 493-498, 2014. https://doi.org/10.3303/CET1438083

BUENO, R.F.; FARIA, J.K.; ULIANA, D.P.; LIDUINO, V.S. Simultaneous removal of organic matter and nitrogen compounds from landfill leachate by aerobic granular sludge. *Environmental Technology*, v. 42, p. 3756-3770, 2020. https://doi.org/10.1080/09593330.2020.1740798

CALLAHAN, B. MCMURDIE, P.J.; ROSEN, M.J.; HAN, A.W.; JOHNSON, A.J.A.; HOLMES, S.P. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, v. 13, n. 7, p. 581-583, 2016. https://doi.org/10.1038/nmeth.3869

CAPORASO, J.G.; LAUBER, C.L.; WALTERS, W.A.; BERG-LYONS, D.; LOZUPONE, C.A.; TURNBAUGH, P.J., *et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, v. 108, p. 4516-4522, 2011. https://doi.org/10.1073/pnas.1000080107

CHUNG, J.; KIM, S.; BAEK, S.; LEE, N-H.; PARK, S.; LEE, J., *et al.* Acceleration of aged-landfill stabilization by combining partial nitrification and leachate recirculation: A field-scale study. *Journal of Hazardous Materials*, v. 285, p. 436-444, 2015. https://doi.org/10.1016/j.jhazmat.2014.12.013

DE KREUK, M.K.; VAN LOOSDRECHT, M.C.M. Selection of slow growing organisms as a means for improving aerobic granular sludge stability. *Water Science and Technology*, v. 49, n. 11, p. 9-17, 2004. PMID: 15303717

DOUGLAS, G.M.; MAFFEI, V.J.; ZANEVELD, J R.; YURGEL, S.N.; BROWN, J.R.; TAYLOR, C.M., et al. PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology*, v. 38 n. 6, p. 685-688, 2020. https://doi.org/10.1038/s41587-020-0548-6

FAN, X.Y.; GAO, J-F.; PAN, K-L.; LI, D-C.; ZHANG, L-F.; WANG, S-J. Shifts in bacterial community composition and abundance of nitrifiers during aerobic granulation in two nitrifying sequencing batch reactors. *Bioresource Technology*, v. 251, p. 99-107, 2018. https://doi.org/ 10.1016/j.biortech.2017.12.038

HAMZA, R.A.; SHENG, Z.; IORHEMEN, O.T.; ZAGHLOUL, M.S.; TAY, J.H. Impact of food-to-microorganisms ratio on the stability of AGS treating high-strength organic wastewater. *Water Research*, v. 147, p. 287-298, 2018. https://doi.org/10.1016/j.watres.2018.09.061

HUBER W.; CAREY, V.J.; GENTLEMAN, R.; ANDERS, S.; CARLSON, M.; CARVALHO, B.S., et al. Orchestrating high-throughput genomic analysis with Bioconductor. *Nature Methods*, v. 12, n. 2, p. 115-121, 2015. https://doi.org/10.1038/nmeth.3252

ILLUMINA. 16S Metagenomic Sequencing Library Preparation. Illumina Guides. 2013. Available at: https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf. Accessed on 06 Mar, 2023.

KOCATURK, I.; ERGUDER, T.H. Investigation of the use of aerobic granules for the treatment of sugar beet processing wastewater. *Environmental Technology*, v. 36, n. 20, p. 2577-2587, 2015. https://doi.org/10.1080/09593330.2015.1039070

LI, D.; WANG, Y.; ZENG, H.; YUAN, Y.; ZHANG, J. Startup and stable operation of advanced continuous flow reactor and the changes of microbial communities in aerobic granular sludge. Chemosphere, v. 243, p. 125434, 2020. https://doi.org/10.1016/j.chemosphere.2019.125434

MURALI, A.; BHARGAVA, A.; WRIGHT, E.S. IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. *Microbiome,* v. 6, n. 140, p. 1-14, 2018. https://doi.org/10.1186/s40168-018-0521-5

NANCHARAIAH, Y.V.; REDDY, G.K.K. Aerobic granular sludge technology: Mechanisms of granulation and biotechnological applications. *Bioresource Technology*, v. 247, p. 1128-1143, 2018. https://doi.org/10.1016/j.biortech.2017.09.131

PARADA, A.E.; NEEDHAM, D.M.; FUHRMAN, J.A. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, v. 18, p. 1403-1414, 2015. https://doi.org/10.1111/1462-2920.13023

PRONK, M.; ABBAS, B.; AL-ZUHAIRY, S.H.K.; KRAAN, R.; KLEEREBEZEM, R.; VAN LOOSDRECHT, M.C.M. Effect and behaviour of different substrates in relation to the formation of AGS. *Applied Microbiology and Biotechnology*, v. 99, n. 12, p. 5257-5268, 2015. https://doi.org/10.1007/s00253-014-6358-3

QUAST, C.; PRUESSE, E.; YILMAZ, P.; GERKEN, J.; SCHWEER, T.; YARZA, P., *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, v. 41, n. 1, p. 590-596, 2012. https://doi.org/10.1093/nar/gks1219

REN, Y.; FERRAZ, F.; KANG, A.J.; YUAN, Q. Comparing young landfill leachate treatment efficiency and process stability using aerobic granular sludge and suspended growth activated sludge. *Journal of Water Process Engineering*, v. 17, p. 161-167, 2017a. https://doi.org/10.1016/j.jwpe.2017.04.006

REN, Y.; FERRAZ, F.; KANG, A.J.; YUAN, Q. Treatment of old landfill leachate with high ammonium content using aerobic granular sludge. *Journal of Biological Engineering*, v. 11, n. 1, p. 42, 2017b. 10.1186/s13036-017-0085-0

REN, Y.; FERRAZ, F. M.; YUAN, Q. Landfill leachate treatment using aerobic granular sludge. *Journal of Environmental Engineering*, v. 143, n. 9, p. 40170, 2017a. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001236

REN, Y.; FERRAZ, F.M.; YUAN, Q. Biological leachate treatment using anaerobic/aerobic process: suspended growth-activated sludge versus aerobic granular sludge. International *Journal of Environmental Science and Technology*, v. 15, n. 11, p. 2295-2302, 2017b. https://doi.org/10.1007/s13762-017-1633-3

ROLLEMBERG, S.L.S.; BARROS, A.R.M.; FIRMINO, P.I.M.; SANTOS, A.B. Aerobic granular sludge: Cultivation parameters and removal mechanisms. *Bioresource Technology*, v. 270, p. 678-688, 2018. https://doi.org/10.1016/j.biortech.2018.08.130

SAXENA, V.; PADHI, S.K.; PATTANAIK, L.; BHATT, R. Simultaneous removal of carbon, nitrogen, and phosphorus from landfill leachate using an aerobic granular reactor. *Environmental Technology & Innovation*, v. 28, p. 102657, 2022. https://doi.org/10.1016/j.eti.2022.102657

SHI, Y.; HUANG, J.; ZENG, G.; GU, Y.; CHEN, Y.; HU, Y., *et al*. Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: An overview. *Chemosphere*, v. 180, p. 396-411, 2017. https://doi.org/10.1016/j.chemosphere.2017.04.042

SILVA, V.E.P.S.G.; ROLLEMBERG, S.L.S.; SANTOS, S.G.S.; SILVA, T.F.C.V.; VILAR, V.J.P.; SANTOS, A.B. Landfill leachate biological treatment: perspective for the aerobic granular sludge technology. *Environmental Science and Pollution Research*, v. 29, n. 30, p. 45150-45170, 2022. https://doi.org/10.1007/s11356-022-20451-3

SILVA, V.E.P.S.G.; ROLLEMBERG, S.L.S., SANTOS, A.B. Step-feeding in aerobic/anoxic cycles enhanced the performance of aerobic granular sludge (AGS) systems treating effluents with low C:N ratios. *Process Safety and Environmental Protection*, v. 171, p. 1031-1042, 2022. https://doi.org/10.1016/j.psep.2022.10.004

TENENBAUM D. Bioconductor Package Maintainer. KEGGREST: Client-side REST access to the Kyoto Encyclopedia of Genes and Genomes (KEGG). R package version 1.32.0. 2021.

WANG, X.; CHEN, Z.; SHEN, J.; KANG, J.; ZHANG, X.; LI, J., *et al.* Effect of carbon source on pollutant removal and microbial community dynamics in treatment of swine wastewater containing antibiotics by aerobic granular sludge. *Chemosphere*, v. 260, p. 127544, 2020. https://doi.org/10.1016/j.chemosphere.2020.127544

WEI, D.; SHI, L.; YAN, T.; ZHANG, G.; WANG, Y.; DU, B. Aerobic granules formation and simultaneous nitrogen and phosphorus removal treating high strength ammonia wastewater in sequencing batch reactor. *Bioresource Technology*, v. 171, n. 1, p. 211-216, 2012. https://doi.org/10.1016/j.biortech.2014.08.001

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