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Microbial interactions and nitrogen removal performance in an intermittently rotating biological contactor treating mature landfill leachate

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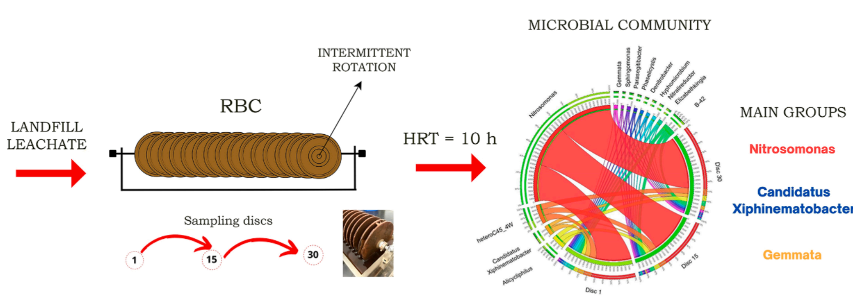
HIGHLIGHTS

- Landfill leachate promoted selective pressure on the RBC microbial community structure.
- PN/A process representatives were identified in the biofilm including denitrifiers.
- The highest average nitrogen removal efficiency was 43.3 ± 8.8 %, with a maximum of 53.1 %.
- The whole biological process occurred within the first 5 cm of reactor length.
- RBC applicability for leachate treatment requires further adapting to complex compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Developing efficient landfill leachate treatment is still necessary to reduce environmental risks. However, nitrogen removal in biological treatment systems is often poor or costly. Studying biofilms in anoxic/aerobic zones of rotating biological contactors (RBC) can elucidate how microbial interactions confer resistance to shock loads and toxic substances in leachate treatment. This study assessed the nitritation-anammox performance in an intermittent-rotating bench-scale RBC treating mature leachate (diluted). Despite the leachate toxicity, the system achieved nitritation with an efficiency of up to 34 % under DO values between 0.8 and 1.8 mg.L^{-1}. The highest average ammoniacal nitrogen removal was 45.3 % with 10 h of HRT. The 16S rRNA sequencing confirmed the presence of Nitrosonomas, Aquamicrobium, Gemmata, and Plantomyces. The coexistence of these bacteria corroborated the selective pressure exerted by leachate in the community structure. The microbial interactions found here highlight the potential application of RBC to remove nitrogen in landfill leachate treatment.

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1. Introduction

Production and consumption of goods and waste generation respond directly to population habits and economic growth. Rampant consumerism has increased the amount and variety of waste products, a global concern for the solid waste sector (Song et al., 2020).

It is estimated that approximately 95 % of the total urban solid waste collected worldwide ends up in landfills (Luo et al., 2020). Landfills produce leachate containing large amounts of refractory organic matter, metal ions, various toxic contaminants, and high ammoniacal nitrogen content. Therefore, it represents a significant environmental risk (Nath et al., 2022). Currently, several leachate treatment technologies are under research. The main goal is to comply with discharge standards while maintaining low cost, simplicity of operation, reliability, and environmental gains (Luo et al., 2020).

The landfill's age is a crucial factor determining the biodegradability of this complex wastewater. For example, the leachate BOD/COD (biochemical oxygen demand/chemical oxygen demand) ratio, average COD levels, pH, and ammoniacal nitrogen concentration change over landfill operation. Old leachates have more recalcitrant organic matter and higher levels of ammoniacal nitrogen (Ilmasari et al., 2022). Hence, parameters conventionally considered in the operation of biological treatment systems may not universally apply to treat mature leachate. In this context, efficiently removing ammoniacal nitrogen at a low cost while meeting the standards established by environmental legislation is still challenging (Costa et al., 2019).

Recent research has focused on treating wastewater with a high concentration of ammoniacal nitrogen and a low carbon/nitrogen (C/N) ratio utilizing the combined process of partial nitritation/anammox (PN/A). The PN/A is a promising process compared to conventional nitrification/denitrification. Savings in aeration costs of up to 60 % and up to 90 % reduction in sludge production are achievable in PN/A. Moreover, it is an entirely autotrophic process (organic carbon is unnecessary) (Bruni et al., 2022). PN/A requires a balance between different types of microorganisms. Thus, the modulation of microbial community composition, structure, and spatial distribution in biofilms is essential for this process (Hubaux et al., 2015). In this regard, inhibition of nitrite-oxidizing bacteria (NOB) is crucial for developing the PN/A process. NOB activity can disturb the ideal balance between AOB and anammox, reducing nitrogen removal efficiency (Li et al., 2018; Sun et al., 2022).

Rotating biological contactors (RBC) are a low-cost technology with simple operation compatible with PN/A. They present biomass growth on the surface of a series of partially submerged discs, resulting in the coexistence of aerobic and anoxic conditions in the same biofilm. Ammonia-oxidizing bacteria (AOB) and anammox can simultaneously produce and consume nitrite, respectively; this prevents nitrite accumulation in the reactor. In addition, the unique characteristics of RBC confer resistance to shock loads and toxic substances from wastewater (Ghalehkhondabi et al., 2021).

Concerning modulation of the microbial ecology in RBC, Bicelli et al. (2020) achieved PN with inhibition of NOB activity by maintaining low DO concentrations through intermittent disk rotation. Moreover, in wastewater with high NH_4^+ levels, such as landfill leachate, inhibition of the NOB may occur even more naturally (Li et al., 2018; Sun et al., 2015). Therefore, investigating the potential efficiency and reliability of PN/A in RBC-treating landfill leachate can improve the sustainability of the waste management sector. Based on a comprehensive investigation of the microbial dynamics, this work aimed to study nitrogen removal in mature landfill leachate treatment by an RBC operated under an intermittent rotation strategy.

2. Materials and methods

2.1. RBC setup and system operation

The RBC reactor comprised a rectangular tank made of acrylic material, measuring 50 × 14 × 7 cm in dimensions, with a total capacity of 4.9 L (and a working volume of 3.9 L). An electric motor connected to a shaft regulated the rotational velocity of 30 polycarbonate discs (10 cm inner diameter). This setup ensured the discs submersion at 40 % of their diameter (Fig. 1). Using a relay module connected to an Arduino UNO R3 microcontroller, short time intervals (measured in milliseconds), as recommended by Bicelli et al. (2020). A diaphragm pump continuously fed the reactor at room temperature (21.4 ± 1.5 °C).

The influent consisted of diluted leachate in a 1:3 ratio with tap water, resulting in similar N concentrations, as previously reported by Bicelli et al. (2020). The leachate was collected from a landfill in Sao Paulo, Brazil. The raw leachate presented: soluble COD, 2852 ± 62 (mg.L^{-1}); total COD, 2923 ± 24 (mg.L^{-1}); dissolved organic carbon, 853 ± 14 (mg.L^{-1}); NH_4^+, 1657 ± 77 (mg N.L^{-1}); TKN, 1826 ± 93 (mg N.L^{-1}); alkalinity, 7900 ± 20 (mg $CaCO_3$.L^{-1}); pH, 8.14; electrical conductivity, 21.3 ± 0.1 (mS.cm^{-1}). The system was previously adapted to high nitrogen concentrations (Bicelli et al., 2020). The results obtained during the adaptation phase supported the disc rotating speed, intermittency, and NH_4^+ concentration definition. The RBC operation and monitoring lasted 131 days, divided into three phases of different hydraulic retention times (HRT). Table 1.. summarizes all operational parameters in this study.

2.2. Analytical methods

APHA et al. (2023) based all chemical and physical analyses, encompassing alkalinity, pH, and nitrogen compounds. Nitrite and nitrate were determined using an ion chromatography system (model ICS-1600 equipped with an As23 column, Thermo Scientific Dionex; the eluent was composed of 4.5 mM Na_2CO_3/0.8 mM $NaHCO_3$ with a flow of 0.25 mL.min^{-1}. COD analyses were performed in triplicate by the dichromate method. A luminescent DO sensor (Hach® multi-digital and multi-parameter HQ430D meter) monitored the internal reactor temperature and dissolved oxygen levels.

2.3. DNA extraction, sequencing of 16S rRNA gene, and FISH analyses

Biofilm samples were collected at the end of phase c to evaluate the structure of microbial communities along the reactor profile in discs 1, 15, and 30. DNA extraction using Wizard® Genomic DNA Purification Kit (Promega®) and verification of integrity and quality was done according to Bicelli et al. (2018). The amplification and sequencing procedure was performed according to Hidalgo et al. (2021). The technique used for quality control, bioinformatics, and taxonomical classification is reported in Oliveira et al. (2023). Amplicon sequencing data are publicly available at the European Nucleotide Archive (https://www.ebi.ac.uk)under accession number PRJEB59877. Complementary, to evaluate the axial distribution of AOB, NOB, and anammox bacteria, in situ hybridization (FISH) was performed in biofilm samples from discs 1,3,5,15, and 30 following with Bicelli et al. (2020) at the end of phase c.

3. Results and discussion

3.1. Nitrogen dynamics

The previous RBC operation with a synthetic effluent lasted 614 days (adaptation phase). Bicelli et al. (2020) reported the RBC performance in the adaptation phase with a continuous and intermittent disc rotation strategy. The average nitritation efficiency was 50.3 ± 3.9 %, indicating the potential applicability of this system to achieve the anammox process (Bicelli et al., 2020).

During phase *a*, the present study used the same operational parameters as Bicelli et al. (2020): 0.5 min rotating/5 min stationary, HRT of 12 h, and concentration of 500 mg NH_4^+-N.L^{-1}. However, when the synthetic effluent was exchanged for diluted landfill leachate, immediate impacts on the system's efficiency were observed. The effluent NH_4^+-N concentration increased, and nitritation efficiency decreased to 29.2 ± 4.0 %. Nitrification inhibition was most likely due to the mature leachate used in this study. Organic matter in mature leachate has high levels of recalcitrant humic-like compounds, adversely impact biological processes (Ramaswami et al., 2018). That also explains the limited organic carbon removal observed in the study (see section 3.2).

A delicate balance between organic carbon, DO, and ammoniacal nitrogen concentration controls nitrification in biological systems. Increasing the HRT may change this balance to favor nitrification. In this regard, Kulikowska et al. (2010) suggested the addition of a second RBC unit to improve the nitrification rates for NH_4^+-N concentrations higher than 243.5 mg.L^{-1}. In the present study, with influent ~ 500 mg NH_4^+-N.L^{-1}, a two-stage RBC system would be necessary to achieve complete nitrification.

The average efficiency of ammoniacal nitrogen removal during phase *a* was 43.3 ± 8.8 %, with a maximum of 53.1 %. Compared with Bicelli et al. (2020), the efficiency decreased to 22.7 %, a reasonable result due to the high complexity of compounds in the leachate. The phase *a* reached a complete nitrification efficiency of 10.0 ± 3.8 %, which is 2.7 % higher compared to the same authors. Moreover, a reduction in the conversion of ammonia nitrogen into nitrite was observed in the first 55 days of operation (Fig. 2a). The highest nitritation efficiency was 34.4 %, much lower than the minimum achieved by Bicelli et al. (2020).

The RBC presented notable instability with the increased HRT in phase *b (*from 12 to 14 h). The average complete nitrification efficiency was 18.0 ± 37.6 %, while the ammoniacal nitrogen removal efficiency was 38.3 ± 39.4 %. The nitritation efficiency was 18.7 ± 25.0 %, remarkably low compared to controlled conditions with synthetic effluent (Bicelli et al., 2020). These results demonstrated that mature leachate characteristics and the HRT applied in phase *b* decreased the RBC capacity to maintain nitritation. During phase *c*, HRT was reduced from 14 to 10 h. Then, after 60 days, the RBC achieved the highest nitritation efficiency of 25.5 %. However, the average nitritation efficiency for the whole period was 17.3 ± 4.5 %, lower than obtained in the previous phases.

Due to the high toxicity of mature leachate generated in landfills, combining different technologies is needed. Considering the operation of RBC in a single unit, the reactor could nitrify in phases *a, b,* and *c* with rates of 18.3 mg N-NH_4^+.L^{-1}.h^{-1}, 12.08 mg N-NH_4^+.L^{-1}.h^{-1} and 21.72 mg N-NH_4^+.L^{-1}.h^{-1}, respectively. These performances are similar to the ones reported by Ramaswami et al. (2018). Although the RBC could not offer the ideal conditions for the PN/A, reducing the HRT provided better conditions for TN removal. In phase *c,* TN efficiency was 15.6 %, 4.7 % higher compared to phase *a* and 10.0 % compared to phase *b.* Other studies reported higher efficiencies combining two or more treatment stages (Jiang et al., 2022; Song et al., 2020), indicating the potential of RBC as an alternative to the pre-treatment of leachate.

Table 1
Description of parameters and operational phases.

Parameter	Units	Phase		
		a	b	c
Duration (start–end)	day	1–55	55–69	69–131
HRT[a]	h	12	14	10
Rotation	rpm	0.5/5[b]	0.5/5[b]	0.5/5[b]
Dilution ratio	–	1:3	1:3	1:3
NH_4^+-N	mg N.L^{-1}	501	440	470
NLR[c]	g N.m^{-3}.d^{-1}	1002	1169	835

[a] hydraulic retention time, [b]x/y (the rotation time of the discs at 5 rpm is represented by 'x', in minutes, and the time in which the discs remained stationary is represented by 'y', also in minutes), [c]nitrogen loading rate.

3.2. Biofilm behavior and detachment

The biofilm sloughing washout along the reactor (see supplementary material) and the presence of denitrifying species in RBC discs (Fig. 3, further discussed in section 3.4) may explain the high variability and low COD removal observed in phases *a, b,* and *c*. The results suggest that the microbial community developed under synthetic effluent feeding did not adapt well to the mature landfill, which has more complex compounds. Inoculation with biomass previously developed within complex substrates and using discs containing more texture and porosity may reduce the detachment. More investigation with leachate dilutions other than 1:3 is recommended as well. Feeding the reactor with increasing leachate concentrations may adapt biofilm to better cope with toxic compounds.

Organic compounds in landfill leachate include a high diversity of recalcitrant and toxic compounds, hampering COD removal. Thus, the sole utilization of biological treatment systems may not be satisfactory for organic matter removal. Nevertheless, it is hypothesized that an RBC with stable and adapted biofilm can achieve COD removal and occurrence of simultaneous nitrification and denitrification (SND) or even simultaneous partial nitritation, anammox, and denitrification (SNAD), using detached organics from the biofilm. As nitrite has a greater affinity with anammox bacteria than denitrifying bacteria, the NO_2^- produced through PN would be consumed immediately by anammox. Then, the nitrate generated by anammox may support heterotrophic denitrification with simultaneous COD removal.

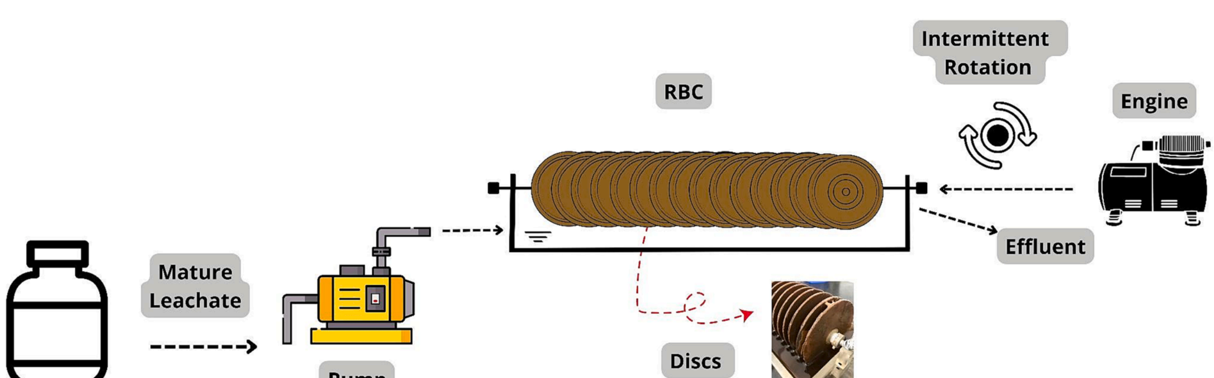

Fig. 1. Schematic diagram of the RBC reactor installation.

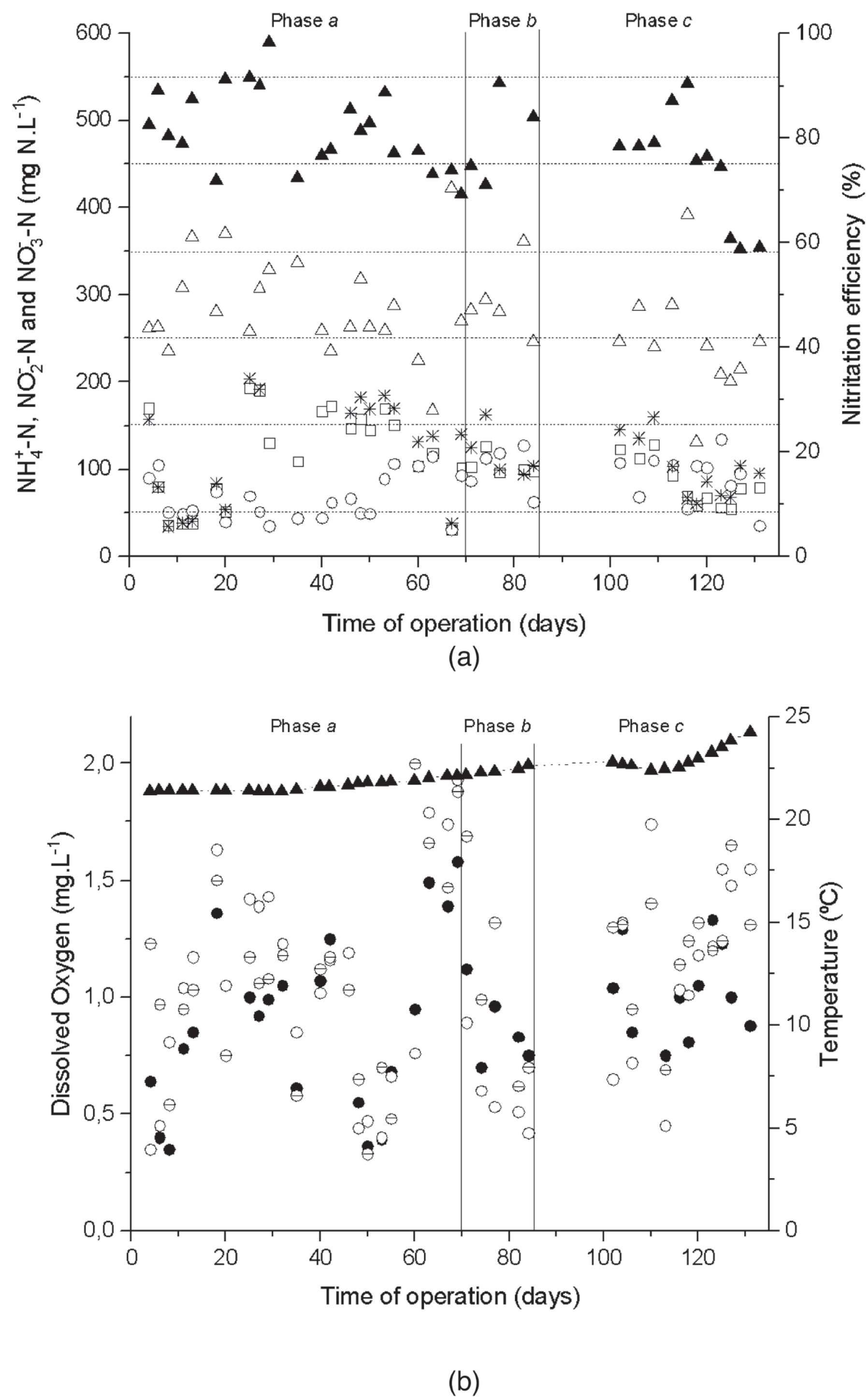

Fig. 2. Temporal results of nitrogen compounds concentrations and efficiency in the reactor: effluent NH_4^+-N (Δ), effluent NO_2^--N (□), effluent NO_3^--N (○), influent NH_4^+-N (▲), and nitritation efficiency () (a) and DO spatial results in the reactor for the sections of: the 1st (○), 15th (), and 30th (○) disc and temperature (▲) (b).

3.3. Dissolved oxygen comparison

In phase *a,* the DO concentration in the RBC was 0.9 ± 0.0 $mg.L^{-1}$, which is higher than reported by Bicelli et al. (2020 under the same operational conditions (DO of 0.3 ± 0.0 $mg.L^{-1}$). Thus, the adverse effects of changing the synthetic effluent for diluted landfill leachate also caused an elevation in DO. This result confirms the complexity of treating wastewater with excessive biodegradable and non-biodegradable products. The leachate impacted the nitrifying activity of the biofilm, leading to the TN removal efficiencies inferior to 16 %. For Yang et al. (2023), a high concentration of organic matter in the leachate impaired autotrophic nitrification, resulting in higher organic matter and nitrogen content in the effluent. The low COD removal also explains the DO values available in the effluent. According to Antwi et al. (2020), TN removal efficiencies of 94.3 % were achieved under DO of 0.2 $mg.L^{-1}$ during leachate treatment in a single-stage PN/A process. In the present study, a DO of 0.6 $mg.L^{-1}$ resulted in nitrogen removal < 20 %.

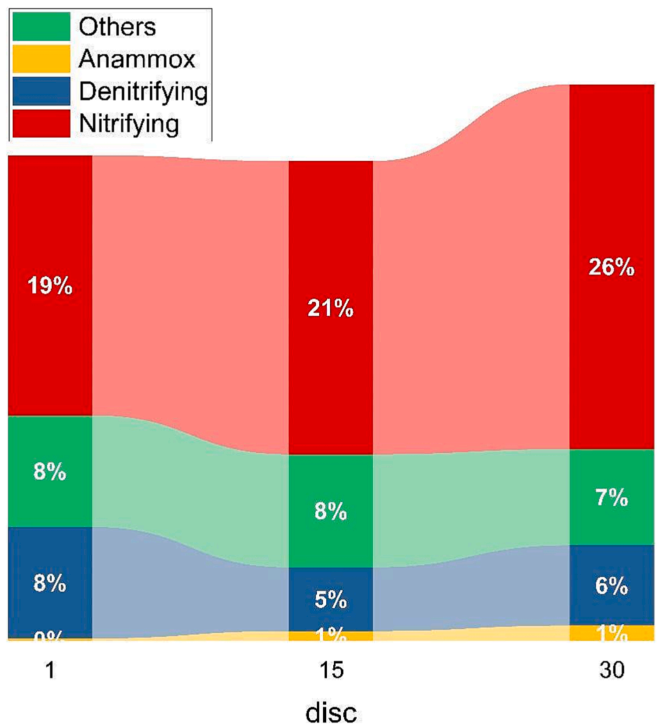

Fig. 3. Microbial distribution of possible metabolic activity in leachate treatment of groups in higher relative abundances in RBC samples (Anammox, Denitrifying, Nitrifying, and Others) of discs 1, 15, and 30.

Intermittent aeration was reported as a suitable strategy for reducing DO when treating synthetic effluent (Bicelli et al., 2020). However, in the present study, the impact of the mature leachate on the biofilm structure hindered the DO consumption, resulting in higher DO concentrations. Song et al. (2020) brought up the difficulty in treating landfill leachate under low DO concentrations and how much this could significantly improve the biodegradability of this wastewater.

Fig. 2b shows the DO concentrations and the average internal temperature on the 1st, 15th, and 30th discs. The phase *a* provided the best performance in oxidizing ammoniacal nitrogen to nitrite and the lowest DO. The reduction of HRT from 12 to 10 h did not translate into a natural decrease in the DO concentration. A gradual inhibitory effect may have hindered the biomass; this revealed the multivariable effect of leachate over biological treatment.

3.4. Microbial community analysis

The 16S rRNA sequencing of biofilm samples generated approximately 60,000 good-quality reads. The Good's estimator was close to 100 % in all studied discs, suggesting a high coverage of the sequencing of all samples (Table 2.). According to the Simpson's index (0.94–0.96), all studied discs had high diversity. In particular, disc 1 presented a slightly higher Simpson's index than the other discs, indicating a higher dominance on the initial discs.

The richness, measured by the Chao-1 index, observed in the RBC discs increased along the length of the reactor, as well as the number of ASVs (311–366). Conversely, the diversity of the microbial community was reduced along the reactor discs (Shannon's index observed was 4.09 in disc 1, 3.97 in disc 15, and 3.96 in disc 30), indicating that the microbial community on the final discs was less diverse. The decreasing diversity was expected, as the spatial monitoring profiles indicated that the biological processes mainly occurred in the first three discs. This spatial tendency was observed in all phases and also in Bicelli et al. (2020). It indicates that all biological processes in the first 5 cm were established until the end of the reactor - without changing the

Table 2
Results of 16S rRNA sequencing.

Parameter	Disc 1	Disc 15	Disc 30
16rRNA data			
Total number of sequences	60,170	60,170	60,170
OTUS	311	328	366
Singletons	0	4	2
Richness Estimation			
Chao-1	311 ± 5	329 ± 8	366 ± 0
Diversity Index			
Shannon	4.09 ± 0.02	3.97 ± 0.02	3.96 ± 0.00
Simpson (1-D)	0.96 ± 0.001	0.948 ± 0.001	0.936 ± 0.000
Coverage			
Good's estimator	100.00 %	99.99 %	100.00 %

ammoniacal nitrogen removal efficiency compared to the final effluent. In future studies, the spatial modulation of the functional microbial groups along the discs may improve the PN/A process – the microbial groups on the first discs may create ideal conditions for anammox organisms to act on the last discs of the system.

Seven phyla were predominant in all studied discs: *Proteobacteria*, *Bacteroidetes*, *Chloroflexi, Verrucomicrobia*, *Planctomycetes*, *Thermi,* and *Actinobacteria*. That indicates a low variation in the community structure along the reactor length and selective action of leachate in the communities since only those that could thrive in toxic conditions prevailed. *Proteobacteria* (59.5 % in disc 1, 61.08 % in disc 15, and 60.11 % in disc 30) and *Bacteroidetes* (10.82 % in disc 1, 11.33 % in disc 15 and 8.65 % in disc 30) are vital phyla for nitrogen removal and COD degradation (Antwi et al., 2017). Their predominance was also observed in other studies focusing on landfill leachate (Song et al., 2020; Zhang et al., 2021).

The diversity of identified phyla indicated a complex community structure that may have carried out many pathways for nitrogen and complex organic matter removal. In addition, the identified groups were also reported in other studies treating landfill leachate (Antwi et al., 2020; Chen et al., 2019; Jiang et al., 2021; Song et al., 2020). These results indicate that the microorganisms in the RBC were resistant and could degrade compounds in this wastewater. Thus, the RBC may be a potential pre-treatment unit for landfill leachate remediation.

The activity of bacteria related to organic nitrogen was observed at the family level mainly in disc 15, with relative abundances of 13.57 % as *Xanthomonadaceae* (Herschend et al., 2017), 7.10 % as *Bradyrhizobiaceae* (Ma et al., 2020) and 2.83 % as *Pirellulaceae* (Kellogg, 2019). At the genus level, a high presence of the ammonia-oxidizing bacteria (AOB) *Nitrosomonas* was observed in all discs (Fig. 4 and Table 3), with a higher relative abundance in disc 30 (17.09 %-25.15 %). These presence and distribution indicate the enrichment of this species along the reactor length. Interestingly, the high presence of *Nitrosomonas* suggests nitritation was the main nitrogen removal pathway despite a reduction in nitritation efficiency from Phase *a* to Phase *c*. *Aquamicrobium* was also identified, mainly in disc 1, and is known for the capability of ammonia oxidation (Duan et al., 2020). Moreover, this bacterial group can decompose refractory organics in leachate into small molecular organics, which other microorganisms can consume (Li et al., 2021). Another genus capable of nitrification (Yun et al., 2019) and denitrification (Tan et al., 2021), *Sphingomonas,* may have also contributed to ammonia oxidation.

Conversely, NOB were not identified in RBC at significant relative abundances. The dominance of AOB over NOB demonstrated that the conditions used in the RBC limited NOB growth, reducing the nitrite-oxidizing activity. Huang et al. (2021) also reported the suppression of NOB growth in landfill leachate treatment. However, in their study, the DO concentration (0.6 mg.L^{-1}) was inferior to the ones in the present study, as discussed in section 3.3. The relatively high DO level can

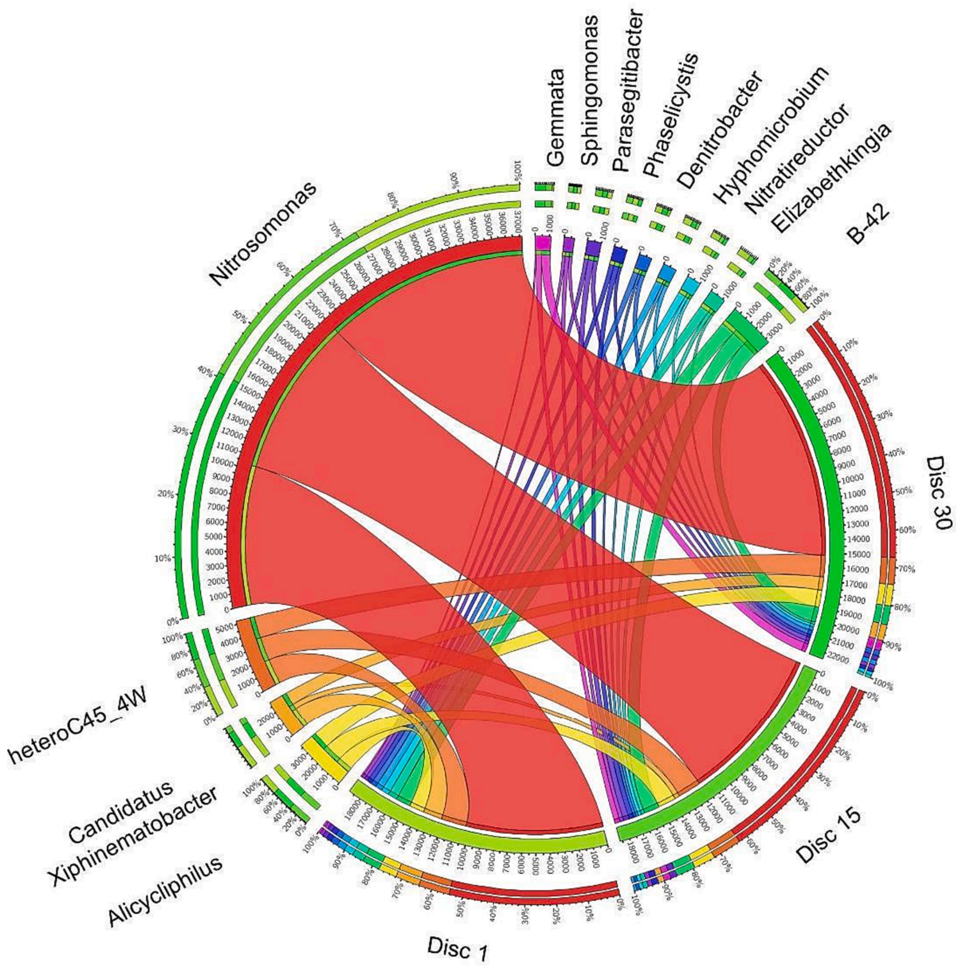

Fig. 4. Circus ideogram of the main genera identified in RBC discs 1, 15, and 30 in phase c.

Table 3
Genus level abundance of main identified species involved in nitrogen removal in the RBC discs.

Genus	Activity	Relative Abundance (%)		
		Disc 1	Disc 15	Disc 30
Nitrosomonas	Ammonia oxidation	17.09	19.54	25.15
Aquamicrobium	Ammonia oxidation	0.62	0.36	0.23
Alicycliphilus	Denitrification	1.84	2.19	2.14
Nitratireductor	Denitrification	0.94	0.39	0.33
Hyphomicrobium	Denitrification	0.85	0.29	0.47
Denitrobacter	Denitrification	0.68	0.31	0.53
Sphingomonas	Denitrification, Heterotrophic and Autotrophic Nitrification	0.37	0.48	0.37
Gemmata	Heterotrophic Nitrification andAnammox	0.17	0.66	1.05

explain the nitrate presence in the RBC effluent, which aligns with the NOB activity observed by FISH analyses (Fig. 5).

Many species related to denitrification were present in the RBC, mainly in disc 1 (Fig. 3), as *Alicycliphilus, Candidatus Xiphinematobacter, Nitratireductor, Hyphomicrobium, Sphingomonas* and *Denitrobacter* genera (Antwi et al., 2020; Solís-González and Loza-Tavera, 2019; Tan et al.,2021) explaining the unbalanced nitrogen removal in the process. Denitrifier populations represented 4.68 %, 3.66 %, and 3.84 % of the total genus in discs 1, 15 and 30, respectively. Similarly, Huang et al. (2021) reported a low relative abundance of denitrification groups during leachate treatment, mainly due to the salinity stress that can shrink these populations.

In addition to denitrification, the genus *Alicycliphilus,* commonly found in landfills, can use oxygen, nitrate, and chlorate as terminal electron acceptors. This variable metabolism allows them to biodegrade xenobiotics under different conditions of oxygen (Solís-González and Loza-Tavera, 2019). Given the leachate toxicity, identifying bacteria that couple denitrification to degradation of xenobiotics highlights the importance of the investigation to public health.

Anammox species were enriched along the reactor length with a population of 0.2 % in disc 1, 0.7 % in disc 15, and 1.1 % in disc 30. The identified anammox genera in the RBC were *Gemmata* and *Plantomyces* (Fu et al., 2020; Oliveira et al., 2022; Sun et al., 2022; Yao et al.,2023). Podder et al. (2020), during the treatment of landfill leachate using single-stage anammox, reported that anammox can co-exist with heterotrophic denitrifies; however, competition for nitrite can also occur. Differently from anammox, the dominance of denitrification groups occurred in disc 1, so competition between these groups may have occurred in RBC. Moreover, the reduction in the abundance of denitrification groups occurred where the participation of anammox species increased (i.e., disc 30). Huang and Lee (2021) reported a similar trend. Enriching of anammox bacteria and AOB groups along the reactor length indicates that the PN/A process occurred and contributed to total nitrogen removal during leachate treatment in RBC. However, the conditions provided in the experiment and leachate toxicity significantly limited anammox growth, resulting in a more intense microbial activity only in the first discs.

The species identified in the RBC acting in many N and C removal pathways can explain the TN removal of 15.6 % in Phase *c*. Despite the

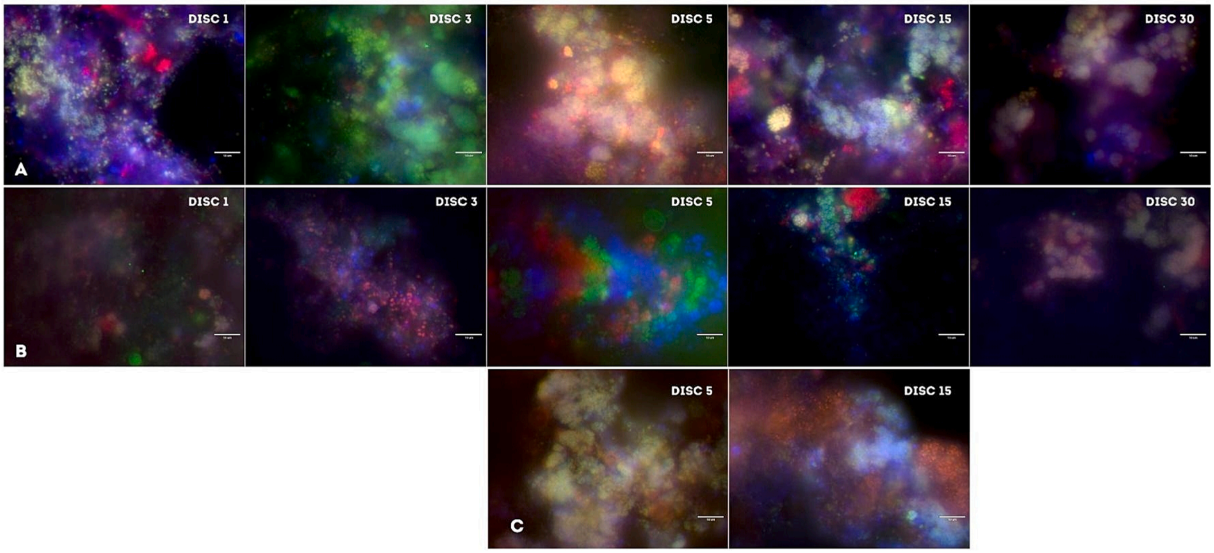

Fig. 5. FISH micrographs 100 × of biofilm samples taken from discs 1, 3, 5, 15, and 30 in phase c: (A) AOB bacteria hybridized with AOB mix in green, EUB mix in red, all DNA hybridized with DAPI in blue, (B) NOB bacteria hybridized with NOB mix in green, all bacteria hybridized with EUB mix in red, all DNA hybridized with DAPI in blue, (C) Anammox bacteria, hybridized with AMX820 probe in red, all bacteria in green and DAPI in blue.

low biodegradability of organic compounds in mature leachate and its toxicity, the sequencing results indicate that RBC can provide conditions to maintain a diverse and complex community structure. Although low ammonia oxidation efficiencies were observed, the reactor provided conditions for nitritation-anammox occurrence, as species capable of PN (*Nitrosonomas*, the dominant genus, and *Aquamicrobium)* were identified, as well as anammox species (*Gemmata* and *Plantomyces*). These results not only corroborate the findings of Bicelli et al. (2020), indicating that RBC can allow the growth of a PN/A microbial community, but also demonstrate its application for leachate treatment. However, in the present study, the adverse characteristics of leachate may have hindered nitritation-anammox growth.

Moreover, denitrifying groups were also present, coupled with recalcitrant compound degradation. These diverse bacterial functional groups highlight the complexity of the microbiota and the importance of further research on RBC as a pre-treatment unit for leachate or using a two-stage RBC. For Yang et al. (2023), using a multi-stage system to treat this wastewater type effectively improved the resilience of ammonia-oxidizing bacteria against complex environments. Another opportunity is co-treating leachate with other wastewater containing organic compounds to increase the denitrification potential and TN removal.

3.5. FISH analyses

FISH analyses demonstrated that AOB and NOB bacteria were present along the reactor profile (Fig. 5). AOB, NOB, and anammox bacteria tended to form clusters, as has been identified by Egli et al. (2003). The study evaluated the microbial composition of an RBC used to treat ammonium-rich wastewater and observed the presence of ammonium and nitrite-oxidizing bacteria, andanammox bacteria. The results complement the ones obtained by 16 rRNA gene amplicon sequencing. The presence of these populations indicated the potential for nitrogen removal during leachate treatment in the RBC. However, the change in the nitrifying community during leachate treatment is evident compared to the results obtained by Bicelli et al. (2020) with synthetic effluent. NOB suppression, which could be achieved in the previous study, was less intense, as seen in Fig. 5b and Table 3, reducing the nitritation efficiency during leachate treatment. In this way, conditions provided to the RBC favored nitrification, denitrification, and activity of anammox bacteria, although they were still lacking to promote high efficiency in the reactor. Nonetheless, the microbial results are strong evidence of the potential of the RBC technology to attain several oxic-anoxic pathways for leachate treatment.

3.6. Perspectives on practical applications

From the results obtained, it was evident the need for a new configuration of the system, with the inclusion of a pre-treatment unit to remove the high content of organic matter in the landfill leachate or the use of a system with two or more stages, thus reducing the adverse effect of these compounds on the activity of the microbial community responsible for nitrogen removal. The main advantage of RBC is the natural aeration, without energy costs, meeting the requirements of economic efficiency. However, it was proven necessary to use intermittent rotation to reach the lowest DO levels. Despite the biomass detachment that occurred during this research, it is known that biofilm-based systems are less sensitive to changes in environmental conditions. It also allows a long biomass retention time, making RBC attractive.

4. Conclusions

This study elucidated how RBC operational characteristics, DO, and complex compounds can influence microbial interactions responsible for nitrogen removal. The findings contribute to achieving more favorable conditions for the PN/A occurrence. The intermittent rotation reduced the difficulty in controlling oxygen transfer. The presence of AOB, NOB, and anammox, as well as denitrifying bacteria, indicate the potential for nitrogen removal and the ability of the RBC to maintain a diverse community structure, including species capable of degrading xenobiotics. Results demonstrated the potential for applying the RBC configuration for landfill leachate treatment, although further research is needed to increase its efficiency.

E-supplementary data of this work can be found in online version of the paper.

CRediT authorship contribution statement

Larissa Garcez Bicelli: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Data curation, Writing - original draft, Writing - review & editing. **Alessandra Giordani:** Formal analysis, Investigation, Writing – review & editing, Methodology. **Matheus Ribeiro Augusto:** Formal analysis, Investigation, Writing – review &

editing, Methodology. **Dagoberto Y. Okada:** Formal analysis, Investigation, Writing – review & editing, Methodology. **Rafael Brito de Moura:** Investigation, Writing – review & editing. **Daniele Vital Vich:** Writing – review & editing. **Ronan Cleber Contrera:** Resources, Supervision, Funding acquisition, Writing – review & editing. **Vitor Cano:** Investigation, Writing – review & editing. **Theo Syrto Octavio de Souza:** Writing – original draft, Writing – review & editing, Resources, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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