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Relatório Final de Pós-doutorado

Título: The influence of micropollutants from domestic wastewater on bacteria-microalgae consortia in an microalgae-based sewage treatment system.

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Agencia financiadora: Capes PrInt Processo 88887.890911/2023-00

Bauru, 2024.

1. Introduction

Despite numerous advances in the treatment of sanitary sewage, the conventional treatment processes have ongoing issues, such as high loads of nutrients, pollutants and micropollutants, which result in the release of a contaminated effluent into water bodies, representing a potential risk to the environment and to the population (Wang and Wang, 2016; Grandclément et al., 2017).

Among the various technologies constantly evaluated, algae-based systems for sewage treatment have been gaining visibility and recognition as a real potential for applications around the world. The use of algae in the treatment of sanitary sewage is promising due to its ability to assimilate macronutrients and micronutrients in its biomass (Fernandes et al. (2017), De Wilt et al. (2016) and Boelee et al. (2011), and their potential to remove micropollutants and other contaminants such as pathogens (Ruas et al., 2023; Pompei et al., 2023; Pompei et al., 2024).

Sewage treatment systems that employ microalgae-bacteria consortia have the potential to increase nutrient removal efficiency through mutual interaction and synergistic effects (Fallahi et al., 2021). Knowledge and control of the mechanisms involved in microalgae-bacteria interaction can improve the system's ability to transform and recover nutrients (Fallahi et al., 2021). Furthermore, they present numerous advantages related to the economy, energy saving, and a more safe environment, due to cooperative interactions between microalgae and bacteria (Zhang et al., 2020).

Several studies cited in reviews on microalgae-bacteria interactions suggest that they are species-specific, sophisticated and determined by high biological diversity in consortia (Ramanan et al., 2016; Fallahi et al., 2021), highlighting the importance of greater exploitation of native species.

It is known that the microorganisms present in these systems are responsible for removing nutrients, organic matter and other compounds harmful to human health present in the effluent (Pompei et al., 2023a, b). These microorganisms, including microalgae, establish a variety of interactions ranging from mutualistic, facultative, parasitic and species-specific associations (Bagatini et al., 2014; Eiler and Bertilsson, 2007). In addition to these interactions, microorganisms also respond to the conditions of the environment in which they are located. Thus, the nutrient recovery potential and biocompound production profile by microalgae are closely related both to biotic interactions and to abiotic growth conditions.

In association with bacteria, algae generally are benefited by remineralized nutrients (Paerl et al., 2001), growth promoters (Croft et al., 2005), vitamins or vitamin precursors (Grant et al., 2014) and even antibiotics production provided by the associated bacteria against other bacteria and viruses (Mayali and Azam, 2004). The bacteria, in turn, feed on the organic matter, mainly polysaccharides, and nutrients produced and released by the algal cells in the phycosphere (Bell and Mitchell, 1972). Some bacteria live immersed in the polysaccharide matrix, being protected against predators (Seymour et al., 2017). It is in the phycosphere that the exchange of compounds between algae and bacteria occurs (Seymour et al., 2017).

While the diversity within microalgae-bacteria microbial communities has been well documented (Astafyeva et al., 2022; Fuentes Cordero et al., 2016; Paddock et al., 2020) none at tropical environment, as Brazil. Also, little is known about the key organisms affected by the presence of micropollutants present into the wastewater. This is the first study showing

that the presence of domestic sewage micropollutants can change the microbial communities (mainly microalgae and bacteria) composition during sewage treatment in microalgae-based system.

2. Materials and Methods

2.1. Experimental set-up

A pilot scale flat panel photobioreactor (FP-PBR) was operated outdoor under real environmental conditions per 42 days, after the reaching a steady-state. The determination of steady-state was performed based on the analysis of all parameters graphically and numerically, and was reached when the variation (dX/dt of most monitored concentrations tended to zero), mainly the P removal close to 95%.

The FP-PBR was operated in batch mode (six) (7 days duration each). For the metabarcoding analyzes the samples (100 mL) were taken at the beginning (day zero) and in the end (day seven) of each batch, totaling 12 samples for phytoplankton and 12 for bacteria and cyanobacteria, collected in duplicate each.

Three batches of the same experimental condition were evaluated (eg., same FP-PBR, and located at the same place outdoor, same inoculum, same sewage from the same collection point) to see if there was a difference in the composition of the communities over the reactors' operating time. Also, into these a total of six batches, three of them contained a mix of 13 selected micropollutants added in the real sewage to be treated by the FP-PBR. So, were evaluated whether there was any effect of the presence of micropollutants on the composition of communities.

2.2. Sanitary sewage microalgae inoculum and FP-PBR information

The sanitary sewage used was previously anaerobically treated by an Anaerobic Baffled Reactor (ABR). It was obtained from the Tibiriçá Wastewater Treatment Plant (WWTP) located in the city of Bauru, São Paulo, Brazil (22°22'7.47"S, 49°21'2.92"W). The samplings were made weekly and the sewage was characterized according to some parameters evaluated (Table 1), and immediately used at FP-PBR.

The microalgae inoculum was obtained through the phycoprospection process (Wilkie et al. 2011) from the same sanitary sewage used to be treated by the FP-PBR. The culture was maintained in batch mode with weekly feedings (10% of inoculum and 90% of sanitary sewage anaerobically treated). After 60 days, a 5 L of the consortium grown at laboratory scale was transferred to the FP-PBR (10% v.v.).

The FP-PBR (of 120 cm width, 60 cm height and 10 cm depth and a thickness of 8 mm on each sidewall) was constructed in acrylic, and had 50L (total volume). Compressed air (10 L min⁻¹) was added at the bottom of the reactor, guaranteeing mixing of the cultures with airflow of 0.2 vvm (volume of air per volume of liquid per minute).

Table 1: Average values of monitored parameters in sanitary sewage anaerobically treated (mg L^{-1} , except for pH).

Parameter	Chemical oxygen demand (COD)	Ammonium nitrogen ($\text{NH}_4\text{-N}$)	Nitrates ($\text{NO}_3\text{-N}$)	Nitrites ($\text{NO}_2\text{-N}$)	Total nitrogen (TN)	Total Phosphorus (TP)	Total suspended solids (TSS)
Mean \pm S	134.42 \pm 46.76	85.67 \pm 13.18	11.98 \pm 4.12	0.70 \pm 1.11	98.35 \pm 10.99	10.97 \pm 1.67	160.98 \pm 33.33

2.3. The 13 selected micropollutants

Thirteen selected micropollutants were added in concentrations commonly detected in WWTPs worldwide as described in the established literature (Pal et al, 2014, Bai et al, 2018, Pompei et al., 2019; Sutherland and Ralph 2019; Derisso et al. 2020). The added compounds were all 99% purity or more, purchased from Sigma-Aldrich. The names and respectively concentration added were: paracetamol ($90.00 \mu\text{gL}^{-1}$), diclofenac ($22.50 \mu\text{gL}^{-1}$), naproxen ($22.50 \mu\text{gL}^{-1}$), ibuprofen ($450.00 \mu\text{gL}^{-1}$), benzophenone ($1.50 \mu\text{gL}^{-1}$), methylparaben ($1.50 \mu\text{gL}^{-1}$), carbamazepine ($6.00 \mu\text{gL}^{-1}$), mefenamic acid ($1.50 \mu\text{gL}^{-1}$), estrone ($0.15 \mu\text{gL}^{-1}$), progesterone ($0.15 \mu\text{gL}^{-1}$), β -estradiol ($6.00 \mu\text{gL}^{-1}$), salicylic acid ($22.50 \mu\text{gL}^{-1}$), and metoprolol ($1.50 \mu\text{gL}^{-1}$).

2.4. Abiotic parameters

The cultivation environmental parameters were not controlled, just monitored daily. Other parameters such as total phosphorus (TP), phosphate (PO_4), total nitrogen (TN), ammoniacal nitrogen ($\text{NH}_4\text{-N}$), nitrite (NO_2), nitrate (NO_3), total kjeldahl nitrogen (TKN), alkalinity, turbidity, cellular density, chlorophyll-a, total suspended solids (TSS), chemical oxygen demand (COD), were also monitored, and the method and the equipment's used are presented at Supplementary material (Table S1).

2.5. DNA extraction, PCR amplification and sequencing

The microorganisms were recovered from 10 to 50 mL (Table S2) of sewage samples (the volume used depended on the turbidity of the sample) under laminar flow by vacuum-filtration. Eukaryotic phytoplankton was collected using a glass fiber filter of $1.2 \mu\text{m}$ pore size (GF/C) and bacteria and cyanobacteria were collected on a $0.22 \mu\text{m}$ pore size cellulose acetate membrane. To avoid contamination, all material used was previously autoclaved. The Genomic DNA was extracted directly from the filters using the kit DNeasy PowerSoil Pro Kit (Qiagen®) following manufacturer's instructions. Extracted DNAs were submitted to electrophoresis in a 1 % agarose gel, stained with GelRed (GelRed Nucleic Acid Gel Stain-Biotium, Hayward, CA, USA), to check the quality and quantity of extracted DNA (Sambrook et al. 1989), using the Bio-Rad 1 kb ladder Molecular Ruler. After, the DNA was quantified with a Nanodrop 2000 UV-Vis (Thermo Fisher Scientific, USA). The amount of total DNA recovered from samples on average was $56 \text{ ng}/\mu\text{L}$ ($\text{A}_{260}/\text{A}_{280} = 1.8$ to 2.3).

The region V3/V4 of the 16S rDNA gene was amplified in order to address the prokaryotic community (bacteria and cyanobacteria) and the region V4 of the 18S rDNA gene to address the eukaryotic community (eukaryotic microalgae). Although the primers for 16S were designed for Eubacteria, we have also used the archaea community recovered by

them in this study. The 16S rDNA was amplified with primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACT ACHVGGGTATCTAATCC-3') (Herlemann et al., 2011) and to amplify the 18S rDNA we used the primers TAREuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3') and TAREukREV3 (5'-ACTTTCGTTCTTGATYRA-3') (Stoeck et al., 2010). The PCR products were sequenced using the Illumina MiSeq sequencing platform to obtain 2x 300 bp paired-end reads at NGS Soluções Genômicas (Piracicaba, Brazil).

In this work, we evaluated as the bacterial community, which included species from the Eubacteria domain, with the exception of Cyanobacteria, which was studied separately.

2.6. Bioinformatic analyses

The ASVs (Amplicon Sequence Variants) were obtained using the pipeline DADA2 (Callahan et al., 2016) at R software (R Core Team, 2022) after the primer's removal with Cutadapt v1.18 (Martin, 2018). The identification of ASVs was carried out using the SILVA database (Quast et al., 2013, Yilmaz et al., 2014). All raw sequencing data obtained from MiSeq were deposited to NCBI SRA database (Project name: PRJNA1089101; <https://www.ncbi.nlm.nih.gov/s>). The exact validated contigs were used in the statistical analysis.

All statistical analyses were performed using R program (R Core team, 2022). The communities (prokaryotic and eukaryotic) in the different treatments were statistically compared using PERMANOVA (Anderson, 2001) and visualized in NMDS (Non-metric Multidimensional Scaling), using "vegan" package (Oksanen et al. 2022). To verify the influence of micropollutants in communities between treatments only the final (i.e., day 7) samples were compared. Indicator species were investigated using the package "indicspecies" (De Cáceres & Legendre, 2009) in R. The correlations between different taxa and environmental variables and micropollutants were compared using PCA (Principal Component Analysis) in R, also were done the mantel test using "vegan" package (Oksanen et al. 2022); and the Spearman. For this, firstly, the normality test was done using "stats-package" package (R Core team, 2022), with the function `shapiro.test`. Then, the correlation tests were done between the percentage of occurrence of each group (algae, bacteria, archaea) with the 14 environmental variables, using "corrplot" package (Wei T, Simko V, 2021).

The alpha diversity and the beta diversity were determined using the 'observed ASVs' using R (R Core team, 2022). For alpha diversity the 'Chao1' (the estimator of species richness), the 'Shannon diversity index' and the 'Simpson Diversity Index' were considered. For beta diversity, were considered the total (Sorensen), the nestedness, and for the turnover, the Simpson.

2.7. Predictive metagenomics for 16S rDNA

The predictive metagenomics was performed to address the prokaryotic community, more specifically for bacteria and archaea. These analyses were done using the web-based taxonomic-to-phenotypic prediction program METAGENassist to identify the putative functional behaviors of the bacteria in the FP-PBR (Arndt et al. 2012). This program was used in coordination with the METAGENassist Tutorial 1

3. Results and Discussions

3.1. Microbial community structure and diversity analysis

The 18S rDNA gene analysis for the eukaryotic population from the FP-PBR revealed the presence of 346 ASVs in total; from these, 85 ASVs affiliated to microalgae genera.

Microalgae

Considering the microalgae composition into the FP-PBR there were no statistical differences between the initial and the end of the batches ($p = 0.57$), nor between treatments at the end ($p = 0.20$). No difference in composition is expected, since the inoculum used for the treatment of sanitary sewage came from the phycoprospecting of the same sewage to be treated, therefore, with the same majority composition. However, it is expected from the treatment that over the period of operation (HRT = 7 days) there will be growth of algal biomass.

Three microalgae ASVs (ASV9 *Dictiosphaerium* sp.; ASV40 *Dicloster acuatius*; and ASV12 *Actinastrum hantzchii*) occurred in all samples with micropollutants, and were less represented in control samples at the end of the experiment. However, they were not a significant indicator of the species of micropollutant samples ($p = 0.06$).

Considering all the samples collected, the calculated alpha diversity indices showed low diversity values of microalgae community (Table S2), with dominance of *Tetradesmus obliquus* (98.10% at the beginning, and 96.01% in the end of the batches). At the end of the batches, we could see the appearance of *Eustigmatophyceae_XX* (1.91%) and *Characiopsis* sp. (1.61%).

The total beta diversity calculated, considering all samples, was 0.85, most of which was due to the turnover process (0.67), rather than nestedness (0.17). It is possible to observe (Figure 2), that most occurrences of greater beta diversity turnover (18S-1 *versus* 18S-12; 18S-12 *versus* 18S-3; 18S-12 *versus* 18S-9; and others) are related to the samples from the beginning and end of the batches, showing that there was a variation in the composition of the community due to the exchange of species, from the initial to the final day of treatment, precisely due to the change in the composition of the sewage, which before was mostly anaerobic and after, becomes aerobic.

Although nestedness was less important for betadiversity, it is also observed from Figure 2 that the occurrences of higher value of beta diversity nestedness (18S-7 *versus* 18S-5; 18S-12 *versus* 18S-2; 18S-12 *versus* 18S-6) occurred between the samples with and without micropollutants, probably showing an effect by the presence of the selected compounds in nestedness the microalgae diversity.

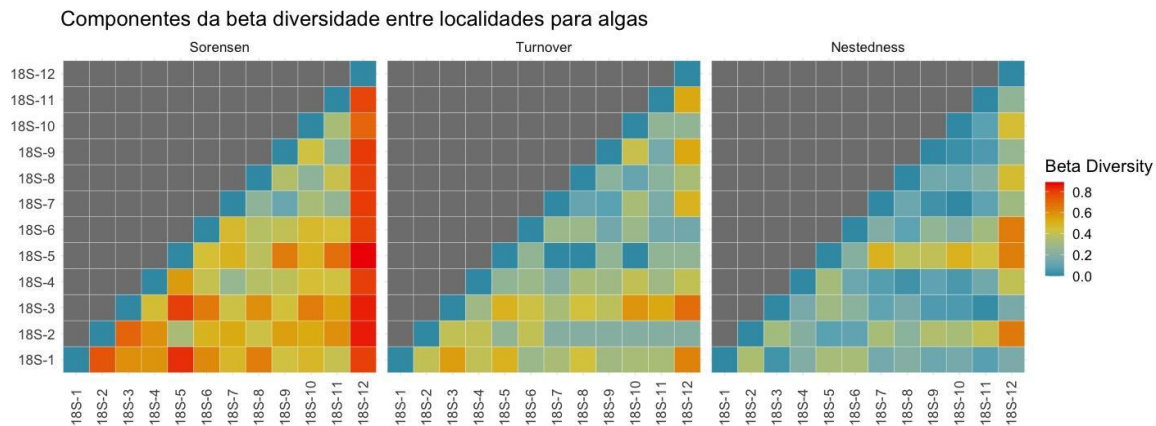
turnover
ASVs.

(Simpson),

and

nestedness

Figure 2: Total Beta
diversity (Sorensen),
for microalgae



The 16S rDNA gene analysis for the prokaryotic population from the FP-PBR revealed the presence of 1127 total ASVs affiliated to 1065 bacterial genera, 40 archaeal genera, and 22 cyanobacterial genera.

Cyanobacteria

Regarding the composition of Cyanobacteria, a large difference in composition was observed between the beginning (71% of *Planktothrix. mougeotii*; 21% of Gastraunidentifiederophilales; 2.9% of *Calenema*; 2.2% of *Synechococcus elongatus*) and the end of batches (29% of *Cyanobacteriales* and 20% of both, *Vampirovibrio* and *Nostocaceae* - which previously had 0% occurrence).

Contrary to what happened with Archaea, for example, which appeared on day zero and disappeared on day 7, some cyanobacteria were not detected in the initial effluent, but appeared at the end of the experiment (day 7), further showing the difference in community composition, the changes that occur over time, mainly due to the change in the predominance of an anaerobic to an aerobic environment.

Despite the few numbers of cyanobacterial ASVs detected, there was a significant difference in the community composition between the initial and final samples (PERMANOVA p -value=0.02, $n=12$), but no difference was detected in the presence of micropollutants (PERMANOVA p -value = 0.4). After adding the mix of micropollutants, only *Vampiriovibrio* and one unidentified *Nostocaceae* remained in one of the replicates – perhaps the most resistant to the presence of contaminants.

The beta diversity calculated for cyanobacteria revealed that from the β_{total} ($\beta_{sorensen} = 0.911$) the majority was $\beta_{sim} = 0.829$, as well as evaluating pairwise (Fig. Xc) showing that the turnover majority explain the variation in different composition between the

treatments in relation to the time (initial and final), and with or without the micropollutants with chance in the species of cyanobacteria in both cases.

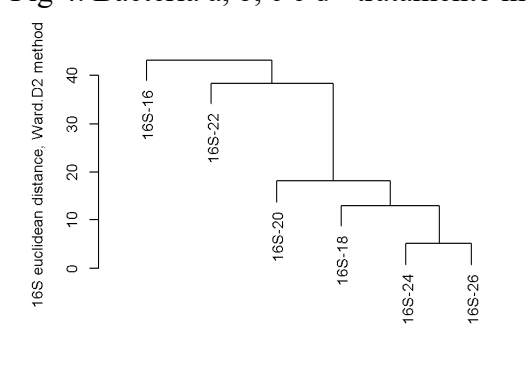
Bacteria and Archaea

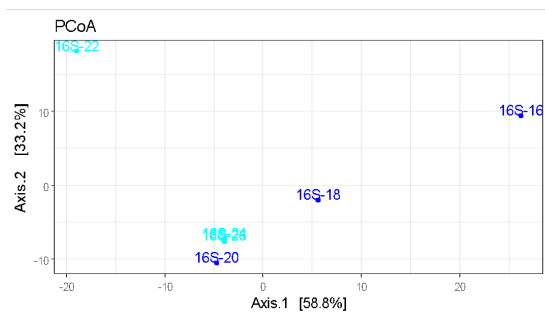
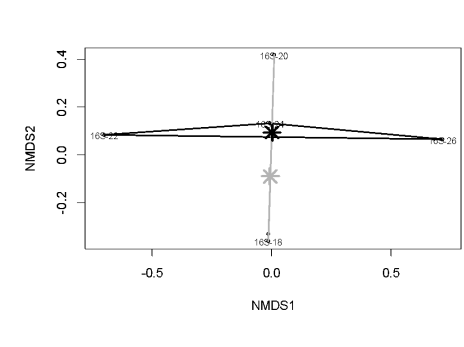
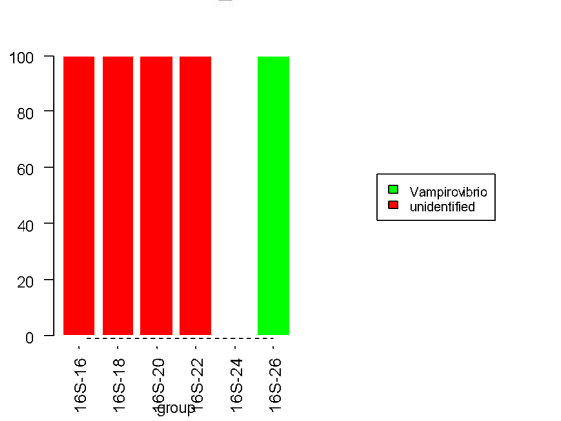
The prokaryotic richness was greater in initial samples (day zero) than in samples collected at the end of the experiment (day seven) (Table S2).

Within prokaryotic community, there was a clear and significant difference in bacterial (i.e., excluding cyanobacteria and archaea) community composition between initial and final samples (days zero and seven) (Fig. 4a), in the NMDS (Fig. 4d) and PCoA (Fig. 4b) (PERMANOVA p -value= 0.00099). The PCoA (Fig 4) also shows the great similarity between bacterial communities at the beginning of the experiment, regardless the replicates or treatments.

Considering the bacterial composition, at the beginning of the FP-PBR operation, we observed a predominance of *Fimicutes* (90.78%), decaying to 62.31% at the 7th day of operation. The opposite occurred to *Proteobacteria*, which abundance was previously 1.86% of the composition, increasing to 30.83%. Our results are in accordance with Ferro et al., (2020), who detected that the most abundant prokaryotic phyla were Firmicutes and Proteobacteria, however, in an open photobioreactor at North-Sweden, using local untreated municipal wastewater. The authors also described that these bacterial groups are ubiquitously found in Wastewater Treatment Plants (WWTPs) plants. Interestingly, a phylogenetic study performed by Ramanan et al., (2016), demonstrated that the *Proteobacteria* and *Bacteroidetes* are more likely to be associated with green algae than other bacterial phylotypes. Similarly, Paddock et al., (2020) describes that the algae bioreactors operated in their study were dominated by *Proteobacteria* (79%).

Fig 4. Bacteria a, b, c e d= tratamento inicio x fim; e,f, g e h: tratamento com e sem fármaco





The discussion of Archaea community composition is hampered since the 16S rDNA primers used are not specific for this group. However, some information can be extracted from our results once considering this bias.

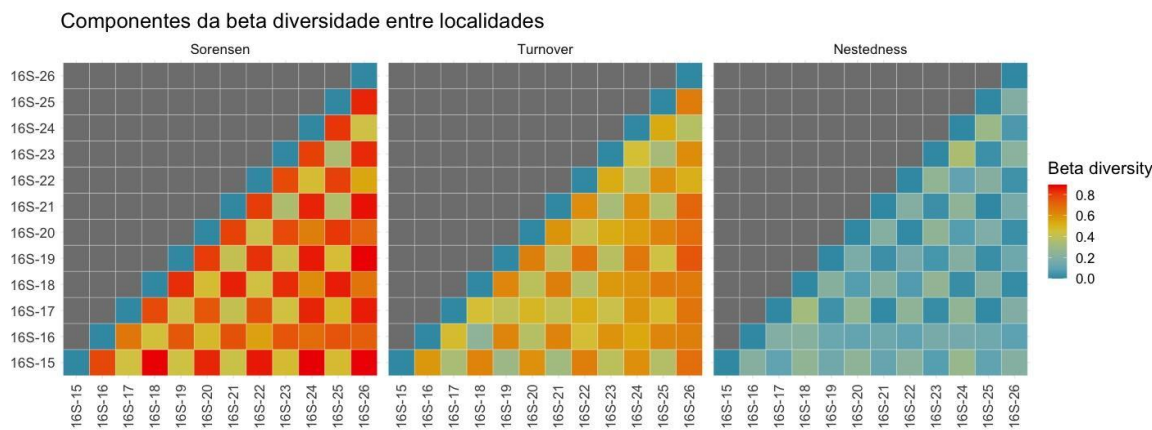
As expected, the Archaea community were present more on day zero (initial of the batches) as it is typical of an anaerobic environment, which was the case of 90 % of the

effluent to be treated at the beginning of the reactor operation, and represented XXX % of prokaryotic community.

Among the Archaea recovered by the primers used, the dominant Phyla was Halobacterota (92 % on day zero, and 95 % on the last day of operation). The *Methanobrevibacter* sp., appeared at the end of the batch (day 7), but in lower concentration compared to day zero (Fig. 5?), and *Methanosaeta* sp. (3 different species). This can also be seen by analyzing total beta diversity, where it is observed from the total (β sorensen = 0.84), the majority were explained by the nestedness (Fig. Xb). This is because the dominant Archaea at the beginning of the batches (Halobacterota - 92%) were typical of the anaerobic environment, and due to the change to an aerobic environment, they no longer found the ideal environment for their survival, giving way to other organisms, such as the community of microalgae that increased in quantity along the operation days (from $\approx 628.2 \mu\text{g L}^{-1}$ to $5998.7 \mu\text{g L}^{-1}$ of chlorophyll-a).

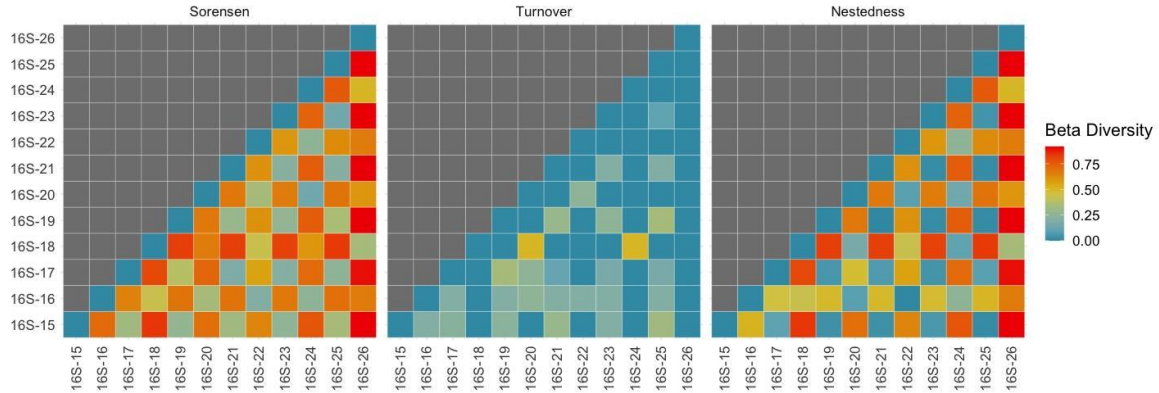
Regarding the composition of the communities in the presence of micropollutants, while the cluster separates the communities (Fig 4e), that is, it seems to have a difference in composition in the presence of contaminants, the PCoA (Fig 4f) does not show much separation, as well as there was no statistically significant difference between the communities regarding the presence or absence of the micropollutants, namely: for bacteria and archaea ($p = 0.1$), and for cyano ($p = 0.4$). However, in samples containing the micropollutants, there is the occurrence of *Patescibacteria*, which could be an indicative of contamination by the selected micropollutants.

Fig: Beta diversity of a) bac, b) arch and c) cyano
 a)



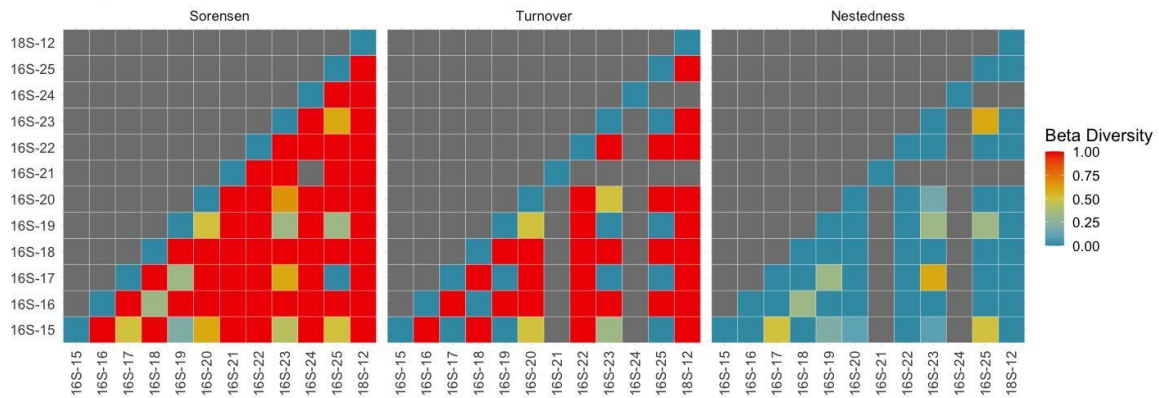
b)

Componentes da beta diversidade entre localidades



c)

Componentes da beta diversidade entre localidades para ciano



3.2. Abiotic versus Biotic

The mantel test was done using the 14 abiotic parameters, and the percentages of occurrence of each ASV in samples with maximum taxonomy provided, which varied in quantity according to the tested group. The analyze indicating that there was an influence of abiotic data on the composition of communities for bacteria ($r = 0.86$, and $p = 0.001$), and for

archaea ($r = 0.82$, $p = 0.001$). However, none significance ($p < 0.05$) was detected for microalgae with or without the 13 selected micropollutants.

More in-depth, other tests correlations were done to see if the most abundant in percentage of most taxonomy were correlated with each parameter. For that, firstly the normality test was done, indicating the needing of Spearman correlation (all data available at Table SX – Supplementary material).

In this approach, the most abundant microalgae (*Tetradasmus obliquus*) don't present significant correlation with any parameter evaluated. Six species showed statistically significant correlations. The parameter that most are related to them were dissolved oxygen, correlated with *Chrysophyceae* ($r = -0.719$; $p = 0.008$); *Ochromonadaceae* ($r = -0.767$; $p = 0.003$); *Polytoma uvella* ($r = -0.770$; $p = 0.003$). The *Actinastrum hantzschii* were positively correlated with $\text{NH}_4\text{-N}$ ($r = 0.58$; $p = 0.04$); *Desmodesmus* presented negative correlation with TP ($r = -0.58$; $p = 0.04$), and positive with NO_3 ($r = 0.67$; $p = 0.6$); *Poteriespumella* sp. presented positive correlation with NO_2 ($r = 0.75$; $p = 0.004$), and negative with pH ($r = -0.65$; $p = 0.02$).

About the cyanobacteria, the two most abundant (*Planktothrix* at the beginning and *Cyanobacteriales* at the end of the batches) were the only ones that showed statistically significant correlations with the parameters considered (Table Sx). The *Planktothrix.NIVA.CYA.15.mougeotii* (71% at initial samples) had positive correlations with PO_4 ($p = 0.0001$), TN ($p = 0.0015$), TKN ($p = 0.0005$), NH_4N ($p = 0.003$), and a negative correlation with NO_2 ($p = 0.013$). Essa análise indica que existe a possibilidade *Planktothrix.NIVA.CYA.15.mougeotii* de não ser sensível aos micropoluentes como indicava análise anterior, mas talvez seja só sucessão por conta da variação de nutrientes ao longo da batelada.

The Cyanobacteriales (29% at final samples) had negative correlations with TP ($p = 0.042$), PO_4 ($p = 0.042$), TN ($p = 0.025$), and TKN ($p = 0.022$).

Em relação as bactérias, 20 apresentaram correlações com os parâmetros. Fimicutes, que dominou nos reatores no início da operação (90%) e no final (62%) apresentou correlações estatisticamente significativas e positivas com PO_4 ($p = 0.026$), TN ($p = 0.011$), TKN ($p = 0.006$), NH_4N ($p = 0.0002$), and turbidity ($p = 0.0016$); and negative correlations with NO_2 ($p = 0.0007$), and DO ($p = 0.032$). Proteobacteria, that occurred in 30% of samples from the end of operations, also had positive correlations with NO_2 ($p = 0.002$), and DO ($p = 0.038$); and also, negative correlations with TN ($p = 0.006$), PO_4 ($p = 0.011$), turbidity ($p = 0.003$), and TKN ($p = 0.004$).

3.3. Predictive Metabolic pathways

Evaluating the samplings of all 16S rDNA sequences, there was a difference in the metabolic pathway predominance considering the operation time of the FP-PR (initial and final of each batch). At initial of batches the ammonia oxidation represents 53%, dehalogenation (52%); and chitin degradation (49%). At the end of batches, despite the same dominant metabolisms, the percentages were different, being 56% of ammonia oxidation, 48% of dehalogenation, and 40% of chitin degradation.

Regarding the presence of micropollutants or not, there was no difference between the types of dominant metabolisms or the percentages of contribution/action of each, with the same metabolisms already mentioned prevailing (ammonia oxidation, chitin degradation,

and dehalogenation). However, due to the reduction in the proportion of one metabolism to the other, based on the ecological tests carried out in the previous items, it is possible that such a reduction occurred due to the change in the community, mainly from anaerobic to aerobic.

Considering just the Archaea group, the metabolic pathway predominant was methanogen (initial = 90%; final = 89%; with micropollutants = 91%; without micropollutants = 88%).

4. Conclusions

The metabarcoding of 16S rDNA and 18S rDNA genes revealed the dynamics of both eukaryotic and prokaryotic communities in a flat panel closed PBR for sanitary sewage treatment. There was a statistically difference in terms of composition both, for eukaryotic and prokaryotic communities, considering the initial and the final time of each batch operation, revealing changes in the composition of the communities, and consequently in their function in the system. However, there were no statistical difference in composition for both eukaryotic and prokaryotic communities, considering the presence of the 13 selected micropollutants added. Although, it is noted a slight difference not statistically proved in changes in terms of composition of bacteria. Furthermore, Patescibacteria seems to be an indicator of the micropollutants presence in the samples.

Into the bacteria group the Firmicutes (%) and Proteobacteria (%) were the most abundant bacterial phyla; in archaea group the XX and XX were the most abundant phyla, being majority at the beginning of the treatment (funcao metabolica exercida pela maioria %). The cyanobacteria was observed in low diversity in the community (%), como descrito por outro auto rem trabalho similar. The dominant microalgae during the treatment was *Tetrademus obliquus* (<90%).

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