Microbiological Diversity in an Aerated Lagoon Treating Kraft Effluent

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The microbiological diversity of cultivable bacteria was analyzed in an aerated facultative lagoon. The removal of specific compounds and measures of pollutant load was evaluated with isolated native bacteria, selected and identified in kraft cellulose effluent. The system was operated with an organic loading rate of 0.2 $kgCODm^{-3}d^{-1}$ for 60 days. Analyses of the fluorescence excitation-emission matrix, acute ecotoxicity, and microbiology were performed. Bioaugmentation tests were done to emphasize the removal of color, using promising species. The removals of biochemical oxygen demand, chemical oxygen demand, and total organic carbon in AFL were 94%, 51%, and 41%, respectively. Regarding color, removal was up to 4%, and the total phenolic compounds were not removed through biological treatment. The treatment also decreased turbidity by 94% and lignin derivatives by 12%. The bacteria identified through NCBI-BLAST and statistical similarity totaled 9 species in the cellulose effluent, three of which have the potential for color treatment: *Bacillus cereus*, *Bacillus thuringiensis*, and *Paenibacillus* sp. The *Bacillus cereus* combined with biomass removed color (69%), total phenolic compounds (37%), and compounds derived from lignin (53%). These species are promising for removing specific parameters combined with biomass from biological AFL treatment systems.

Keywords: Aerated facultative lagoon; Bioaugmentation; Biological treatment; Kraft effluent; Pulp and paper mill; Recalcitrant compounds

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INTRODUCTION

The pulp and paper industry is important for the global and Brazilian economies (Ibá 2020). Pulp and paper production requires high water consumption, using 22 to 40 m^3 per ton of pulp produced (Hubbe *et al*. 2016; Ibá 2020). The resulting effluents lead to environmental contamination due to the concentration of organic matter and compounds that are difficult to degrade (Kamali and Khodaparast 2015; Peitz and Xavier 2020). Cellulose effluent is treated in various ways, including biological systems, physical-chemical processes, adsorption, advanced oxidation, and membrane filtration (Majumdar *et al*. 2019). Except for the biological ones, these methods are expensive, which often makes their application unfeasible (Kamali and Khodaparast 2015; Kamali *et al*. 2019).

Table 1. Bacterial Species Identified in the Biological Treatment of Effluent from the Pulp and Paper Industry

Treatment system	Operational conditions	Microorganisms	Parameters	Efficiency (%)	Authors
N.i.[1]	pH: 7.0 to 8.0 Temperature: 35 °C Agitation: 140 rpm HRT: 7 d	*Serratia marcescens* *Serratia liquefaciens* *Bacillus cereus*	LC Color COD BOD_5	95 65 63 64	Chandra *et al.* 2012
N.i.[1]	pH: 7.6 Temperature: 34 °C Agitation: 120 rpm HRT: 6 d	*Paenibacillus* sp.	TPC LC Color BOD_5 COD	86 54 68 83 78	Raj *et al*. 2014
Semi-batch reactor[2]	pH: 6.5 Temperature: 45 °C Agitation: 150 rpm HRT: 2.6 d	*Bacillus cereus*	Color COD BOD_5	90 61 66	Saleem e*t al.* 2014
Aerated lagoon[1]	pH: 7.4 to 7.8 Temperature: 20 °C HRT: not informed	*Bacillus thuringiensis* *Bacillus subtilis* *Runella* sp. *Legionella* sp.	-	-	Bailón-Salas *et al*. 2017
Semi-batch reactor[2]	pH: 7.0 to 8.2 Temperature: 35 °C HRT: 1.3 d	*Brevibacillus parabrevis*	LC Color COD	42 51 60	Hooda *et al.* 2018
Sequential batch reactor[1]	pH: 7.0 Temperature: 37 °C HRT: 3 d	*Bacillus* sp.	AOX TPC LC Color TOC COD BOD_5	75 88 64 73 82 86 93	Sonkar *et al*. 2019

[1] Bacteria identified in the effluent treatment system [2] Isolated bacteria used in the treatment process AOX – absorbable organohalogen compounds, N.i. – not informed, TOC – total organic carbon.

Activated sludge and aerated facultative lagoon (AFL) processes are the most used systems in the biological treatment of effluents from the pulp and paper industry (Kamali and Khodaparast 2015; Bailón-salas *et al*. 2017; Lewis *et al*. 2018). In this sense, AFLs are widely used in Brazil due to the country's favorable climatic conditions and the large availability of area for the construction of these lagoons (Von Sperling 2016).

In addition, AFLs are simple to maintain, have low cost, and remove the biochemical oxygen demand (BOD_5 between 80 and 95%) and the chemical oxygen demand (COD between 40 and 60%) in different types of effluents. Aerated facultative lagoons are stable in relation to shock loads, distributing the excess over their length, and have long hydraulic retention time (HRT) (2 to 10 days) (Swamy *et al*. 2011; Subashini 2015). In kraft effluents, lignin compounds and their derivatives persist in the cellulose effluent because of their recalcitrance. Therefore, efforts have been made to optimize this process for the removal of these specific compounds (Machado e*t al*. 2018).

The bioaugmentation is based on the spontaneous and controlled action of microorganisms to increase their quantity and be able to degrade pollutants from soil, water bodies, and industrial effluents process (Ardeleanu 2011). This process is an alternative to improving the performance of AFL treatment in the removal of specific compounds, and it is a sustainable technology with a good benefit-cost ratio (Hossain and Ismail 2015). The challenge is to select the best microorganism to degrade the specific compounds in pulp industry effluents (Ghribi *et al*. 2016; Bailón-salas *et al.* 2017). Table 1 lists studies with specific bacteria used to remove recalcitrant compounds present in cellulose effluents, such as lignin compounds (LC) and their derivatives, aromatic compounds (AC), total phenolic compounds (TPC), and color, which show the efficiency in the use of bacteria for such treatment.

Among the microorganisms found in aerobic biological treatment systems are *Bacillus* sp., *Brevibacillus parabrevis*, *Paenibacillus* sp., and *Serratia liquefaciens*, which are efficient in the degradation of specific and hard-to-degrade compounds contained in cellulose effluents (Hooda e*t al*. 2018; Singh *et al*. 2019; Sonkar *et al.* 2019).

The objective of this research was to evaluate the bacterial diversity in an AFL system with an organic loading rate (OLR) of 0.2 $kgCODm^{-3}d^{-1}$ and the removal of specific compounds through bioaugmentation with isolated native bacteria, selected and identified contained in kraft cellulose effluent.

EXPERIMENTAL

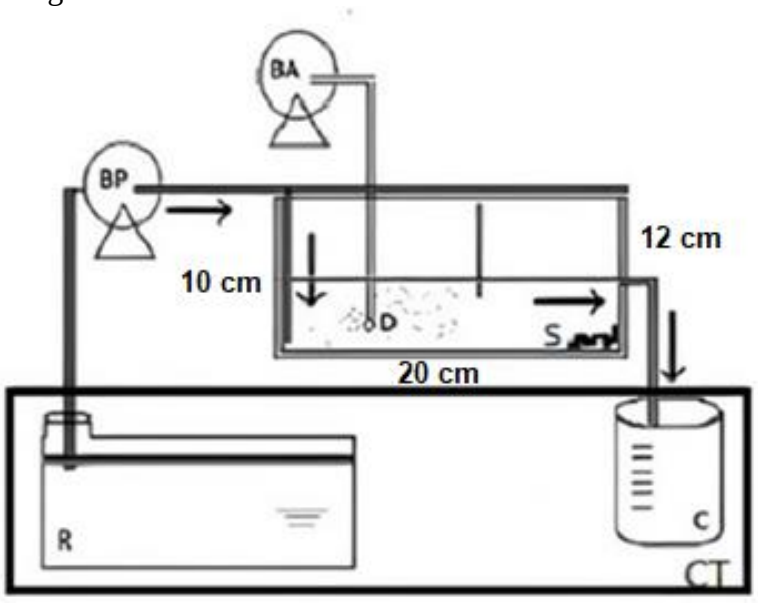
Effluent Sample Collection

The AFL influent was provided by an unbleached kraft pulp mill based in the metropolitan region of Curitiba, in the state of Paraná, Brazil. The samples were collected at the entrance of the treatment system, before primary settling basin, which is followed by biological treatment through aerated facultative and maturation lagoons. The samples were transported in 20 L containers and stored at 4 °C in the absence of light (Apha 2017). For treatment in the AFL, two sample collections were performed (Sample 1), and for bioaugmentation tests, a third sample (Sample 2) was collected.

Laboratory-scale aerated facultative lagoon

The AFL treatment was operated for 60 d with an OLR of 0.2 $kgCODm^{-3}d^{-1}$, and the addition of nutrients to the AFL influent remained in the proportion of 100:0.5:0.1 for

COD:N:P, based on the ratio applied by the mill that provided the samples. The concentration of inoculated sludge was 70 mgL^{-1} (Von Sperling 2014). A Lutron Oxygen Meter DO-5519 (Taipei, Taiwan) was used in order to measure dissolved oxygen (DO), ICEL OR-2300 (Haifa, Israel) was used for redox potential (RP), and pH Meter CienlaB mPA-210 (Piracicaba, Brazil) for the pH. The system developed in laboratory conditions is represented in Fig. 1.



SUBTITLE

BA – Aeration pump	**CT –** Cool box	**S** – Sedimentation zone
BP – Peristaltic pump	**D –** Air diffuser	→ - Flow direction
C – Treated effluent collector	**R** – Affluent reservoir	

Fig. 1. Bench AFL scheme

Characterization of cellulose effluent samples

The sample provided by the industry and also the influent and effluent from AFL treatment were analysed. The samples were filtered with a nitrocellulose filter of 0.45 μm pore size and analyzed using the parameters of BOD_5, COD, TOC, color (Vis_{440nm}), turbidity, TPC, aromatic compounds (UV_{254nm}), and compounds derived from lignin, that is, lignins (UV_{280nm}) and lignosulfonic ones (UV_{346nm}) (Çeçen 2003; Chamorro *et al*. 2009; Apha 2017). The color sample was analyzed at pH 9.0, and the sample of aromatic compounds and lignin derivatives was analyzed at pH 7.0 (Çeçen 2003), both using a Varian UV-VIS Cary-50 Spectrophotometer (Santa Clara, United States of America). All analyses were performed in triplicate.

In addition, the following analyses were also performed when the AFL reached steady-state: Fluorescence excitation-emission matrix (EEM), total and volatile suspended solids (TSS and VSS) on the biomass, and acute ecotoxicity of both the influent and effluent of the treatment with *Daphnia magna* (ABNT NBR 12713 2016).

Microbiological Diversity Analysis

Microbiological analyses were performed on biomass samples taken from the AFL at steady-state and guided by molecular identification based on 16S rRNA gene sequence analysis. First, bacteria were isolated from biomass using nutrient agar medium (Himedia,

Mumbai, Índia) after incubation of plates at 30 °C for 48 h. Genomic DNA was extracted and purified from isolates in the kraft effluent (Vicente *et al*. 2008) and 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal primers 968F/1392R for the bacteria domain. Afterwards, PCR fragments were analyzed by electrophoresis through 1% agarose gels for 16S rRNA products followed by purification and sequencing on a Illumina MiSeq platform using the MiSeq Reagent Kit v3 600 (San Diego, USA).

Bacteria were identified by sequence alignment using the nucleotide Basic Local Alignment Search Tool (BLASTN) against the nucleotide collection database (NCBI database, https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Bioaugmentation Test

The bioaugmentation test was performed as presented by Saleem *et al*. (2014). Initially, *Bacillus cereus*, *Bacillus thuringiensis*, and *Paenibacillus* sp. were plated; subsequently, 2 mL of the solution of each isolated species were added to a 250 mL Erlenmeyer flask containing 20 mL of nutrient broth. These were stirred at 40 rpm in a shaker at 30 °C, and their growth was monitored every 1 h through the optical density analysis of the samples using a UV-Vis spectrophotometer at a wavelength of 600 nm (Bombardi *et al*. 2018). The results of these measurements were analyzed using the Chem Agilent software to determine the concentration of the colony-forming unit (CFU mL^{-1}).

In the bioaugmentation process, the combinations shown in Fig. 2 were under aeration and agitation at 70 rpm in a shaker, at a temperature of 25 °C, for 2.1 d in order to have OLR conditions similar to those of the AFL with 0.2 $kgCODm^{-3}d^{-1}$. In experiments containing biomass, 70 mg VSS L^{-1} was used in each system. The influent of each system was 100 mL of the sample from the kraft pulp mill, with pH adjusted to 7.0 (0.02) and addition of nutrients for COD:N:P of 100:0.5:0.1. Figure 2 shows a flowchart with the layout of the tests for the bioaugmentation treatment.

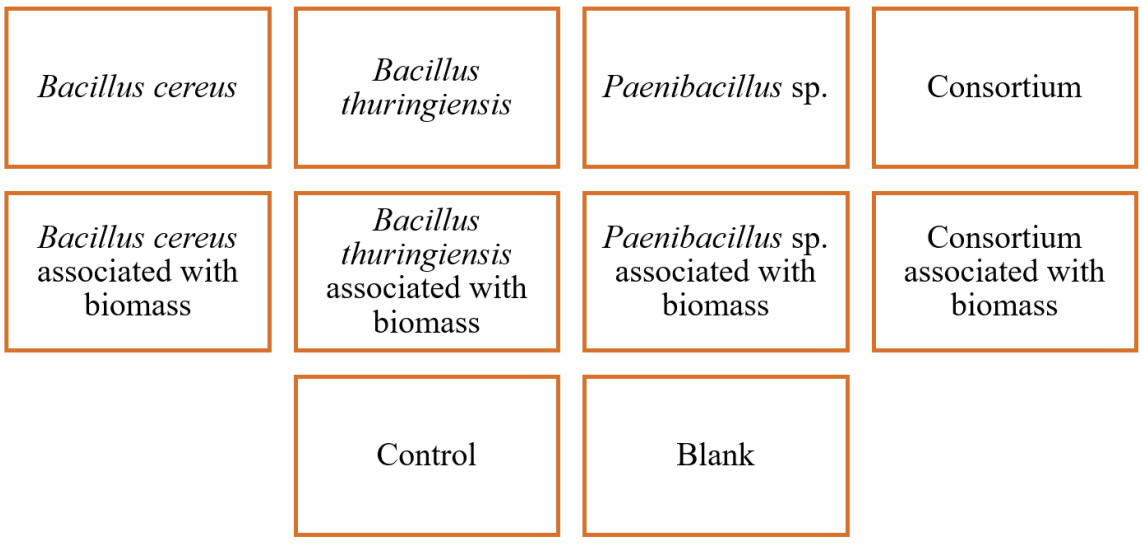


Fig. 2. Layout of bioaugmentation tests

The isolated bacteria, isolated bacteria combined with biomass, consortium, and consortium combined with biomass were tested in triplicate. A blank was carried out containing only neutralized influent with the addition of nutrients, while in the control, in

addition to the aforementioned content, 70 mgVSSL^{-1} of biomass were present.

The efficiency of the treatment was evaluated according to the removal of BOD_5, COD, TPC, color, aromatic compounds, and compounds derived from lignin, that is, lignin and lignosulfonic compounds.

RESULTS AND DISCUSSION

Sample Characterization

Results for characterization of Samples 1 and 2 provided by the pulp mill are shown in Table 2. Sample 1 was used during the treatment with OLR of 0.2 kg COD m^{-3} d^{-1}, and Sample 2, in the bioaugmentation test. The influent of Sample 1 presented a BOD_5 / COD ratio of 0.28 and Sample 2 of 0.33. According to Jordão and Pessoa (2016), the values above 0.30 suggest good biodegradability, being favorable for biological treatment.

Table 2. Characterization of the Cellulose Industry Sample

Parameters	Sample I (n = 2)	Sample II (n = 1)
pH	7.50 (0.20)	7.32 (0.10)
COD (mg L^{-1})	440.89 (4.80)	360.00 (3.50)
BOD_5 (mg L^{-1})	124.74 (2.70)	120.40 (2.10)
BOD_5/COD	0.28	0.33
TOC (mg L^{-1})	102.47 (2.19)	-
TPC (mg L^{-1})	174.82 (3.40)	295.11 (3.24)
Color (Vis_{440nm})	0.31 (0.10)	0.28 (0.01)
AC (UV_{254nm})	2.65 (0.24)	2.83 (0.01)
LC (UV_{280nm})	2.85 (0.30)	2.50 (0.49)
LSC (UV_{346nm})	1.05 (0.25)	0.78 (0.09)
The values presented in the table are averages of the results obtained from the characterization analyzes of the tributary used during the treatment, carried out in triplicate. In parentheses are the standard deviations of these results. n - number of samples, LSC – lignosulfonic compounds.		

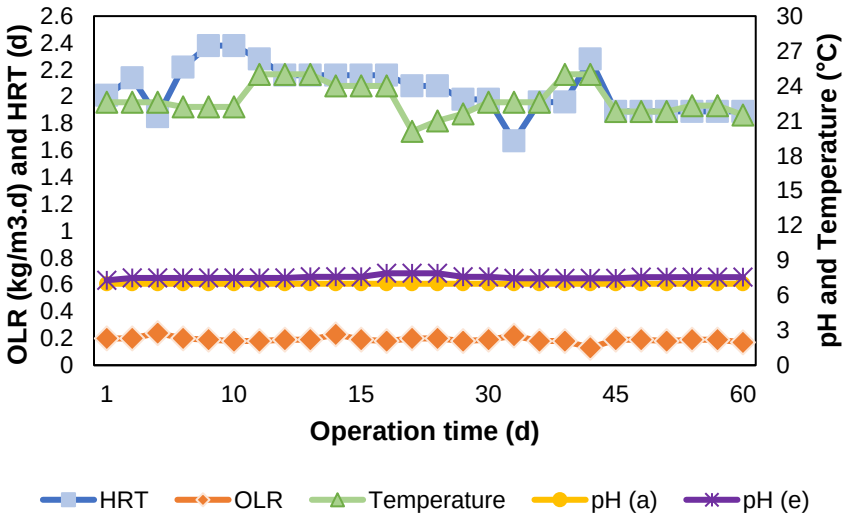


Fig. 3. Control parameters of AFL. OLR, organic loading rate; HRT, hydraulic retention time

AFL operating parameters

Figure 3 contains data on the AFL control parameters in relation to organic loading rate, pH, temperature, and hydraulic retention time. The average organic loading rate was 0.19 (0.02) kgCODm^{-3}d^{-1}, which is close to the predicted value that resulted in an average HRT of 2.1 d. The average temperature was 23.0 °C. The influent pH was set at 7.0 to enter the AFL treatment system; however, the average pH measured in the treatment effluent was 7.4. The RP measured in the AFL aerated zone was 42.0 mV, while in the sedimentation zone it was -23.5 mV, which is in line with the AFL system, in which an anoxic environment is observed in the sedimentation zone (Metcalf and Eddy 2016).

Evaluation of organic matter removal

Figure 4 shows the organic matter removal in terms of BOD_5 and COD. The average values of BOD_5 removal were greater than 90%, which is in line with previous reports, 50 to 95% of BOD_5 removal in systems and conditions similar to those employed in this study (Machado *et al*. 2018; Peitz and Xavier 2020).

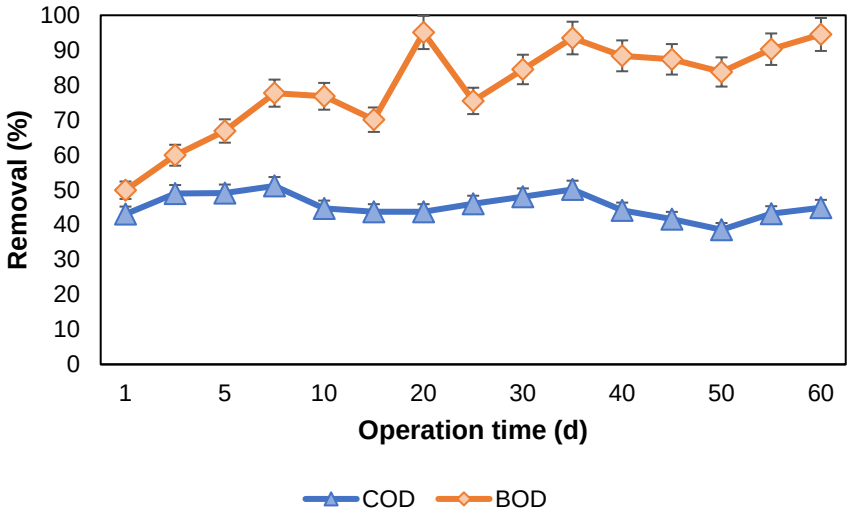


Fig. 4. Removal of organic matter in relation to BOD_5 and COD

The COD removal ranged between 40 and 60% during the 60 d of operation. This result was similar to that obtained by Machado e*t al.* (2018) using the organic load rate of 0.2 kgCODm^{-3}d^{-1} in an aerated lagoon. Hubbe *et al*. (2016) and Kamali *et al*. (2019) reported the difficulty that biological systems present in treating cellulose effluents because the efficiency of COD removal is usually around 50%.

The analysis of TOC removal averaged 49% for the AFL with OLR of 0.2 kgCODm^{-3}d^{-1}. The results obtained in such OLR were similar to the result found by Lewis *et al*. (2018) in an aerated facultative lagoon.

In the state of Paraná, Brazil, the *Conselho Estadual do Meio Ambiente* [State Council for the Environment] (CEMA) establishes that for the pulp and paper industry, the limit of BOD_5 in the effluent discharged into water bodies should be 50 mg L^{-1}, and the COD should be 300 mg L^{-1} (CEMA Resolution 070/2009). Thus, the treatment performed was effective in adapting the effluent to the discharge criteria based on global organic matter.

Evaluation of the AFL specific compounds, color, and turbidity

Figure 5 presents the analysis of specific compounds, namely: total phenolic compounds, aromatic compounds, lignin compounds, lignosulfonic compounds, in addition to the AFL color and turbidity parameters.

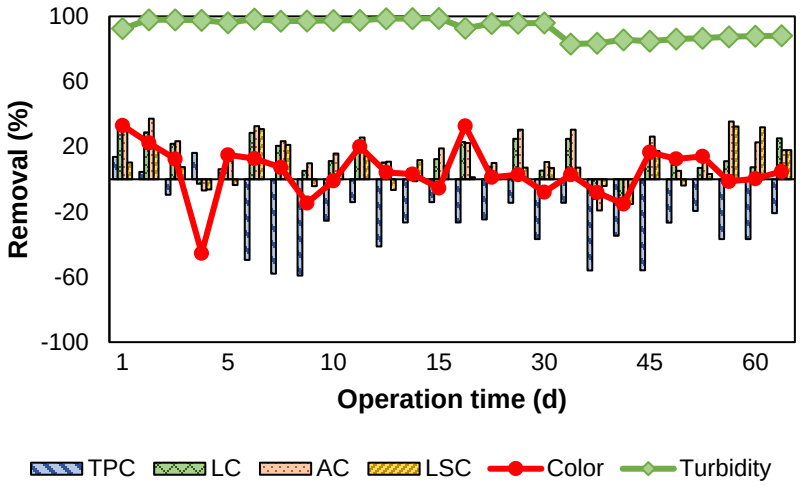


Fig. 5. Evaluation of removal of specific compounds, color, and turbidity

Figure 5 shows that the TPC increased during the AFL treatment with an average of 26%. Some studies with kraft effluent showed an increase in total phenolic compounds in aerated biological systems (Chamorro *et al*. 2009; Duarte e*t al*. 2018; Machado e*t al*. 2018; Melchiors 2019; Peitz and Xavier 2020).

In relation to the other specific compounds of the kraft cellulose effluent, the removal of lignin compounds was around 13%. For aromatic compounds, the average removal was 16%, and the lignosulfonic compounds had an average removal of 8%.

The data verified that there was no color removal. The increase observed in the first 10 d of operation is related to the stabilization of the treatment system. The increase in color may be related to the process of biotransformation of chromophoric units and the condensation of color-forming compounds, without mineralization of the effluent (Lewis *et al*. 2018; Peitz and Xavier 2020). Low color removal has been observed during treatment by aerated lagoons (Kamali and Khodaparast 2015; Peitz and Xavier 2020).

Regarding the removal of turbidity, the system showed an average removal of 94%. In general, the AFL system showed good removal in this parameter, indicating potential for clarification of the effluent in the AFL sedimentation zone.

C:N:P ratio and the performance of aerated lagoons

In relation to the C:N:P ratio used, the AFL efficiency used in this study was compared with studies using different nutrient ratios. Table 3 shows the performance of aerated lagoons treating kraft cellulose effluent with different COD:N:P ratios.

In the studies by Machado *et al*. (2018) and Peitz and Xavier (2020), the nutrient ratio was 100:5:1; in the present study, it was 100:0.5:0.1, which is more similar to that of an AFL and to what is actually employed by the industry. The removal of biodegradable organic matter (BOD_5) was greater in this study than in those with more use of nutrients. The lower color removal, however, may be associated with low redox potential in the AFL

sedimentation zone. In general, it was observed that the demand for nutrients can be optimized, which can result in savings in the treatment process.

Table 3. Comparison of Removal of Parameters

Removal (%)			
Parameters	**100:0.5:0.1**[1]	**100:5:1**[2]	**100:5:1**[3]
BOD_5 (mg L^{-1})	94	87	75
COD (mg L^{-1})	51	52	50
TOC (mg L^{-1})	49	-	-
TPC (mg L^{-1})	-26	24	-20
Color (Vis_{440nm})	4	7	12
LC (UV_{280nm})	13	18	16
Turbidity (NTU)	94	97	-
DO (mg L^{-1})	3.9	6.3	4.0

[1] in this research, [2] Machado e*t al.* (2018), [3] Peitz and Xavier (2020). The values presented in the table are averages of the results obtained from the analyzes during the treatment. NTU – nephelometric turbidity units, Negative values indicate an increase in the parameter.

Analysis of fluorescence excitation-emission matrix (EEM)

Figure 6 shows the fluorescence excitation-emission matrices (EEM) of the treatment system. Fluorescence intensity (FI) is expressed in arbitrary units (a.u.).

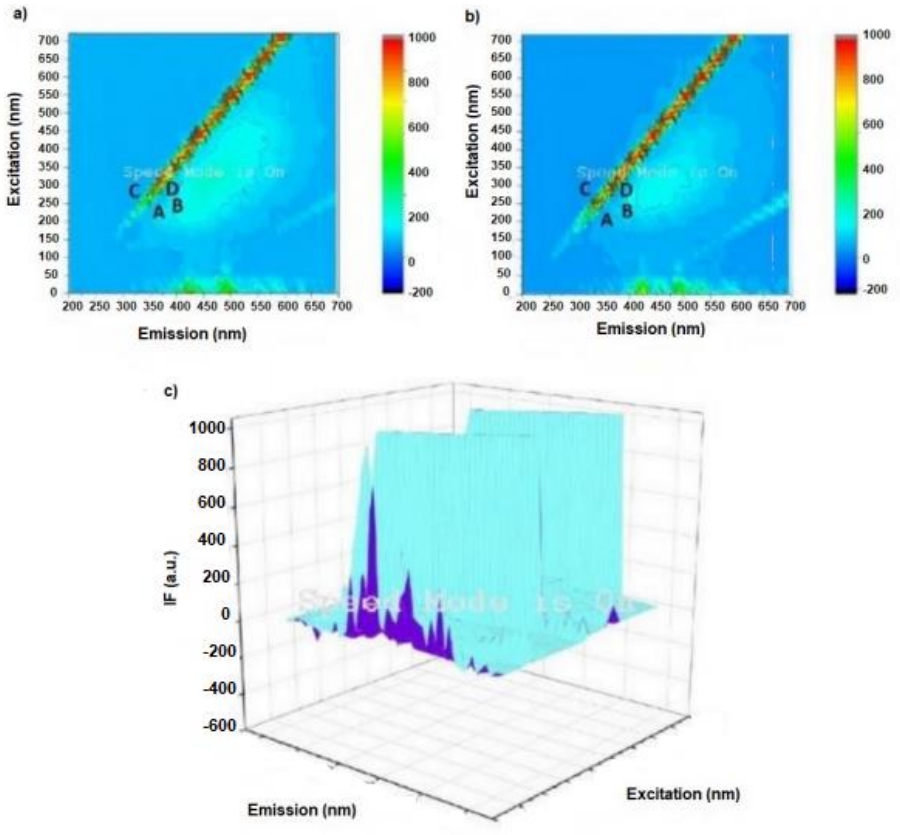


Fig. 6. Fluorescence excitation-emission matrices of the treatment system by LAF a) EEM's affluent, b) EEM's effluent, c) 3D spectrum of fluorescence intensity (FI) of the fluorogenic compounds removed and increased during treatment in the AFL system

Figures 6a and 6b show different fluorescence peaks, represented by excitation (λ_{EX}) and emission (λ_{EM}) wavelengths. The observed peaks were named A, B, C, and D. Peaks A and B, located in the λ_{EM} < 380 nm region, were identified by Carstea *et al*. (2016) in several EEMs obtained from wastewater. These peaks are associated with by-products of organic matter biodegradation (Bridgeman *et al*. 2013). Peak C is related to the common chemical characteristics of cellulose industry effluents, such as compounds derived from lignin (Managó 2019). Peak D is related to fluorogenic compounds. Comparing Fig. 6a and 6b, it is possible to observe a small removal of the aforementioned compounds.

Figure 6c shows the 3D spectrum of the difference between the fluorescence intensity emitted by the AFL influent and effluent. The peaks of positive FI represent the fluorogenic compounds removed in the treatment, and negative FI indicates the intensity increased during the treatment (Melchiors 2019). The presentation of this spectrum corroborates the results of the present research, such as color production in the anoxic zone, TPC in the aerated zone, and the low removal of lignin derivatives. Other authors have also encountered the removal of fluorogenic compounds in cellulose effluents (Janhom *et al*. 2011; Murphy *et al*. 2011; Carstea *et al*. 2016; Melchiors 2019).

Biomass analysis

Regarding the biomass of the treatment system, it was observed that after 60 d of operation in the lagoon, the VSS reached 770 mg L^{-1} and the TSS was 1181 mg L^{-1}. The average VSS/TSS ratio was 0.67, which indicates a stabilized biomass (Von Sperling 2014). The observed growth rate was 1100% and is comparable to that observed by Peitz 2018, in which the biomass grew from 70 mg L^{-1} of VSS to 1783 mg L^{-1} in 60 days of operation in an aerated lagoon, containing support medium with an OLR similar to the one in the present study.

Ecotoxicity analysis

The results of acute ecotoxicity were carried out with *Daphnia magna* with a 48 h exposure to the influent and the effluent treated by AFL. The toxicity factor obtained was 1 (TF = 1), which shows that the sample of effluent obtained from the pulp and paper industry did not present acute toxicity even at 100% concentration. These results are in line with those found by Machado *et al.* (2018) and Peitz and Xavier (2019) with *Daphnia magna* exposed to the same type of effluent. Thus, these data comply with the current state legislation by CEMA, resolution Nº 081/10 (CEMA Resolution 081/2010 (2010).).

Identification of Bacteria Contained in the Effluent

The comparison of the 16S rRNA gene sequences of the bacteria were carried out against the NCBI database to find regions of identity with statistical significance between deposited sequences. Table 4 shows the isolated bacteria identified (≥97% of query coverage) in the biomass of the AFL treating kraft pulp effluent. The total number of microorganisms collected from the aerated and sedimentation zones was 9, which were species of bacteria, as shown in Table 4.

The microorganisms identified in the AFL biomass have also been found in other studies with bacteria, such as the ones by Chandra *et al.* (2012), Raj *et al*. (2014), Saleem *et al.* (2014), Bailón-Salas *et al*. (2017), and Sonkar *et al*. (2019). Among such microorganisms, there was an emphasis on *Bacillus cereus* as promising for color removal in cellulose effluent, as suggested by Saleem e*t al*. (2014).

Table 4. Bacteria Identified in the AFL

Anaerobic Zone	NCBI Accession Number	Percent Identity (%)	Aerated Zone	Percent Identity (%)	NCBI Accession Number
Acinetobacter junii	AJ786647.1	98	-	-	
Bacillus anthracis	NR_041248.1	99	-	-	
Bacillus cereus	AB050631.1	98	-	-	
Bacillus thuringiensis	NR_114581.1	98	*Bacillus thuringiensis*	97	NR_114581.1
Cytobacillus kochii	MW358143.1	98	*Brevibacillus choshinensis*	98	NR_115590.1
Paenibacillus sp.	NR_115597.1	97	*Lysinibacillus mangiferihumi*	98	NR_118146.1
Sphingomonas koreensis	NR_024998.1	97	-	-	

Bioaugmentation Test

Subsequently, bioaugmentation tests were performed to emphasize the removal of color, using the following species: *Bacillus cereus*, *Bacillus thuringiensis*, and *Paenibacillus* sp. This parameter was considered to be a challenge for biological treatment due to the processes of depolymerization and molecular repolymerization in the different conditions employed. Following the increase in the concentration of bacteria, it was observed that the maximum growth occurred in 1.5 h for *Bacillus cereus* and *Bacillus thuringienses*, and in 6 h for *Paenibacillus* sp. under incubation conditions. The bacteria concentration was 6.2×10^8, 6.4×10^8, and 6.3×10^8 CFU mL^{-1} for the three species, respectively.

In the bioaugmentation, both organic matter and specific compounds were removed. In Fig. 7, these results are presented from the tests with *Bacillus cereus*, *Bacillus cereus* coupled with biomass, *Bacillus thuringiensis*, *Bacillus thuringiensis* coupled with biomass, *Paenibacillus* sp., *Paenibacillus* sp. coupled with biomass, mixed (*Bacillus cereus*, *Bacillus thuringiensis*, and *Paenibacillus* sp.), mixed coupled with biomass, blank and control.

Despite the performance of *Paenibacillus* sp. combined with biomass having been the best system for the removal of global organic matter, the treatment of specific compounds, especially color, was better in the system in which *Bacillus cereus* combined with biomass was used. Under these conditions, besides color (69%), TPC (37%), LC (53%), AC (50%), and lignosulfonic compounds (49%) were also removed. In the control test, the use of aeration alone caused an increase in the TPC in the medium, as observed in other studies (Chamorro *et al*. 2010; Melchiors 2019; Peitz and Xavier 2020).

Regarding the bacteria used in the present study, it is worth mentioning that Saleem *et al.* 2014 used *Bacillus cereus* alone for the treatment of cellulose effluent, removing BOD_5, COD, and color by 66%, 61%, and 90%, respectively, with pH 6.5 in a batch reactor. Chandra e*t al.* (2012) also employed *Bacillus cereus* in the treatment of cellulose effluent; however, they combined it with *Serratia marcescens* and *Serratia liquifaciens*, which had been identified in a cellulose effluent treatment system. They obtained the removal of 65% for color, 63% for TPC, 63% for COD, and 64% for BOD_5 for a 7-day HRT. With these results, it is possible to affirm that the present bioaugmentation study using *Bacillus cereus* combined with biomass was close to that observed in the literature.

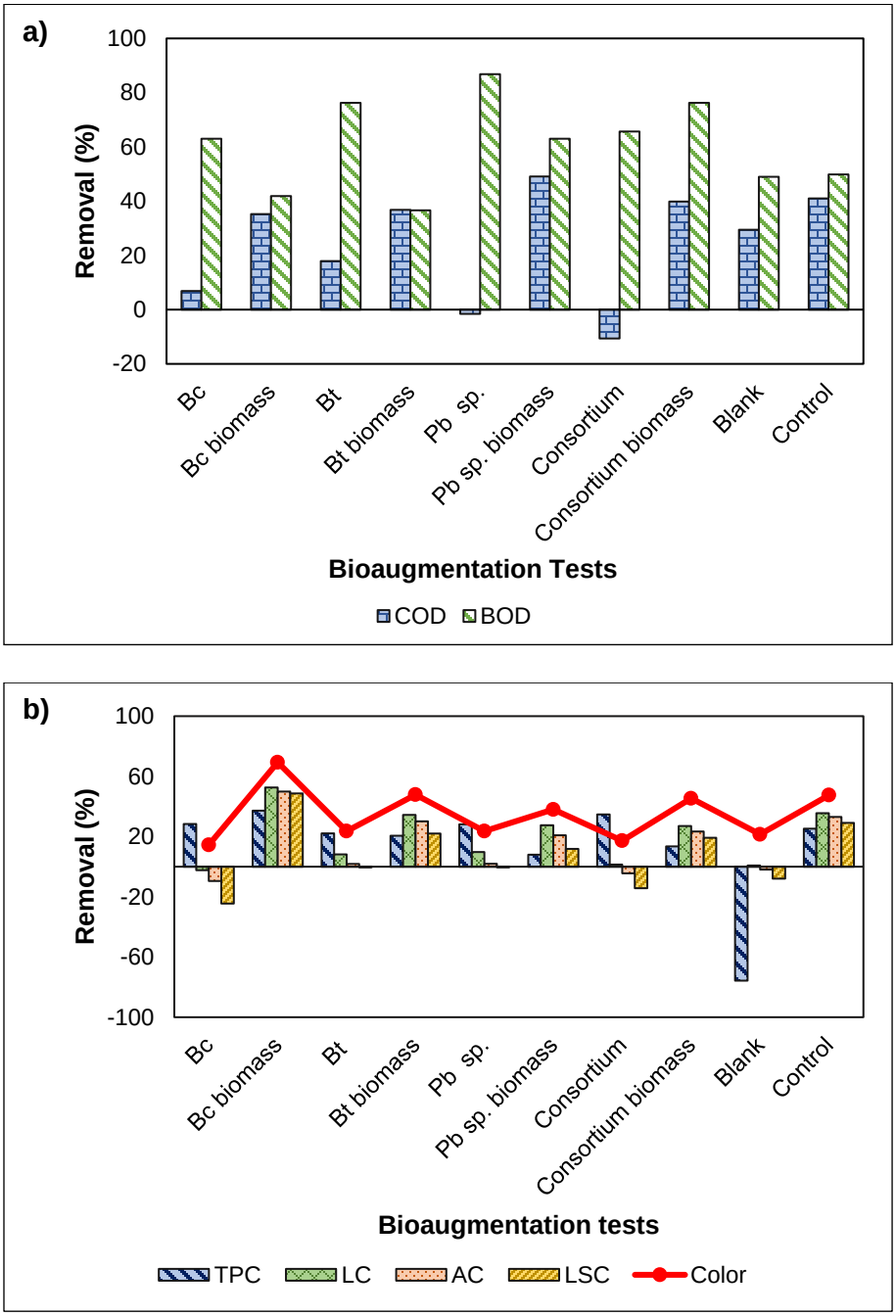


Fig. 7. Evaluation of removal of organic matter and specific compounds in bioaugmentation a) removal of organic matter in BOD_5, and COD b) removal of specific compounds from kraft effluent, BC – *Bacillus cereus* Bt – *Bacillus thuringiense* Pb – *Paenibacillus* sp.

The bacterium *Paenibacillus* sp. was identified by Raj *et al*. (2014) in a batch reactor in a 6-day HRT in the treatment of cellulose effluent, in which removals of 68%, 54%, 86%, 83%, and 78% were obtained for color, lignin compounds, total phenol, BOD_5, and COD, respectively. Chandra *et al*. (2008) identified the bacteria *Bacillus* sp. and *Paenibacillus* sp. in a cellulose effluent treatment system operating with 6-day HRT, pH of 7.6 and temperature of 30 °C, in which color was removed by 65% and 48% for the bacteria used separately in treatment by bioaugmentation.

Sonkar *et al*. (2019) identified the bacterium *Bacillus thuringiensis* in a cellulose effluent treatment system and found a 99% similarity to it in a batch reactor. The removal of BOD_5, COD, TOC, and color was by 93%, 89%, 82%, and 73%, respectively, with a 3-day HRT. These species (*Bacillus cereus*, *Bacillus thuringiensis*, and *Paenibacillus* sp.), which were identified and used in the bioaugmentation tests, proved to be promising for removal of specific parameters combined with biomass from AFL biological treatment.

CONCLUSIONS

1. In this study, the microbiological diversity of a kraft effluent treatment system by an aerated lagoon was analyzed, and 9 species of bacteria were identified, three of which have the potential for color treatment: *Bacillus cereus*, *Bacillus thuringiensis*, and *Paenibacillus* sp.
2. There was efficiency by AFL in removing specific compounds from the kraft effluent with an OLR of 0.2 $kgCODm^{-3}d^{-1}$ being in the parameters of BOD_5 (94%), COD (51%), TOC (49%). Regarding color, removal was up to 4%, and the total phenolic compounds were not removed through biological treatment. The treatment also decreased turbidity by 94% and lignin derivatives by 12%.
3. In bioaugmentation tests, the treatment of specific compounds and especially the color was better in the system with *Bacillus cereus* associated with biomass, in which the removal were to color (69%), TPC (37%), LC (53%), AC (50%) and lignosulfonic compounds (49%).
4. The bioaugmentation of the *Bacillus cereus* with the biomass of the treatment system is a sustainable and innovative alternative for the treatment of kraft effluent in OLR of 0.2 kg $CODm^{-3}d^{-1}$.

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