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INGENIERÍA AMBIENTAL

Identificación de un consorcio microbiano en humedales construidos de flujo sub-superficial alimentados con aguas residuales industriales coloreadas

ENVIRONMENTAL ENGINEERING

Microbial consortium identification in constructed wetlands of horizontal subsurface flow fed with industrial wastewater colored

Erika Y. Alzate*, Laura V. Castrillón-Cano*/, Luisa F. Rúa-Vásquez*, Dania L. Rojas*, Nancy J. Pino*/**, Ruth M. Agudelo C.*§, Gustavo A. Peñuela*/****

**Grupo Salud y Ambiente GISA, Departamento de Ciencias Específicas, Facultad Nacional de Salud Pública, Universidad de Antioquia. Medellín, Colombia.*
***Grupo Diagnóstico y Control de la Contaminación GDCON, Facultad de Ingeniería, Universidad de Antioquia. Medellín, Colombia.*
erika.alzate@udea.edu.co, fernanda.rua@udea.edu.co, laura.castrillon@udea.edu.co, dania.rojas@udea.edu.co, nancy.pino@udea.edu.co, §ruth.agudelo@udea.edu.co, gustavo.penuela@udea.edu.co

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Resumen
En esta investigación se aislaron microorganismos de un consorcio microbiano, presentes en humedales construidos a escala piloto de flujo sub-superficial horizontal, empleados en el tratamiento de aguas residuales de una industria textil. Los humedales estaban plantados con *Phragmites australis* y se monitorearon parámetros fisicoquímicos como DQO, DBO_5, ST, SST, SDT, nitratos, fósforo total y metales (hierro, manganeso, zinc, cobre, plomo). Los consorcios microbianos presentes en los humedales fueron aislados por técnica de enriquecimiento selectivo a partir de una mezcla de cuatro colorantes y el consorcio que presentó una mayor degradación de color en el laboratorio fue elegido para su identificación por métodos moleculares. Del aislamiento microbiano, se realizó la evaluación de 24 consorcios, en los cuales se encontraron tasas de decoloración de más del 50%, el consorcio que mejor resultados arrojó estaba conformado por hongos y una bacteria (*Rhodotorula mucilaginosa, Galactomyces pseudocandidum, Rhodotorula* sp. y *Escherichia coli*). En promedio se presentaron remociones mayores del 50% de DQO y del 80% de DBO_5. De los cinco metales estudiados solo se encontraron trazas de manganeso y plomo.

Palabras clave: *Degradación de color, mezcla de colorantes, tratamiento de aguas residuales industriales.*

Abstract
In this research, a microbial consortium was isolated from pilot-scale horizontal subsurface flow-constructed wetlands treating rich dye textile wastewater. Wetlands were planted with *Phragmites australis*, and some parameters,including COD, BOD_5, total solids, total suspended solids, total dissolved solids, nitrates, total phosphorus, and metals (Fe, Mn, Zn, Cu, Pb), were monitored in water. Microbial consortia were isolated using an enrichment technique, featuring a mix of four dyes. A total of 24 consortia were isolated with removal percentages above 50%. The consortium with the best dye removal percentage was selected for identification by molecular techniques. This microbial consortium was composed of three different fungus strains and one bacterial strain (*Rhodotorula mucilaginosa, Galactomyces pseudocandidum, Rhodotorula* sp., and *Escherichia coli,* respectively). On average, removals of COD above 50% and BOD_5 above 80% were obtained. Of the five metals evaluated, only Mn and Pb traces were found.

Keywords: *Degradation color, dye mixture, treating rich dye textile wastewater.*

1. Introduction

Industrial processes generate a wide range of pollutants, including toxic compounds with low degradability. In developing countries such as Colombia, industrial wastewateris discharged into water resources; this is a special concern for textile and clothing industries. The dyes are compounds that are used in many textile industries; for this reason,the generated wastewater can be colored. Medellin (Colombia) is a textile city, and theindustries have an environmental obligation to treat water before it is discharged, thus avoiding pollution to water resources. In the textile industries, 2% of dyes are discarded directly into water sources and 10% are lost in the textile dyeing process (Garzón, 2009). The presence of dyes in water bodies hinders the diffusion of oxygen and light, thus altering the photosynthesis processes. Additionally, some of these compounds are considered persistent and some of their precursors or byproducts are carcinogenic, mutagenic, and genotoxic (Morgan et al., 1994). The alternatives for the treatment of colored wastewater include physical, chemical, and biological treatments; the latter is considered environmentally friendly and less expensive (Crini, 2006) given that dye removal requires the metabolism of organisms (Kalyani et al., 2008).

As an alternative to the treatment of pollutants in colored wastewater, various techniqueshave emerged that use anaerobic or aerobic processes,depending on the characteristics of the wastewater. However, thelarge investment in time and resources required in conventional treatment plants has been one of the main drawbacks to providing a satisfactory solution to this problem. Therefore, the idea to study natural,especially economic, mechanisms to depurate wastewater through biological filters (constructed wetlands) has emerged (Delgadillo et al., 2010). The first experiments to test the purification capacity of these wetlands were carried out at the Max Planck Institute in Germany in 1952 by KätheSeidel. Later, in 1972, other surface flow-constructed wetlands in Florida, Michigan, and California were studied, but the first reports of subsurface flow constructed wetlands to full scale were announced in 1974 in Wolverton (United States) and Othfresen (Germany) (Kadleck et al., 2000). The use of wetlands to remove color from the wastewater of the textile industry was explored by Bulc and Ojstršek (2008). The researchers described efficiencies in color reduction of 90% in pilot wetlands, which renders this technology an attractive option for the treatment of colored wastewater; however, knowledge of plant–microbe symbiosis between these systems makes the study of the individual components an attractive scenario.

The use of microorganisms in the removal of dyes from wastewater has been widely reported in bacteria, molds, yeasts, actinomycetes, and algae; however, theirabilities must be explored further (Chen et al., 2003; Daneshvar et al., 2007; Srinivasan and Viraraghavan, 2010). The effectiveness of microbial discoloration depends on the adaptability and selective activity of microorganisms,and it has been reported thatisolated strains of microorganisms are not effective in the degradation of dyes until complete mineralization. In contrast, microbial consortia are much more effective due to the metabolic synergy of various microorganisms when attacking various organic dyes and metabolites until complete mineralization (Chang et al., 2004; Forgacs et al., 2004; Haug et al., 1991). Therefore, studies to identify those microbial consortia that possess the ability to degrade dyes and their secondary metabolites are a subject of current interest. These studies can ultimately helpimprove the efficiency of biological treatment systems.

2. Methodology

2.1 Installation and operation of the SSF CW

Three artificial pilot-scale horizontal subsurface flow constructed wetlands (SSF CW) were built according to the design by Kadleck et al. (2000) in a textile company in eastern Antioquia, using fiberglass (length: 1.20 m; width: 0.4 m; and height: 0.6 m) and river gravel with a porosityof 53%, a coefficient of uniformity <1.7, HCL solubility <5,

and a specific gravity of 2.5 from ½" to 1" (Industrial Arenas SPC), which was placed evenly in the three modules to a height of 0.50 m. According to Delgadillo et al. (2010), in full-scale wetlands, plants should be located every 0.5m; however, we use pilot scale constructed wetlands, thus a distance of 0.20m between plants was used.A total of 9 plants/m^2 of *Phragmites australis* were planted and supplied withcolored residual water from a cooling tower at a constant flow of 35 mL/minute, reaching a water sheet of 0.45cm with a retention time of 2 days.

The modules were called H1, H2, and H3. H1 and H2 were duplicates of the treatment and H3 was a control without plants. The wetlands were fed for five months, with two previous months of acclimatization, in order to achieve good adaptation among both microbial populations and plants. Two samplings were conducted to determine the initial maturation conditions in the wetlands, and five monthly samplings were performed later, in which water samples were taken in the influent, effluent, and gravel in the inlet and outlet pressure gauges.

2.2 Equipment and methods

During each sampling,in situ parameters were determined using portable equipment to measure dissolved oxygen (DO), pH, electrical conductivity, and water temperature. COD digestion was performed in a thermal reactor (Hanna, Model C 9800), and measured in an ultraviolet–visible (UV-Vis) Evolution 300 spectrophotometer, which was also used for total phosphorus determination.BOD_5 samples were incubated at room temperature in an incubator (WTW TS 606/3-I). Nitrate (NO_3) and chlorides (Cl–) were analyzed on an ion chromatograph (Dionex, Model ICS-1000), with a conductivity detector, anion self-regenerating suppressor (ASRS) membrane 300, anion and loop 25 µL, and a column Ion Pac AS14A 4×250mm. Team plasma atomic emission-induced microwave (4100 MP-AES Agilt brand) was used for the analysis of metals (Al, Fe, Mn, Zn, Cu, and Pb). All parameters were evaluated according to standard methods (APHA, 2012).

2.3 Isolation of microbial consortia

The isolation of microbial consortia was conducted from a selective enrichment medium, taking initial samples from biofilm gravel in the inlet and outlet of piezometers of the H1, H2, and H3 as inoculums, for a total of six samples. The biofilm was obtained by stirring 10 g of gravel with 90 mL of phosphate buffer at a pH of 7.2 at 250 rpm for 20 minutes at room temperature. Then, 125 mL Erlenmeyer flasks were used containing 18 mL of medium with the following composition: 1 g/L of K2HPO4 (Merck KGaA, Darmstadt, Germany); 0.5 g/L of MgSO4.7H2O (Honeywell, Offenbach, Germany); 1 g/L of (NH4) 2SO4 (Mallinckrodt, Mexico City, Mexico), 4 g/L of anhydrous glucose (PanReac, Barcelona, Spain), 2 mL of inoculum, and a mixture of textile dyes given by the textile company (Rainofix yellow, blue, red, and black Everzol) at individual concentrations thatwere gradually increased from 40 mg/L to 100 mg/L. Two different incubation conditions at room temperature were created: shaking at 100 rpm and still; the lattercondition was differenced with an (i) that accompanied the name of the consortium. Enrichment was performed in darkness with successive additions every 3 days to reach a maximum concentration. The isolation was performed by seeding the dilutions from each flask in Luria broth (LB) agar plates; seeding was also performed on malt extract agar (Merck KGaA). The different morphotypes were isolated, purified, and pooled to build the consortia from each Erlenmeyer flask.

2.4 Removal of color consortia

A total of 24 consortia were obtained to evaluate their ability to remove dyes; they were grown in LB broth in constant agitation for 48 hours at room temperature, and they were then centrifuged at 5,000 rpm at 5°C for 10 minutes to obtain the biomass (centrifuge Boeco U510). They were subsequently washed twice with saline solution (0.9%) and re-suspended in the same solution. The concentration of microorganisms in each culture was determined at OD 620 nm. Dye removal assay was carried out in the culture medium described

To determine the percentage of color removal, the following equation (Gou et al., 2009) was used:

$$\frac{Ao - A}{Ao} x100 \quad (1)$$

where A0 is the initial absorbance and A is the final absorbance.

Measurement of absorbance A and A0 was made to 566 nm, given that this was the wavelength at which the dye mixture exhibited the greatest absorbency.

2.5 Molecular identification

The isolated microbial consortium was identified by sequencing. To achieve that aim, DNA extraction was performed with the DNA Tissue EZNA® commercial kit, and polymerase chain reaction (PCR) fragments of the 16S rRNA with primers 27f (5 'AGAGTTTGATCMTGGCTCAG 3') were amplified using 1492r (5'TACGGYTACCTTGTTACGACTT 3 '), ITS1 (5'-TCC GTA GGT GAA CCT GCG G 3'), and ITS4 (5'-TCC GCT TAT TGA TAT GC 3 ')(White et al., 1990). The amplified fragment was observed on 1.2% agarose gel. DNA sequencing was performed on Macrogen (Korea), and the sequences were compared using the Basic Local Alignment Search Tool (BLAST).

2.6 Data analysis

Statistical analysis of the data was performed using the statistical program, Statgraphics Centurion XVI, to identify significant differences between the variables and sampling points; hypotheses were established, and analysis of variance (ANOVA) was performed to test them. The level of significance was defined as 5% (*P=0.05*), and correlations between the physicochemical variables were identified through the correlation matrix with the statistical program, Stata version 12. The sequences derived from the identification of microorganisms were edited and aligned in the Geneious® software (version 8.1.3).

3. Results and discussion

3.1 Characterization of influent and effluent water

The water pH had an average minimum value of 8 and 7.7 in the influent and the effluent water, respectively 7 (CV 6% and 4%, respectively) (Table 1). These are suitable values for treatments using wetlands, as they offer an optimal pH of between 6.8 and 8.5 to support the existence of biological life (Quezadas et al., 2009).

The dissolved oxygen values were found to be low in both the influent and effluent water (1.3 mg/L in both cases) (Table 1). High content of organic matter promotes microbial growth; therefore, the value of oxygen may decrease significantly (Ong et al., 2009). This is consistent with the results obtained for both COD in the influent of 419.18 mg/L and that for the effluent of 179.69 mg/L; a decrease in oxygen concentrationwas observed when compared with the current regulation that defines a maximum value of COD discharge for industrial wastewater of 150 mg/L (Ministry of Environment, Housing and Territorial Development, 2015). Likewise, the dissolved oxygen concentration of the influent to the effluent decreases in the control wetland; the results also suggest a poor contribution of emerging plants in the oxygen supply through its root system in the gravel bed, particularly since concentration OD in the planted and unplanted wetlands are not significantly different (Ong et al., 2009).

The conductivity values vary between 4 and 5 ms/cm (CV 53% and 33%, respectively) (Table 1). This has coherence when it is compared with the high values of chlorides obtained (1,000 mg/L and 1,500 mg/L, respectively) in both the influent and effluent water of the three wetlands, as this is an inorganic ion that promotes electrical conductivity. According to the literature, a high amount of chlorides in wastewater could adversely affect biological processes in treatment systems (Calheiros et al., 2012) when taking into account the values in the influent and effluent of wetlands. It might be perceived that microorganisms were not able to degrade the analyte; however, there

is evidence that the biological processes were not affected by the high amount of chlorides in the water supply, thus leading us to obtain high percentages in the removal of organic matter.

Table 1. *Average results of In situ analysis, in constructed wetlands Riotex-Rionegro 2013-2014.*
Source: Study Results. Statgraphics Centurión XVI statistical program

Wetland	Site	pH	OD mg/L	T °C	Conductivity (ms/cm)
Wetland 1	**Affluent**	7.90	1.40	21.50	4.50
	Effluent	7.50	1.40	22.10	5.30
Wetland 2	**Affluent**	7.80	1.32	21.30	4.10
	Effluent	7.60	1.35	20.50	5.00
Wetland 3	**Affluent**	8.20	1.40	20.80	3.70
	Effluent	8.00	1.30	20.50	4.02

3.2 Physicochemical parameters

Wetlands have the great ability to remove organic matter (COD and BOD5) and SST. With respect to metals, the wetlands did not show high removal efficiency, as manganese and lead were the predominant metals in the affluent. Regarding various nutrients (nitrates and phosphorus), heterogeneity in the concentration of some sampling sites, whichexhibited accumulation in the wetlands, was observed. Therefore, it was evident that the maturation stage of the system favored its efficiency, increasing contaminant removal.

According to the statistical analysis, the concentration of COD in the wetlands averaged 419.18 mg/L in the influent and 179.69 mg/L in the effluent, with high deviation percentagesfound for the two sampling points, showing heterogeneity in the data. The average COD in the three wetlands decreased 53%.

ANOVA (Table 2) was performed to evaluate the difference between the influent and effluent, where the null hypothesis was that there are no differences between the sampling sites, with a significance level of 5%; if the P-value is <0.05, the null hypothesis is rejected. Thus, it is concluded that there are significant differences for COD in the influent and effluent.ANOVA was also performed to assess differences in the affluent of the three wetlands, where the null hypothesis was that there is no difference for the three wetlands (P=0.8823); it can be concluded that the COD concentration in the effluent of the three wetlands do not exhibit significant differences. In fact, identifying the plants had no effect on COD removal, presenting similar averages in the effluent (130.30, 145.24, and 163.54 mg/L) (Table 2). However, we can show that there was a slightly higher concentration in wetland number three, which has no plants, because the transformation of COD is essentially affected by microorganisms whose presence and activity is potentiated by the both the presence and mediated processes of the plants in the wetlands (Vymazal, 2014).

Regarding BOD5 removal, close to 80% was observed; wetland H1 had a concentration of 44.40 mg/L, wetland H2 had a concentration of 49.60 mg/L, and wetland H3 had a concentration of 52.60 mg/L, with coefficients of variation of 15%, 28%, and 67%, respectively, showing that wetland three exhibited a larger dispersion of the data (Table 2).The removal of BOD5 in the constructed wetland is mainly due to the persistence of stable levels of heterotrophic microorganisms in the influent, effluent, and gravel (Cooper et al., 1996; cited in Delgadillo et al., 2010) (Figure 1B). The concentration of organic matter is important for the efficiency of a system of wetlands; the majority of domestic wastewater has a BOD5/COD ratio >0.5, but many industrial wastewaters do not reach this value. Therefore, it is necessary to treat these wastewaters using biological processes as a primary treatment (Vymazal, 2014). The water used in this study came from a conventional treatment plant, which favored the efficiency of wetlands, because harmful contaminants were previously removed and did not affect the microbial community. This could explain why the efficient removal of organic matter yielded a BOD5/COD ratio of 0.3, which is consistent withwhat was reported by Bulc and Ojstršek (2008), who reported achieving BOD5 removals of 66% in constructed wetlands.

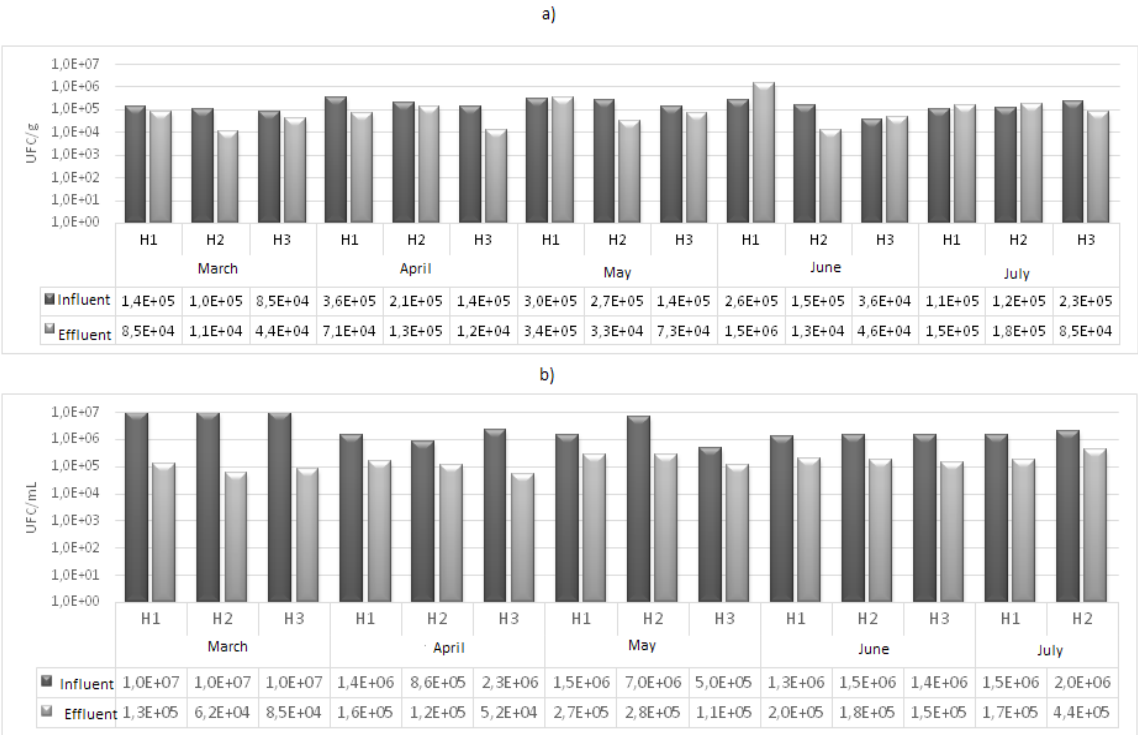

Figure 1. *Results count of heterotrophic, a) In influent and effluent of H1, H2 and H3 wetland, and b) Inlet and outlet Count of Heterotrophic in gravel of piezometer of H1, H2 and H3 wetlands.Source: Study Results.*

Regarding the solids, the highest removal was obtained for the total suspended solids (TSS) across all samples; however, it is evident that the total solids (TS) and total dissolved solids (TDS) increased in effluent, except during the fourth sampling. This could indicate that the TSS were transformed to TDS, but they were not completely removed. The TSS showed a significant trend of removal across all samples, reaching 95% in the third sampling (Table 2).

Table 2. *Concentration of COD, BOD5 and Solids of each wetland in influent and effluent.* *Source: Study Results. Statgraphics Centurión XVI statistical program *concentration in mg/L*

Humedal	Sitio	DQO	% Desviación	DBO_5	% Desviación	ST	% Desviación	SST	% Desviación	SDT	% Desviación
Humedal 1	Afluente	424.38	42	214.20	32	1569.60	30	46.80	44	1522.80	31
	Efluente	230.30	70	44.40	15	1834.80	37	16.15	46	1818.80	36
Humedal 2	Afluente	435.78	32	193.20	35	2170.80	36	45.60	55	2125.20	37
	Efluente	145.24	37	49.60	28	2236.00	28	14.40	47	1864.00	35
Humedal 3	Afluente	397.40	49	157.20	60	2074.40	24	49.60	53	2024.80	25
	Efluente	163.54	57	52.60	67	2353.60	40	12.15	26	2341.60	40

On average, in the influent of the wetlands, a manganese concentration of 0.45 mg/L was found, but the heterogeneity of the data was constant with a 73% coefficient of variation. In the effluent, the average concentration was 0.36 mg/L, with a high coefficient of variation of 85%. For lead, a concentration of 0.08 mg/L was observed in the influent, with a coefficient of variation of 71%.Furthermore, in the effluent, a concentration of 0.12 mg/L with a variation of 68% was noted.The data indicate that there was lead accumulation in the wetland given that plants and microorganisms were likely unable to remove it.

During the sampling,concentrations of iron and zinc were not detected in the effluent. Copper was found at concentrations of 0.40mg/L and 0.41 mg/L in the effluent of wetland H1 in the third and fourth sampling, respectively. Although cadmium was included in the last two samplings, it did not showsignificant concentrations.

Nitrates had a concentration of 0.52 mg/L in the effluent of wetland H2 in the fourth sampling. At the last sampling, only wetland H3 exhibited nitrate removal, which reduced from 1.96mg/L to 1.32mg/L. The limited nitrogen removal in the wetlands can be explained by its construction. According to the literature,nitrate removal is more efficient in surface-flow wetlands because ammonia is oxidized by nitrifying bacteria; conversely, the SSFCW wetlands are less efficient because aerobic conditions exist in very limited spaces,such as around the roots, or a very thin top layer, which can be given atmospheric oxygen transfer. This situation is consistent with the results observed in the study by Vymazal (2014). The removalobtained during the fifth sampling for the H3 wetland could be due to physical factors, particularly since the oxygen concentration was<2 mg/L (1.58 mg/L), but it was slightly higher than that presented in the H1 and H2 wetlands (1.56 mg/L and 1.44 mg/L). However, the amount of oxygen available in the system was not enough to sustain a process of nitrification by bacteria.

A clear trend in the behavior of phosphorus was not observed because at samplings one, three, and five, the effluent had a higher concentration of phosphorus than the influent. This means that phosphorus was not adequately adsorbed, absorbed,or precipitated. According to the literature, the removal of phosphorus is not efficient in constructed wetlands built with filter materials such as gravel and sand, because the phosphorus is mostly removed by adsorption, and both materials do not have the sufficient capacity to do it. Furthermore, there are other industrial byproducts that – based on their chemical properties – can adsorb the nutrient more efficiently than gravel. It has also been shown that precipitation is another way through which to remove contaminants in these types of wetlands (Vymazal, 2014).

Resolution 631 of March 17, 2015 (Ministry of Environment, Housing and Territorial Development, 2015) establishes the maximum permissible limits for some pollutants. According to the analysis presented in this study, the maximum allowable limits for COD, BOD5, and TSS was not fulfilled. The following levels were found: COD, 150 mg/L;and BOD5 and TSS, 50 mg/L. However, the pH level fell in a range between 6 and 9 units,which is in line with regulations. Given that lead accumulation was observed, this is not in compliance with the maximum permissible limit of 0.10 mg/L, while copper met the requirement of being 1 mg/L. The other metalsevaluatedin this study were not covered by current regulations, as the other variables were not mentioned.

3.3 Molecular identification of microbial consortium

Microorganisms were identified as *Rhodotorula mucilaginous, Galactomyces pseudocandidum, Escherichia coli*, and Rhodotorula sp., which have been previously reported in the literature as dye degrading. The yeast *Rhodotorula mucilagenosa* has been reported by Li et al. (2014) in bleaching crystal violet at different pH values and temperatures. Faryal and Hammad (2005) reported *Rhodotorula* yeasts isolated from effluent from an industry of dyes featuring high metal concentrations, while Rovati et al. (2013) isolated yeast from extreme climates. The genus *Galactomyces* was reported in dye degradation by Jadhav et al. (2008), and *E. coli* has been reported in association with *Pseudomonas aeruginosa* in the degradation of azo dyes (Isik and Sponza, 2003). The *E.coli* strain NO3 was also reported by Chang and Kuo (2000) in the degradation of azo dyes.

3.4 Evaluation of the percentage discoloration

The color removal of 24 isolated microbial consortia was evaluated. In Figure 2A,the results for the 12 consortia E3 are shown, with a percentage of

discoloration of almost 70% obtained by the consortium E9i, while the consortium E8 has the lowest percentage (less than 10%). The coefficient of variation obtained is very high after two days (55%), while after five days, it is 62%. The percentage of discoloration obtained by the E4 (Figure 2B) consortia did not exceed 60%, but all of the assessed consortia had percentages of discoloration higher than 17%. The consortium E48i, which had a higher percentage of discoloration, as well ashigher coefficients of variation, did not exceed 23%. The microorganisms identified in consortium E48i may have developed the mechanisms necessary to degrade further due toits constant exposure to pollutants, especially since the joint action of microorganisms can simultaneously attack several sites ofthe dye molecule (Su and Lin, 2013). Lade et al. (2012) compared the color removal rates obtained with a dye and textile industry effluent using a microbial consortium of microorganisms and, individually, they found better color removal percentages with a consortium mixture in both industrial wastewater and with dye. Using individual microorganisms, lower rates of discoloration, as well as the formation of toxic aromatic amines, were found.

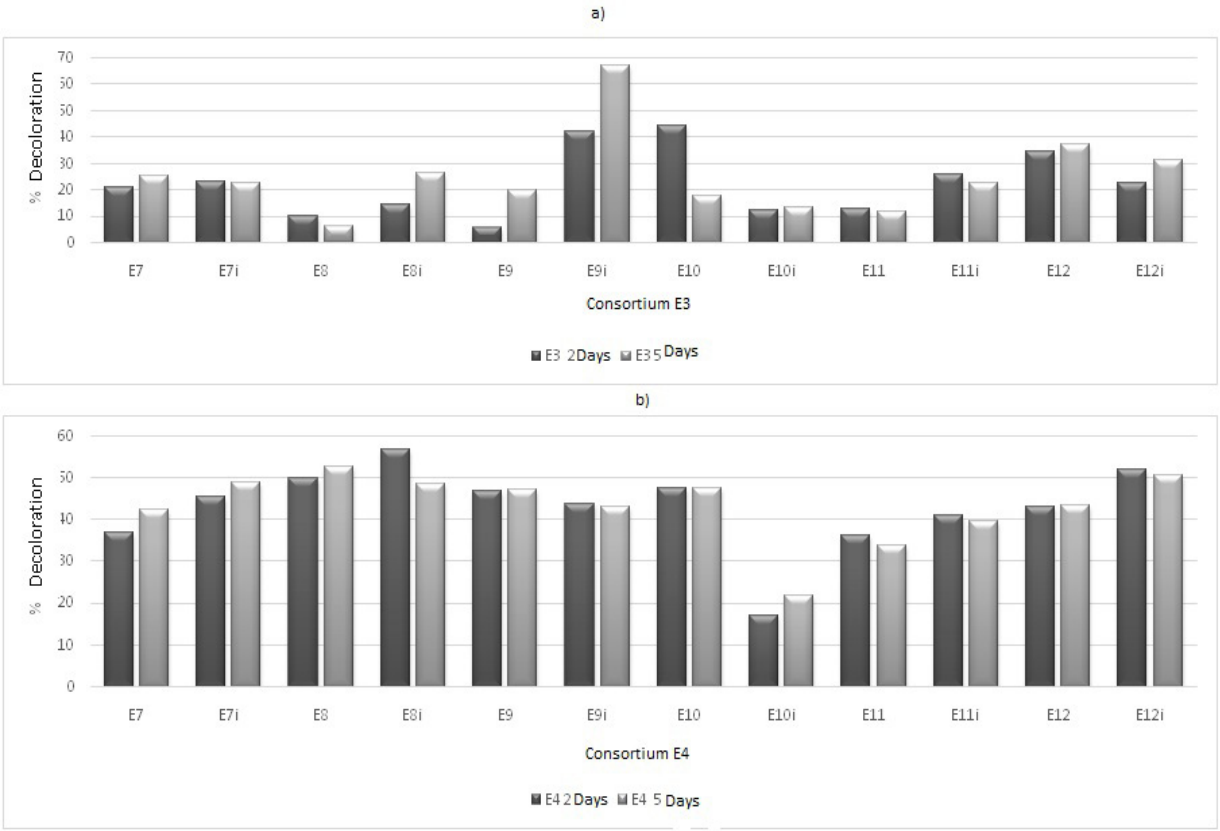

Figure 2. *Results discoloration average, a) discoloration obtained for 12 consortia E3 at 2 days and 5 days, and b) discoloration obtained for 12 consortia E4 at 2 and 5 days. Source: Study Results.*

Discoloration with microbial consortia was evaluated at two different times (days 2 and 5) in order to find out if there was an effect on the rates of discoloration. The results are shown in Figure 3A; small differences between the media were observed at 2 and 5 days, and extreme data emerged in both cases (17% and 22%, respectively). It was also observed that the minimum and maximum values (17% and 57%, respectively, at 2 days, and 22% and 52%, respectively,at 5 days) differed significantly, indicating that the percent degradation was higher at 2 days. With respect to the dispersion of the data, it was shown that after 2 days, the majority of the data were greater than 44%, which corresponds to

the median. Following degradation after 5 days, the data were grouped under the median equivalent to 45% of discoloration. The scattering data can be explained by the metabolic and structural diversity of the microorganisms that comprise each consortium.The fact that the evaluated consortia were obtained from two different samples, the called E4 correspond to the mature stage of wetlands, and therefore was further adaptation. This fact is reflected in the low dispersion rate obtained from the E4 consortium when compared with the E3 consortium.

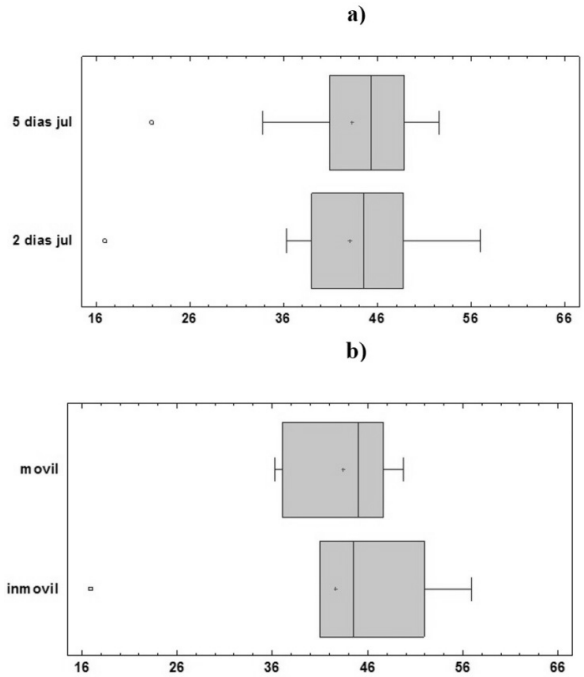

Figure 3. *Box plot incubation time at consortia, a) consortia E4 with respect to discoloration percentage and b) shaking and still incubation consortia E4, with respect to discoloration percentage.Source: Study Results.*

The effect of incubation conditions on discoloration by microorganism consortia (Figure 3B) demonstrated that there are no significant differences between the incubation conditions. Furthermore, the percentage of discoloration of the consortia was also evaluated due to the similarity in their means and medians; however,when the consortia were incubated withoutshaking, the minimum and maximum discoloration values differed significantly when compared to the conditions of shaking,having a similar behavior with data rate of fading at 2 days, since most of the data were above to median(44%). The literature reports successful cases of discoloration using both conditions. For instance, Waghmode et al. (2011) observed discoloration rates of 88% in the evaluation of a fungus with a mixture of structurally different dyes at a maximum concentration of 70 mg/L. This percentage was obtained when incubated under stirring, but the culture without stirringonly had a discoloration percentage of 49%.This could be explained bythe rapid diffusion of oxygen and substrate. Conversely, Saratale et al. (2009) evaluated the removal of a consortium formed by two microorganisms. The authors evaluated the consortium and the microorganisms individuallyfor the discoloration of a mixture of industrial dyes and azo (Scarlet R) dye under anoxic conditions without the use of stirring cultures. The researchers reported the complete discoloration of the mixture in both cases, but only the combined action of the consortium was able to act over 200 mg/L of the mixture due to increased activity of the NADH–reductase and riboflavin DCIP reductase enzymes. Jadhav et al. (2009) reported the activation of the NADH–enzyme complex in geotrichum DCIP galactomyces MTCC strain type 1360 in the presence of methyl red azo dye, which achieved complete discoloration within 1 hour of agitation. An environment with low oxygen content facilitates the breakdown of the azo group links due to a reduction mechanism, which can result in the formation of colorless solutions, as demonstrated in the study by Chen et al. (2003). In their study, the bacterium *Aeromonas hydrophila* exhibited better color removal percentages under both anoxic and anaerobic conditions, achieving 90% removal of RBN RED dye in 8 days with an initial concentration of 3,000 mg/L. Based on previous studies, we can say that both the stirring conditions, as well as the immobility test cultures, may promote discoloration under aerobic and anoxic conditions. However, it should be noted that although the reductive mechanisms seem to be most viable for breakbonds, a toxicity study must accompany the degradation kinetics to monitor the formation of toxic byproducts such as aromatic amines, whose formation occurs under anaerobic conditions (Imran et al., 2015; Franciscon et al., 2010; Saratale et al., 2011;. Solis et al., 2012).

4. Conclusions

The efficiencies obtained for organic matter removal were satisfactory, reaching 50% of COD and 80% BOD, although nutrient removal did not present with high efficiencies and no significant differences were found in planted and unplanted wetlands. In general, this treatment system the wetlands effluent accomplish with the stipulate for current regulations for dumping. This indicates that this method is useful as an optimal secondary treatment, but design changes must be made to implement it in full-scale environments, particularly in relation to the filter material, allowing for better removal of phosphorus. This can also reduce the number of plants that would yield maintenance benefits.

Additionally, the 24 consortia isolated from biofilm gravel wetlands showed bleaching percentages between 17% and 57% after 2 days of incubation. The consortium with the highest percentage of discoloration was formed by Rhodotorula mucilaginous E48i, *Galactomyces pseudocandidum*, E. coli, and *Rhodotorula* sp. The bleaching time evaluation of 2 days/5 days proved to have no effect on the percentage of discoloration, which shows promise in reducing treatment times. At the same time, evaluating incubation conditions with mobile and stationary cultures exhibited no differences; however, further research should be conducted to assess the occurrence of toxic products linked to such conditions, and to explore whether the combination of alternate aerobic–anaerobic treatments may be more efficient to not only reduce, but also remove,toxic byproducts. Finally, reducing discoloration times to 2 days, and the use of cultures, still have positive effects on reducing time and costs, particularly if future studies are expected to evaluate the behavior of the consortium isolated in a real environment.

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6. References

APHA (American Public health Association), AWWA (American Water Works Association),& WEF (Water Environment Federation). (2012) *Standard Methods for Examination of Water and Wastewater*, 22 ed. Washington D.C., USA.

Arenas Industriales LTDA (2012). *Ficha Técnica Arenas Industriales. Medellín*, Colombia.

Bulc, T.G., & Ojstršek, A. (2008).The use of constructed wetland for dye-rich textile wastewater treatment. *Journal of Hazardous Materials* 155 (1-2), 76–82.

Calheiros,C.S., Quitério, P.V., Silva, G., Crispim., L.F., Brix H., Moura, S.C., & Castro, P.M. (2012). Use of constructed wetland systems with Arundo and Sarcocornia for polishing high salinity tannery wastewater. *Journal of Environmental Management* 95 (1), 66-71.

Chang, J.S., Chen, B.Y., & Lin, Y.S. (2004). Stimulation of bacterial decolorization of an azo dye by extracellular metabolites from Escherichia coli strain NO3. *Bioresource Technology* 91 (3), 243-248.

Chang, J.S. & Kuo T.S. (2000). Kinetics of bacterial decolorization of azo dye with Escherichia coli NO3. *Bioresource Technology* 75 (2), 107-111.

Chen, K.C., Wu, J.Y., Liou D.J. & Hwang S.J. (2003). Decolorization of the textile dyes by newly isolated bacterial strains. *Journal of Biotechnology* 101 (1), 57-68.

Crini, G. (2006). Non-conventional low-cost adsorbents for dye removal: A review. *Bioresource Technology* 97 (9), 1061–1085.

Daneshvar, N., Ayazloo, M., Khataee, A.R. & Pourhassan, M. (2007). Biological Decolorization

of dye solution containing Malachite Green by microalgae Cosmarium sp. *Bioresource Techonology* (98), 1179-1182.

Delgadillo, O., Andrade M., Camacho, A., Pérez, L. & Argote, R. (2008) *Zonas húmedas construidas, una tecnología natural para la depuración de aguas residuales con fines de riego en municipios rurales y periurbanos ponencia presentad*a. En la Reunión sudamericana para manejo y sustentabilidad de riego en regiones áridas, Salvador, Bahía, Brasil, p. 24-50.

Delgadillo, O., Camacho, A., Perez, L.F. & Andrade, M. (2010). Centro Andino para la Gestión y Uso del Agua (Centro AGUA). *Depuración de aguas residuales por medio de humedales artificiales*. [online] Disponible en: http://www.aguasresiduales.info/revista/libros/depuracion-de-aguas-residuales-por-medio-de-humedales-artificiales [Accesado el día 28 de julio de 2014].

Faryal, R. & Hameed, A. (2005).Isolation and characterization of various fungal strains from textile effluent for their use in bioremediation. *Pakistan Journal of Botany* 37 (4), 1003-1008.

Forgacs, E., Cserháti, T. & Oros, G. (2004). Removal of synthetic dyes from wastewaters: a review. *Environment International* (30), 953-971.

Franciscon, E., Piubeli, F., Fatinatti-Garboggini, F., Menezes, C.R., Silva, I.S., Paulo, A.C., Grossman, M.J. & Durrant, LG. (2010). Polymerization study of the aromatic amines generated by the biodegradation of azo dyes using the laccase enzyme. *Enzyme and Microbial Technology* (46), 360-365

Garzón, R. (2009). *Cinética de degradación de colorantes textiles de diferentes clases químicas por hongos y bacterias inmovilizados sobre fibra de Agave tequilana webbwer var azul*. Trabajo de grado para optar por el título de Microbiólogo Industrial, Facultad de Ciencias, Universidad Javeriana, Bogotá D.C, Colombia.

Gou, M., Qu, Y., Zhou, J., Ma, F. & Tan, L. (2009). Azo dye decolorization by a new fungal isolate, Penicillum sp. And fungal-bacterial cocultures. *Journal of Hazardous Material* 170 (1), 341-319.

Haug, W., Schmidt, A., Nörtemann, B., Hempel, D.C., Stolz A. & Knackmuss H.J. (1991).Mineralization of the sulfonatedazo dye Mordant Yellow 3 by a 6-aminonaphthalene-2-sulfonate-degrading bacterial consortium. *Applied and Environmental Microbiology* 57 (11), 3144-3149.

Imran, M., Shaharoona, B., Crowley, D.E., Khalid, A., Hussain, S. & Arshad, M. (2015). The stability of textile azo dyes in soil and their impact in microbial phospholipid fatty acid profiles. *Ecotoxicology and Enviromental safety* (120), 163-168.

Isik, M. & Sponza, D.T. (2003). Effect of oxygen on decolorization of azo dyes by Escherichia coli and Pseudomonas sp. and fate of aromatic amines. *Process Biochemistry* 38 (1), 1183-1192.

Jadhav, S.U., Kalme, S.D. & Govindwar, S.P. (2008). Biodegradation of Methyl red by Galactomyces geotrichum MTCC1360. *International Biodeterioration & Biodegradation* (62), 135-142.

Jadhav, S.U., Ghodake, G.S.,Telke, A.A., Tamboli, D.P. & Govindwar, S.P. (2009). Degradation and Detoxification of Disperse Dye Scarlet RR by Galactomyces geotrichum MTCC 1360. *Journal Microbiology Biotechnology* 19 (4), 409–415.

Kadleck, R., Knight, R., Vymazal, J., Brix, H., Cooper, P. & Haberl R. (2000). *Constructed wetlands for pollution control: Processes, performance, design and operation*. IWA Specialist Group on use of Macrophytes in Water Pollution Control, London: IWA Publishing.

Kalyani, D.C., Patil, P.S., Jadhav, J.P. & Govindwar, S.P. (2008).Biodegradation of reactive textile dye Red BLI by an isolated bacterium Pseudomonas sp. SUK1.*Bioresource Technology* 99 (11), 4635–4641.

Lade, H. S., Waghmode, T.R., Kadam, A.A. & Govindwar, S.P. (2012). Enhanced biodegradation and detoxification of disperse azo dye Rubine GFL and textile industry effluent by defined fungal-bacterial consortium. *International Biodeterioration & Biodegradation* 72 (1), 94-107.

Li, G., Gao, J., Ding, Z., Liu, Y., Zhang, L., Gu, Z., Shi, G. & Zhang, K. (2014). Biodegradation and

decolorization of Triphenylmetano dye by *Rhodotorula mucilagenosa JB401*. *Chinese Journal of Bioprocess Engineering* 12 (3), 26-31.

MADS (Ministerio de Ambiente y Desarrollo Sostenible). (2015). *Resolución 631 del 17 de marzo de 2015. Por la cual se establecen parámetros y los valores límites máximos permisibles en los vertimientos puntuales a cuerpos de agua superficiales y a los sistemas de alcantarillado público*. Bogotá, D.C., Colombia.

Morgan, D.L., Dunnick, J.K., Goehl, T., Jokinen, M.P., Matthews, H.B., Zeiger, E. & Mennear, J.H. (1994). Summary of the national toxicology program Benzidinedye initiative. *Environmental Health Perspectives* 102 (12), 63-78.

Ong, S.A., Uchiyama, K., Inadama, D. & Yamagiwa, K. (2009). Simultaneous removal of color, organic compounds and nutrients in azo dye-containing wastewater using up-flow constructed wetland. *Journal of Hazardous Materials* 165 (1-3), 696–703.

Presidencia de la Republica de Colombia (1984). *Decreto 1594 de 1984.por el cual se reglamenta parcialmente el Título I de la Ley 09 de 1979, así como el Capítulo II del Título VI - Parte III - Libro II y el Título III de la Parte III Libro I del Decreto 2811 de 1974 en cuanto a usos del agua y residuos líquidos*. Bogotá, D.C., Colombia.

Quezadas, M. & Rodríguez, E. (2009). Evaluación tecnológica de lagunas de estabilización de Cárdenas, Tabasco. *División académica de ciencias biológicas* 26 (29), 16-29.

Rovati, J.L., Pajot, H.F., Ruberto, L., Mac Cormack, W. & Figueroa, L.I. (2013). Polyphenolic substrates and dyes degradation by yeasts from 25 de Mayo/ King George Island (Antarctica).*Wiley Online library* 30 (1), 459-470.

Saratale, R.G., Saratale, G.D., Kalyani, D.C., Chang, J.S. &. Govindwar, S.P. (2009). Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR. *Bioresource Technology* 100 (9), 2493-2500.

Saratale, R.G., Saratale, G.D., Chang, J.S. & Govindwar, S.P. (2011). Bacterial decolorization and degradation of azo dyes: A review. *Journal of the Taiwan Institute of Chemical Engineers* (42), 138-157.

Solís, M., Solís, A., Pérez, H.I., Manjarrez, N. & Flores, M. (2012). Microbial decolouration of azo dyes: A review. *Process Biochemistry* (47), 1723-1748.

Srinivasan, A. & Viraraghavan, T. (2010). Decolorization of dye wastewaters by biosorbents: A review. *Journal of Environmental Management* 91 (10), 1915-1929.

Su, W.T. & Lin, C.H. (2013). Fungal-bacterial synergism enhanced decolorization of reactive red 120 by response surface methodology. *International Biodeterioration &Biodegradation* 82, (1) 1-8.

Vymazal, J. (2014), Constructed wetlands for treatment of industrial wastewaters: A Review. *Ecological Engineering* 73 (1), 724–751.

Waghmode, T.R., Kurade, M.B. & Govindwar S.P. (2011).Time dependent degradation of mixture of structurally different azo and non azo dyes by using Galactomycesgeotrichum MTCC 1360. *International Biodeterioration& Biodegradation* 65 (3), 479-486.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990). *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: PCR Protocol: A guide to Methods and Applications (ed. M.A Innis, D.H. Gelfand, J.J Sninsky & T.J White), Academic Press: San Diego USA. (Capítulo 38).

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