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Evaluation of a Microbial Consortium and Selection of a Support in an Anaerobic Reactor Directed to the Bio-Treatment of Wastewater of the Textile Industry

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Abstract: The dyeing processes of the textile industry generate waste products such as unfixed dyes, phenolic surfactants and heavy metals. These constitute an environmental problem for the bodies receiving their wastewater due to the interruption of the lighting in the aquatic environment and the release of toxic molecules by the decomposition of the dyes. There are several treatment methods, of which biological methods are the most feasible. In the current study, the I5-ESPE microbial consortium was obtained and evaluated on the components of textile wastewater, in addition to the selection of a support for an anaerobic reactor that is directed to the treatment of effluents from the textile industry. Two microbial consortia were achieved by exposure to air in *Pseudomonas* culture medium modified with direct dyes Red 23 and Blue 106, evaluating their removal capacity of the reactive dyes Navy 171, Red 141 and Yellow 84. The consortium I5-ESPE was selected for its greatest action, yielding approximately 95% removal. Its tolerance to phenol was also determined; we reached 98% removal of chromium(VI) and 67% of total chromium under anaerobic conditions and some 25% zinc in aerobiosis. The reduction in the chemical oxygen demand (COD) was evaluated with (57.03%) and without (31.47%) aeration. The species *Staphylococcus xylosus*, *Saccharomyces cerevisiae* and *Candida tropicalis* were identified prior to treatment of textile wastewater, as well as *Enterobacter cloacae* and *Bacillus megaterium* after treatment. *Bacillus subtilis* was present throughout the process. We evaluated coconut shell as a support for an anaerobic reactor, and it demonstrated better physical characteristics than plastic and common rock, in addition to similar results in the reduction in COD of 50%, volatile suspended solids of 2545.46 mg/L and total suspended solids of 282.82 mg/L.

Keywords: textile dyes; microbial consortium; phenols; heavy metals; coconut shell; COD

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1. Introduction

Textile industries are the main users of dyes worldwide, and use 70 m^3 to 150 m^3 of water per ton of cloth dyed [1]. Thus, pollutants are discharged into the environment and directly into bodies of water without prior treatment [2]. The wastewater treatment of the textile industry constitutes an important field of scientific and technological development due to the significant environmental problems generated by these wastes due to their high oxygen demands [3], with the subsequent interruption of lighting in the aquatic environment and the release of toxic molecules by the decomposition of dyes [4]. Harmful effects occur in degumming (15%) and maceration (20%) processes such as bleaching, dyeing and washing (65%) [5]. The wastewater is characterized by being alkaline and having high levels of biochemical oxygen demand (BOD) and chemical oxygen demand

(COD). The BOD to COD ratio is typically 0.5:1 for raw domestic wastewater, and may drop to as low as 0.1:1 for a well-stabilized secondary effluent. There is no official value for the BOD/COD biodegradability index for different types of wastewaters [6]. However, reported values for the biodegradability index vary from 0.4 to 0.8 for municipal raw wastewater. The ratio can exceed 10 for industrial wastewater. Its chemical composition depends on organic compounds such as phenol; halogenated solvents; heavy metals such as chromium, copper, zinc, lead and nickel [7]; and dyes not fixed in fabrics [8].

The concentrations of dyes in the wastewater have been able to reach values between 20 and 200 mg/L. Some 60% of the textile industries use mainly reactive dyes that only fix 50% of the fabrics, and the rest exit to the wastewater [8,9]. The metals, salts, organic surfactants, sulfides and formaldehydes are added as auxiliaries to improve the adsorption of the dye to the textile fibers [10]. Phenol is the most widely used, and surfactants with nonilfenol and alkylphenol ethoxylate improve the wetting of the fabric by reducing surface tension [11]. There are microbial strains with the ability to degrade phenol in low concentrations. However, in high concentrations, it is toxic and inhibits its growth, requiring a period of prior acclimatization [12]. Metallic concentrations higher than 75 mg/L are found in raw cotton fibers, but increase when the cotton or cloth thread enters the machinery for processing [13]. Heavy metals are unable to be degraded, while biological treatment consists of detoxification and immobilization in order to reduce their biological toxicity and delay their transport. Zinc is one of the direct emitters of inorganic contaminants in water, mainly from the textile industry [14].

The processes generally used in wastewater treatment are chemical and/or physical such as precipitation, oxidation, reduction and coagulation, ion exchange, filtration, adsorption, electrochemistry, reverse osmosis, evaporation removal and solvent extraction, with different percentages of effectiveness, but have the disadvantage of being generators of new waste even more dangerous than the original [15]. Biological treatment is characterized by easy-to-treat, economically viable secondary products and reduced sludge [16]. Azo dyes, being recalcitrant due to their complex structures and xenobiotic nature, require anaerobic treatments for their mineralization [8]. Studies have been carried out on the removal of dyes from wastewater of the textile industry using anaerobic bacteria, resulting in 100% removal of the dyes from synthetic water and real contaminated water [17].

The use of support media in biological reactors is one of the most common alternatives for industrial wastewater treatment with COD greater than 1500 mg/L, as it allows the retention of solids through superficial biofilms and in the interstices of the bed [18]. The optimal characteristics of materials used as supports are (1) structurally resistant, (2) chemically and biologically inert, (3) light, (4) possess high surface area and porosity, (5) allow rapid proliferation of microorganisms, (6) without smooth surface and (7) low cost [19]. They are mainly used to improve the contact between the substrate and the biological solids contained in the reactor, facilitating a uniform flow, accumulating a large amount of biomass, acting as a physical barrier preventing the solids from being dragged out of the treatment system and separating solids from gases [18]. The support material may be stones, ceramic blocks, foams, plastic materials, PVC core blocks, granite, polyethylene spheres or bamboo, among others [19].

The main aim of the current study was to achieve a microbial consortium with the capacity to remove COD and typical contaminants from textile wastewaters (dyes, phenols, chromium and zinc) and to identify some of the microbial consortium species as well as select a support medium for anaerobic reactor for treatment.

2. Materials and Methods

2.1. Microbial Consortia

The microbial consortiums were obtained by exposure to air for 10 min of two test tubes with 100 mg/L of direct textile dyes [9], Red 23 (I5-ESPE) and Blue 106 (I6-ESPE) and modified Pseudomonas medium (MP) (4 g/L de $(NH_4)_2SO_4$; 1.4 g/L of $MgSO_4.7H_2O$; 0.7 g/L of NaCl; 0.08 g/L of $CaSO_4.2H_2O$; 1 g/L of K_2HPO_4; 2 g/L of KH_2PO_4; 0.3 g/L

de EDTA-Fe), modified in the source of calcium, magnesium and iron, and finally, 5 g/L of glucose as a carbon source [20]. They were covered and incubated at laboratory temperature (between 15 and 22 °C) until a color change was obtained. Some 0.1 mL of the cultures was seeded in 10 mL of thioglycolate [20,21] and incubated for 48 h at 35 °C. An aliquot was seeded in modified MP medium as a principal consortium for the following experimentation phases.

2.2. Selection of the Microbial Consortium

The color reduction was evaluated until reaching 90% in order to determine the time of removal for each microbial consortium. Some 0.1 mL (3×10^8 CFU/mL) was inoculated in 10 mL of modified MP medium, with 100 mg/L of the reactive dyes Navy Blue 171 ($C_{40}H_{23}Cl_2N_{15}Na_6O_{19}S_6$), Red 141 ($C_{52}H_{26}C_{l2}N_{14}Na_8O_{26}S_8$) and Yellow 84 ($C_{52}H_{38}C_{l2}N_{18}O_{26}S_8$), separately, at pH 6. We determined by spectrophotometry [21] wavelengths of 620 nm, 410 nm and 530 nm for Navy Blue [22], Yellow 84 [23] and Red 141 [24], respectively.

2.3. Kinetics of Microbial Growth and Removal of Textile Dyes

The microbial growth kinetics were established with 100 mg/L of dye in 10 mL of the I5-ESPE consortium (2×10^8 CFU/mL) and 390 mL of modified MP medium incubated at 35 °C under anaerobic conditions. The growth curve was realized over 15 days with the method of counting by deep sowing. The removal of each textile dye was determined by spectrophotometry, at the aforementioned specified wavelengths, in the supernatant after centrifuging 2 mL for 5 min at 10,000 rpm [21–24].

2.4. Removal of Phenol, Chromium(VI), Total Chromium, Zinc and Evaluation of Microbial Growth

The phenol removal was determined at 10 and 100 mg/L [25] and for chromium VI (K_2CrO_4) [26] at 10, 50 and 100 mg/L [27,28], and added to the modified MP medium under anaerobic conditions. The concentrations of zinc ($ZnSO_4$) in the modified MP medium were 5, 8 and 10 mg/L [29] with constant agitation of 150 rpm at 35 °C. The consortium I5-ESPE was inoculated at 1×10^4 CFU/mL [30], 1×10^5 CFU/mL [31] and 7×10^6 CFU/mL for phenol, chromium and zinc, respectively.

Then, 2 mL was taken after 30 days for phenol and chromium and 16 days for zinc. The samples were centrifuged at 14,000 rpm for 5 min. We determined in the supernatant microbial growth the pH, the removal of phenol and chromium(VI), as well as the total chromium and zinc. Calibration curves to yield the removal of phenol in modified MP medium were realized with the direct technique of 4-aminoantipyrine and potassium ferricyanide at 500 nm [32] with standard solutions between 0.1 and 3 mg/L. Chromium(VI) was determined by the diphenyl carbazide method at 540 nm [33,34] using standard solutions between 0.04 and 0.4 mg/L. We accepted curves with R^2 higher than 0.995. The absorbances obtained from the experimental tests were compared with the standard curves in order to determine the concentrations of both phenol and chromium(VI) and calculate the percentage of removal. Simultaneously, the total chromium and zinc were measured by atomic absorption spectrophotometry with VARIAN AA 240 FS [33,35].

2.5. Reduction in COD in Textile Wastewater by the Microbial Consortium

The reduction in COD was performed in wastewater from a textile facility at the first discharge after dyeing. A total of 800 mL COD was determined before and after inoculating 80 mL of the I5-ESPE microbial consortium with 3×10^8 UFC/mL [36] by the closed flow colorimetric method [37]. The reduction in COD was evaluated with and without aeration over 31 days.

2.6. Microbiological Identification before and after Textile Wastewater Treatment

The microbiological identification of some strains of the I5-ESPE consortium was conducted through the isolation of colonies by serial dilutions of 10-1–10-6 in sterile saline

solution [14], and 0.1 mL of each dilution was placed in Petri dishes [38] with nutrient agar and PDA for the first isolation. A second isolation was performed in Man-Rogasa-Sharpe (MRS) and Mannitol Salt Agar (MSA) media at 30 °C for 48 h for bacteria, and for 21 days for fungi [14,38,39]. The purification of yeast and yeast bacteria was conducted with stretch marks on TSA agar [14], PDA [40] and Gram stain [41]. For the biochemical identification, bibliographic keys were followed [42], confirmed by the Profile Analytical Index (PAI®) revealed on the APIweb virtual platform [43].

2.7. Selection of Support for Anaerobic Reactor at Laboratory Level

The selection of a support for an anaerobic reactor was performed in synthetic water consisting of a mixture of Red reactive textile dyes ED7B, Yellow 3GL and dark Blue ED at a concentration of 100 mg/L [9]. The materials used were common rock, polyethylene terephthalate (PET) bottle plastic and sterilized coconut shell. The parameters of selection were physical aspects such as porosity [44], density and specific weight, and the chemical properties were the reduction in COD, the removal of dyes and biomass. The stabilization of the I5-ESPE consortium was achieved by placing it in a 2:1 ratio with synthetic water in a volume of 4 L [45]. In three glass bioreactors, the supports were placed with plastic mesh at 3.5 cm from the bottom and 5 cm high with semicontinuous flow.

The adaptation of the microorganisms was supervised every 24 h. Once installed, sampling was carried out daily for 30 days, extracting 300 mL of treated water and incorporating 300 mL of synthetic water in order to maintain a constant volume of 4 L in the bioreactor. The COD was determined by the closed flow colorimetric method [37]; SST by gravimetry [46]; SSVL with the protocol of the Standard Methods for the Examination of Water and Wastewater, without modifications; and the removal of dyes by spectrophotometry [21], controlling pH between 6.5 and 7.6 and temperature at 35 °C [47]. The parameter control was executed by sterilizing the materials in order to avoid external microorganisms with a pH between 6.5 and 7.5, a temperature at 35 °C ± 1 with a minimum of 32 °C at the time of recirculation, and dissolved oxygen between 0.5 and 2 mg/L.

2.8. Statistical Data Analysis

In analyzing the results of the removal of textile dyes, phenol, chromium and zinc, we applied the Levene statistic, ANOVA and Tukey test at a 0.05 significance level [48–52]. In analyzing the reduction in COD, Duncan's test was applied [53,54]. For the microbiological identification, we used the chi-square of independence, Fisher's test, *coefficient* $_{\varphi}$, probability ratio (OR), Jaccard's statistical index and the Sørensen–Dice coefficient [55–64]. In the study of the selection of a support for the anaerobic bioreactor, ANOVA variance analysis and Duncan tests were performed to compare means. We used the statistical program SPSS 15.0.

3. Results

3.1. Selection of the Microbial Consortium

We obtained two microbial consortiums, I5-ESPE and I6-ESPE. The two consortia (Figure 1) demonstrated significant differences in the percentage of removal ($p \leq 0.00$), classifying them into different groups of homogeneity according to the Tukey test. Due to the higher percentage of removal (Figure 2), the I5-ESPE consortium was selected for the subsequent phases of the study.

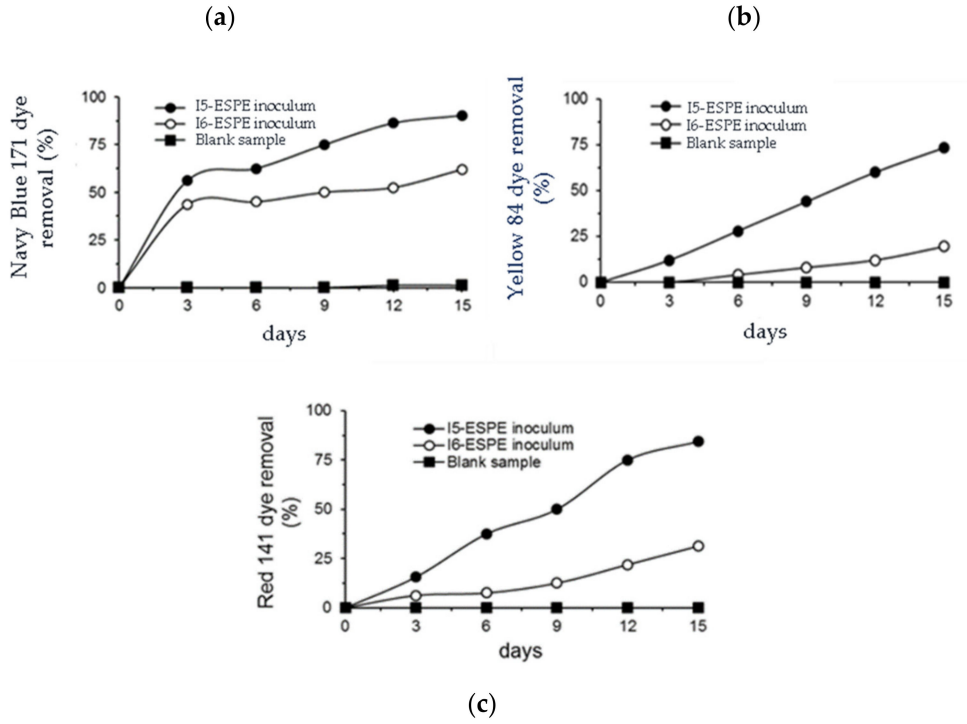

Figure 1. Average percentage (n = 3) of removal of 100 mg/L of textile dyes (**a**) Navy Blue 171, (**b**) Yellow 84 and (**c**) Red 141 for 15 days by consortia I5-ESPE, I6-ESPE and negative control (blank sample).

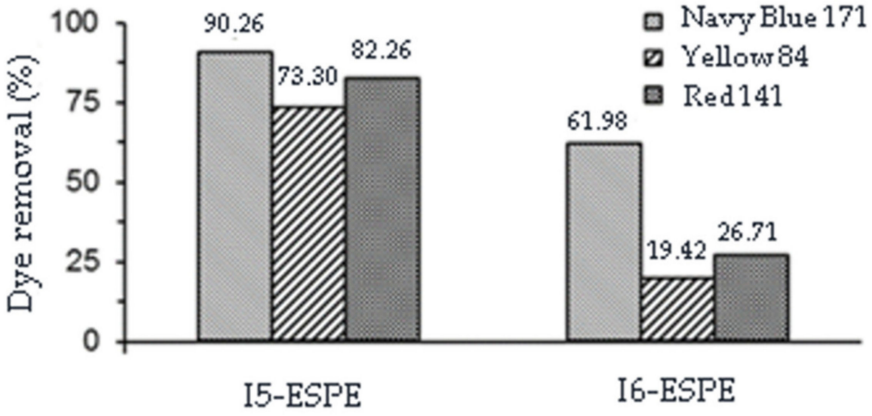

Figure 2. Average percentage of dye removal by the two studied microbial consortia. The ANOVA determined that there were significant differences in color reduction for each inoculum ($p \leq 0.00$). The Tukey test classified the three dyes into two homogeneity groups.

3.2. Kinetics of Microbial Growth and Removal of Textile Dyes

With the same trend, at pH 6, cell density increased from an initial value of 10^5 CFU/mL to 10^{10} CFU/mL for Yellow 84 and Red 141, and to 10^9 CFU/mL for Navy Blue 171. The microbial population decreased to 10^8 CFU/mL in Red 141 and to 10^7 CFU/mL in Navy Blue 171 and Yellow 84. Some 50% removal of Navy Blue 171 and Yellow 84 was reached in the first four days of testing, while for Red 141, this occurred on the sixth day (Figure 3).

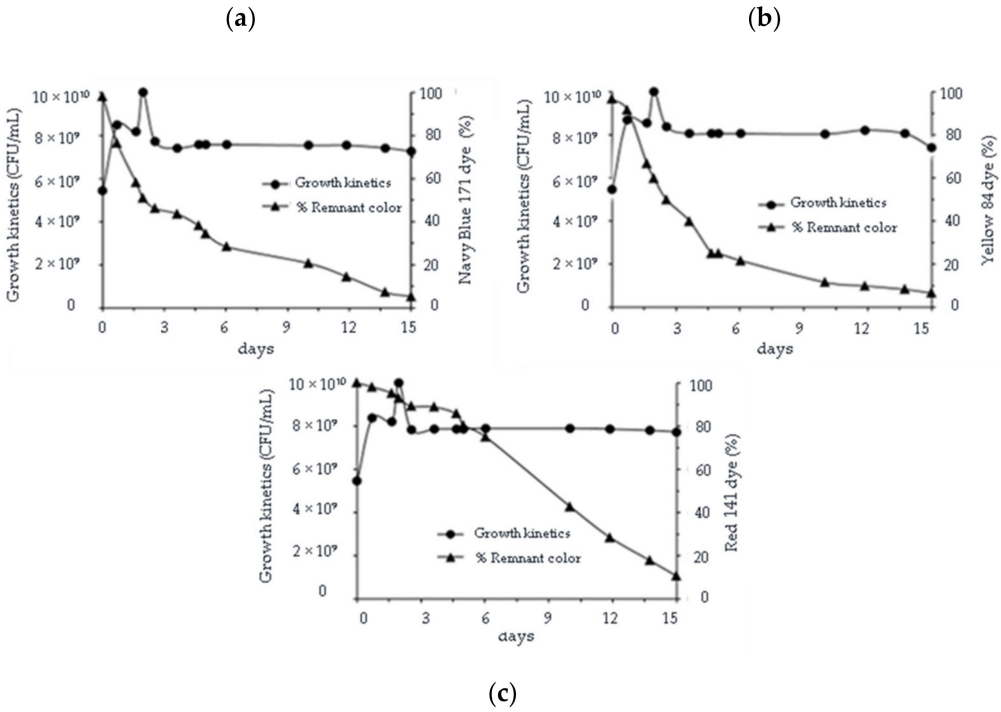

Figure 3. Ratio of microbial growth and percentage of remaining color for dyes (**a**) Navy Blue 171, (**b**) Yellow 84 and (**c**) Red 141 during the 15 days of the test. For the three dyes, at the highest cell densities, there was an obvious decrease in pH.

3.3. Removal of Phenol, Chromium(VI), Total Chromium and Zinc and Evaluation of Microbial Growth

In the first 10 days, the initial cell density increased for the elimination of phenol from 10^3 CFU/mL. From day 20, it remained constant until the end of the trial. There was no evidence of any phenol removal (Figure 4).

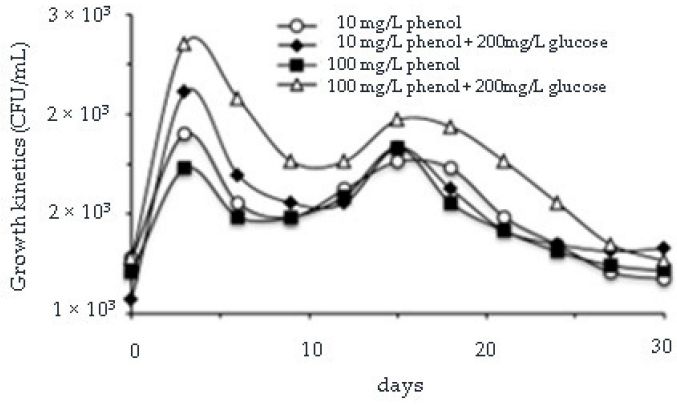

Figure 4. Growth kinetics of the I5-ESPE microbial consortium at different concentrations of phenol. During the test time, no changes were observed in the phenol concentration.

For chromium(VI), the cell density increased on the second day from 10^5 CFU/mL to 10^8 CFU/mL and decreased until the end (Figure 5). In addition, a 98% removal of chromium(VI) at 10 mg/L was observed on day 16 (Figure 6). The total chromium removal was 67% for the same concentration (Figure 7). The pH remained at 6. There was no variation in 50 and 100 mg/L of chromium(VI).

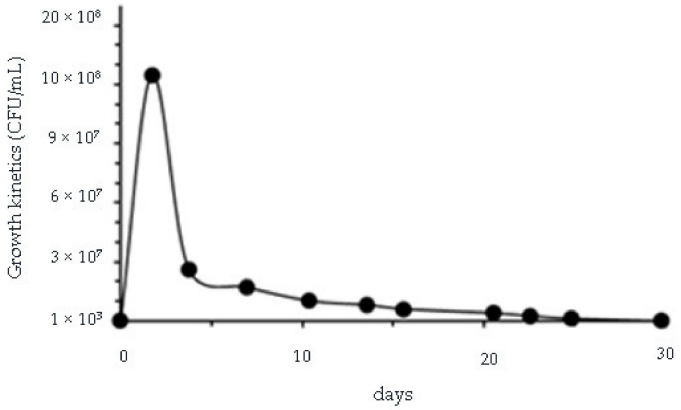

Figure 5. Growth kinetics of the microbial consortium I5-ESPE without chromium(VI) concentrations.

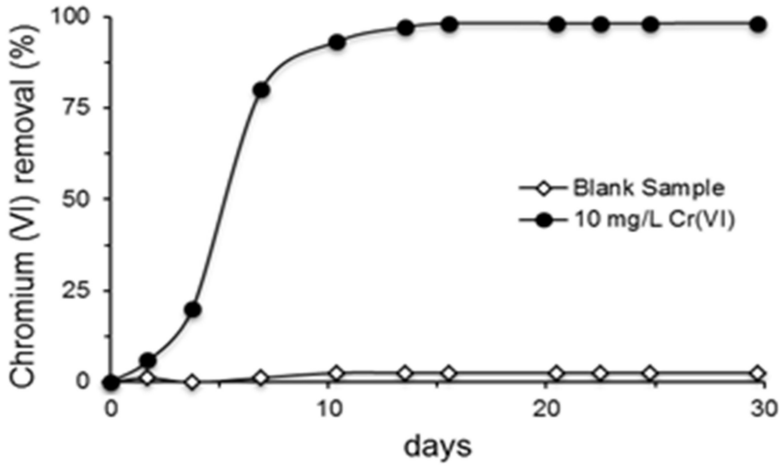

Figure 6. Removal percentage by the I5-ESPE microbial consortium for an initial concentration of 10 mg/L of chromium(VI).

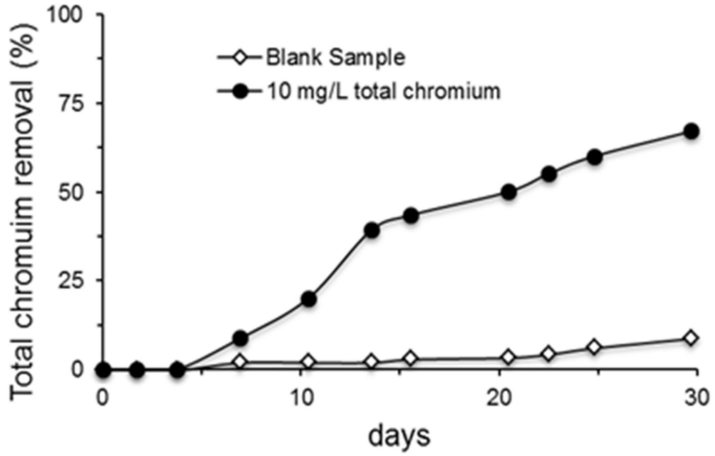

Figure 7. Removal percentage of total chromium by the I5-ESPE microbial consortium for an initial concentration of 10 mg/L.

For the removal of zinc, the cell density of 10^6 CFU/mL of the I5-ESPE consortium increased to 10^9 CFU/mL in contact with the metal (Figure 8). The removal percentages for the concentrations of 5, 8 and 10 mg/L of zinc were 22%, 25% and 25%, respectively (Figure 9). The pH values remained in the range of 5 to 5.5.

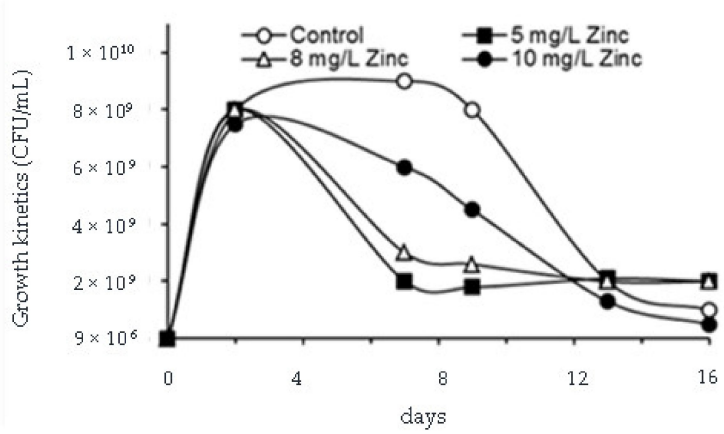

Figure 8. Growth of the I5-ESPE microbial consortium with each zinc concentration.

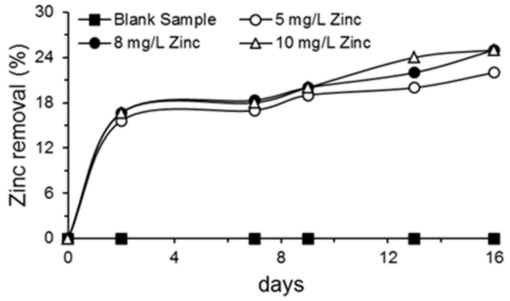

Figure 9. Removal percentage for 5, 8 and 10 mg/L zinc by the microbial consortium I5-ESPE.

3.4. Reduction in COD in Textile Wastewater by the Microbial Consortium

The COD reduction in textile wastewater by the consortium I5-ESPE without aeration was about 31.47%, and with aeration, it was 57.03% (Figure 10). The ANOVA indicated statistically significant differences between the treatments, highlighting the treatment with aeration.

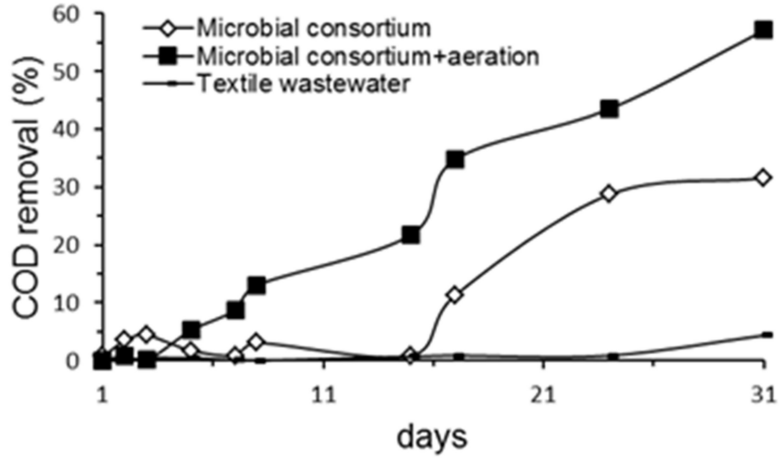

Figure 10. COD reduction percentage by the I5-ESPE microbial consortium.

3.5. Microbiological Identification before and after Textile Wastewater Treatment

Thirteen microbial colonies were obtained in the first isolation and nine in the second isolation. At 21 days, the growth of filamentous fungi was not yet evident. By biochemical analysis, confirmed by the API® system, we identified the species *Staphylococcus xylosus*,

Saccharomyces cerevisiae and *Candida tropicalis* before the treatment, and the species *Enterobacter cloacae* and *Bacillus megaterium* were identified after the treatment of textile wastewater. *Bacillus subtilis* was isolated in both cases (Figure 11).

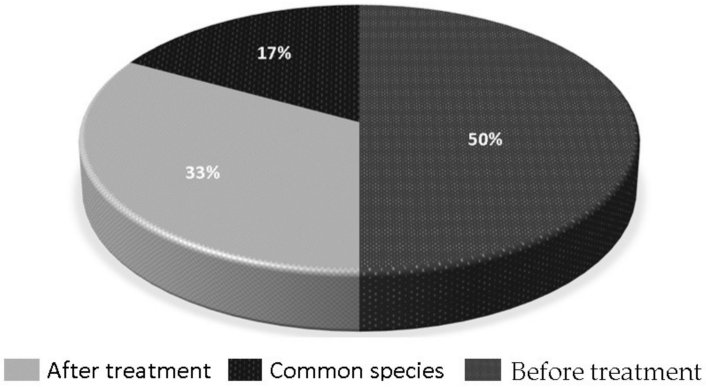

Figure 11. Some microbial species present in the I5-ESPE microbial consortium before and after the treatment of textile wastewater.

The comparison by means of Boesch contingency tables indicated statistically significant differences of the I5-ESPE consortium before and after the textile residual water treatment, with bilateral Fisher values of −0.75 and $p > 0.05$ [65]. A moderate intensity of relation was deduced (V Cramer = 0.5; K = 0.58; $_{\varphi}$ = −0.71) and nullity of the difference of the consortia in the change of one of the variables (OR1/2 = 0.00). The Jaccard index expressed a low proportion of common species (1-I_J = 0.86) and the Sørensen–Dice index (1-S = 0.75) indicated dissimilarity in the consortium before and after the treatment.

3.6. Selection of Support for Anaerobic Reactor at Laboratory Level

The coconut shell presented the highest percentage of porosity (44.24%), as well as a low density (1.03 g/mL), a specific weight of 10,084 N/m^3, a lower amount of biomass (1.6×10^7 CFU/mL) and a greater removal of textile dyes (45.92%) with respect to rock and plastic (Table 1), being all statistically different values according to the Duncan test. The three supports indicated similarity in the reduction in COD (500.66 mg O2/L of approx. 50%), volatile suspended solids (VSS) (2545.46 mg/L) and total suspended solids (TSS) (282.82 mg/L), the coconut shell having greater reduction (Table 2). For the three supports, the number of microorganisms decreased with respect to time, until stabilizing on day 13.

Table 1. Physical characteristics of the support media.

Material	Porosity (%)	Density (g/mL)	Specific Weight (N/m^3)
Common stone	44.24	1.81	17,738
Plastic	13.96	1.26	12,348
Coconut shell	83.06	1.03	10,084

Table 2. Evaluation of the support media with the microbial consortium I5-ESPE in the treatment of textile wastewater.

Material	Dye Removal (%)	COD Reduction (mg/L)	Growth Kinetics (CFU/mL)	VSS (mg/L)	TSS (mg/L)
Common stone	34.48	674.05	3.9×10^7	2498.09	281.0
Plastic	32.42	540.66	2.1×10^7	2284.36	282.82
Coconut shell	45.92	500.66	1.6×10^7	2545.46	282.18

4. Discussion

The microbial consortiums I5-ESPE and I6-ESPE indicated the removal capacity of Navy Blue 171, Yellow 84 and Red 141. The I5-ESPE reached higher percentages of removal. It was evident that with a redox intermediate in the culture medium, the Red 23 direct dye removal reaches 90% [66]. The I6-ESPE consortium was possibly less able to use the redox potential of the medium to break the azo bond of the chromophore, so the removal values remained lower. The use of a cosubstrate for the removal of textile dyes under anaerobic conditions has been indicated as indispensable [67], but in our study, by not being added, the result was null. However, this also depends on the structure of the dye [9,68], indicating that the discoloration is affected by the molecular weight, substitution groups and intramolecular hydrogen bond between the azo and hydroxy groups of the coloring. This possibly explains the greater time of action required by the consortium I5-ESPE on Red 141. At the end of the trial, similar removal percentages were obtained for the three dyes. The elimination of structurally different azo dyes demonstrates that the anaerobic process is nonspecific [9].

Although the concentration of phenol does not vary in the time of the test, the cell density remains constant, evidencing tolerance of the consortium (10 and 100 mg/L) [69]. We determined that the strains used for the decontamination of wastewater with phenol must not only be active, but also sufficiently resistant to their presence. The growth of the species Vibrio nereis and Arthrobacter mysorens was demonstrated in a medium with 800 mg/L of phenol, indicating tolerance to high concentrations, although they are incapable of metabolizing it [70]. In the trial, the reduction of chromium(VI) (10 mg/L) to chromium III probably occurred, being expelled from the cell, to be immobilized by the mechanism of bioadsorption [71,72]. Studies on the species *Termitomyces clypeatus* determined that the bioadsorption of chromium(VI) is produced by amino, carboxyl, hydroxyl and phosphate groups with which they form chemical bonds [73], independently of metabolism [74]. Bacteria such as *Pseudomonas fluorescens* and *Enterobacter cloacae* with the ability to conduct the reduction of chromium(VI) by oxidation–reduction reactions, under anaerobic conditions, may use it as electron acceptor of the transport chain.

At 100 mg/L of chromium(VI), the same failed to occur after 30 days, a result similar to the study of [31] with *Bacillus* sp., where the microbial growth was significantly affected. This is probably due to the fact that at this concentration, the I5-ESPE consortium has not yet developed the capacity to protect itself from the toxicity of metals through mechanisms such as adsorption, methylation, bioaccumulation, oxidation and reduction [74]. However, other studies indicate an 89% reduction of 100 mg/L of chromium(VI) after 144 h of incubation by *Bacillus* sp. JDM-2-1 and *Staphylococcus capitis* [75]. At the zinc concentrations of 5, 8 and 10 mg/L, the I5-ESPE consortium indicated removal levels of 24.5%, 23.5% and 22.8%, respectively, maintaining a constant growth rate of 109 CFU/mL. This possibly occurred due to the development of resistance to toxicity by detoxification mechanisms generated by direct exposure to metal [76]. Several studies presented that the effect of zinc toxicity varies in microorganisms. Thus, in *Escherichia* sp. PLK1, concentrations $\leq$ 26 mg/L inhibit dehydrogenase activity [77], unlike *Arthrobacter* sp. SED4, in which the total enzymatic inhibition is reached with 78.45 mg/L, and for *Bacillus* sp. DISK1 and *Escherichia* DISK2, with 52.3 mg/L [78]. The sulfate source present in the modified MP medium eventually allowed the growth of sulfate-reducing anaerobic bacteria, generating hydrogen sulfide (H_2S), which precipitates divalent cationic metals of low solubility [79].

Oxygen tolerance studies indicate that sulfate reduction is not affected during the first nine hours in the presence of oxygen, and subsequently, the inactivation of bacterial metabolism occurs without causing cell death [80]. That may explain the constant cell densities of 109 CFU/mL. An oxygen tolerance study on the sulfate-reducing bacteria Desulovibrio oxyclinae presented cell agglutination due to the lack of mechanisms to deal with oxygen radicals, forming a space of internal anoxia, allowing the anaerobic reduction of sulfates in the presence of high concentrations of dissolved oxygen [81].

The reduction capacity of the I5-ESPE consortium of the COD values has been demonstrated, with the aeration treatment having the highest value (57.03%). Without aeration, we obtained approximately 32%. A similar study in wastewater from textile effluents yielded 100% removal of dyes by anaerobic treatment. However, COD decreased by only 24.5% compared to 68.2% in a later aerobic phase [9]. The identified species before treatment of textile wastewater were *Staphylococcus xylosus*, *Saccharomyces cerevisiae* and *Candida tropicalis*, while after the treatment, we identified *Enterobacter cloacae* and *Bacillus megaterium*. The common species was *Bacillus subtilis*. Ref. [82] mentioned certain bacteria that may be able to mineralize reactive dyes under aerobic conditions by sulfate reduction and the secretion of aerobic azoreductases [23] catalysts of NADPH reduction of the azo bonds in the aromatic rings, fragmenting the molecule and favoring the removal of the dye [83] at temperatures between 30 °C and 40 °C [84].

The difference in the consortium before and after the wastewater treatment is possibly due to changes in pH, temperature, oxygen, nutrients, number of microorganisms and diversity. Ref. [85] pointed out that only some microbial species are able to tolerate high concentrations of chemical agents and to assimilate the byproducts created by the initial degrading microorganisms, *B. subtilis* being the species that adapted to the change in this case. Microorganisms handle the biological treatment of wastewater in different stages. In the first stage, they assimilate organic matter, generating secondary products which favor the growth of others in the second phase, continuing with the elimination of contaminants. In addition, there is no single microorganism capable of metabolizing all the compounds present in wastewater [86].

Several studies indicate that the species present in the I5-ESPE microbial consortium before and after the treatment of textile wastewater have the capacity to be applied. *B. subtilis* has been reported as one of the first isolates [82] for its CotAlaccase enzyme, allowing the reduction of the azo bond [87]. *B. megaterium* 96.88% of Red azo dye 3BN [88], E. cloacae removed 92.6% of Black reactive dye 5 and together with Bacillus spp. removed 75% COD in wastewater with Red reactive dye RR [89]. *S. xylosus* decreased aromatic compounds (1,2-dichlorophenol and 4-Cl-m-cresol) in biological wastewater treatments [90]. *S. cerevisiae* allowed the elimination of Blue dye 19 due to the presence of laccases [91]. *C. tropicalis* removed up to 97% of the synthetic dye RB 5, azo reactive dyes and anthraquinone dyes due to the enzyme manganese peroxidase (MnP), as well as due to being a phenol degrader [92].

Anaerobic biological treatments have been proven to be effective in the treatment of textile wastewater. Removals of up to 92% have been obtained in water contaminated by reactive dyes in an anaerobic up flow reactor on a laboratory scale [93]. Using a semi-continuous reactor, under anaerobic conditions, the reactive dye Orange 16 reached 90% efficiency at concentrations above 320 mg/L [94], and a 100% removal of textile dyes was decreased in synthetic wastewater using an anaerobic up flow reactor at a laboratory scale [95]. They have been reduced by 88% [96] for mono and diazo dyes using acetate as an alternate source of carbon in an upstream methanogenic reactor. In the current study, anaerobic filters were used as reactors in which there is a medium of support, with ascending or descending flow and a basic flow rate-type piston. This is one of the alternatives of greater application for the treatment of industrial wastewater with concentrations of COD greater than 1500 mg/L [18]. The use of support means in bioreactors allows the retention of solids, thereby forming a biofilm [18], or sets of microorganisms in a layer whose extracellular polymers are attached to a solid surface to hold and accumulate biomass without the need for other solid separation systems [97]. The main advantage of biofilm reactors is the ability to retain ten times more biomass per unit volume of the reactor than suspended biomass systems, with a more stable operation and reduced washing process [36].

Among the supports commonly used are rock and plastic. Rock in sizes smaller than 3 cm can cause efficiency losses due to clogging, while plastic allows the adherence of anaerobic microorganisms since methanogenic populations are able to associate with stable solid materials to form beds [98], and it has been proven that plastic as a support medium

can achieve a COD removal efficiency of 70–90% [99,100]. An alternative material as a support medium in anaerobic filter is coconut shell due to its large specific surface for adherence of microorganisms, high percentage of voids facilitators of flow (83%), low specific weight with less complex containment infrastructures and long useful life [18]. In our study, the porosity obtained for coconut shell (83.06%) was higher than for rock (44.24%) and plastic (13.96%). The distribution of the pores and their size guarantees a contact surface for the generation of the biofilm and transfer of the contaminant mass, favoring the microbial adhesion conditions from the initial stages of the process. The specific weight of the supports determines the construction costs, so if the support material is lighter, no external structural effort will be required to sustain the filter medium, and the transportation and installation of the material will be lower [98].

The removal of dyes (45.92%) and COD (50%) by the microbial consortium I5-ESPE in the support medium with coconut shell was higher and statistically different from rock and plastic in the case of dyes. However, an anaerobic–aerobic combined treatment may increase the COD reduction. It has been demonstrated that the elimination of dyes with azo bond in the anaerobic stage and the elimination of COD in the aerobic stage allow the mineralization of the azo dye Mordant Yellow [99]. Although the anaerobic reduction of azo dyes is more satisfactory than the aerobic degradation, the products generated as aromatic amines must be degraded, as they present greater toxicity. A wide variety of aromatic amines are biodegradable by aerobic means, producing oligomers and possibly dark-colored polymers of low solubility easily separated from the aqueous phase [101].

The anaerobic filter does not require biomass recycling because it remains attached and is not lost with the effluent [102]. Over the 30 days of treatment, it was not necessary to add microorganisms due to their adhesion to the supports. The anaerobic filter is not indicated in the treatment of wastewater with high concentrations of solids in suspension due to obstructions [102].

The TSS in the effluent is a parameter that allows a projection of the amount of organic matter substrate for anaerobic digestion in the wastewater [103]. As expected, in the used wastewater, both the TSS and COD demonstrated variations due to the difference in organic load. However, the reported values of TSS in the effluent represented the substrate availability for microorganisms in the system.

The adaptation of the I5-ESPE consortium to the required conditions was conducted by progressive scaling until obtaining an average concentration of the VSS of approximately 2000 mg/L. Normally, the value ranges between 1500 and 10,000 mg/L. In this study, it was between 1500 and 3000 mg VSS/L, being in an acceptable range since most of the microorganisms are adhered to the support, decreasing the COD.

Anaerobic treatment is a process of degradation or oxidation of organic matter by coordinated action of microorganisms in four sequential stages, namely, hydrolysis, acidogenesis, acetogenesis and methanogenesis [18], obtaining a biogas byproduct whose composition is methane, carbon dioxide, nitrogen, hydrogen, ammonia and hydrogen sulfide [104]. To reach the stage of methanogenesis, we should control the temperature, pH, amount of oxygen, alkalinity and microorganisms.

When the amount of oxygen dissolved in values is lower than 2 mg/L, its solubility decreases at a higher temperature, although this also depends on the composition of the water, as it is reduced in seawater. The temperature should be between 20 °C and 40 °C [104]. In our study, it was maintained at 35 °C. It is possible to operate anaerobic treatments at temperatures lower than those required, due to the high concentration of biomass in the filter [105]. However, in the recirculation, the minimum temperature was about 32 °C. The stability of the pH needs to be controlled since the methanogenic activity is highly vulnerable to changes compared with the other populations present in the consortium. At low pH values, acid fermentation prevails over methanogenic fermentation, resulting in acidification of the reactor content. The acidogenic agents act at a pH between 5.5 and 6.5, and the methanogenic agents between 7.08 and 8.02. However, when there is coexistence of both microorganisms, the optimum pH range is 6.8 to 7.5 [106]. Finally, it is evident

that the dyeing processes of the textile industry generate waste products which constitute major environmental problems in wastewater, similar to other anthropogenic pollution, including personal hygiene products, hormones, illegal drugs and decomposing corpses, among many others [107–111].

5. Conclusions

The microbial consortium I5-ESPE achieved greater removal of the textile dyes Navy Blue 171, Yellow 84 and Red 141 compared to the microbial consortium I6-ESPE, also demonstrating tolerance to the presence of phenol.

The same microbial consortium removed chromium(VI) and then total chromium under anaerobic conditions and three concentrations of zinc (5, 8 and 10 mg/L) in 23.6% and COD in 57.03% under aerobic conditions. Microbiologically, it proved to be different before and after the aerobic treatment of textile wastewater with pure cultures consisting of yeasts (41%), Gram-negative bacilli (36%), Gram-positive bacilli (18%) and Gram-positive cocci (5%).

The use of coconut shell as a support for an anaerobic reactor achieved the highest results of color removal and COD, offering advantages such as a broad specific surface for the adherence of microorganisms, low specific weight that allows the use of simple containment structures, and prolonged shelf life compared to common rock and plastic.

Our study proves that the I5-ESPE consortium has the potential to be applied in the treatment of wastewater from the textile industry together with coconut shell as a support for anaerobic bioreactors.

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