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Decolourization of Textile Dyes with Biotechnological Potent Bacterial Isolates

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ABSTRACT

The discharge of effluents from the textile industries to aqueous bodies is currently one of the biggest concerns of environmentalists. Dyes used in the textiles industries create pollution in the environment. The application of biological treatments is one of the most economically viable systems to decolorized effluents using bacterial isolates (TBSP, TBSP2). In this sense, studies were carried out tests for the removal of colour of Congo red, Methyl orange, Brilliant Blue, using bacterial strain. Firstly, all two bacterial strains were isolated from different environments. Then, tests were performed to verify which concentration of the dye would be limit for the growth of each microorganism. In addition to decolourization tests, other parameters such as pH, biomass, total proteins and toxicity of the metabolites formed were also monitored. The bacterial strain, isolated from the river environment, was able to decolourization concentrations of 100ppm to upto 700ppm respectively for the, Coomasie Brilliant blue, Methyl orange and Congo red. TBSP1 and TBSP2 discoloured respectively at concentrations 100ppm to 700 ppm , while the intercropping (consortium) of the two bacteria discoloured at a concentration of 700ppm for the three dyes tested individually. In these cultivation conditions, the decrease in the quality rate varied with the lowest rate observed in the assay containing Congo Red and Congo red dye and the highest removal rate in the assay containing the intercropping and the CBBR dye. The results shows that the bacteria biotechnological potent bacteria present initial pot in the decolourization of textile dyes, initial pot were shows the ability to decolorize azo dyes.

Keywords- Bacterial strains (TBSP1 and TBSP2), Biodecolourization, Biotechnologocical Potent Bacteria, Azo Dyes and Dyes toxicity.

I. INTRODUCTION

The main characteristic of the human being to make use of the forces of nature, such as solar energy, wind forces, and materials provided with the environment, for his own benefit. Due to the rapid growth of the population worldwide, there was a need to increase this production. With this demand, industrial growth

was inevitable to meet the needs of the human being. Industrial growth, although it contributed to the economic and social development, was also responsible for the increase in environmental problems, making them increasingly critical and frequent, mainly because industries were emerging in a disorderly manner, compromising soil, air, and water quality. The way to eliminate toxic substances is one of the most worked subjects today, which has led researchers to seek new techniques and more powerful tools that can decrease and/or eliminate the toxicity of liquid and gaseous effluents formed in their distinct processes, leaving them in. regulations and legislation on environmental protection. Among the various existing industries, the textile sector has generated a lot of concern regarding the release of its effluents in water bodies, due to the use of synthetic dyes to dye your fabrics. The textile sector is currently responsible for much of the developed countries and one of the main economic activities of some emerging countries. The textile industry currently consumes 15% of all water destined to the Brazilian industry, and the stages of washing and dyeing are the ones that consume the most water in textile processing. The liquid effluents generated in the process of the textile industries constitute a wide variety of components, such as dyes, additives, high organic load, and strong staining, which can cause damage to the environment if not properly treated.

In general, in textile industries, the treatment processes of their effluents are based on the operations of physical-chemical systems of precipitation-coagulation, followed by biological treatments with activated sludge system. The system has a relatively high treatment efficiency, ranging between 85 and 95%, allowing a good removal of the dye load. However, the problem related to the accumulation of slum becomes critical, since the content of adsorbed dyes is quite high, preventing any possibility of reuse. For all these reasons, there are using microorganisms capable of efficiently degrading a large number of pollutants at a low operational cost for the proper treatment of textile effluents. In practice, it is known that this treatment is very difficult mainly due to the diversity, concentration and composition of chemical substances present in each effluent. The use of microorganisms is widely known in the effluent treatment process. This process is one of the most economically viable, and therefore is one of the most used, making the study very interesting mainly due to ease of work. Based on information on the biotechnological potential of bacteria to discolor some dyes, this work had the following objectives:

- To evaluate the Decolourization capacity of two strains of TBSP1and one of TBSP2, alone and in intercropping, against the anthraquinone dye Coomasie brilliant blue R and the azo dyes Congo red and Methyl Orange.
- Use different concentrations of dyes to test the maximum tolerated concentration of each microorganism and its intercropping.
- Evaluate the toxic potential of metabolites formed during the Decolourization process.

II. THE USE OF WATER IN THE TEXTILE INDUSTRY

The textile sector in the industrial area is one of the oldest sectors in the world, being one of the precursors to the period of the Industrial Revolution at the end of the 18th century. This occurred mainly due to the incorporation of new methods of production of synthetic compounds and new technologies in the production process of all water available for world consumption, about 88% is used in agriculture. The industry accounts for only 7%, with the remaining 5% for home use. In industry, the textile sector consumes about 15% of the water, returning it after the highly polluted textile production processes. This water is used as a solvent medium for various chemicals that enter textile processes, such as washes, b ranking and dyeing of yarns and fabrics.

The environmental problems caused by the textile industry are well documented, being associated with problems such as the high volume of solid and liquid waste, its high organic load, chemical factors of the effluent and problems related to the release of dyes that have not been fixed in the processing or those that have not been removed by conventional treatment. The dye has a high potential for environmental impact because interferes in photosynthetic processes, and many of them have mutagenic characteristics.

Most of the contaminant load consists of impurities inherent to the raw material, products added to facilitate the processes of spinning and weaving, auxiliaries and dyes eliminated during the different finishing steps. The quality and quantity of the pollutant load are closely related to the fibers used in the preparation of tissues, whether natural or synthetic. The amount of water consumption by the textile industry depends directly on the raw material to be processed. Some processes, such as dyeing of the wires, bleaching and washes generate a large volume of water, so that, when emitted to the effluent, it carries quite polluting, among which stand out the corantes.

2.1 DECOLOURIZATION OF DYES BY BACTERIA

In aerobic systems, the degradation of dyes is usually done by bacteria, as in the activated sludge system, but this system presents low degradation efficiency of these compounds due to the lack of isolation of bacteria with metabolization capacity of dye molecules although Decolourization assays have been reported in the literature using some bacteria. The use of microorganisms in the biodegradation of synthetic dyes has become attractive because it is of simple and economical methodology, although biological mechanisms tend to be complex. Many microbial species foil tested in the Decolourization and mineralization of various dyes and showed that unfortunately most of these compounds are chemically stable and resistant to microbiological attack. Meanwhile, when studying the Decolourization of textile dyes using bacteria isolated from a textile effluent that contained 24 different types of dyes of three different classes found high Decolourization potential for some of the isolated strains.

The efficiency of the Decolourization process depends on the adaptation of the selected microorganism. Microorganisms related to the Decolourization of textile dyes are bacteria, fungi, algae and more recently, microbial consortia.The difficulty in removing these pollutants lies in the isolation of microorganisms, period of adaptation and the ability to discolor various structures. The use of aerobic bacteria such as Bacillus gordonae, Bacillus benzeovorans and Pseudomonas putida, showed good results in the Decolourization of the anthraquione acid dye Tectilon Blue used in carpet dyeing. Total Decolourization, 19% occurred by bio-sorption of the dye to bacterial biomass.

III.MATERIAL AND METHODS

The work was developed at the Environmental Microbiology VBSPU, the isolation of TBSP1 was performed according to the Bacteriological Analytical Manual using the colorimetric method. Approximately 800 mL of the effluent was collected from the ETE supply canal of a textile industry, located in the metropolitan region of Tanda in a sterile amber bottle. Likewise, a water collection was carried out at the river site, in front of stream, at a depth of ± .5 m. In the laboratory, the bottles were stirred to homogenize the material contained in the water and proceed to a series of dilutions of the samples (10-1 to 10-4). After the dilution stage, an aliquot of 1 mL was added in Lauril Tryptose broth containing inverted Durham tube and subsequently incubated in a bacteriological greenhouse at 35°C for 48 h. Tube positivity was observed by turbidity and gas production in durhan tube. Then an inoculum of the positive tubes was transferred to tubes

containing nutreient broth and incubated at 45ºC in a water bath for 24 hours. After this period, the positive EC tubes were showed in Petri dishes containing nutrient agar and incubated at 35ºC for 24 hours. The characteristic colonies of TBSP1, that is, with a diameter of 2 to 5 mm, white center, then isolated in test tubes. The NA tubes were incubated in an oven at 35°C for 24 hours and after this period, the isolated strains were identified under a microscope through their morphology and biochemical tests of IMViC (Indol, Methyl Red, Voges-Proskauer and Citract) according to FENG et al. (2001). The strains which presented, in the biochemical tests, characteristics such as + + - or - + - were classified as TBSP1, TBSP2.

3.1 COLORANTES

The dyes of the reactive azo type Congo red (C.I. 22120) and methyl Orange (C.I. 13025) and the Coomasie Brilliant Blue R (C.I. 42660) (Figure 9) were purchased from LABOGENS (AHMEDABAD GUJRAT) HI MEDIA (VADHANI MUMBAI). Initially, a stock solution of each dye was prepared at the final concentration of 500 mg L-1. Each solution was previously filtered in a sterile polyethylene filter of the Brand UHMW (UPP®, De zhou he sheng Plastic Products, China) with polyethersulfonic membrane of 0.22 μm. The final dye concentration used in the Decolourization assays was 100PPM to 800ppm mg L-1.

3.2 CULTIVATION

The test microorganism was grown in culture medium containing nutrient broth of the brand Himedia, with the following composition (g L-1): Peptone, Beef extract 5, Sodium Chloride 10. For the tests of decolourization used NB broth supplemented with glucose pH was adjusted in 7.0±0.2.

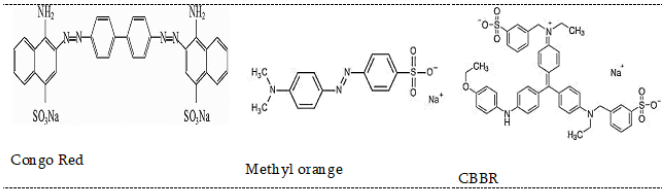


Figure-1. Chemical structure of the Azo dyes used in Experiment

3.3 METHODS AND BIOLOGICAL ANALYSIS

Each Decolourization assay was carried out after an over-night cultivation of the test microorganism in 50 mL of LB broth in Erlenmeyer vials of 125 mL capacity, kept under 100 rpm agitation and temperature 35ºC±0.2, resulting in a culture of approximately 106 CFU mL-1 (EVANGELISTA-BARRETO et al., 2009).

3.4 DECOLOURIZATION TESTS

Initially, the Decolourization capacity of the microorganisms (individual and intercropping of the two bacteria) was observed for each of the dyes. Decolourization assays were performed in 120 mL of LB broth supplemented with glucose (5 g L-1), in vials of 250 mL capacity, plus dye at final concentration from 100 to 800 ppm. Glucose was used to choose glucose due to a previous experiment using the anthraquinone dye CBBR. After the addition of the dye (v v-1) then inoculum of the test microorganism was added. The vials were kept under agitation of 100 rpm in shaker (Vortex mixer,) for up to 96 h. Control vials without the microorganism were kept in the same conditions. All experiments were carried out in triplicate, under controlled activities. Then, aliquots of the solution in the intervals of 0, 12, 24, 48, 72 and 96 h were removed and centrifuged at 16,000rpm for 8 minutes for Decolourization analysis in UV/VIS spectrophotometer (SYSTRONICS TYPE 117) through absorbance (ABS). The reading was performed at the specific wavelength for each dye (495 nm for Congo red, 464 nm for Methyl Orange and 595 nm for Coomasie brilliant blue R). The Decolourization efficiency was obtained using the following formula:

$$DE\ \% = \frac{(\text{Final ABS} - \text{Initial ABS})}{\text{Initial ABS}} \times 100$$

Where % DE represents Decolourization efficiency, Initial ABS represents the absorbance value of the L B medium containing the dye and Final ABS represents the absorbance value after treatment with the bacterium.

After verifying the capacity of the microorganism, and the intercropping, in discoloring the dye in the test concentrations, a new test was carried out under the same conditions described above, higher dyes concentrations 800ppm did not discolor by the microorganism but the dyes concentrations between 100ppm and 600ppm is the best concentration for the isolated bacterial species were used. Best cultivation condition (microorganism / Decolourization / time) was observed to analyze the variables pH, biomass, WFD, total protein and toxicity.

3.5 HYDROGEN ION POTENTIAL (pH)

Ph meter Model HTLP 081 was used to determine the Ph of the cell free metabolic fluid

3.6 TOTAL PROTEIN DETERMINATION

Protein determination was performed by biuret colorimetric method using Total Protein Kit (Beacon). In test tubes, 1 mL of biuret reagent and 0.01 mL of the metabolic liquid free of cellulase were added. After mixing the content was incubated at 26°C for 5 minutes. Next, the absorbance of the test were determined at 546 nm and with these values, the calculation was performed with the following formula, according to the kit manufacturer:

$$\text{Total Proteins} = \frac{\text{Test Absorbance}}{\text{Absorbance of the Pattern}} \times 4\ (\text{g/dL})$$

3.7 TOXICITY OF THE METABOLITES FORMED

The toxicity test was performed according to the technique described Artemia saline eggs were placed to hatch in artificial seawater (NaCl solution at 3.8%, pH: 8.5±0.2) and left at room temperature for 48 hours, using artificial light and under sufficient aeration for the movement of cysts within the container. To perform the bioassay, 10 nauplius of Artemia salina were transferred with the aid of a micropipette to flat-bottomed microplates with a lid containing 12 holes. In each orifice and placed 5 mL of the metabolic liquid, cell-free, diluted in artificial seawater (5, 25, 50 and 75%), with pH ranging between 7.5 and 8.0. Only artificial seawater was used to control the test. After 24 hours, the live and dead larvae were counted and then the LC50 was calculated, that is the lethal concentration for 50% of the larvae, using the mathematical method Trimmed Spearman-Karber. All trials were performed in triplicate. All those that did not present any movement within 10 seconds of observation with the aid of magnifying glass were considered dead larvae. The diagram below illustrates the sequence of assays and procedures adopted with the three different bacteria and for their intercropping.

IV.RESULT AND DISCUSSION

4.1 DECOLOURIZATION EFFICIENCY

The analysis of the individual Decolourization efficiency of TBSP1 and TBSP2 bacteria isolated from the River Environment, of Textile Effluent and experiment was performed using a concentration of non-inhibiting dye for crop growth. From the Decolourization efficiency observed at the lowest concentration (100ppm L-1) the other Decolourization tests using higher dye concentrations (100ppm 200ppm 300ppm 400ppm 500ppm 600ppm L-1) for up to 96 h, aimed to evaluate the maximum concenteration tolerated by microorganisms. According to the literature, minimum dye concentrations (<100ppm) are sufficient to compromise and impair the aquatic environment mainly affecting photosynthesis and gas. In the textile industry the concentration of dyes used in dyeing the fabrics ranges from 10 to 1000 mg L-1 depending on the reaction power of the dye and the dyeing process used, being absorbed only about 50 to 70% of the dye molecules by hydrolysis and the remainder lost to the effluent.

It is also worth mentioning that according to the literature, anthraquinonic dyes are widely watered in textile industries, and may be present in effluents in a wide variety of colours and in high concentrations. Nevertheless, investigations on the physical-chemical and biological treatment applied to the removal of

color of this dye class are not as frequent when compared with trials conducted with azo- dyes.

Table 1 shows that in the period of 96 h the bacterium TBSP1 presented a 100% decolourization efficiency for the anthraquinoe dye Coomasie brilliant blue R (CBBR) at the concentration 300 ppm L-1 and only 70% at the concentration of 500ppm L-, however, the same period using the azo dye Methyl Orange the bacterium reached 100% decolourization at the concentration of 400 ppm . In higher concentrations (500, 600, 700 and 800ppm L-1) of Methyl Orange dye, the bacterium presented a low percentage of removal, i.e., 19, 12, 3 and 1%, respectively. When Congo red was used, TBSP1 presented decolourization like that obtained for CBBR (Table 1). In this assay, no higher concentration was tested because the bacterium presented decolourization of only 55% at the concentration of 500 ppm of the dye.

When the TBSP2 Effluents Textile strain was tested, the decolourization efficiency was much better, reaching up to 100% removal for both CBBR and Congo red dyes (Table 2) at the concentration of 500ppm with 72 h of cultivation. When higher concentrations were tested, the bacterium did not present the same behavior, and the removal values were 58, 42, 28 and 3% for CBBR and 10, 5, 3 and 1% for Congo red with concentrations.

100, 200, 300, 400 ppm, respectively (Table 2). For Methyl Orange dye the microorganism was efficient only at the lowest concentration tested, reaching 94% Decolourization with 96 h of assay, although with 48 h of cultivation the bacterium had already removed more than 50% of the dye.

CBBR with –TBSP1						
Concentration (mgL^{-1})	**Time**					
	Decolourization (%)					
	0h	**12h**	**24h**	**48h**	**72h**	**96h**
100ppm	**0**	**62**	**78**	**95**	**100**	-
200	0	**36**	69	84	96	100
300	0	26	68	84	95	100
400	0	20	49	52	80	80
500	0	15	30	40	60	70
600	NR	NR	NR	NR	NR	NR
700	NR	NR	NR	NR	NR	NR
800	NR	NR	NR	NR	NR	NR
Mo						
100	**0**	**61**	**100**	-	-	-
200	0	**53**	95	100	-	-
300	0	47	74	95	100	-
400	0	48	71	90	99	100
500	0	10	60	65	70	70
600	NR	5	20	30	40	50
700	NR	NR	10	12	15	20
800	NR		NR	NR	NR	5
CR						
100	**0**	**61**	**100**	-	-	-
200	0	**53**	95	100	-	-
300	0	30	42	61	86	100
400	0	19	30	40	50	55
500	0	10	15	30	40	60
600	0	NR	NR	15	20	20
700	NR	NR	NR	NR	NR	NR
800	NR	NR	NR	NR	NR	NR

Table 1 - Percentage from decolourization Of bacterium TBSP1 isolated from the river Environment CBBR, Methyl Orange and Congo red dyes, using different Concentrations.

The TBSP1 strains promoted the decolourization of the dyes, but there are few data in the literature on the efficiency of biodegradation of this bacterium without any genetic improvement. Despite the low dye removal rates, it was expected that the TBSP1 strain isolated from the textile effluent would present better efficiency because it came from an environment that contained chemical structures like the dyes tested, unlike the TBSP1 strain isolated of the river environment.

The difference in the behavior of microorganisms, even being of the same species, occurs due to the environment from which they were isolated. Aerobic bacteria need an adaptation period for the reductive process to be significant. This adaptation involves a

long period of exposure of the culture to the dyes so under controlled conditions, the bacterium can synthesize specific enzymes and thus promote the cleavage of the compounds. It is believed that part of the decolourization occurred by cellular absorption, since the bacterial biomass was coloured when cultivated with CBBR. Cultures in contact with the Congo red and Methyl Orange dyes also showed staining, but with less intensity. The accumulation of dye in the biomass may indicate chronic toxicity to the cell, and bioaccumulation is a possibly necessary condition for cell survival.

Figure- 2. Decolourization of dye Congo red by Biomass of TBSP1

The authors observed that there was maximum decolourization of dyes 99% in 72 h of cultivation, although the same percentage was observed with 24h of cultivation under aerobic conditions. Biodegradation processes occur in aerobic or combination systems. In decolourization systems under aerobic conditions, the generated electrons are transferred to oxygen because their reduction potential is high, why it does not bind to the dye. In anaerobic systems, electrons are transferred directly to the dye molecule, promoting their cleavage, and forming by-products such as aromatic amines that are potentially toxic and carcinogenic requiring a post-treatment before residues are disposed of in the environment, despite the efficient color removal observed in anaerobic cultures.

Several authors also report the decolourization of textile dyes in aerobic crops, demonstrating that the presence of oxygen in the medium does not directly inhibit the activity of azo reductase an enzymes, but yes, that the removal process is an oxygen-dependent event by bacterial metabolism.

The decolourization efficiency by TBSP2 against CBBR dye reached 100% removal in 72 h of cultivation at the concentration of 500 ppm of the dye. Although in the 24-hour time interval, removal rates of 91, 82, 77 and 42% were achieved by this bacterium at concentrations of 100, 200, 300, 400, 500, 600, ppm, respectively. Removal of 42% of the dye was also observed when using 600ppm from the dye. For Methyl Orange and Congo red dyes, the removal efficiency was lower, presenting 100% decolourization when 400 ppm of the dyes was used (Table 2). The results were observed for Methyl Orange, the bacterium TBSP2 also promoted the removal of 51% of Congo red dye at the concentration of 500 ppm in 96 h of cultivation. When concentrations of 200, 300, 400 and 500 ppm were used, removal rates of 100, 98 and 96% were obtained in the case of Methyl Orange and 100, 100, 98% of different concentration of Congo red with in 48 h of cultivation, respectively (Table 2) To increase the efficiency of decolourization by the bacterium, nitrogen sources (yeast extract and peptone) were added to the medium under these conditions similar to the experiment of this research and using different types (anthraquinones), obtained removal in colour from 60 to 96% with 12 to 96 h of incubation for these dyes. The Decolourization capacity of TBSP1 against different dyes, varied between 80 and 100% in color removal, for practically all the dyes studied. Decolourization was observed of both dyes found 5, 9, 45%, respectively in the case of TBSP2, within 4 days of exposure to the dye. Also in this work, the decolourization by the bacterium was observed higher in case of the anthraquinone CBBR dye than the Congo red and Methyl Orange.

in the observation of consortia TBSP1 and TBSP2 gave the best result in comparison to individual microorganism, which showed 100% decolorization of synthetic textile dyes (CBBR, MO and CR) of 300ppm, 400ppm and 500ppm. in 72h respectively.

Ghazali et al. also reported that the process of decolourization of dyes by individual microorganisms is a limited process, however application of consortia is much more effective. So we can say Decolourization of synthetic textile dyes by microorganisms is an attractive method for sustainable environment.

CBBR						
Concentration	Time					
	Decolourization (%)					
	0h	12h	24h	48h	72h	96h
100ppm	0	71	100	100	100	100
200	0	65	91	100	100	-
300	0	65	91	100	-	-
400	0	54	82	98	100	-
500	0	49	77	93	100	-
600	0	30	42	65	72	78
700	0	10	18	26	28	31
800	0	04	05	09	11	12
		02	03	04	04	04
Mo						
100	0	58	93	100	-	-
200	0	51	93	98	100	-
300	0	43	72	92	100	-
400	0	39	67	93	100	-
500	0	08	19	28	36	40
600	0	04	10	12	14	15
700	0	02	03	05	06	06
800	0	01	01	01	01	02
CR						
100	0	75	100	-	-	-
200	0	58	93	100	-	-
300	0	51	87	100	-	-
400	0	48	71	92	100	-
500	0	32	44	49	51	53
600	0	28	35	40	41	42
700	0	07	20	23	26	26
800	0	03	06	08	08	09

Table 2- % Decolourization of the TBSP2 against the dyes CBBR, Methyl Orange and Congo Red using different concentrations in (PPM)

The position of structural substitutes in the dye molecule is very important in the microbial decolourization process. Hao; Kim; Chiang (2000) observed that the position of the structural substituants of the acid dye Orange -20 was easily discolored by Pseudomonas sp. when compared to the “ortho" position of the Congo red mono azo dye.

There are cases in which the microorganisms involved have greater enzymatic capacity to degrade complex pollutants, in the case of dyes, and produce intermediate compounds, such as sulphuric acid. This metabolite was found in the cleavage of azo dye Congo red when used a consortium in an aerobic reactor. Despite this, the consortium was able to make about90% of the dye in 18 days a reactor cultivation. The use of the intercropping involving the two bacteria strains of TBSP1 and TBSP2 promoted 100% removal in the concentraation of 500ppm with 72 h of cultivation for each dye tested alone. However, when the intercropping was submitted to higher concentrations, a decrease in the efficiency of marked decolourization in Methyl Orange dye was observed, followed by Congo red and CBBR, respectively. When compared with the individual Decolourization behavior of bacteria, it is observed that bacteria removed higher concentrations of CBBR and Congo red dyes when compared with TBSP1. However, it showed low efficiency when compared to TBSP2 individually and CBBR dye. TBSP2 achieved removal of 600 ppm from the dye. It is possible that there was competition between the bacteria occurring to decrease in decolourization efficiency when used in intercropping.

These data are like the results found by Khehra et al. that decolourization efficiency ranging from 78 to 100% for various azo dyes when using a consortium of bacteria composed of Stenotrophomonas sp, Pseudomonas putida, Pseudomonas fluorescence and Bacillus cereus. According to the authors, the efficiency comes from the natural adaptation that occurred between bacteria, since the isolates were isolated from the effluent treatment plant located in a

textile industry in the city of Tanda district Ambedkar nagar Uttar pradesh .

Sharma et al. investigating the removal of color against blue acid 15 with the intercropping of Bacillus sp., Alcaligenes sp. and Aeromonas sp., observed a Decolourization percentage of 94%. Several authors report the potentiality of synergistic action of microorganisms when applied in wastewater treatment of the textile industry. This synergism was observed in the degradation of the dye diazo Remazol Black B by the bacteria Pseudomonas aeruginosa, Rhodobacter sphaeroides, Proteus mirabilis, Bacillus circulene, with rates obtained from up to 84% Decolourization. When studying the degradation of azo direct dye Fast Scarlet 4BS as the only carbon source and found Decolourization of 90% in 24 h of incubation.

CBBR						
Concentration In ppm	Time					
	Decolourization (%)					
	0h	12h	24h	48h	72h	96h
100	0	68	87	100	-	-
200	0	40	75	90	98	100
300	0	36	70	88	100	-
400	0	30	50	60	90	95
500	0	25	35	45	70	70
600	0	20	30	40	60	65
700	0	10	15	20	25	30
800	0	nr	nr	nr	nr	nr
MO						
100	0	70	95	100	-	-
200	0	60	95	98	100	-
300	0	40	85	95	100	-
400	0	30	80	85	93	100
500	0	20	72	85	95	65
600	0	15	50	60	60	45
700	0	10	20	30	40	30
800	0	nr	nr	nr	nr	NR
CR						
100	0	65	95	100	-	-
200	0	60	95	100	-	-
300	0	50	60	90	100	-
400	0	40	55	70	90	100
500	0	30	40	55	75	90
600	0	20	31	45	65	80
700	0	10	20	30	30	50
800	0	nr	nr	nr	nr	NR

Table 3- % Decolourization of the intercropping of TBSP1and TBSP2 against the dyes CBBR, Methyl Orange and Congo Red using different concentrations in (PPM)

4.2 THE ANALYSIS OF PH, BIOMASS, WFD AND PRODUCTION OF TOTAL PROTEINS

Bacterial Decolourization is directly influenced by some factors that affect the production of total proteins, such as temperature, inoculum size and pH of the culture medium. Regarding pH, the two strains of TBSP1showed a similar behavior in the cultivation,

starting from an initial pH of 7.3 to 4.5 at the end of cultivation. TBSP2 and the intercropping, in turn, presented a slightly higher final pH, around 5.3. The decrease in pH is the result of the accumulation of organic acids from glucose degradation present in the medium. Table- 4 shows that the TBSP1 decrease of 0.1233 and 0.0703 g L-1 in its biomass when cultivated in

Bacterium	Dye	Concentration in PPM	Ph		WFD (mg O2 $^{L-1}$)			Biomass without dye (g $^{L-1}$)	Biomass na the end of the test (g $^{L-1}$)	Yield (g $^{L-1}$)
			Initial	Final	Initial	Final	Removal (%)			
TBSP1	CBBR	200	7.34	4.48	39.393	15.589	**60**	0.1719	0.0486	- D 0.1233
	METHYL ORANGE	500	7.24	4.49	36.619	15.285	58		0.2757	0.1038 I
	Congo red	200	7.35	4.41	23.781	11.283	53		0.1016	- D 0.0703
TBSP2	CBBR	100	7.08	5.42	39.393	15.787	**60**	0.1927	0.1146	- D 0.0781
	METHYL ORANGE	500	7.24	5.45	36.619	15.087	59		0.0768	- D 0.1159
	CONGO RED	500	7.21	5.10	30.200	16.672	45		0.1295	- D 0.0632
Consortium	CBBR	500	7.34	5.09	38.497	12.107	**69**	0.2557	0.1174	- D 0.1383
	METHYL ORANGE	500	7.27	5.47	34.118	12.454	63		0.1430	- D 0.1127
	CONGO RED	500	7.32	5.12	30.027	11.748	61		0.1329	- D 0.1228

Table 4 - Findings of the ph Code biomass in process from Decolourization Using TBSP1, TBSP2 and the consortium in the face of CBBR dyes, Methyl Orange e Congo red.

Presence of CBBR and Congo red, respectively. However, in the presence of Methyl Orange the bacterium showed an increase of 0.1038 g L-1. On the other hand, the Strain of TBSP1 showed an increase in biomass of 0.02757g L-1, respectively, in the cultivation containing the dyes CBBR, Methyl Orange and Congo red, showing better adaptation in the presence of these dyes and the possible use of these dyes as a carbon source for growth. The opposite situation occurred with the biomass production of the bacterium TBSP2 and consortium, demonstrating competition between bacteria for nutrients, since the biomass was not coloured. Ren et.al.2006 studying the Decolourization efficiency of Aeromonas hydrophila against different dyes (triphenylmethans, azo and anthraquiones) observed good efficiency when the cultivation was at 25 to 37°C and the pH ranging from 5.0 to 10.0, confirming the results of this

research. In this study, the authors observed that efficiency increased when the assay was submitted to agitation, indicating that Decolourization depended on the presence of oxygen. Regarding biomass, the authors reported that the presence of violet crystal dye as a carbon source favored an increase of approximately five times in biomass after 72 h of cultivation.

An increase in the biomass of Pseudomonas aeruginosa cultivation when the bacterium was adapted to the presence of black pyrazole dye and its use as the only carbon source.

Increasing the pH from 4.0 to 10.0 there was an increase in Decolourization efficiency by TBSP1 in assays using azo dye methyl orange . However, at pH above 10.0 there was a significant reduction in efficiency. The effect of pH on the Decolourization of azo dyes by bacteria occurs due to differences in genetic determinants responsible for the production of enzymes (mainly of the type azoreductase) or responsible for part of bacterial physiology, such as exemple, the mechanism of transport of dye molecules. The molecular weight of azoreductase enzymes varies from species to species, indicating the diversity of genes in the production of these enzymes used in the Decolourization process..

Regarding the quality OFR, several authors report the high VALUES of WFD found in textile effluents due to the presence of dyes. In this study, when the bacteria were used individually (TBSP1, and TBSP2), there was a decrease in the RATE of QUALITY DQ. The highest removal rate was obtained in the trial of the intercropping of the three strains against the CBBR dye (69%), suggesting that there was biodegradation of the dye, while the lowest removal rate (45%) was observed for the assay containing TBSP2 and the Congo red dye. The low decrease in WFD suggests the occurrence of biotransformation in the dye molecule or the presence of recalcitrant compounds. According to the authors, the efficiency in the reduction was not higher due to the presence of from the breakdown of the dyes or extracellular metabolites released by the bacterium. Reduction into WFD ranging from 56 to 85% was observed in the degradation of Magenta, Violet Crystal, and Malachite Green dyes.

In the total protein tests analyzed from the metabolic liquid, a decrease in the concentration of total proteins was observed in almost all trials, differing only in the assays containing TBSP1and Methyl Orange, which showed an increase in protein percentage (Table 5). It may be suggested that this increase in protein concentration in both trials may have occurred due to the presence of enzymatic compounds, interfering in the reading of OD.

Species	Initial	Total Protein gL-1						
		Trial	24 h	48 h	72 h	96 h	yield	
TBSP1	CBBR	0.4848	03724	0.2763	0.2342	0.2042	D	-58%
	MO		0.7658	0.8274	0.9094	0.9436	I	95%
	CR		0.2894	0.2532	0.2326	0.1860	D	-62%
TBSP2	CBBR	0.4041	0.2625	0.1732	0.1312	-	D	-68%
	MO		0.2849	0.2258	0.1667	-	D	-59%
	CR		0.3506	0.2762	0.1965	-	D	-51%
Consortium	CBBR	0.6415	0.6164	0.5472	0.4654	-	D	-27%
	MO		0.5967	0.5053	0.4444	-	D	-31%
	CR		0.4383	0.3951	0.3086	-	D	-52%

I = Prtotein increment D = Protein decrease

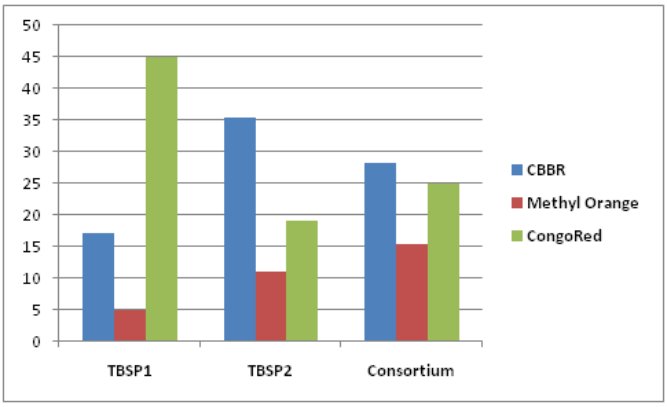
Table 5 - Percentage of total protein in metabolic fluid after the Decolourization step using microorganisms TBSP1 , TBSP2 and intercropping against CBBR, Methyl Orange and Congo Red dyes.

Researcher used TBSP1in the Decolourization of Methyl Orange dye as a carbon source, observed increase in protein production suggests an adaptation of the bacterium during the assays. Absorption of nutrients by microorganisms often promotes the reduction of proteins in the medium, demonstrating the classic biochemical behavior of nutrient absorption.

4.3 TOXICITY OF METABOLITES FORMED IN THE DECOLOURIZATION PROCESS

The harmful effects of intermediary substances of dyes was examine during degradation process when introduced into aquatic ecosystems can cause acute or chronic toxicity. These tests consist of the observation of adverse effects on organisms used depending on the variation in the concentration of the substance studied and the time of exposure of the organism to the substances. shows the percentage of LC50 (lethal concentration in 50% of the population) of the metabolic fluid of the assays, compared to the microcrustacean Artemia salina, calculated by the Trimmed Spearman-Karber method, when exposed for 24 h. All samples showed toxicity to the test organism. The values of LC50 ranged from 11.18 to 46% (v v-1), while in the tests performed with the dye without the microbiological treatment the mortality was low, not reaching 15%. In most cases the dyes is not toxic to the environment but during biodegradation process so many intermediates compounds formed were toxic to the aquatic ecosystems. When comparing the values of LC 50 of the metabolic liquids of the isolates with the intercropping, it was observed that for some cases, this concentration increased, configuring a decrease in the toxicity of the compounds produced during decolourization. In the case of TBSP1 strains Lethal concentration was observed 17.22, 50.0 and 44.98% in CBBR, MO, CR dyes respectively and in the case of strain TBSP2 it was observed 35.56,11.18 and 19.20% in CBBR, MO, CR dyes respectively . In the case of consortium lethal concentration was decreased due to less production of intermediary compounds.

LC 50 (%)



	TBSP1	TBSP2	Consortium
CBBR	17.22	35.36	28.28
Methyl Orange	5.00	11.18	15.49
CongoRed	44.98	19.20	25.01

Figure3. Percentage of LC50 using Artemia salina after the Decolourization process of the dyes CBBR, Methyl Orange and Congo red using TBSP1, TBSP2 and the bacteria intercropping.

In this study, it was observed that the dye methyl orange without treatment shows low toxicity, after microbial treatment the toxicity to the Microcrustacean Artemia salina amounted to LC 50=60% ±5. Fontana et al (2007) also observed that the acute toxicity tests using the textile effluent showed different results, i.e., the bioindicators Artemia salina, Daphnia magna, and onion root growth (Allium cepa), showed that a reduction of the toxicity of the textile effluent. It is important to note that each bioindicator responds differently when contacting the test solution he observed that the toxicity of the remazole Turqueza Blue G and Lanaset Azul 2R dyes was similar to the toxicity of the products formed after the enzymatic degradation of using the Artemia saline bioindicator and obtaining a LC50 between 55% and 58.33%, respectively.

V. CONCLUSION

In the case of observation the results obtained in the present study led to the following conclusions: The TBSP1 isolated from the river environment was able to decolorize the CBBR, Methyl orange and Congo red dyes at the maximum concentration upto 700ppm. TBSP2 showed better decolourization efficiency when compared to the other bacteria it removed 100% CBBR, MO and CR dyes up to500ppm, respectively. The microbial intercropping (consortium) showed better decolourization efficiency when compared to TBSP1 and TBSP2. In most cultures, microorganisms have promoted a decrease in OD of around 60%. Microorganisms both TBSP1 and TBSP2 alone and in consortium in the cleavage process of the molecule produce metabolites with recalcitrant characteristics.

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