CHEMICAL ENGINEERING TRANSACTIONS
VOL. 37, 2014
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Eliseo Ranzi, Katharina Kohse- Höinghaus

ISBN 978-88-95608-28-0; **ISSN** 2283-9216
DOI: 10.3303/CET1437141

Determination of Strains from Tropical Culture Collection for Biological Treatment of Effluents from Textile Industry

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The azo dye is the most widely used in the textile industries. This type of dye is considered recalcitrant due the presence of aromatic ring in the molecule. When it inserted in the environment, cause environmental damage, like the interference in the absorption and reflection of light in the aquatic environment, and can to be carcinogenic and mutagenic, molecule with both the full and after decolonization. Research with the degradation of dye used microorganisms, especially bacteria, it have result in excellent reduction of compound, and the environmental benefit overlaps the treatment and / or physical chemical. In this study, a facultative anaerobic bacterium was used to decolorize nine dyes of azo type. The bacteria in the Tropical Culture Collection was certified how Shewanella putrefaciens and reference it is CCT 1967. This was a fractional factorial planning in order to observe the effects of variables (temperature, pH, microorganisms, culture medium and agitation) for the decolourization of dyes. The better condition analysis was determined for temperature 25 °C and pH 8.5. The result, under these conditions, was the discoloration of seven dyes, and in a dye was the precipitation of compound and, in other, not decolorized. The analysis in condition aerobic was also performed, and the result was not satisfactory for any of the dyes in question.

1. Introduction

Due the complex structure and origin synthetic the textile dyes are of difficult discoloration. And so, the discoloration of textile dye in municipal sewage systems, by treated aerobically, not occur (Willmott et al., 1998; Robinson et al., 2001).
A low concentration of dyes in effluent is visible and undesirable (Nigam et al., 2000; Robinson et al., 2001). The presence of dyes in the aquatic environment interferes in light absorption, both vegetal and animal life, in the accumulation potential and in the transport of the contaminant in the source water and the water distributed to the population (Guarantini; Zanoni, 2000).
The azo dyes, considered recalcitrant to biodegradative processes, are produced as a by-product at a rate of 100,000 to 150,000 liters per ton of treated fabric (Li and Guthrie, 2010; Bezerra dos Santos, 2005).
An alternative to chemical and physical methods, that are not widely used due to their high costs and secondary pollution, is the biological approach, which is considered environmental friendly, and sometimes can make a complete mineralization of the organic pollutants (Pandey et al; 2006).

Please cite this article as: Casellato A.M.F., Coelho D.D.F., Silveira E., Tambourgi E.B., 2014, Determination of strains from tropical culture collection for biological treatment of effluents from textile industry, Chemical Engineering Transactions, 37, 841-846
DOI: 10.3303/CET1437141

However, there are several microorganisms that are able, under certain environmental conditions, to degrade azo dyes to non-colored products or even to completely mineralize them (Stolz, 2001).
There are many studies in this area using fungi, bacteria, yeast and algae that, under certain environmental conditions, are able to degrade azo dyes to non-colored products and show satisfactory results (Pandey et al., 2006). Chen (2008) showed that the degradation of dye with Shewanella decolorationis had a higher rate and greater extent than any other microorganisms reported previously.
In this study, Shewanella putrefaciens (CCT1967) was used to evaluate the effects of culture medium, temperature and pH on the color removal from aqueous solutions containing azo dyes.

2. Material and Methods

2.1 Microorganisms

The microorganisms were obtained from the Tropical Culture Collection of Foundation Andre Tosello. They were preserved in nutrient broth, with 10% glycerol (v/v) in cryotubes.

2.2 Dyes and reagents

The textile dyes were obtained with kind permission from Clariant of Brazil (Sao Paulo, Brazil). The commercial names will be omitted. The dyes received the following codenames: B31 (C.I. 20460); B36 (C.I. 11825); B86 (C.I. 26400); Methyl orange (C.I. 13025); R34 (C.I. 14714); R91 (C.I. 18800); Y15 (C.I. 13950); Y79 (C.I. 13065) and Y87 (C.I. 22910). They were filter-sterilized on a 0.22-µm filter (Millipore, SA). All other reagents are from analytical grade.The medium used contained Nutrient Broth (Meat extract 1.5 g.L-1 and Peptone 2.5 g.L-1) and was sterilized at 121ºC for 15 min.

2.3 Dyes' Calibration Curves

Calibration curves were obtained by optical spectrophotometry. Each dye solution was dilute from 100 $mg.l^{-1}$ using distilled water to the following concentrations: 0.75, 1.25, 2.5, 5.0, 7.5 , 10.0, 20.0, 30.0, 40.0 and 50 $mg.l^{-1}$.

2.4 Decolourization Experiments

50 mL of each culture medium were added in 50 mL of each dye at the concentration of 100 mg.L-1 (initial averaged concentration of 50 mg.L-1) in a 250 mL Erlenmeyer flask. One mL of 1.0 McFarland was added in 100 mL of solution, which corresponds to 107 cfu.mL-1 of Shewanella putrefaciens, and inoculated in the previous solution. The experiments were cultivated in static condition for 24 and 48h. Aliquots (3 mL) of the culture media were centrifuged after 24 and 48h at 3500 rpm for 10 min to separate the supernatant from the cell mass. Decolorization was determined by measuring the absorbance of the supernatant and the percentage was calculated. The analysis were performed at room temperature (25°C) and pH 8.5.

3. Results and Discussion

3.1 Bacteria selection

The chosen microorganisms are shown in table 1. These bacteria are level 1 of biosafety, except the *Pseudomonas* aeruginosa, that is level 2, according the American Biological Safety Association, Mundelein (ABSA, 2014). Among the seventeen microorganisms whose biodegradation efficiency was evaluated, the strain which showed the best result was *Shewanella putrefaciens* (CCT 1967) (see tables 1 and 2).

Table 1: Microorganisms choose to analyse of biodegraded

Species	Strain Designations	Isolation
Brevundimonas diminuta (Pseudomonas diminuta)	CCT 0594 / ATCC 11568	Freshwater
Pseudomonas fluorescens	CCT 0595 / ATCC 13525	Pre-filter tanks
Pseudomonas mendocina	CCT 0597 / ATCC 25411	Soil enrichment with ethanol as carbon source
Sphingomonas sp.	CCT 1899 / ATCC 31461	Lake
Pseudomonas aeruginosa	CCT 1915 / ATCC 13388	Uninformed
*Pseudomonas rubsescens**	CCT 1967 / IMI 12202 (CABI)	Uninformed
Pseudomonas oleovorans	CCT1969 / IMI 6576 (CABI)	Uninformed
Pseudomonas putida	CCT 2357 / ATCC 12633	Uninformed
Pseudomonas aeruginosa	CCT 2738 / ATCC 15442	Animal room water bottle
Pseudomonas mendocina	CCT 2956 / ATCC 25411	Soil enrichment with ethanol as carbon source
Pseudomonas fluorescens	CCT 3178 / ATCC 13525	Pre-filter tanks
Pseudomonas aeruginosa	CCT 4392 / ATCC 15442	Animal room water bottle
Pseudomonas fluorescens	CCT 4973 / CCT isolation	Wastewater treatment
Pseudomonas acidovorans	CCT 4976 / CCT isolation	Wastewater treatment
Pseudomonas aeruginosa	CCT 5448 / ATCC 15442	Animal room water bottle
Pseudomonas aeruginosa	CCT 5587 / ATCC 15442	Animal room water bottle
Pseudomonas stutzeri	CCT 7544 / ATCC 31258	Soil

- According Culture Collection DSMZ (2014), *Pseudomonas rubsescens* is *synonymous of Shewanella putrefaciens*

3.2 Discoloration

The bacteria efficient are CCT 1915 (*P. aerugionosa*) and CCT 1967 (*Shewanella putrefaciens*). They degraded 7 azo dyes. The bacterium S. putrefaciens was a good microorganism to this work because it not pathogenic and it has ideal growth conditions (mesophilic, facultative anaerobic, simple medium, etc.). As the bacteria CCT 0597, CCT 2956, CCT 4973, CCT 4976 and CCT 5587 degraded 2 azo dyes and CCT 2357, CCT 3178 degraded only 1 azo dye, they were not regard in the experiment.

Table 2: Result of the selection of microorganisms for discoloration of azo dyes.

CCT	B31	B36	B86	MO	R34	R91	Y15	Y87	Y79
0594	-	-	-	-	-	-	-	-	-
0595	-	-	-	-	-	-	-	-	-
0597	+	+	-	-	-	-	-	-	-
1899	-	-	-	-	-	-	-	-	-
1915	+	+	-	+	+	+	+	+	-
1967	+	+	-	+	+	+	+	+	-
1969	-	-	-	-	-	-	-		-
2357	-	+	-	-	-	-	-	-	-
2738	-	-	-	-	-	-	-	-	-
2956	+	+	-	-	-	-	-	-	-
3178	-	+	-	-	-	-	-	-	-
4392	-	-	-	-	-	-	-	-	-
4973	+	+	-	-	-	-	-	-	-
4976	+	+	-	-	-	-	-	-	-
5448	-	-	-	-	-	-	-	-	-
5587	+	+	-	-	-	-	-	-	-
7544	-	-	-	-	-	-	-	-	-

+ Average biodegradation visible, - biodegradation not visible

The tests were performed within 48 hours in a spectrophotometer. The absorbance values were converted into concentration using equations from calibration curves (Figure 1).

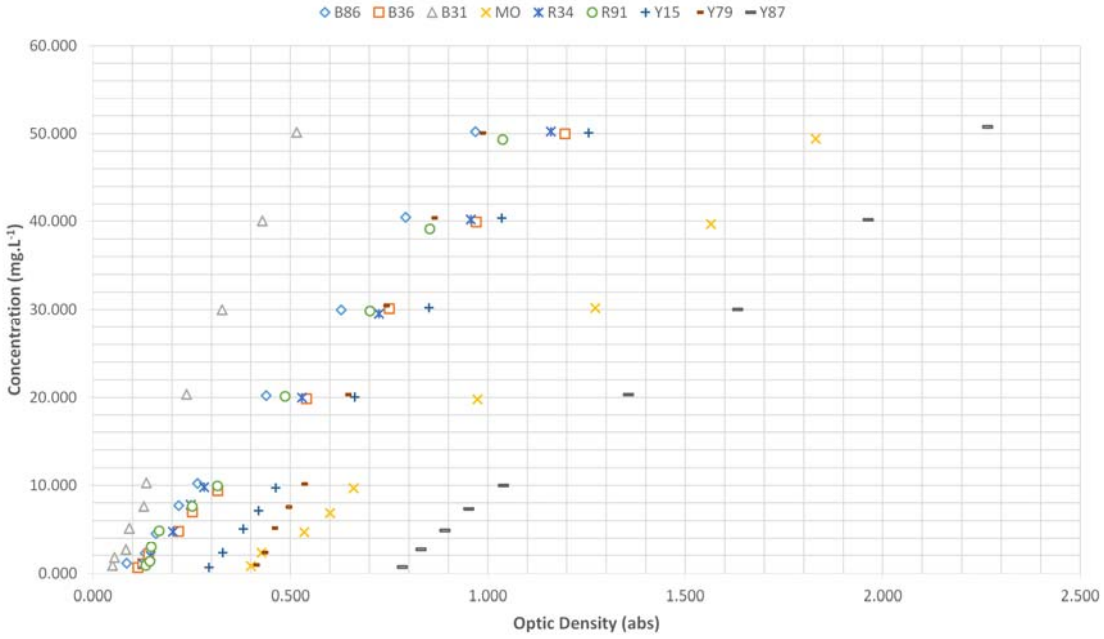

Figure 1: Calibration curve for Dyes used in this study

The decrease on rate of removal of colour observed in some dyes (figure 2) after 24 hours of treatment may be due to the synthesis, at the process end, of metabolite and by-products.

All dyes were studied in conditions previously established. In this work, both known phenomena (bioaccumulation and biodegradation) related to colour removal in water were observed. The dyes Methyl orange, R34, B36, R91, B31, Y87, and Y15 were biodegraded, once produced biomass were white-clear. The biomass formed using dye B86, on the other hand, yielded a blue precipitate and therefore was bioaccumulated. Dye Y79 was not biodegraded at all.

The dyes were removed from synthetic solution at rates of 1-80%, whereas S. putrefaciens showed the best efficiency in color removal of the dye B3.

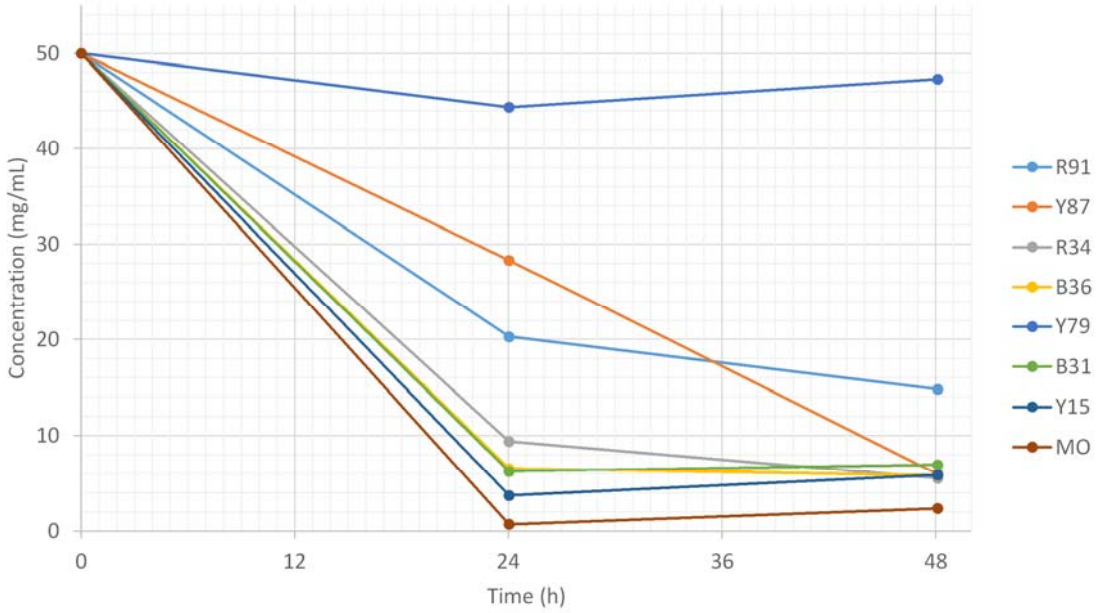

Figure 2: Decolourization of dyes in times in the 24 and 48 hours.

Although the full design comprises a larger number of experiments, table 3 shows the best results achieved. In those experiments, S. putrefaciens CCT1967 was able to reduce dye initial concentration from 50 mg.mL-1 to concentration below 12 mg.mL-1. This result was obtained in the assay 6, at pH 8.5 and 25°C. The better culture medium was the nutrient broth, which provides the minimum of some nutrients: carbohydrates, mineral, protein and nitrogen source.
Li and Guthrie (2010) found a result similar, they used in yours research the bacteria Shewanella where the best conditions were pH 8-9 and temperature 30 °C.

Table 3: Results of last Design of Experiments

Assay	Culture Medium	Temperature	pH	Results	
				Dye Concentration (mg.L-1)	Degraded Dye (mg.L-1)
1	½ CN	20 °C	8.0	21.142	28.858
2	Pept NaCl	20 °C	9.0	37.075	12.925
3	½ CN	30 °C	9.0	32.353	17.647
4	Pept NaCl	30 °C	8.0	22.983	27.017
5	CN	25 °C	8.5	11.331	38.669
6	CN	25 °C	8.5	11.880	38.120
7	CN	25 °C	8.55	11.731	38.269

4. Conclusions

Further investigations concerning dye removal rate are still necessary, but the importance of this study is to provide a new option of microorganism capable to sustain biological treatment of dye to reduce, or virtually eliminate, azo dyes that contaminate water bodies and make it safe for life consumption. Under that perspective, the bacterium CCT 1967 is a very promising agent, since that only dye B86 was not degraded.

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