

## Biodegradation of Textile Azo Dyes by *Shewanella putrefaciens* (CCT 1967)

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Dyes are toxic to aquatic environment and for human because they are mutagenic and carcinogenic. Due to the large amount of effluent that the textile industry discharges in aquatic environment, the concern with better and more economic treatment is growing. Dyes are recalcitrant to conventional biological treatment, so the research for using the method of biodegradation is to apply selected bacteria capable of degrading these compounds, mineralize the dye, thus reducing or even eliminating the toxicity. The aim of this study is to test and optimize the degradation of nine azo dyes using the bacterium *Shewanella putrefaciens* (CCT 1967). The discolorations of the dyes were performed in anoxic condition, pH 8.5, temperature 25 °C and addition of carbon source: meat extract 1.5 g.L<sup>-1</sup> and peptone 2.5 g.L<sup>-1</sup>. The dye concentration used was 50mg.L<sup>-1</sup> and the incubation times were 24 hours and 48 hours. Seven dyes were biodegraded in a percentage of 29 to 81% and two dyes were not biodegraded.

### 1. Introduction

Dyes are inserted into aquatic environment carried by water that was used in coloring and washing processes in textile industry. This effluent is 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). Concentration of 1 mg.L<sup>-1</sup> or low dye can easily be detected in waterbodies (Guaratini and Zanoni, 2000).

The presence of dyes in the aquatic environment interferes with the absorption of light and the oxygen transfer in the water. It also leads to aesthetic problems, as affect the water appearance (Pandey et al., 2006, Rajagur et al., 2002, Umbuzeiro et al., 2005).

When compared with some industrial discharges, the textile and other dye-related industries effluents produce frequently genotoxic effluents (Umbuzeiro et al., 2005). Studies report that azo dyes are known to be mutagenic and carcinogenic when there is cleavage of azo bond, or when it comes along with the dye commercial products as contaminants (Oliveira et al., 2007, Guaratini and Zanoni, 2000).

The azo dyes are considered recalcitrant to biodegradative processes. 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 that uses fungi, bacteria, yeast and algae that

show satisfactory results (Pandey et al., 2006). Physical-chemical methods are not widely used due to its high costs and secondary pollution that is generated by excessive use of chemicals (Silveira et al., 2009, Jadhav et. al., 2007). Hence, the physical-chemical treatment of effluent typically results in formation of secondary sludge (Pearce et al., 2008).

An alternative to chemical and physical methods is the biological approach, which is considered environmental friendly, and sometimes a complete mineralization of the organic pollutants is achieved (Pandey et al; 2006). Studies report that species *Shewanella putrefaciens* is able to mobilize and reduce toxic pollutants and radioactive metals, including Co, Cr, Te and U. There is also research showing the use of this bacterium in dehalogenated reduction and transformation of organic pollutants (Brenner et. al., 2005). 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* (CCT1987) was used for the removal of color from aqueous solutions containing azo dyes. The effects of culture medium, temperature, pH on the color reduction were also studied.

## 2. Materials and Methods

### 2.1 Microorganism

The microorganism was obtained from the Tropical Culture Collection of Foundation Andre Tosello, previously identified as *Shewanella putrefaciens* (CCT 1967). The bacterium was certified in Vitek<sup>®</sup> (automated system of identification - bioMérieux). It was preserved in nutrient broth with 10% glycerol (v/v) in suspension 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- $\mu$ m filter (Millipore, SA). All other reagents are from analytical grade.

### 2.3 Culture Medium

Two medium were used: Nutrient Broth (Meat extract 1.5 g.L<sup>-1</sup> and Peptone 2.5 g.L<sup>-1</sup>) and minimum medium described by Silveira (2009). The media were sterilized at 121°C for 15 min.

### 2.4 Decolorization Experiments

50 mL of each culture medium were added in 50 mL of each dye at the concentration of 100 mg.L<sup>-1</sup> (initial averaged concentration of 50 mg.L<sup>-1</sup>) in a 250 mL Erlenmeyer flask. One mL of 1.0 McFarland was added in 100 mL of solution, which corresponds to 10<sup>7</sup> cfu.mL<sup>-1</sup> of *Shewanella putrefaciens*, was 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.

### 3. Results and Discussion

#### 3.1 Biodegradation Condition Optimization

The decolorization by *Shewanella putrefaciens* was observed in different liquid medium, pH and temperature. These first set of experiments were performed to optimize the biodegradation conditions. The dyes utilized at this set of experiments were B36 and Methyl orange. Table 1 shows the dyes degradation under different conditions, proving the capability of bacteria to grow and decolorize azo dyes.

Table 1: Degradation of the dyes under different conditions

Dyes	Nutrient Broth Medium, pH 8,0, temperature 30°C	Nutrient Broth Medium, pH 8,5, temperature 25°C	Minimum medium, pH 7,0, temperature 30°C	Minimum medium, pH 8,5, temperature 30°C
B36	+	++	-	-
Methyl Orange	+	++	-	-

++ best biodegradation visible, + average biodegradation visible, - biodegradation not visible

The best results achieved were with nutrient broth, pH 8.5, temperature 25°C at static conditions. The fact of the process being static was important too because the microorganism is a facultative anaerobic.

#### 3.2 Decolorization

In set of experiments, all dyes were submitted in the condition described previously. The results are shown in Figures 1 and 2. According to Silveira et al. (2009) there are two ways in which microorganism can remove color in the water: (1) bioaccumulation, in which dyes adsorb into the cell walls of the microorganism and (2) biodegradation, in which the dyes are oxidized by the enzymatic systems of the microorganism.

In this work both phenomena were observed, the dyes B31, B36, Methyl orange, R34, R91, Y15, and Y87 were biodegraded because the biomass produced were white-clear. In the other hand, the dye B86 bioaccumulated because within the decolorization process the dye and biomass formed a blue precipitate. When the suspension was centrifuged, and the dye Y79 not was biodegraded at all.

The decolorization rate of the dyes ranged of 2-81%, the dye B36 was that show best color removal by *S. putrefaciens*. In some dyes, the results showed a decrease of color removal rate from 24 hours to 48 hours, it should be occurred due the production of by-products and metabolite at the process end. There is also a possibility that azo dyes were used as an alternative energy source by the microorganism, which would reduce the mass transport problem.

Similar conditions were described by Kalyani (2008) when decolorizing Red BLI dye by *Pseudomonas sp.*, which resulted in 99.28% of decolorization in 1 hour, with initial concentration of 50 mg.L<sup>-1</sup>. Some *Pseudomonas*, as described by Silveira (2009),

showed dye degradation rates higher than those described here for Methyl Orange, B86 and B31 dyes, however, Y87, R91, B36, Y15 and R34 dyes showed a higher degradation rate by *Shewanella putrefaciens* (CCT1967).

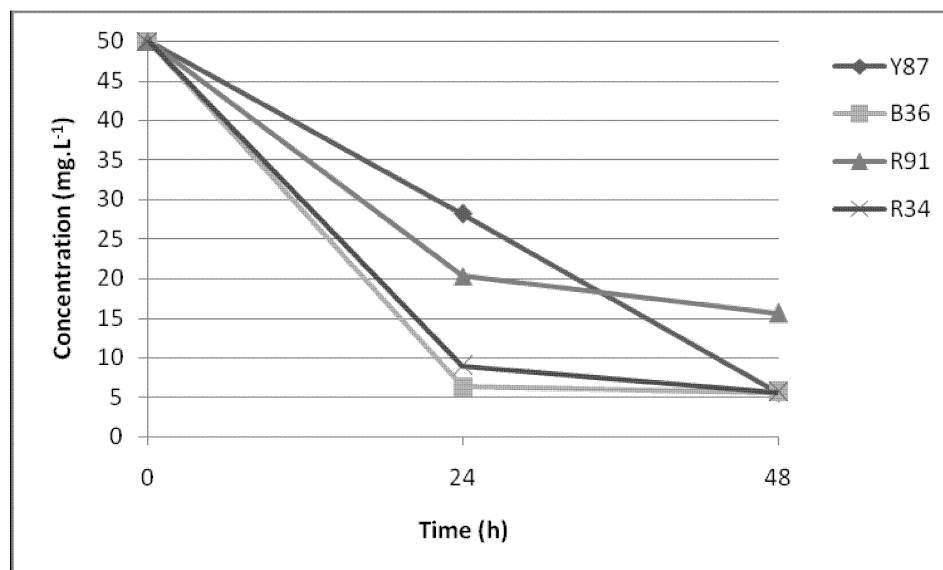


Figure 1: Decolourization of dyes in times in the 24 and 48 hours.

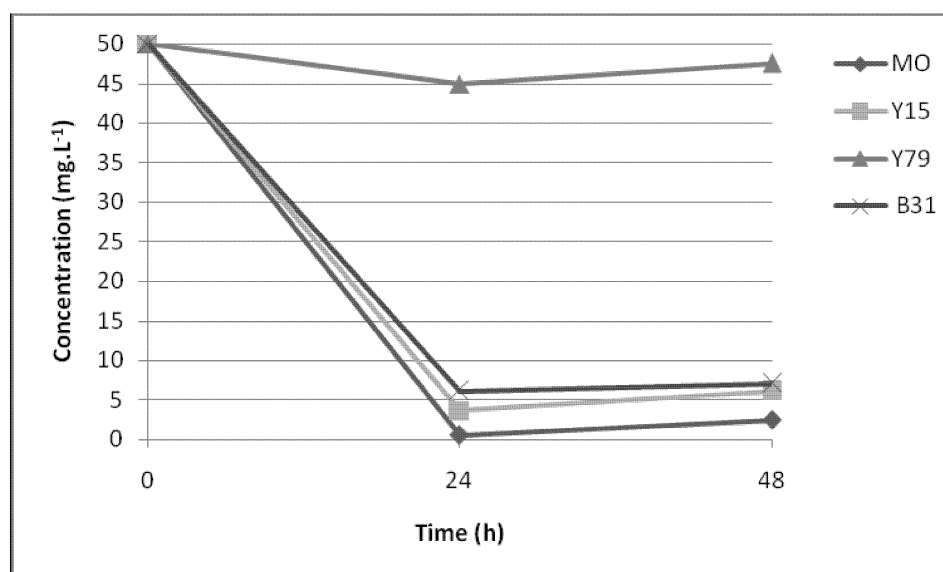


Figure 2: Decolourization of dyes in times in the 24 and 48 hours

The utilization of alkaline pH in this research was substantial because in the textile final process wastewater is very alkaline. Chen (2008) in his study used a *Shewanella decolorationis* in static anaerobic conditions, optimum pH 8 to 9 and temperature 30 to 40°C for decolorization of crystal violet and the result was high effective (100%).

#### 4. Conclusion

The *Shewanella putrefaciens* (CCT1987) had shown high ability for the degradation of the dyes B31 B36, Methyl orange, R34, R91, Y15, and Y87, although the dyes B36 and Y79 were not biodegraded. Further investigations concerning dye concentration could therefore increase the decolorization rate, as the studies Chen (2010) that used the concentration of 24.4 to 1,345 mg l<sup>-1</sup> h<sup>-1</sup>.

The importance of this study is to provide a new source of microorganism as a biological treatment tool to reduction or a possible elimination of azo dyes of water bodies to be safe for human and animal consumption.

#### References

- Bezerra dos Santos A., 2005, Reductive decolourisation of dyes by thermophilic anaerobic granular sludge. PhD thesis, Wageningen University, The Netherlands.
- Brenner D.J., Krieg N.R., Staley J.T., 2005, Bergey's Manual of Systematic Bacteriology. Vol 2: The *Proteobacteria*. Part B: The *Gammaproteobacteria*.
- Chen C.H., Chang C. F., Liu S. M., 2010, Partial degradation mechanisms of malachite Green and methyl Violet B by *Shewanella decolorationis* NTOU1 under anaerobic conditions. *Journal of Hazardous Materials*, 177, 281-289.
- Chen C.H., Chang C.F., Ho C.H., Tsai T.L., Liu S.M., 2008, Biodegradation of crystal violet by a *Shewanella* sp. NTOU1. *Chemosphere*, 72, 1712-1720.
- Guaratini C.C.I., Zanoni M.V.B., 2000, Corantes Têxteis. *Quím Nova*, 23, 71-78.
- Jadhav J.P., Parshetti G.K., Kalme S.D., Govindwar S.P., 2007, Decolourization of azo dye methyl red by *Saccharomyces cerevisiae* MTCC 463. *Chemosphere*, 68, 394-400
- Kalyani D.C., Patil P.S., Jadhav J.P., Govindwar S.P., 2008, Biodegradation of reactive textile dye Red BLI by na isolated bacterium *Pseudomonas* sp. SUK1. *Bioresource Technology*, 99, 4635-4641.
- Li T., Guthrie J.T., 2010, Colour removal from aqueous solutions of metal-complex azo dyes using bacterial cells of *Shewanella* strain J18 143. *Bioresource Technology*, 101, 4291-4295.
- Oliveira D.P., Carneiro P.A., Sakagami M.S., Zanoni M.V.B., Umbuzeiro G.A., 2007, Chemical characterization of a dye processing plant effluent-Identification of the mutagenic components. *Mutation Research*, 626, 135-142.
- Pandey A., Singh P., Iyengar L., 2006, Bacterial decolorization and degradation of azo dyes. *International Biodeterioration & Biodegradation*, 96, 73-84.
- Pearce C.I., Guthrie J. T., Lloyd J. R., 2008, Reduction of pigment dispersors by *Shewanella* strain J18 143. *Dyes and Pigments*, 76, 696-705.

- Rajaguru P., Vidya L., Baskarathupathi B., Kumar P.A., Palanivel M., Kalaiselvi K., 2002, Genotoxicity evaluation of polluted ground water in human peripheral blood lymphocytes using the comet assay. *Mutation Research*, 517, 29-37.
- Silveira E., Marques P.P., Silva S.S., Lima-Filho J.L., Porto A.L.F., Tambourgi E.B., 2009, Selection of *Pseudomonas* for industrial textile dyes decolourization. *International Biodeterioration & Biodegradation*, 63, 230-235.
- Stolz A., 2001, Basic and applied aspects in the microbial degradation of azo dyes. *Appl Microbiol Biotechnol*, 56, 59-80.
- Umbuzeiro G.A., Freeman H.S., Warren S.H., Oliveira D.P., Watanabe T., Claxton L.D., 2005, The contribution of azo dyes to the mutagenic activity of the Cristais River. *Chemosphere*, 60, 55-64.