

# Creating connections between biotechnology and industrial sustainability

August 25 to 28, 2024 Costão do Santinho Resort, Florianópolis, SC, Brazil

**ENVIRONMENTAL BIOTECHNOLOGY** 

# DECOLORIZATION OF SYNTHETIC AND NATURAL DYES IN BIODEGRADATION PROCESS

Eduarda L. Neumann<sup>1\*</sup>, Quethelen E. A. G. Rodrigues<sup>1\*</sup>, Ariane C. V. Oliveira<sup>2</sup>, Joel Dias Silva<sup>1</sup>, Aline C. Alves<sup>1</sup>, Vanderléia Botton<sup>3</sup>, Marcela K. Silva<sup>3</sup>, Tania M. Costa<sup>2</sup>, Carolina K. Souza<sup>3</sup>, Marcel J. Gonçalves<sup>1,3</sup>

Postgraduate Program in Environmental Engineering, Regional University of Blumenau (FURB), Blumenau, SC, Brazil.
Chemical Engineering Department, Regional University of Blumenau (FURB), Blumenau, SC, Brazil.
Postgraduate Program in Chemical Engineering, Regional University of Blumenau (FURB), Blumenau, SC, Brazil.
\* Corresponding author's email address: elneumann@furb.br; grodrigues@furb.br

# **ABSTRACT**

This study aimed to evaluate the biodegradability efficiency of the synthetic dyes Rhodamine B (Rh B) and Flavine 8G (FV), as well as the natural dye annatto (control), using the biological activated sludge process, commonly applied in textile industries. Two experiments were conducted with biomass collected on different dates, using duplicate samples for each dye. The decolorization was evaluated at 24, 48, and 72 hours. The average decolorization efficiencies of experiments 1 and 2 were similar for all dyes but generally higher in the second experiment. The best performances obtained were 62.9%, 80.2%, and 89.6% for Rh B, FV, and annatto dyes, respectively, all at 72 hours. The results for the synthetic dyes indicate varied efficiency in dye removal, with FV being more easily removed compared to Rh B. This difference can be attributed to the distinct chemical and structural properties of each dye, which influence their interaction with the biomass present in the activated sludge and the effectiveness of the decolorization process.

Keywords: Rhodamine B. Flavine 8G. Textile effluents. Microrganisms caracterization. Toxicyti. Fluorente dyes

#### 1 INTRODUCTION

The textile industry uses large volumes of water in its production processes, making it one of the industries with the highest water consumption and, consequently, one of the largest generators of liquid effluents <sup>1,2</sup>. Textile effluents are known for having high loads of organic and inorganic pollutants, heavy metals, and dyes, making them one of the main sources of environmental contamination when discharged without effective treatment. These contaminants are harmful to human health and other living beings, because they are often toxic, carcinogenic, mutagenic, and difficult to degrade <sup>3,4</sup>.

Dyes represent one of the main pollutants, with an estimated 15% of these products being lost in the textile processing due to non-adherence to the fibers, and thus being directly released into the effluents <sup>5,6</sup>. Among the dyes used in this sector, the fluorescent acid dyes Rhodamine B and Flavine 8G stand out, having been recently introduced in various fashion collections due to their vibrant coloration. One of the main characteristics of fluorescent dyes is their ability to absorb light at one wavelength and emit it at a longer wavelength, producing intense and colorful fluorescence <sup>7</sup>. According to textile industries, these dyes present significant challenges in their removal from effluents, which limits the daily production of fabrics that use them. The dyes are responsible for the intense coloration of the final effluents and, when discharged into nature without effective treatment, they prevent light from passing through water bodies, hindering the photosynthesis of aquatic organisms <sup>3,8</sup>.

Therefore, it is imperative that proper treatment is applied to these effluents, ensuring better quality of the receiving bodies and even enabling the reuse of the treated effluent, thus reducing the consumption of water drawn from natural water bodies <sup>3,4</sup>. In this context, the present study aimed to evaluate the efficiency of the biodecoloration of the dyes Rhodamine B, Flavine 8G, and the natural dye annatto (control) through the biological activated sludge process, commonly used in textile industries.

# 2 MATERIAL & METHODS

For this research, two synthetic dyes were used: Rhodamine B (Rh B) and Flavine 8G (FV), and a natural annatto dye (biodegradable) as a control. For the synthetic dyes, the concentration was 40 mg/L, as according to <sup>9</sup> this value falls within a range of expected average concentration of dyes in the final raw effluent. For the natural dye (control), the chosen concentration was 0.32 g/L (liquid dye). Two assays were conducted with biomass collected on two different dates, with each assay performed with duplicate samples for each dye.

The activated sludge biomass was collected at the wastewater treatment plant (WWTP) of a large textile company in the region of Blumenau, SC, Brazil, which manufactures woven fabrics and has an average effluent flow rate of 110 m³/h. On the first day of collection, in February 2024, the system showed an efficiency of 88.8% in biochemical oxygen demand (BOD) removal. On the second collection, in March 2024, the efficiency was at 91.3%. Microscopic analyses revealed a wide variety of microorganisms in both collections, including several rotifers and other micrometazoans, indicating that the effluent treatment system was operating stably, with a propensity for good biodegradation efficiency and low toxicity present <sup>10</sup>.

After the three days of biodecoloration assays, a new sludge sample was analyzed under the microscope, revealing similarity in the types of microorganisms initially found. This proved that there was no significant (visual) loss of biomass diversity present in the aerobic reactor.

The biomass collected from the sludge return from the settler to the aerobic reactor, with suspended solids ranging from 4,400 mg/L to 4,700 mg/L, was centrifuged and washed with distilled water, aiming to eliminate the maximum impurities present in the effluent. The filtered biomass was resuspended in an equal initial volume of effluent, and to each dye solution, a biodegradable carbon source (1 g/L of soluble starch) was added <sup>11</sup>. Samples were collected every 24 hours over a total period of 72 hours. After collection, the samples were centrifuged to sediment the suspended solids, and then absorbance measurement was carried out.

The determination of the percentage of decolorization was based on the reduction of absorbance at the maximum wavelength of each dye: 433.5 nm for the FV dye, 484 nm for the Rh B dye, and 400 nm for the natural annatto dye. The absorbance at the maximum wavelengths was measured using a Shimadzu UV-VIS spectrophotometer (UV-1650pc) to obtain the linear relationship between the dye concentration and the absorbance values. The decolorization capacity was determined based on the absorbance (A) of each sample, calculated using Equation 1.

Decolorization capacity (%)= 
$$\frac{(A_0 - A_1)}{A_0} \times 100$$
 (1)

Where  $A_0$  is the initial absorbance, and  $A_1$  is the final absorbance of each sample.

# **3 RESULTS & DISCUSSION**

The information from textile industries indicates difficulties in treating effluents with activated sludge when processing colors containing Rh B and FV dyes. The results obtained from the decolorization of the Rh B, FV, and Annatto dyes in the two experiments (1 and 2) can be observed in Figure 1. These results corroborate the difficulties faced by industries, especially with the Rh B dye.

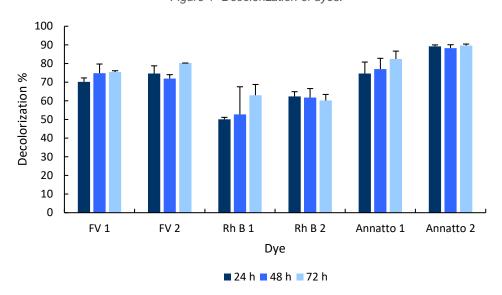


Figure 1- Decolorization of dyes.

Note: The vertical bars represent the standard deviation of each sample.

The average decolorization efficiencies of experiments 1 and 2 were similar for all dyes, but generally higher in the second experiment. During this one, the data from the ETP indicated an efficiency of 91.3% in COD removal and a solids concentration of 4.7 mg/L at the time of collection. According to<sup>3,9</sup> higher sludge concentrations increase adsorption capacity and the presence of microorganisms, resulting in greater efficiency in contaminant removal.

The best performances obtained were 62.9%, 80.2%, and 89.6% for the Rh B, FV, and Annatto dyes, respectively, all within the 72 hour. It is noteworthy that Annatto achieved the highest decolorization percentage as it is a natural dye, confirming its biodegradability. The results obtained for the synthetic dyes indicate varied efficiency in dye removal, with FV being more easily removed compared to Rh B. This difference can be attributed to the distinct chemical and structural properties of each dye, influencing their interaction with activated sludge and the effectiveness of the decolorization process. Studies indicate that the complex ring structure of Rh B hinders its direct biodegradation, which may reduce biological activity <sup>12</sup>. A similar result to that of

Rh B was obtained in a study on textile effluent color removal through the mixed aerobic process after 72 hours of aeration, achieving an efficiency of 58.57% <sup>13</sup>. Another study achieved a 30% color removal from a textile effluent through aerobic-anoxic treatment <sup>14</sup>.

The decolorization of dyes by microorganisms mainly occurs through their adaptive capacity and can be carried out in two ways: (a) dye adsorption onto microbial biomass and (b) biodegradation by microorganisms. Adsorption occurs through ion exchange, wherein the dye structure remains unaltered but is only adhered to the present biomass, being effective when conditions for microbial growth and population maintenance are not favorable. In the case of biodegradation, the dye molecule is decomposed by microbial cells, undergoing complete mineralization or generating by-products. It is believed that dye removal under aerobic conditions occurs primarily (though not exclusively) through dye fixation onto biomass, due to the complexity of biodegradation in oxygen-rich environments and the initial exposure of biological flocs to the dye <sup>15,16</sup>.

# 4 CONCLUSION

Given the presented results and the need to preserve water resources, it becomes evident that optimizing the use of the activated sludge process for the decolorization of textile effluents containing Rhodamine B dye is necessary. Combining activated sludge with other complementary techniques, such as the combination of aerobic and anoxic processes<sup>17</sup>, fungal application <sup>18</sup>, physical-chemical post-treatment, or electrochemical treatment<sup>19</sup>, can assist in decolorization and more effectively eliminate the non-biodegradable part of the dyes present in wastewater.

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### **ACKNOWLEDGEMENTS**

The authors gratefully financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES [finance code 001], Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina - FAPESC, and the Regional University of Blumenau (FURB).