



## ORIGINAL ARTICLE

# Biostimulation to improve the dye biodegradation of organic dyes by activated sludge

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## KEYWORDS

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**ABSTRACT:** In this work, biodegradation of organic pollutants by activated sludge (AS) in the presence of glucose (2 g/L) as an additional carbon source was studied. The AS (without pre-acclimation) was freely suspended under aerobic conditions. Three organic dyes representative of the Algerian textile industry were selected: Cibanon Navy (CN), Solophenyl Scarlet (SS) and Cibacron Green (CG). The results showed that after 10 days of incubation, AS displayed good biodegradation capabilities achieving removal percentages ranging from 50.3% to 89.4% and reduction in COD ranging from 93.1% to 98.3%. Particularly, the textile dye CN was removed up to 89% with high reduction in COD (94.7%). The microbial development stimulated by glucose achieved therefore efficiently the discoloration of contaminated solutions and pollutant degradation. Although it is assumed that dyes can be degraded only under anaerobic conditions, the wastewater treatment using AS appears therefore suitable to the removal of different types of textile dyes before final discharge.

## INTRODUCTION

The textile industry is one of the highest consumers of water and then considered as one of the biggest producers of liquid effluents. Approximately 280 000 tons of

dyes and auxiliaries are discharged with these effluents every year [1]. The release of the textile effluents in aquatic ecosystems without adequate treatment and/or appropriate quality cause serious problems to various

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aquatic organisms because of their high concentration in pollutants mainly dyes [2, 3]. Scientists, environmentalists and politicians are always interested in biotechnological approaches that are eco-friendly, efficient and low operation cost for the treatment of such effluents [4]. The main compounds in wastewater textile industry are the azo dyes which are characterized by the presence of at least one azo bond (N=N) bearing aromatic rings. Microorganisms which can decolorize azo dyes are mainly focused on bacteria and fungi. Isolated strains found a large application on decolorization of azo dyes. Song et al. [5] worked with an isolated salt-tolerant yeast strain *Pichia occidentalis* for biodegradation of azo dyes. Biological treatment with single strain was used for biodegradation of leather dyes by a native isolate of *Trametes villosa* [6].

In this context, the biodegradation of organic pollutants using activated sludge (AS) could be an interesting and sustainable solution to problems associated with textile industry pollution. Due to their wide spectrum of metabolic properties, mixed cultures (e.g. AS) has been recommended in biological degradation to prevent the production of toxic intermediates [7, 8]. This technology uses natural biota and their processes for pollution reduction and becomes even more interesting if it leads to a complete destruction of the organic pollutants [9]. Toxic compounds are then converted to harmless products (e.g. carbon dioxide and water). Consequently, it can complete the treatment of the effluent that was previously treated by another physico-chemical process such as photocatalysis, Fenton, sonication, chemical oxidation, etc. [1, 10-14]. For example, Zeghioud et al. [15] investigated the degradation of recalcitrant industrial textile dye (Reactive green 12) in aqueous solution by TiO<sub>2</sub> impregnated polyester at room temperature and observed total decolorization for low concentration. But in some cases, the metabolites produced from dye degradation are, in many cases, more toxic than the parent dye [16].

Activated sludge, largely used as a pollution bioremediation agent, is not pathogenic and not dangerous for the environment. The microbial aggregates appear as flocs composed of colonies (filamentous, protozoa and metazoa bacteria) trapped in a cloud of extracellular polymeric substances (EPS) and other constituents namely organic and inorganic compounds and adsorbed particles [17, 18]. In a wastewater treatment plant (WWTP), the effluent wastewater constitutes the main source of the bacterial communities (diversity, composition and dynamics) which oxidize pollutants leading to the removal of organic matter and nutrients from the wastewater, if oxygen is supplied [19]. This kind of processes is considered as a complex ecosystem that is influenced by both biotic and abiotic parameters. Although it is assumed that dyes can be degraded only under anaerobic conditions [20], some studies showed that some dyes are less biorecalcitrant in aerobic environment than others [21,22].

In the present work, we selected three industrial dyes, Cibacron Navy (CN), Solophenyl Scarlet (SS) and Cibacron Green (CG) representative of different commercially important dye types widely applied in the textile industry of eastern Algeria. The main objective was to investigate the ability of AS selected from an urban wastewater plant to remove and degrade these dyes present in synthetic textile wastewaters.

## MATERIALS AND METHODS

### *Chemical reagents*

Three organic dyes, procured by the textile industry factory of Constantine (Algeria) were used in the present work. They are listed in Table 1 with their physico-chemical data. A 1 g/L synthetic solution of each dye was prepared in distilled water and maintained at 4 °C. The chemical reagents used for preparing the mineral

salt medium were purchased from Merck, Germany. All used chemicals were of the highest available purity and of analytical grade. The composition of this culture medium was similar to that of Bajaj et al. [23].

**Table 1.** Physicochemical data of dyes under study

Propriety	Cibanon Navy	Solophenyl Scarlet	Cibacron Green
	DB-01 MD	BNLE	LS-3B
Empirical formula	C <sub>34</sub> H <sub>16</sub> O <sub>2</sub>	C <sub>44</sub> H <sub>22</sub> N <sub>10</sub> Na <sub>4</sub> O <sub>16</sub> S <sub>4</sub>	C <sub>60</sub> H <sub>29</sub> Cl <sub>3</sub> N <sub>16</sub> NiO <sub>21</sub> S <sub>6</sub> Na
Molecular weight	456	1372	1837
Physical aspect	Dark blue powder	Bright red powder	Bluish green powder
CI name	Vat blue 20	Direct Red 89	Reactive Green 12
Chemical class	Anthtraquinoid dye	Azo dye	Phtalocyanin dye
Dyer class	Vat dye	Direct dye	Reactive dye
λ <sub>max</sub> (nm)	580	494	615
pH*	7.60	7.10	6.90

\* pH of the aqueous solution at 50 mg/L.

**Activated sludge**

Activated sludge (AS) was collected from the aeration tank of the municipal wastewater treatment plant located in the city of Guelma (Algeria). It was transported to the laboratory in plastic containers and placed under aeration and moderate stirring in a plastic flask at room temperature. AS suspension was then let to settle for 30 minutes in 1 L-test tube to recover the pellet. The latter was centrifuged at 4000 rpm for 15 min by washing each time with distilled water. Finally, the pellet recovered at the end of the operation was mixed with 10 mL of distilled water for further use in biodegradation, biosorption and photodegradation assays.

**Biodegradation assays**

In four thermostated stirred reactors of 1 L capacity, treatment and control essays were carried out in duplicate during 10 days. The treated volume, continuously aerated by an air pump, was 900 mL and the experimental conditions were as follows: temperature: 20±1 °C, pH: 7.0±0.1, AS dosage: 20 mL/L and initial dye concentration: 50 mg/L. Control treatments (without dyes) were elaborated in the same conditions. Samples were collected at regular interval (each day) from each reactor for analyzing Chemical Oxygen Demand (COD), dye absorbance and dry matter (DM). To assess the efficacy of dye biodegradation in the presence of AS,

reduction in COD (% of degradation) was calculated as follows:

$$\% \text{ degradation} = \frac{\text{COD}_{\text{initial}} - \text{COD}_t}{\text{COD}_{\text{initial}}} \times 100\% \quad (1)$$

Where,  $\text{COD}_{\text{initial}}$  and  $\text{COD}_t$  are the COD before and after treatment at time  $t$ , respectively.

Growth index representing the microbial development was determined on the basis of initial and final AS dry matter (DM); it was expressed as:

$$\text{Growth index} = \frac{\text{DM}(t=10 \text{ days})}{\text{DM}(t=0)} \quad (2)$$

DM (g/L) was determined using the following equation:

$$\text{DM} = \frac{(\text{Weight of filter with AS} - \text{Weight of blank filter})(g)}{\text{Volume of AS}(L)} \quad (3)$$

The percentage of removal of target compounds was calculated according to the equation (4):

$$\text{PR}(\%) = \frac{\text{Concentration}_{\text{initial}} - \text{Concentration}_{\text{final}}}{\text{Concentration}_{\text{initial}}} \times 100\% \quad (4)$$

#### 2.4. Photodegradation and biosorption assays

In order to highlight the role of AS on the biodegradation phenomenon, photodegradation and biosorption experiments were carried out at  $20 \pm 1$  °C, pH of  $7.0 \pm 0.1$  and an initial dye concentration of 50 mg/L. Photodegradation duration was 3 days while biosorption was achieved after 1h of stirring the dye solution at 150 rpm in the presence of 20 ml/L AS.

#### 2.5. Analytical methods

The concentration of the three dyes in the supernatant was analyzed using a Hach-Lange DR 3900-spectrophotometer after centrifugation at 5000 rpm for 10 min. Dye concentration was evaluated by measuring the absorbance at the maximal wavelength of each dye (Table 1) and extrapolation by the calibration curve which were carried out by using solution with known Cibanon Navy (CN), Solophenyl Scarlet (SS) and

Cibacron Green (CG) concentrations (in the range of 0-15 mg/L). Chemical Oxygen Demand (COD) was analyzed using NFT 90-101 method to measure the oxidation degree of pollutants. A calibration curve was established using a standard solution. For determining dry matter of the biomass a precise volume of sample was filtered, placed in an oven and dried at a temperature of 105 °C for 48 h (NFT 90-029); it was then weighed and the dry matter was used for calculating the growth index.

All analytical experiments were done at least in duplicates and the values reported correspond to the average of the measured values.

## RESULTS AND DISCUSSION

### Microbial development

As a first result, the three dyes at 50 mg/L tested in the present study were not toxic for AS community since in all cases the presence of these compounds did not affect biomass growth in the bioreactors and the growth index increased (Growth index > 1). The biostimulation by the addition of glucose as a co-substrate improved cell growth and biodegradation of the target molecules (Table 2). Growth indices ranged from 1.68 to 2.34 can be noticed showing that, whatever its nature, the dye molecule constitutes a source of nutrients for AS microorganisms. These results corroborate those of Brossillon et al. [10]. Glucose, an easily biodegradable organic nutrient, is a source of carbon and energy for the microorganisms. It was added to the culture medium in the presence of the target compounds to stimulate the production of enzymes for the co-metabolism of glucose and dye compounds [24]. In the study of Tan et al. [2], it was shown that the microbial community collected from the sea mud of a beach that was close to an industrial harbor zone (India) was able to degrade azo dyes by utilizing them as a carbon source and energy. In the present study, of the three dyes tested, SS was the best substrate for AS with a growth index of 1.84 in the absence of

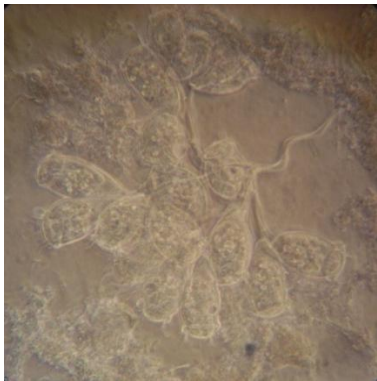
glucose (Table 2), showing a higher assimilation of the azo dye if compared to the anthraquinoid and phthalocyanin dyes. According to our visual observations, microbial growth started from the second day of treatment as well as for the controls. Microscopic observation of AS samples, before and during treatments, revealed the presence of diverse types of microorganisms mainly *Vorticella*, *Rotifera* and *Euplotes sp* genes (Figure 1). According to Duchene and Cotteux [25] *Euplotes sp* is present in an aqueous medium only if the carbon removal is ensured

while the presence of *Vorticella* is a sign of a satisfactory oxygenation; in addition, the abundance of *Rotifera* in AS reveals a stable operating of the AS treatment. Relative low growth indices (1.42-1.83) obtained in the case of treatments without glucose supports the fact that glucose is a necessary substrate for the development of activated sludge microorganisms, which show ability to degrade organic contamination present in the contaminated water [26].

**Table 2.** Growth indices of AS during biodegradation experiments

Dye	Growth index		
	Controls	Without glucose	With glucose
Cibanon Navy	1.64	1.42	1.68
Solophenyl Scarlet	1.93	1.83	2.31
Cibacron Green	2.03	1.62	2.34

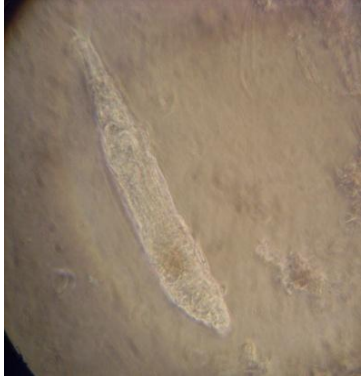
\* Growth index was calculated according to the equation 2.



*Vorticella*



*Euplotes sp*



*Rotifera*

**Figure 1.** Microscopic observation of the activated sludge, Differential Interference Contrast (DIC) microscopy (X500, X400, X100)

**Dye abatement**

For the three compounds tested, dye concentration during photodegradation and biosorption

essays did not change significantly (Table 3); the two phenomena accounted for only 0.5-2% and 4-7% of the total color removal, respectively.

**Table 3.** Percentage removal (PR) of dyes during photodegradation and biosorption experiments

Dye	Photodegradation (%)	Biosorption (%)
Cibanon Navy	0.81	4.92
Solophenyl Scarlet	2.18	5.10
Cibacron Green	0.56	7.43

\* The percentage removal was calculated according to the equation 4.

However, significant decrease in dye concentration was observed during biodegradation (Figure 2). This suggests that color removal was mainly caused by biodegradation rather than biosorption and/or photodegradation. A color removal was achieved after 2-5 days of treatment period. This was visually confirmed with the inspection of the different solution colors after biodegradation experiments (Figure 3). The highest percentage removal (PR) was recorded towards CN; this dye was then more easily removed from water than the two others. This might be due to the molecular weight and/or the structure of the dye. Apparently, there is a link between the degradation rate and the structure of the organic molecule but this cannot be confirmed at this stage. Further investigation is needed to understand the degradation mechanism involved in such phenomenon and to conclude about this assumption. Several studies demonstrated that AS (or bacteria isolated from AS) successfully biodegrade organic dyes including diverse types of dyes under aerobic or anaerobic conditions [27-29]. Acid Red B dye was removed by AS (90%) within 3 days [30]. Consortium of microorganisms isolated from three different sources (domestic,

paper mill and tannery effluents) was able to decolorize eight reactive azo dyes within 24 h with a high reduction in COD [27].

Additionally, from the same essays, the influence of the growing medium was considered. AS cultured on media containing 2 g/L of glucose as carbon source showed significantly higher PR with respect to the AS cultured without glucose. The additional carbon substrate enhanced the growth of the microorganisms, as shown from Table 2, resulting in the degradation of the dyes. This is in accordance with the work of Yang et al. [26] on the decolorization of an azo dye by yeast isolates which demonstrated that glucose had a cometabolic role in the degradation of the dye. Various carbon sources are generally used such as glucose, sucrose and starch. Padmavathy et al. [31] used glucose, starch, lactose, sewage and whey water for studying their effects on dye decolorization in the presence of different mixed bacterial cultures. The authors observed that starch was the best source of carbon for the decolorization of reactive azo dyes; in the presence of 250 mg/L of starch, all the reactive dyes (used in their work) were decolorized within 24 hours with 75.15 -95.9% reduction in COD.

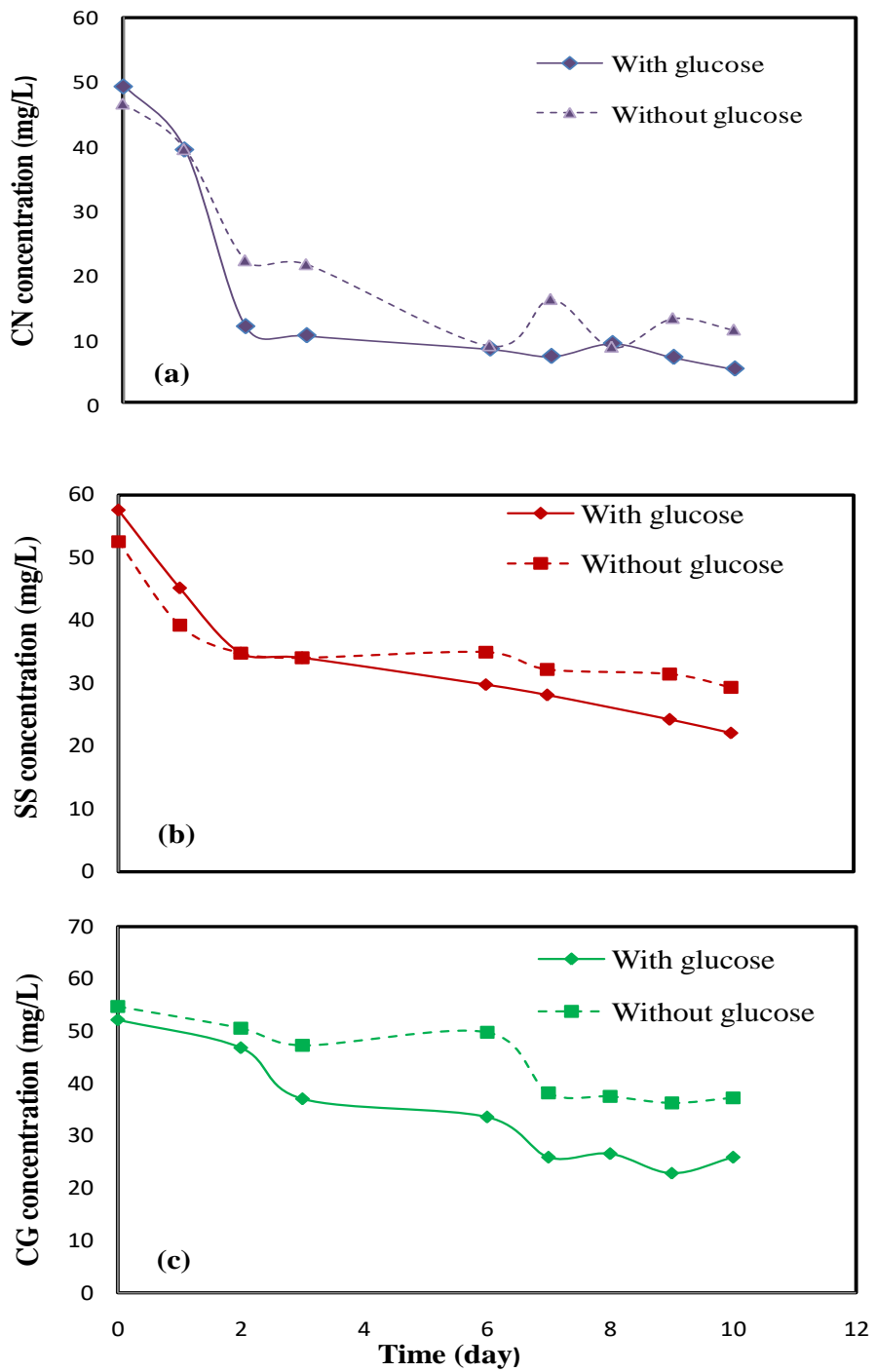
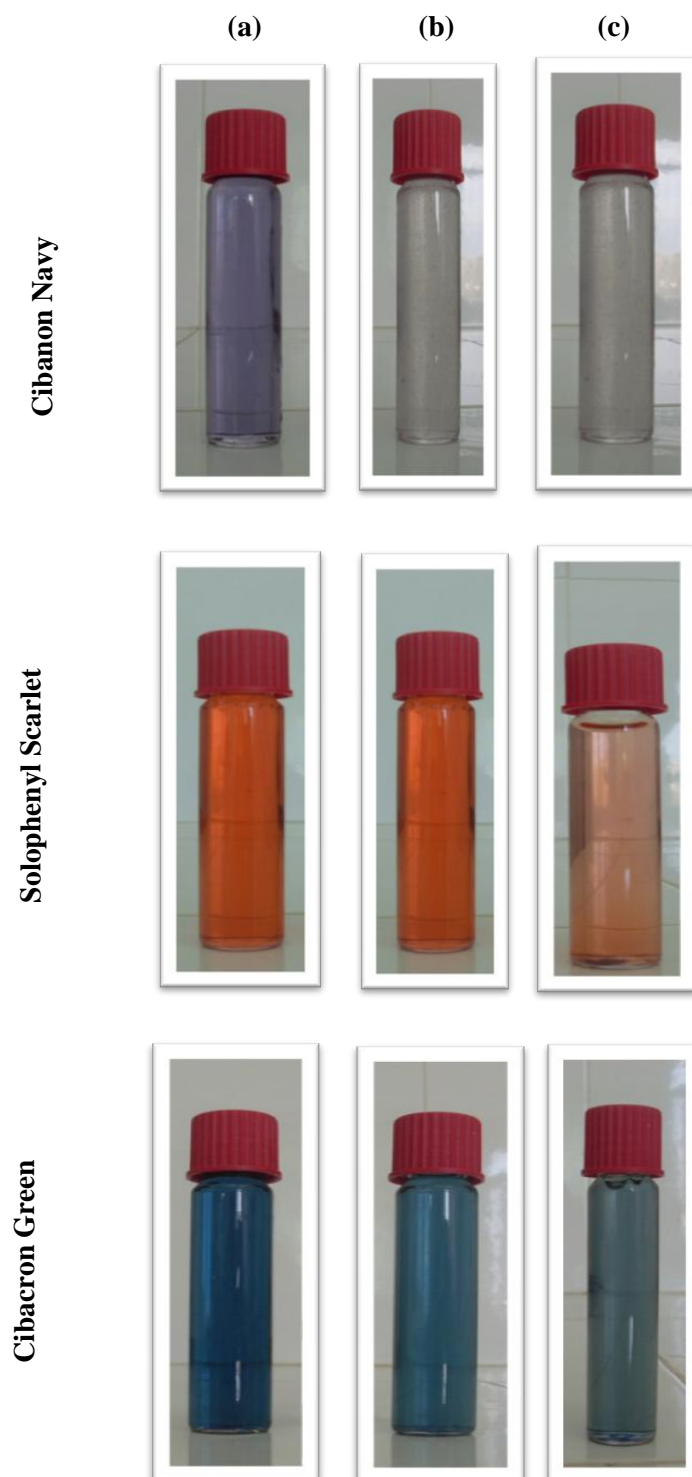


Figure 2. Dye concentration profile with respect to time during biodegradation with AS, (a) CN, (b) SS, (c) CG



**Figure 3.** Discolorisation of dye solution by the activated sludge. (a) Before treatment, (b) After treatment without glucose, (c) After treatment with glucose

Reduction in COD

To understand the degree of biodegradation of the three dyes, reduction in the chemical oxygen demand (COD) after 10 days incubation with AS in the presence of 2 g/L of glucose was determined. The different COD profiles were depicted in Figure 4. In most cases, a significant decrease in COD was observed during the treatment. After treatments, the values of COD decreased until 55, 36 and 35 mg O<sub>2</sub>/L for CN, SS and GC, respectively. These values are largely lower than the maximum acceptable concentration in contaminated waters (120 mg O<sub>2</sub>/L). The high biomass concentration (§ 3.1) was probably the result of this significant COD reduction since the different values of COD were maintained within a stable range [32]. It can be noted that the increase of the COD values observed in the absence of added glucose could be explained by the release of organic matter due to cell lysis (data not shown).

For a complete mineralization of the organic compounds (COD → 0), an adequate microbial population capable of metabolizing such compounds is needed for an effective and sustainable solution to textile wastewater treatment [33]. Alternatively, toxicity tests of treated waters may be useful to verify the detoxification of the medium. Indeed some of the derived metabolites of dyes could be responsible of genotoxicity and metabolic toxicity [34, 35].

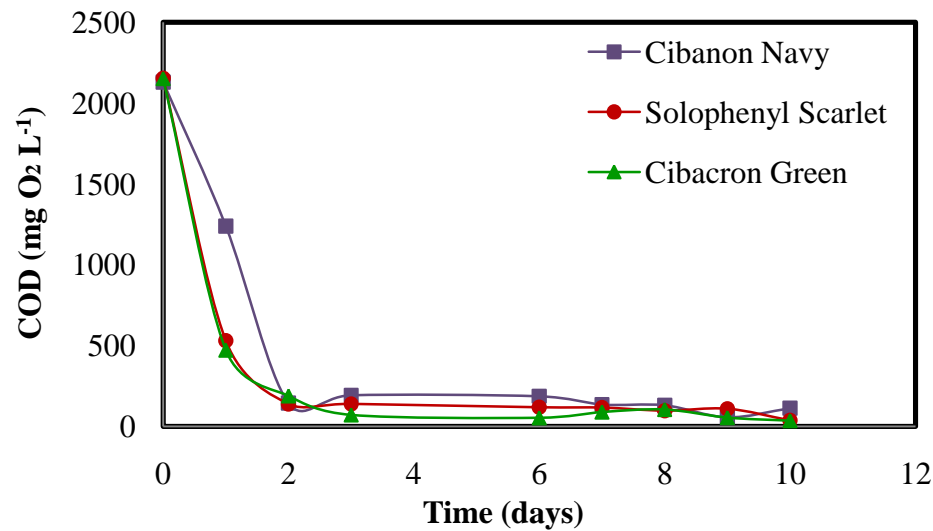


Figure 4. Percentage of degradation of dyes with AS under aerobic conditions

## CONCLUSIONS

This paper illustrated the degradation capacities of activated sludge (AS) in aerobic conditions towards three organic pollutants commonly used in textile dyeing processes. The main results obtained in this study showed that the presence of dyes in the simulated wastewaters did not affect the growth of AS community. The abatement and degradation of the organic pollutants from contaminated waters were possible by AS microbial communities. The COD removal reached near 100% of degradation after 3 days of biological treatment, in the experimental conditions of this study.

Environmental aspects, mainly temperature and pH should be taken into account to improve the efficacy of pollutant biodegradation. Additionally, biodegradation with AS should be further tested for its performance to degrade dyes in a bioaugmented system by co-culturing with bacterial communities, to determine if the selected strains are able to enhance dye degradation rates and/or if the biological system is able to minimize the wastewater treatment duration.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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