

Malachite green decolorization by marine strain isolated from Bou-Ismaïl Bay (Algeria)

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ABSTRACT - Decolorization of malachite green dye by marine strain was studied, results obtained demonstrated a good ability to decolorize malachite green dye after 72 h of incubation at 30°C and 150 rpm. The decolorization efficiency was found to be affected by initial dye concentration, pH and salinity of medium. UV-Vis spectroscopy analysis of samples before and after decolorization confirmed the ability of the tested strain to degrade MG.

Keywords: Triphenylmethane dye, Bioremediation, pollution, Degradation.

1. Introduction:

Triphenylmethane are an important group of synthetic colorants used extensively in industries [1]. Malachite green (MG), a triphenylmethane dye, is used for coloring a variety of materials, such as cotton, wood, silk, leather, jute and paper [2]. It is also been used in aquaculture industry as a fungicide, parasiticide and disinfectant [3].

MG has properties that make it difficult to remove from aqueous solutions [4].

Therefore, the environmental pollution caused by the MG remains a difficult problem.

To date, a wide range of methods has been developed for the removal of synthetic dyes from wastewater: adsorption, coagulation / flocculation, precipitation, oxidation, advanced

oxidation, membrane separation and biodegradation [5].

Several MG-decolorizing microorganisms have been reported from fungus [6, 2], microalgae [4] and bacteria [7, 8, 3]. Dyes biodegradation can be affected by operational parameters such as: pH, temperature, initial dye concentration, N content in medium, salts, shaking and aerobic and anaerobic [9].

This study aims to investigate the potential of marine microorganism for decolorization Malachite Green dye.

2. Materials and methods

2.1 Microorganism and culture conditions

Marine strain was isolated from sediment contaminated by industrial wastewater in Bou-ismail bay (north of Algeria), strain was isolated using green malachite as carbon source. Basal medium was used for microorganism isolation

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and dye biodegradation, the composition of the basal medium was (g/l) 0.1 yeast extract, 0.4 NH₄Cl, 0.3 K₂HPO₄, 30 NaCl, 0.33 MgCl₂·6H₂O, 0.05 CaCl₂·H₂O, and 1 ml of trace element solution [10]. The pH of the medium was adjusted at 7.0 ± 0.2.

2.2 Decolorization of green malachite

Decolorization studies were conducted in 250 ml Erlenmeyer flasks, 2 ml of inoculum was added to 100 ml of basal medium and green malachite was added at final concentration of 50 mg/l. Flask cultures were then incubated at 30 °C, on a rotary shaker at 150 rpm. Glucose (5 g/l) was added in order to evaluate co-substrate effect on dye biodegradation.

In order to evaluate the effect of pH, salinity and initial dye concentration on dye decolorization, the pH of the medium was adjusted in the range between 5 and 9 and the sodium chloride was added in medium to achieve final concentrations of 0 to 50 g/l. initial dye concentration was varied from 25 to 200 mg/l. Basal medium containing dyes solution without bacteria was served as control.

2.3 UV–visible analysis

Decolorization extent was determined by measuring the absorbance of the culture supernatant at 618 nm, decolorization percentage was calculated according to the following formula:

$$\text{Decolorization} = 100 * (A_i - A_f) / A_i$$

where A_i is the initial absorbance and A_f is the final absorbance.

3. Results and discussion

3.1 Microorganism growth on MG

MG was used as carbon and energy source by microorganism isolated from sediment contaminated by industrial effluents

Strain SG1 showed the ability to decolorize malachite green (86.76 %) with initial concentration of 50 mg/l after 3 days of

incubation at 30°C, Chen and Ting, (2015) reported that 98% of MG at initial concentration of 50 mg/l was degraded after 5 days of incubation by *Corioloopsis* sp. [11]. Ali et al, 2009 reported that 96% of MG was degraded by *Aspergillus flavus* and *Alternaria solani* after 6 days of incubation at 30°C [12]. MG (50 mg/l) was completely decolorized within 5 h by *Pseudomonas aeruginosa* NCIM 2074 [13]

Simple substrate like glucose was added to medium to evaluate the effect of co-substrate, the results showed that decolorization of MG was affected by co-substrate addition, glucose was used preferentially by SG1 strain. Parshetti et al, 2006 reported that MG degradation by *Kocuriarosea* MTCC 1532 was not affected by molasses addition as co-substrate but significantly affected when Urea, sucrose, peptone and glucose were added, degradation rate was decreased from 100% to 31% when glucose (3%) was added as co-substrate [14]. Ali et al, 2009 study showed that MG degradation by *Aspergillus flavus* and *Alternaria solani* was not affected by co-substrate addition (sucrose) [12].

3.2 Effect of initial dyes concentration on MG decolorization

Initial dye concentration can affect the decolorization processes, MG concentration was varied from 25 mg/l to 200 mg/l (Fig 1).

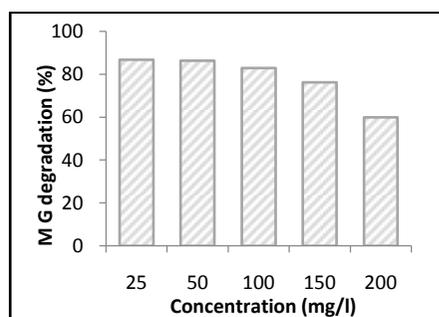


Fig. 1 Effect of initial concentration on MG decolorization (after 3 days of incubation)

Results showed that decolorization of MG (25–100 mg/l) reach 82.97–86.84%, which was decreased to 60% when the MG concentration increased to 200 mg/l.

Several studies reported inhibition of MG decolorization at high concentration. Olukanni et al, 2013 study showed that MG decolorization by *B. thuringiensis* RUN1 increased when initial dye concentration from 10 to 40 mg/l but dropped when dye concentration increased to 80 mg/l [3]; similar observations were noted by Parshetti et al, 2006, MG degradation by *K. rosea* decrease from 100% (50 mg/) to 13% (70

mg/l) and only 6% (100 mg/l) [14]. However, *Pseudomonas* sp. strain DY1 could efficiently decolorize MG of high concentrations (100 to 1000 mg/l) [8]

The results of this study showed that marine strain SG1 tolerate high MG concentration.

Table 2: Biodegradation of MG dyes by different bacteria/fungi.

Bacteria/fungi	Dye concentration	Removal efficiency	References
<i>Shewanella decolorationis</i> NTOU1	100 mg/l	Complete in 2h	[7]
<i>Pseudomonas aeruginosa</i>	50 mg/l	Complete in 5 h	[13]
<i>Pseudomonas</i> sp. strain DY1	100 to 1000 mg/l	More than 90% in 24 h	[8]
<i>Sphingomonas paucimobilis</i>	50 mg/l	75% after 24h	[11]
<i>Kocuria rosea</i> MTCC 1532.	50 mg/l	Complete in 5h	[14]
<i>Bacillus cereus</i> strain DC11	55 μ M	More than 90 % in 4h	[15]
<i>Dietzia maris</i> NIT-D	15 mg/l	72.05 % in 240 min	[16]
<i>Deinococcus radiodurans</i> R1	50 mg/l	99.9 % in 30 min	[17]
<i>Staphylococcus epidermidis</i>	750 mg/l	39 % after 14 h	[18]

3.3 Effect of NaCl concentration on MG decolorization

NaCl concentration effect was determined at concentrations ranging from 0 to 50 g/l. the results (Fig 2) indicated a high decolorization capacity in a wide range of NaCl concentration (0-40 g/l). decolorization rate declined (65,4%) at high NaCl concentration of 50 g/l.

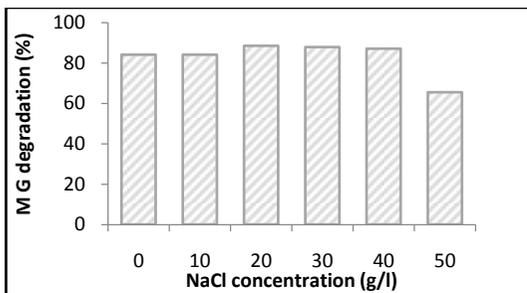


Fig. 2 Effect of NaCl concentration on MG decolorization (after 3 days of incubation)

Most of work on MG degradation has been studied in low salinity medium [4, 13, 1]. However, wastewaters from different industries contain substantial amounts of salts; Thus, microbial species capable of tolerating salt stress will be beneficial for treating such wastewaters. [19].

3.4 Effect of pH medium on MG decolorization

Initial pH medium is an important parameter in dye decolorization studies, pH value of medium may influence the decolorization process by impacting the transport of dye molecules through the cellular envelope and may exert a strong influence on the activity of extracellular redox enzymes that are often involved in decolorization processes [20].

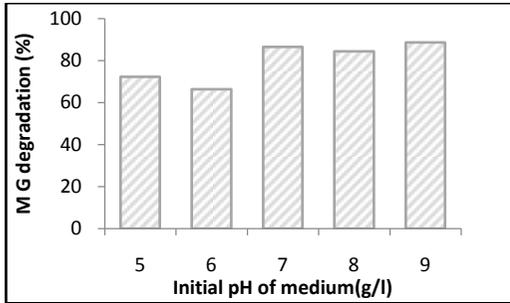


Fig. 3 Effect of a pH on MG decolorization (50 mg/l after 3 days of incubation)

The results (Fig 3) revealed that dye decolorization was most effective in basic medium and reached the maximum at pH 9. Decolorization rate decrease in acidic medium. Ayed et al. (2009) reported that maximum MG decolorization by *Sphingomonas paucimobilis* was observed at pH 9 and increase in pH value from 4 to 9 led to a threefold increase in decolorization rate [1].

The MG decolorization efficiency by *P. pulmonicola* YC32 increased when pH medium increased from pH 4 to 7, leveled off between pH 7 and 9, and slightly increased to 95.6% at pH 10 [21].

3.5 Biodecolorization of MG

Decolorization of dyes by microorganisms could be due to adsorption by microorganisms cells or to biodegradation. In the case of adsorption, the UV-Vis absorption peaks decrease approximately in proportion to each other, whereas in biodegradation, either the major visible light absorbance peak disappears completely, or a new peak appears [13].

UV-Visible spectral scan (Fig 4) showed a reduction in the absorbance peaks of MG at its λ max, 618 nm indicated a rapid degradation of the dye. Therefore, the color removal was attributed to biodegradation, and was not due to physical change or adsorption.

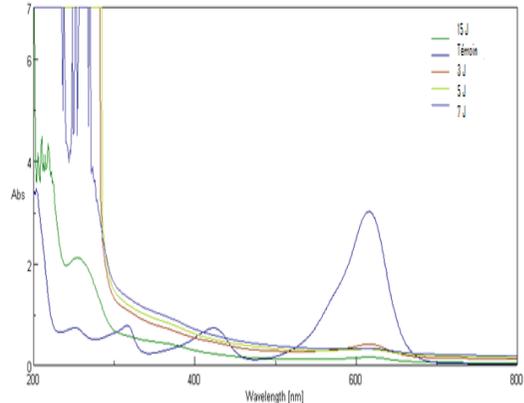


Fig. 4. UV-Vis spectra of Malachite green (50 mg/l) biodegraded by SG1 strain.

Conclusion

The present study revealed the ability of marine microorganism to decolorize Malachite Green. The effects of operational parameters on decolorization were determined, and the results showed that this strain exhibited excellent MG biodegradation ability in wide range of pH (4-9) and salinity (0-50 g/l). This study suggests that SG1 strain might be an ideal candidate for dyes wastewater treatment.

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