Lyallpur Khalsa College, Jalandhar-144001, Punjab

Research & Reviews in Biotechnology & Biosciences
Homepage: www.biotechjournal.in
Volume: 5.Issue: 2, Year: 2018 PP: 53-58

Review Article

Short communication Microbial Degradation of Malachite green Dye

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DOI: https://doi.org/10.6084/m9.figshare.8977325.v1

Article History

Received: 09/07/2018
Revised: 29/07/2018
Accepted: 25/08/2018

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Abstract

The microbial decolorization of dyes has been of considerable interest in biological treatment of the wastewater containing dyes. The bacterial isolate, Bacillussp.was isolated from the textile effluent sample. Different parameters such as various carbon sources, nitrogen sources, temperature andpH were optimized for decolorization of malachite green by using this bacterial isolate.Bacillussp. showedmaximum dye decolorizationof about86% at 30°C and pH 7 after 8 days of incubation.Maximum decolourization of malachite green (75.80%) was observed when urea was used as nitrogen source. High decolorization extent showed the potential of the bacterial strain to be used in the decolourization of malachite green dye.

Keywords: Bacillus sp., malachite green, decolourization.

Introduction

Dyes are synthetic and aromatic molecular

structural compounds. These are basically chemical compounds that can attach themselves to fabrics or surfaces to impart colour.Over 100,000 commercially available dyes exist and morethan 7 × 10^5 metric tonnes of dyestuff are produced worldwide annually. During the dyeing processes about 10-90% of the dyestuff do not bind to the fibres and therefore, released into the sewage treatment system or the environment. Colored industrial effluents from the dyeing industriesrepresent major environmental problems(2). Dyeing industry effluents are one of the most problematic

wastewaters to be treated not only for their high chemical oxygen demand, but also for high biological oxygen demand, suspended solids, turbidity, toxic constituents but also for color, which is the first contaminant discernible by the human eye.(4).Therefore, industrial effluents containing dyes must be treated before their discharge into the environment. Not all dyes currently used can be degraded or removed with physical and chemical processes and sometimes the degradation products are more toxic.Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Dyes can be degraded and decolorized by various microorganisms such as bacteria, fungi, yeast etc. Bacteria could also degrade synthetic dyes at a faster rate but at the same time releases carcinogenic aromatic amines as degradation products which severely effects human and animal health and water. So, to overcome such problems posed by bacteria, filamentous fungi have been used for degradation and decolourization of

dyes(3).Filamentous fungi are advantageous because most of them are adapted to contamination already and they have the ability to extend through the soil. Therefore the biological methods possess many advantages over chemical and physical methods also such as possibility of degradation of dye molecules to carbon dioxide and water, formation of less sludge in addition to being environment friendly.(1).

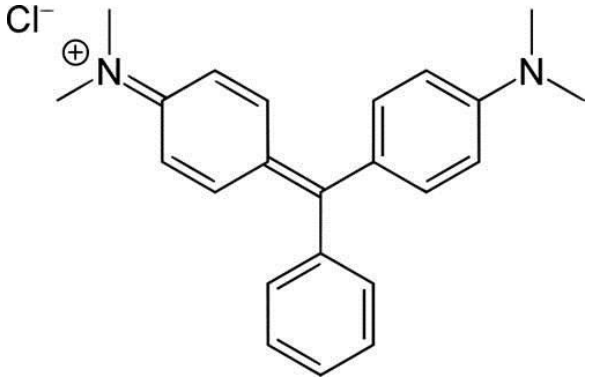
Materials and Methods

Effluent samples

Textile effluent samples were collected from the vicinity of textile industries. The effluent samples were collected aseptically in a plastic container and stored under refrigerated conditions in a refrigerator to avoid further changes.

Chemicals

The commonly used dye for dyeing, malachite green was used in this experiment. The other chemicals used were of analytical grade and highest purity.(5).



(Malachite Green)

Medium

Nutrient Agar MediumComponents(g/l)Peptone(5.0), Beef extract(3.0), Sodium chloride(5.0),Agar-agar(20.0),pH(7.2)

Composition of nutrient broth was same as above except that agar-agar was omitted.

All other chemicals used were of analytical grade.(6).

Bacterial isolation and cultivation-

Isolation was carried out by "shake flask soil slurry methods"(7) Ten gram of dye house effluent soil was added to 90 ml of Zhou and Zimmermann liquid medium with 5 µg/ml Malachite green dye and 1.0 grams of yeast extract(8). The flasks were incubated on orbital shaker at 120 rpm, at 30°C temperature for 10 days. After 10 days the flasks were removed and 10 ml of enriched broth was transferred to another set of above flasks containing 90 ml liquid medium having 10 µg/ml of dye and 0.5 g of yeast extract. The flasks were again incubated on orbital shaker (at 120 rpm) at 30°C. Similarly, enrichment was carried out for two more times (9) regarding isolation and enrichment on plates containing Zhou and Zimmermann medium.

Determination of minimum inhibitory concentrations

One loopful of individual pure cultures from slants were transferred into one ml sterile water blank, and uniform suspension was made by using a vortex mixer. Fifty µl of this suspension was then spotted on Zhou and Zimmermann medium plates having

different concentrations (viz. 6.25, 12.50, 25, 50 and 100 µl/ml) of Malachite green dye with the help of micropipette(10) Plates were incubated at 30°C in a BOD incubator for three days in inverted positions. Growth of bacterial isolates was compared with the growth on plate containing no dye and noted down the minimum concentration of dye at which no growth was observed(11)

Estimation of decolourizing activity

Decolourization activity was expressed in terms of percentage decolourization by the modified method given by(12) and determined by monitoring the decrease in absorbance of Malachite green at 625 nm(13)

A loopful of culture was inoculated in 30 ml nutrient medium broth and incubated on shaker at 30°C for 2-3 days. Culture suspension (100 µl) containing about 10^8cells per millilitre was transferred to the Zhou and Zimmermann liquid medium and incubated at 30°C on orbital shaker (120 rpm). After every two days, one ml sample was taken out aseptically and centrifuged (10,000 rpm for 5 min) (14) To one ml supernatant, two ml of water saturated 1-Butanol was added, mixed thoroughly by vortex mixer, allowed to stand for one hour till two layers of water and butanol get separated. Upper layer of butanol containing the dye was separated and absorbance was measured. Percent decolourization was calculated. Uninoculated flasks were treated as control.

Percent decolourization was calculated using formula:

$D = 100*[A_{ini}-A_{obs}]/A_{ini}$

Where,

D = Percent decolourization

A_{ini} = Initial absorbance

A_{obs} = Final absorbance

Decolourization of malachite green dye in the presence of carbon sources , Nitrogen Sources , Temperature and pH.

To test the ability of the isolates to decolourize dyes in the presence of various carbon sources like glucose, sucrose, sodium malate and mannitol. Flasks were prepared using mineral medium, 0.05% yeast extract and 1 % carbon source. The flasks without additional carbon source served as control. The flasks were inoculated as described earlier. The flasks were incubated at 30°C on an orbital shaker (120 rpm). Percent decolourization was determined after six and eight days as described earlier.

For optimization of various conditions like pH, temperature and nitrogen sources for maximum decolourization of dyes, only selected isolates was used. To find the alternate nitrogen source in place of yeast extract, compounds like ammonium chloride, urea and sodium nitrate at 1 % concentration were used in Zhou and Zimmermann broth without yeast decolourization was compared with that of yeast extract at 6 and 8 days. For optimization of temperature another set of flasks containing Zhou and Zimmermann broth were prepared. The flasks were incubated at different temperatures like 20, 25, 30, 35 and 40°C on orbital shakers (120 rpm) and percent decolourization was determined. For optimization of pH for maximum decolourization pH 6.0, 6.5, 7.0, 7.5 and 8.0 each were prepared in Zhou and Zimmermann broth using 0.1 N NaOH or HCl. To 30 ml broth 100 µl culture suspension containing 10^8cells of selected isolate The flasks were incubated at 30°C on orbital incubator shaker. The samples were removed at 6 and 8 days and percent decolourization was determined as described earlier.

Results and Discussions

Isolation of bacterial cultures

Few bacterial cultures were isolated from the prolonged cultures in Textilewastewater.The isolate H was isolated as the most active dye-decolorizing bacteria. Selected bacterial isolate was identified on the basis of gram reaction, shape and sporulation. The bacterial isolate was gram positive, spore forming and rod shaped. The bacterial isolates were further tested for various biochemical characteristics such as oxidase test, catalase test, Voges-Proskauer reaction, citrate utilization, nitrate reduction, methyl red and urease production. Based on morphological, physiological and biochemical characteristics the isolate was tentatively grouped in the genus *Bacillus*(15)..

Determination of optimum temperature for decolourization of Malachite green:

Optimization of temperature at which maximum decolourization could occur was also determined by incubating flasks at 20, 25, 30, 35 and 40° C and percent decolourization was determined at 6 and 8 days. In case of dye Malachite green, maximum decolourization of about 79percent was seen when the flasks were incubated at 30°C followed by 40°C at 8 days of incubation as shown in table-I.

Table-I: **Determination of optimum temperature for decolourization of Malachite green. (* standard deviation, n=3)**

Temperature (° C)	**Decolourization**	
	6 days	**8 days**
20	62.3 (±2.094)*	67.6(±2.241)
25	75.0 (±4.082)	75.0(±4.082)
30	79.0 (±2.944)	79.0 (±2.944)
35	72.3 (±2.0551)	75.0(±4.082)
40	73.0 (±2.160)	76.0(±1.632)

Table-II: **Determination of optimum pH for decolourization of Malachite green**

pH	**Decolourization**	
	6 days	**8 days**
6.0	72.0 (±1.632)*	72.0(±1.632)
6.5	67.6 (±2.241)	73.0 (±2.160)
7.0	85.0 (±4.082)	86.0 (±3.262)
7.5	76.0 (±1.632)	76.0 (±1.632)
8.0	72.3 (±2.055)	73.0 (±2.160)

(* standard deviation, n=3)

(To find out optimum pH for maximum decolourization of malachite green experiments were carried out at different pH values ranging from 6.0, 6.5, 7.0, 7.5 to 8.0. The results showed that maximum decolourization was obtained at pH 7.0 after 8 days. Maximum decolourization with isolate was found to be 86 percent in case of malachite green dye as shown in table-II

Effect of different carbon and nitrogen sources on malachite green decolourization:

To find out the best carbon source in the presence of which maximum decolourization of Malachite green dye could be obtained, Zhou and Zimmermann medium was supplemented with different carbon sources like glucose, sucrose, sodium malate and mannitol and percent decolourization at 8 days was determined.

Table-III: **Effect of Different Carbon Source on Dye Decolourization. (* standard deviation, n=3)**

Carbon Sources	Decolourization	
	6 days	8 days
Without Carbon	67.6 (±2.231)*	67.6(±2.231)
Glucose	82.0 (±1.632)	86.0 (±3.262)
Sucrose	75.0 (±4.082)	76.0 (±1.632)
Sodium Malate	72.0 (±1.632)	72.0 (±1.632)
Mannitol	72.3 (±2.0551)	73.0 (±2.160)

Table-IV: **Effect of Nitrogen Source on Dye Decolourization**

Nitrogen Sources	Decolourization	
	6 days	8 days
Yeast Extract	72.3(±2.055)*	75.0 (±4.082)
Ammonium chloride	65.0 (±4.082)	73.0 (±2.160)
Urea	84.0 (±1.332)	86.0 (±3.262)
Sodium Nitrate	73.0 (±2.160)	79.0 (±2.944)

(* standard deviation, n=3)

Maximum decolourization was observed with glucose as carbon source as compared to others. Maximum decolourization of 86.0 percent was obtained in Malachite green dye. Different inorganic sources of nitrogen like ammonium chloride, urea, sodium nitrate and complex organic nitrogen source like yeast extract, were used at one percent concentration. Maximum decolourization of malachite green (86.0%) was observed when urea was used as nitrogen source by isolate followed by sodium nitrate (about 79%) at 8 days of growth.(16)as shown in table-III & IV

Conclusion

Maximum decolorization was observed

inMalachite green by bacteria isolated from textile dye effluent**.** Different parameters such as various carbon source, nitrogen source, temperature, pH showed significant effect on dye decolourization.*Bacillussp.*

showed highest decolorization of Malachite green dye.

ACKNOWLDGEMNTS

Source of Support: Authors want to thank president governing council for this project work
Conflict of interest: No

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