Decolorization of Malachite Green Using SR0 Strain

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Abstract:- **Microbial degradation of malachite green was investigated by performing isolation of potential microbes from the dye contaminated soil samples. Bushnell-Haas medium embedded with 100ppm filter sterilized malachite green(pH 8.0) was used for the isolation. Among different isolates bacterium coded as SR0 was found to be more potential. Biodecolorization study indicates that 68 to70% of decolorization was found with SR0. Studies with physico-chemical parameter indicates that the presence of nitrogen source, pH 7.0, 8.0 and shaking conditions at 37^{o}C temperature were more suitable for the higher decolorization of malachite green.**

I. INTRODUCION

Dyes are the chemical compounds used in textile industries. Malachite green, a triphenylmehtan dye is widely used in various applications (Pershetti et al., 2006). Dye containing effluent has been treated using either chemical or physical methods, which are expensive and difficult to operate (Shah et al., 2013). Economic and safe removal of dyes from an effluent is still a bigger challenge and issue. In an environment, microorganisms have an indigenous capacity to grow in the presence of complex compounds. Literature review indicates numerous bacteria, fungi were able to decolorize as well as degrade the dye compounds. According to this, biological method must be an alternative method of choice because of its eco-friendly products (Acuner et al., 2004; Akshu et al., 2003; Anjali et al., 2004). In recent days, researchers are mostly interested to isolate and identify the microbes which can degrade as well as decolorize the dye components. Thus, the present study deals to isolate potential dye decolorizing and degrading bacteria from dye containing effluent, optimization of physic-chemical parameters to stimulate the process.

II. MATERIALS AND METHODS

A. Enrichment and Isolation of dye decolorizing microbes

Acclimatization of microbes from the samples was carried out by inoculating 5.0ml of prepared sample in Bushnell Haas medium (Magnesium suphate, 0.2; Calcium chloride, 0.02; Monopotassium phosphate,1.0; Dipotassium phosphate, 1.0; Ammonium nitrate, 1.0; Ferric chloride, 0.05; distilled water 1000ml) embedded filter sterilized 10ppm dye , pH 8.0 (Bhatt et al., 2012). It was kept at 120 rpm at room temperature for two days. This enriched culture was streaked on Bushnell Haas agar medium mentioned as above. Plates were kept at room temperature for two days. Each of the bacterial strains was isolated and purified on nutrient agar medium and preserved at 4^{o}C temperature.

B. Screening of potential dye degrading bacteria

Screening of potential microbes were carried out by tube and plate method described by Syed et al.,(2009) and respectively. For, tube method, Bushnell Haas medium was embedded with different concentration ranging from 10 to 400ppm of filer sterilized dye, pH 8.0. 5.0ml of above mention medium was inoculated with 1.0ml of each bacterial suspension. All tubes and plates were kept for 24 hours at room temperature.

Plate method

Cup made by using cup borer on Bushnell Haas Agar embedded with 100 ppm dye and microorganism solution added in cup and incubate at 37^{0}C for 24 hr .Bacterial strain capable to decolorize high concentration of dye was used for further studies.

C. Dyes and Chemicals

All media component and chemicals are analytical grade and purchased from Hi-media laboratories(Mumbai, India). Malachite green was purchased from Loba chemie Pvt. Ltd.

D. Sample collection and its preparation

Soil contaminated with dye effluent was collected from . Each sample was preserved in sterile glass bottles at 4^{o}C temperature. Sample preparation was carried out by inoculating 1.0gm of soil samples in 100ml of sterile distilled water. Keep it for 3 to 4 hours for homogenization. Centrifuge it at 10,000 rpm for 10 minutes. Collect the supernatant and used it for further studies.

E. Decolorizing experiment

Decolorizing study was performed according to Parsetthi et al., 2006. Enriched culture grown (24 hours old) in Bushnell Haas medium embedded with 100 ppm dye, pH

8.0 was used for the study. 5% (v/v) culture was added into the 250ml flask containing 100ml above mentioned sterile medium. It was kept at 37^0C temperature at 120 rpm for 48 hours. At specific time interval (24 hours), 10ml sample was withdrawn and centrifuged at 10,000rpm for 10 minutes. Supernatant was collected and used for the analysis of pH, optimal density to measure the dye concentration etc. Measurement of cell biomass was performed by collecting the cell pellet and washed twice with sterile normal saline.

Percentage of dye decolorization was calculated according to the following formula (Sahasrabudhe et al., 2011);

$$\% \text{ of dye decolorization} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

F. Effect of physico-chemical factors on biodecolorization

Decolorization is based on the environmental conditions provided to the microbes. Thus, experiment was carried out by performing the effect of various physic-chemical parameters. The design of the experiment was done using the parameters like effect of pH(5.0,6.0,7.0,8.0,9.0), temperature (37,55^{o}C) addition of 1% different carbon(glucose, maltose, lactose, starch sucrose), nitrogen sources(yeast extract, peptone, urea, casein, ammonium sulfate) and static as well as shaking conditions (Ramizani et al., 2013).

G. Analytical methods

Dye concentration was measured by its optimal density at 610 nm. Growth behavior was observed by taking its OD at 600nm. pH was measured using pH electrode. Each experiment was performed in triplicate with its respective controls.

RESULTS AND DISCUSSION

Collection of the samples and isolation of the bacterial strains

All the effluent samples collected from the industrial sites of Ankleshwar, Surat, Navsari and Vapi gave the growth of varied type of bacteria. Ten different isolates exhibiting different morphological and cultural characteristics were selected and further isolated. Isolate AK3, AK7 VP4, VP9, SR4, SR7, SR0, NV2, NV5 and NV8 were further screened for their efficiency of dye decolourization.

Screening for dye decolorizing isolates

The isolates were screened for their dye decolorizing capabilities for malachite green on solid media by observing the zone of decolorization. Isolate no. AK3, VP4, VP9, SR7 and SR0 gave positive results (Table 1). The isolate SR0 showed efficient decolourization of dye and was further screened for the optimum decolourization of malachite green.

Table 1 : Screening of Bacterial isolates for dye decolorization

Sr. No.	Isolates	Decolorization of dye
1.	AK3	+
2.	AK7	-
3.	VP4	+
4.	VP9	+
5.	SR4	-
6.	SR7	+
7.	SR0	+
8.	NV2	-
9.	NV5	-
10.	NV8	-

Screening for Maximum dye decolorization

Isolate SR0 was screened for its capability of dye decolourization at a varied dye concentrations ranging from 10 ppm to 400 ppm after 24 hrs and 48 hrs. The highest decolourization was seen maximum at a dye concentration of 100 ppm at 24 hrs (56%) as well as 48 hrs (61%). Decolourization upto 50 % was also seen at 200 ppm as well as 400 ppm.

Table 2 : Qualitative data of dye decolourization

		Concentration of dye (ppm)											
Isolate	Time (hrs)	10	20	30	40	50	60	70	80	90	100	200	400
SR0	24	+3	+3	+3	+3	+2	+2	+2	+2	+2	+2	+1	+1
	48	+3	+3	+3	+3	+2	+2	+2	+2	+2	+3	+1	+1

Optimization of Parameters for dye decolourization

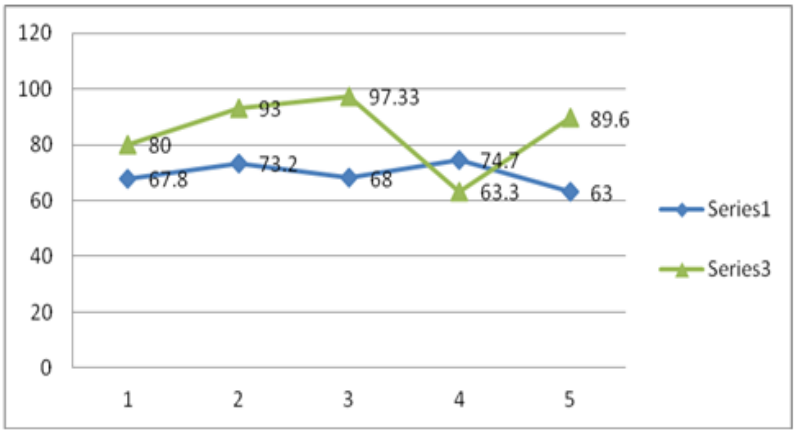
Effect of substrate variation on dye decolourization efficiency

Table 3 indicates the results of optimization for the dye decolorization efficiency for the C source, N source, physical parameters of pH, Temperature as well as static and shaking conditions (Panewad et al.,2000). On varying the carbon source the % decolorization showed a varied result (Oranusi et al.,2005). Maximum decolorization was obtained when the carbon source used was Lactose (74.7%) Sucrose (73.2 %) compared to the other carbon sources Fructose, Glucose and maltose. Compared to the alternative C sources, the variation in Nitrogen sources gave greater decolorization efficiency. Caesin gave maximum % decolorization of 97.33% while yeast extract showed 93%.

Table 3 : Effect of Carbon and Nitrogen source on dye decolorization

Sr. no.	C sources	% decolorization	N sources	% decolorization
1.	Glucose	67.8	Peptone	80.0
2.	Sucrose	73.2	Yeast extract	93.0
3.	Maltose	68.0	Casein	97.33
4.	Lactose	74.7	Amm. sulfate	63.3
5.	Fructose	63.0	Urea	89.6

Graph 1 :



Series 1 - Carbon Source
Series 3 – Nitrogen

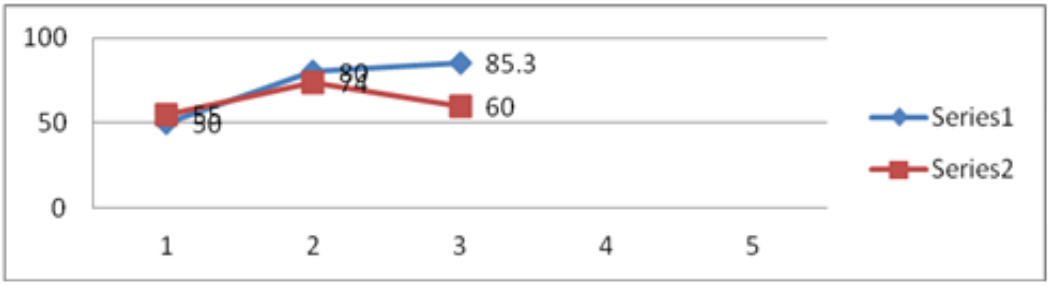
Effect of Physical parameters on decolourization efficiency

The variation in pH seems to have a profound effect on the decolourization efficiency of the isolate SR0. Maximum dye decolourization (85.3%) is observed at an alkaline pH 8 (Le Goff et al.,2008) as well as neutral pH 7(80%) while acidic pH doesn't seem to increase the decolourization. The % dye decolorization showed variation with variation in temperature (Cetin et al., 2006 ; Varel et al., 1980). The optimum temperature for efficient decolourization of malachite green is 37c.

Table 4 : Effect of physical parameters on dye decolorization

Sr. no.	pH	% decolorization	Temperature (˚c)	% decolorization
1.	6.0	50	27	55
2.	7.0	80	37	74
3.	8.0	85.3	55	60

Graph 2 :



Series 1 - pH
Series 2 – Temperture

Effect of static and shaking conditions

The static and shaking conditions showed a profound effect on the dye decolourization efficiency (Chen et al.,2009) Variation in the dye decolourization efficiency was also seen when the SR0 isolate was kept in static and shaking conditions keeping the pH 7 and 37c. The efficiency of decolourization was observed higher when the medium was kept on shaking conditions for 24 hrs as well as 48 hrs.

CONCLUSION

The above results of decolourizing the malachite green varying the different parameters would lead to an effective bioremediation of dye. Combining the lactose as a carbon source, casein as nitrogen source and keeping alkaline pH would be an effective decolourization means of malachite green. The mesophilic range of temperature is suitable for dye decolourization is indicative of no additional need for maintaining higher temperature proving to be more economical measure. The further characterization of SR0 strain as well as optimization will prove an effective means of bioremediation and an ecofriendly measure of removing the toxicity of dye from industrial effluent.

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