The Bioscan 5(4) : 591-594, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

MICROBIAL DECOLOURIZATION AND DEGRADATION OF CRYSTAL VIOLET BY AEROBIC BACTERIA

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KEY WORDS
Crystal violet
Bacteria
Degradation
Decolourization

Received on :
20.08.2010
Accepted on :
19.11.2010

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ABSTRACT
In present study, it was observed that, 92% decolourization of Crystal violet dye by the unidentified acclimatized bacterial cultures isolated from soil, collected from near by area of dye industry. The decolourization was achieved when dye solution was incubated at room temperature for 24 h. The decolourization was effective at neutral pH. No significant increase in decolourization was observed when the bacteria were supplemented with co-substrate (1% Glucose). Gas chromatography- mass spectroscopy analysis was carried out for confirmation of degradation. Five degradation products were isolated and identified.

INTRODUCTION

Contamination of surface water with dyes released from Textile and Dye industries represents a serious problem. These dyes are recalcitrant to the microbial degradation and affect the usual biological treatment of the industrial effluents (Swamy and Ramsay, 1999). Most of the azo dyes, which are released in to the environment originate from the textile industry and dyestuff manufacturing industry (Carliell *et al.*, 1995). Physico-chemical treatment methods are not economically feasible as they produce large volume of sludge. Besides the conventional Physico-chemical methods, microbial degradation of Azo dyes has been attracted significant attention. Microbial degradation of azo dyes has been reported using different microorganisms (McMullan *et al.*, 2001; Jadhav *et al.*, 2007), bacteria, yeasts, and white rot fungi. Several triphenylmethane dye-degrading microorganisms have reported (Naggar *et al.*, 2004; Chiing-Chang Chen *et al.*, 2007; Sarnaik and Kanekar, 1999). Most biological degradations of azo dyes are carried out by anaerobic bacteria (Raffi *et al.*, 1990, Raffi and Cerniglia, 1993, Carliell *et al.*, 1995, Zissi and Lyberatos, 1996, Tan, 2001, Yoo, 2000).

Generally azo dyes are resistant to attack by bacteria under aerobic conditions. Reports of Chang and Kuo, 2000 and Chang *et al.*, 2001 showed that bacteria could degrade azo under aerobic conditions. Azoreductase catalyzes reductive cleavage of azo bonds to produce aromatic amines while in aerobic conditions the initial step of azo bond cleavage is typically followed by hydroxylation and ring opening of aromatic intermediates (Sheshadri *et al.*, 1994 and Flores *et al.*, 1997).

In present study, the attempts were made to decolourize and degrade the crystal violet dye by aerobic bacteria and to detect and analyze the degradation. Products.

MATERIALS AND METHODS

Acclimatization and isolation of crystal violet degrading bacteria

Soil samples were collected from nearby area of textile and dye manufacturing industries. All these samples were homogenized on rotary shaker at 175 rpm by taking 10 g amount from each. To these homogenized samples, crystal violet dye solution (50mg/L) was added in increasing concentration *viz*.- 20%, 40%, 60%, 80% and 100% for two months. After period of two months, acclimatized soil sample was then added in sterile 100ml nutrient broth containing dye solution from stock solutions and kept for three to four days for enrichment purpose. One ml of enriched sample was serially diluted with saline and acclimatized bacteria were isolated on nutrient agar containing crystal violet dye.

Decolourization of crystal violet by selected isolate

The decolourization experiments were performed by growing the microbial culture isolated from acclimatized soil samples in nutrient medium containing crystal violet dye solution. The flasks were incubated aerobically on rotary shaker at 175 rpm at room temperature for 24 hr. After incubation the sample was centrifuged at 7000 rpm in cooling centrifuge (Remi) to remove the bacterial cell mass. The optical density of the supernatant was measured at 580 nm (λmax) by using Spectrophotometer (Systronics 106 model). The percentage

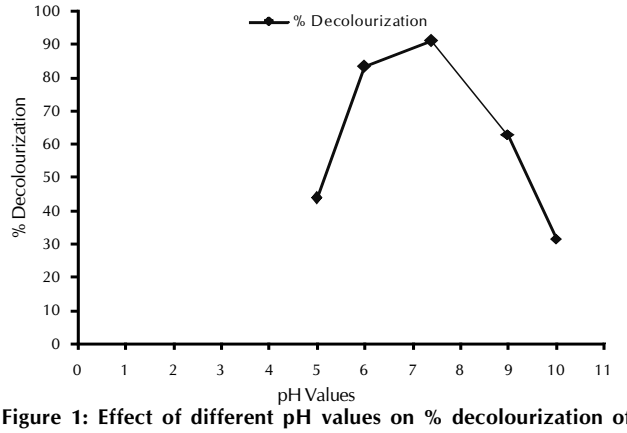


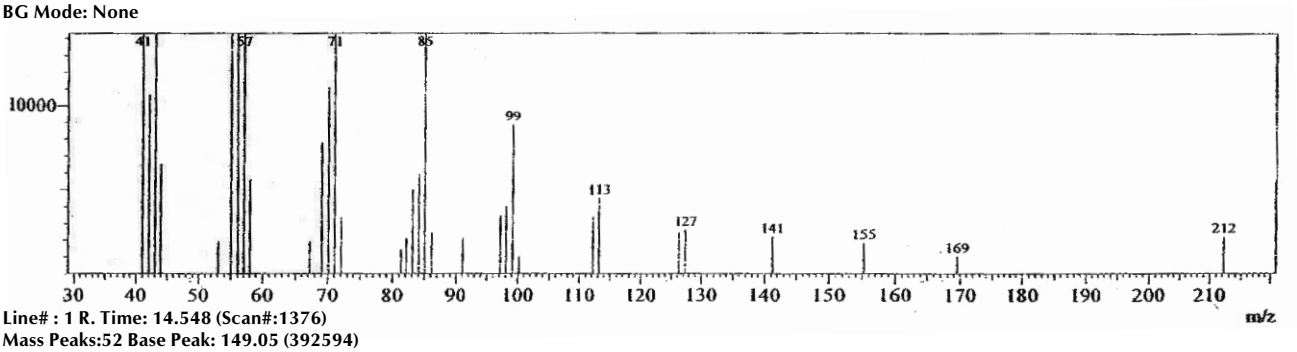
Figure 1: Effect of different pH values on % decolourization of Crystal Violet

of decolourization was calculated from the difference between initial and final absorbance values.

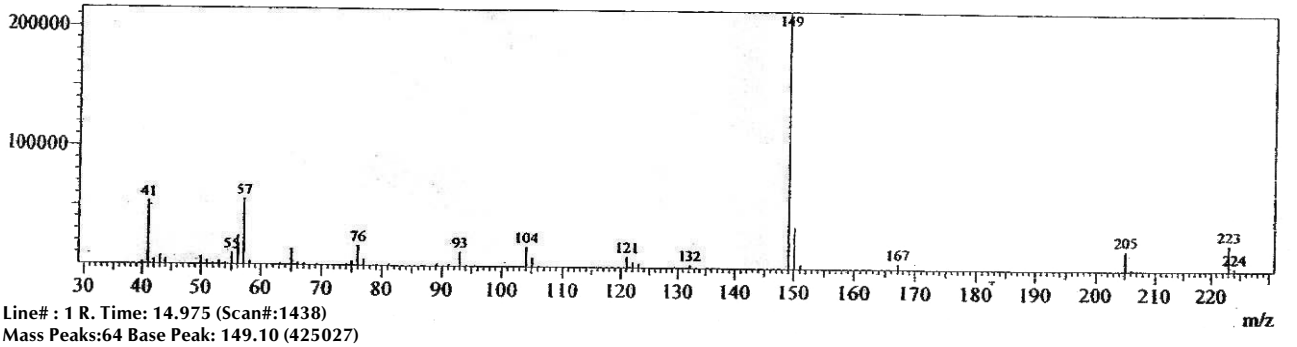
RESULTS AND DISCUSSION

In all, total 7 cultures were isolated on the basis of crystal violet decolourization potential. The results of decolourization studies of crystal violet by these cultures are given in Table 1. The percent decolourization of crystal violet after 24 hr incubation was in between 90.72-92.03. The highest decolourization was shown by unidentified culture CD-11 while least decolourization was given by CD-14 isolate. These results indicate that effective decolourization is achieved within 24 hr of incubation. Highest decolourization by CD-11 indicates the effectiveness of this culture in crystal violet decolourization. Hence this isolate was selected for further study.

Line# : 1 R. Time: 9.350 (Scan#:763)
Mass Peaks:33 Base Peak: 57.05 (104187)
Raw Mode:Single 9.350 (763)
BG Mode: None



Line# : 1 R. Time: 14.548 (Scan#:1376)
Mass Peaks:52 Base Peak: 149.05 (392594)
Raw Mode:Single 14.458 (1376)
BG Mode: None



Line# : 1 R. Time: 14.975 (Scan#:1438)
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BG Mode: None

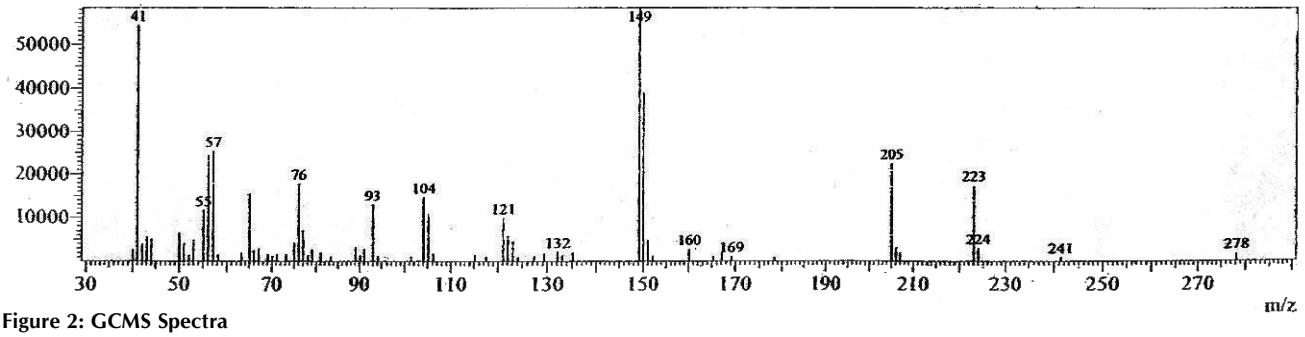
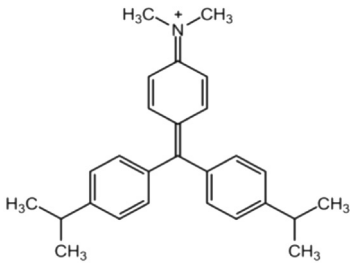
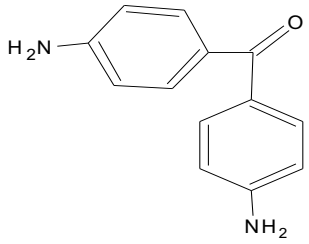
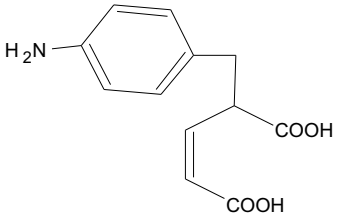


Figure 2: GCMS Spectra

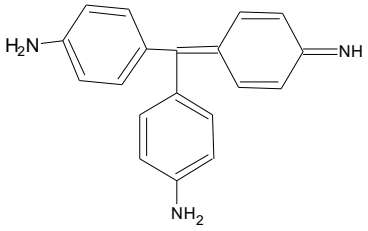




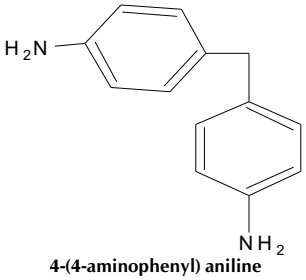
b is (4-am inophenyl) methanone



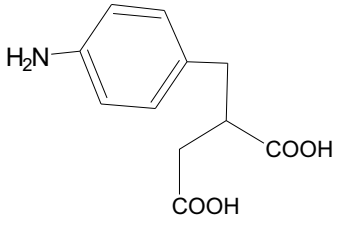
(2z)-4-(4-am inobenzyl)pent-2-enedioic acid



4-[(4-aminophenyl)(4-iminocyclohexa-2,5-dien-1-ylidene)methyl]aniline



4-(4-aminophenyl) aniline



2-(4-aminobenzyl) succinic acid

Figure 3: Structures of the products produced during the biotransformation of Crystal Violet Dye

Table 1: Percent decolourization of Crystal Violet by CD-11 isolate in nutrient broth

S.N.	Culture Code	% Decolourization
1	CD-2	90.94
2	CD-5	91.51
3	CD-6	90.98
4	CD-11	92.03
5	CD-14	90.72
6	CD-15	91.72
7	CD-17	91.99

It was observed that this strain could decolorize crystal violet dye at its maximum level when incubated at neutral pH. The % decolourization obtained at pH 5.0, 6.0, 7.0, 9.0, and 10.0 were 43.80, 83.40, 92.03, 62.90 and 31.50 respectively (Fig. 1).

The study of effect of co-substrates (1% glucose) was also performed. It was observed that there was no much increase in % decolourization of crystal violet when 1.0% glucose was used. The % decolourization was 91.03 and 91.90 when CD-11 culture used without and with co-Substrate (1% glucose) respectively.

It was observed that this isolate (CD-11) could decolourize crystal violet azo dye, which is normally considered to be more recalcitrant than other azo dyes (Haug *et al.,* 1991). The azo dyes are easily decolourized as the penetration through the cell membrane is rate limiting step during bacterial reduction (Mechsner and Wuhrmann, 1982). Such decolourization could have occurred by an oxygen insensitive azo reductase (Zimmerman *et al.*, 1982).

After GCMS analysis (Fig. 2), it was observed that isolate CD-11 could carry out degradation of of crystal violet and able to produce 4, [(4-amino phenyl) (4- imminocyclohexa-2, 5 dien-1-ylidene) methyl] aniline, bis (4- amino phenyl) methane, 4-(4- amino phenyl) aniline, (2Z)- 4-(4-aminobenzyl) pent-2 enedioic acid and 2-(4- amino benzyl) succinic acid products. The names of these products were determined by using ACD Labware Chem Sketch, and their structures are shown in Fig. 3.

Thus the isolate CD-11 is most promising in azo dye decolourization and degradation. This organism can decolorize crystal Violet even aerobic condition, which is in contrast to the reports given by (Raffi *et al.,* 1990; Nigam *et al.,* 1996a, 1996b; Vander Zee, 2002). Such properties of this organism can solve problem of pollution caused due to textile industry wastewater where mostly azo dyes are used for dyeing purpose.

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