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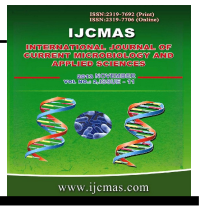

Original Research Article

Bioremediation of structurally different textile dyes by a novel bacterial consortium PVN-5

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K e y w o r d s

Azo Dye; Decolourization; Tri-phenylmethane dye.

A B S T R A C T

The potential of the consortium PVN-5 constituted by mixing 3 isolates (CR1,CR2 & CR3) were compared with that of individual isolates in decolourizing, 2 azo dyes (Direct Red-28,Direct Blue-8) & a triphenylmethane dye (Basic Green-4) which were used in this study.The individual bacterial isolates were not able to completely decolourize these dyes. The consortium PVN-5 was able to decolourize all the 3 dyes used at an initial concentration of 100 mgl^{-1} at a significantly higher rate as compared to that achieved by individual isolates.

Introduction

Textile industries have been using synthetic dyes intensively because of their ease and cost effectiveness in synthesis (Raju *et al*., 2008). Synthetic dyes are extensively used in the textile, food, cosmetics, Pharmaceutical, Paper, leather, Photographic and aquaculture industries (Chen *et al*., 1999). The main classes of dyes are azo, anthraquinone and triarymethane dyes based on their chromophoric groups and application technologies (Oranusi and Mbah 2005). Over $7x10^5$ tons of these dyes are produced annually world wide (Zollinger, 1987). It is estimated that about 10,000 different types of dyes are used industrially worldwide (Carliell *et al*., 1995) and about 10-15% of the dyes are lost in the effluent during such dyeing processes (Vaidya and Datye, 1982). Commercially useful dyes must possess a high degree of Chemical and photolytic stability which implies that removal from effluent is difficult. Stability to microbial attack is also a required characteristics of dyes (Pagga and Brown, 1996). Thus they are less amenable to biodegradation and contribute to contamination of the environment (Riu *et al*., 1998). Although the dyes impart only a minor fraction of organic load,colour removal is required not only for aesthetic reasons but also due to the toxicity to aquatic life and mutagenicity of few dyes and carcinogenicity of their degradation products (Coughlin *et al*., 1997).

Most Physio-Chemical dye removal methods, which are generally used for the effluent treatment, have many limitations (Balcioglu and Arslan, 2001;Ghoreishi Haghighi, 2003). These methods are not only economically unattractive because of high cost and high energy input but are environmentally unfriendly because they merely transfer the pollutant from one phase to another. For example, chlorination generates toxic organochlorides (Saras *et al*., 1998). Biological treatments of dye waste waster under aerobic and/or anaerobic conditions have been reported (Cripps *et al*., 1990; Banat *et al*., 1996; Chen *et al*., 1999). Some dyes and/or their degradative by products (aromatic amines from azodyes and lecuco triphenylmethane from triphenylmethane dyes) are potentially toxic mutagenic and carcinogenic (Houk *et al*., 1991; Burchmore and Wilkinson,1993; Ganesh *et al*., 1994; Henderson *et al*., 1997)**.**

Mixed culture studies may be more appropriate for decolourization of dyes.About 80% of colour removal in an effluent sample containing mixture of azo and diazo – reactive dyes was observed by using mixed bacterial culture (Nigam *et al*., 1996). Higher degree of biodegradation and mineralization can be expected when co-metabolic activities of mixed cultures within a microbial community complement each other. The advantages of mixed cultures are apparent as some microbial consortia can collectively carry out biodegradation that cannot be achieved by pure culture culture (Nigam *et al*., 1996; Sharma *et al*., 2004)**.**

In the present study, we report a novel bacterial consortium named PVN-5 compared with 3 individual isolates having decolourizing ability for three structurally different dyes.

Materials and Methods

Dyes and Chemicals

The azo dyes Direct Red-28 (DR-28), Direct Blue-8 (DR-8) and triphenylmethane dye Basic green-4 (BG-4) were obtained from a textile company. The chemical structures of dyes DR-28, DB-8 & BG-4 are shown in (Fig.1). The dyes were selected on the basis of their structural diversity and frequent use in local textile industries. The media components and chemicals were purchased from Himedia Labs,Bombay,India. All chemicals used were of analytical grade.

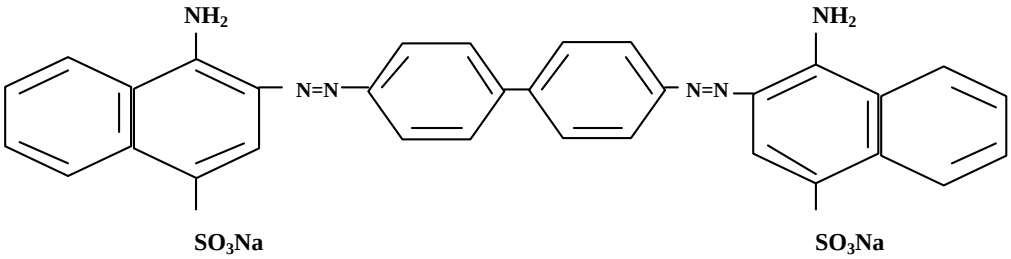
Growth medium

The pure cultures of CR1,CR2 &CR3 isolated from soil samples collected from dye polluted areas and textile mill effluents were grown in 250 ml Erlenmeyer flask,containing 100 nutrient broth[(g l^{-1}) : Peptone 10,NaCl 5, Yeast extract 2 and beef extract] at 30±2˚C for 24 hrs under static condition.

Development of Bacterial Consortia

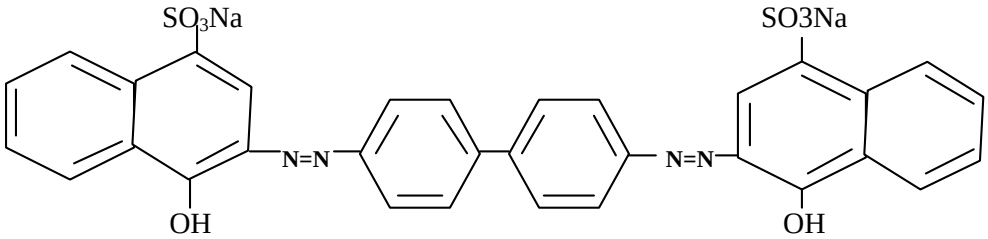
The consortium was developed with the selected isolates by aseptically transferring 1ml of 24hrs cultures ($3.6x10^{9}$ $Cfuml^{-1}$) of each in a 250 ml conical flask contaioning 100 ml of nutrient broth, P^{H} 7.0 and incubated at 37˚C for 24 hrs under static conditions. A 1% v/v aliquots of the culture mix were then transferred into a 250 ml Erlenmeyer flasks containing 100 ml of nutrient broth with100 ppm of dyes DR-28,DB-8 and BG-4 respectively. The cell suspensions of individual isolates was also run along with the consortium with the respective dyes. Suitable control was maintained without any inoculum.

Figure.1 Chemical Stucture of Direct Red -28



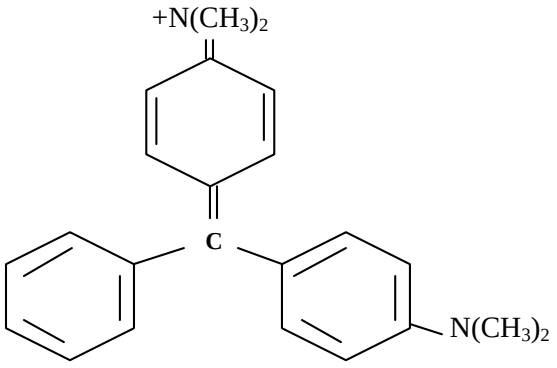
C.I.Direct Red-28(DR-28).C.I. 22120 λ_{max} = 500nm

Chemical Stucture of Direct Blue -8



C.I.Direct Blue-8(DB-8).C.I. 24140 λ_{max} = 560nm

Chemical Stucture of Basic Green-4



C.I.Basic Green-4(BG-4).C.I. 42000 λ_{max} = 560nm

Decolourization Assay

The conical flasks containing the cell suspension of individual isolates and consortium PVN-5 were completely sealed, so as to achieve anoxic conditions. The uninoculated control was also incubated under same conditions to check the abiotic decolourization of the dye. The autoclaved cells of consortium PVN-5 were also incubated to study the decolourization due to adsorption of dye. The samples were withdrawn at every 12 hrs for 6 days, centrifuged at 10,000 rpm for 15 min.

The supernatant of the centrifuged samples were read at absorbance maximum (λ max) of the dyes as per Colour Index International(1992) ie.,500nm for DR-28, 560nm for DB-8 and 614nm for BG-4 using spectrophotometer. The cell pellet was resuspended in equal volume of ethanol to extract the dye absorbed to cell surface. The methanol sample was centrifuged and supernatant aws read at λ max of dyes used. The uninoculated dye free medium was used as blank. All assays were performed in triplicates and compared with respective uninoculated control.The flasks were observed for 10 days to assess the maximum decolourization (data not shown). The decolourization percentage was calculated as follows :

$$\text{Decolourization (\%)} = \frac{\text{Dye (i) - Dye (r)}}{\text{Dye (i)}} \times 100$$

Where,(I) is the initial dye concentration and Dye (r) is the residual dye concentration

Result and Discussion

Decolourization by living cells

The results in Fig.2-4 shows that the living cells of PVN-5 were able to completely decolourize DR-28, DB-8 and BG-4 in 24, 24 & 36 hrs respectively.Since the eventual fraction of methanol-extractable dye is very negligible, the results clearly indicate that the overall decolourization of DR-28, DB-8 and BG-4 is due to biological mechanisms.

Decolourization by autoclaved cells

The results in Fig.2-4 show initial decolourization of DR-28,DB-8 & BG-4 by autoclaved cells of PVN-5 (5%,60% & 2%) respectively during first 4 hrs; but no further significant variation in decolourization was observed during the next 20 hrs. Extraction with methanol recovered the major part of the decolourized dye indicating that DB-8 decolourzation by autoclaved cells of PVN-4 is mainly due to adsorption.Decolourization by abiotic control was not significant in all the 3 dyes (Fig.2-4).

Decolourization of Direct Red-28

The results presented in **Fig.5** show the ability of individual isolates , viz CR1,CR2,CR3 & consortium PVN-5 to decolourize azo dye DR-28. The pure cultures of the strains CR1 CR2 &CR3 showed a maximum of 85.50%,77.88% & 75.79% respectively at 72 hrs. On the other hand consortium PVN-5 achieved 97% decolourization of DR-28 in 12 hrs and complete decolourization in 24 hrs. The average decolourization rate of dye DR-28 for the consortium PVN-5 was significantly higher than that observed for

Figure.2 Decolourization of Azo dye DR-28 by living & autoclaved cells of Consortium PVN-5

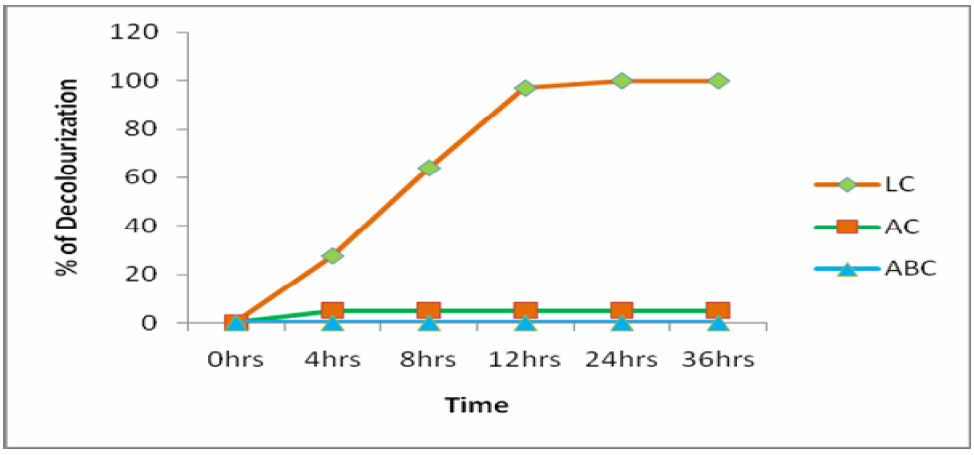


Figure.3 Decolourization of Azo dye DB-8 by living & autoclaved cells of Consortium PVN-5

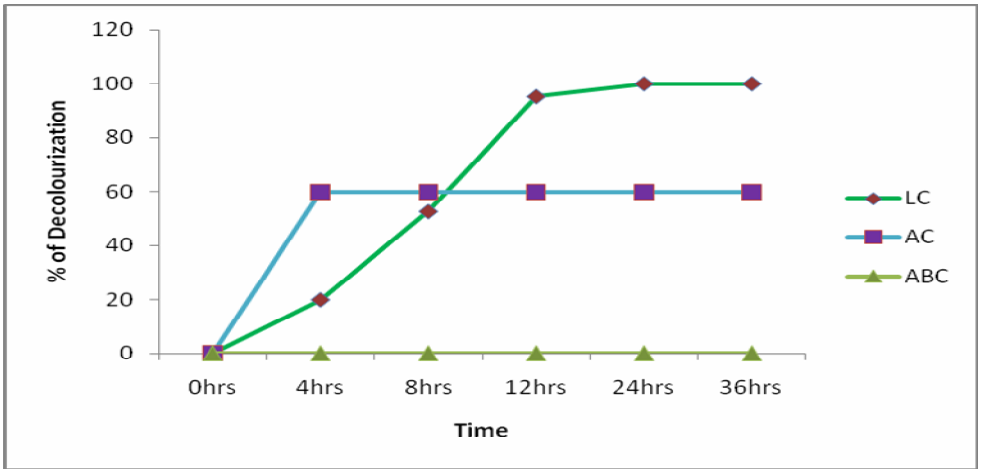


Figure.4 Decolourization of Azo dye BG-4 by living & autoclaved cells of Consortium PVN-5

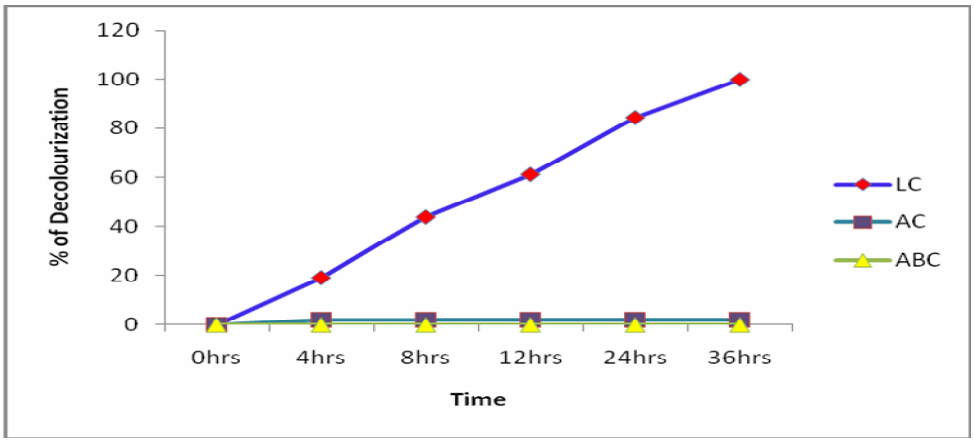


Figure.5 Decolourization profile of Azo dye DR-28(100ppm) by individual isolates & the Consortium PVN-5

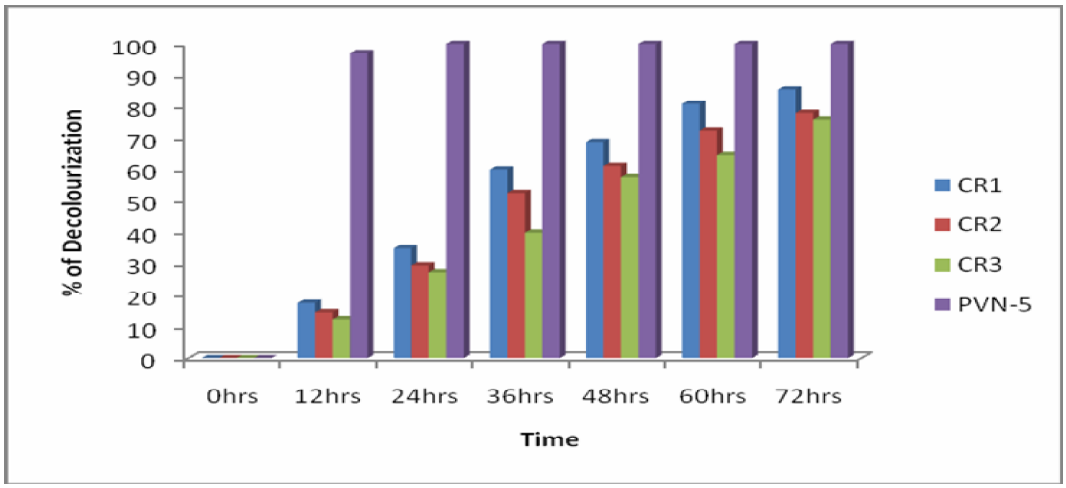


Figure.6 Decolourization profile of Azo dye DB-8(100ppm) by individual isolates & the Consortium PVN-5

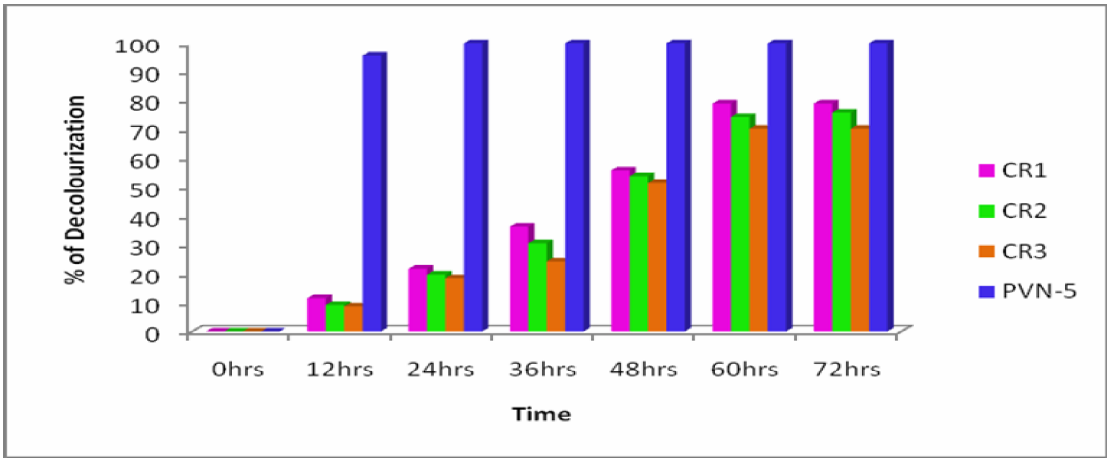
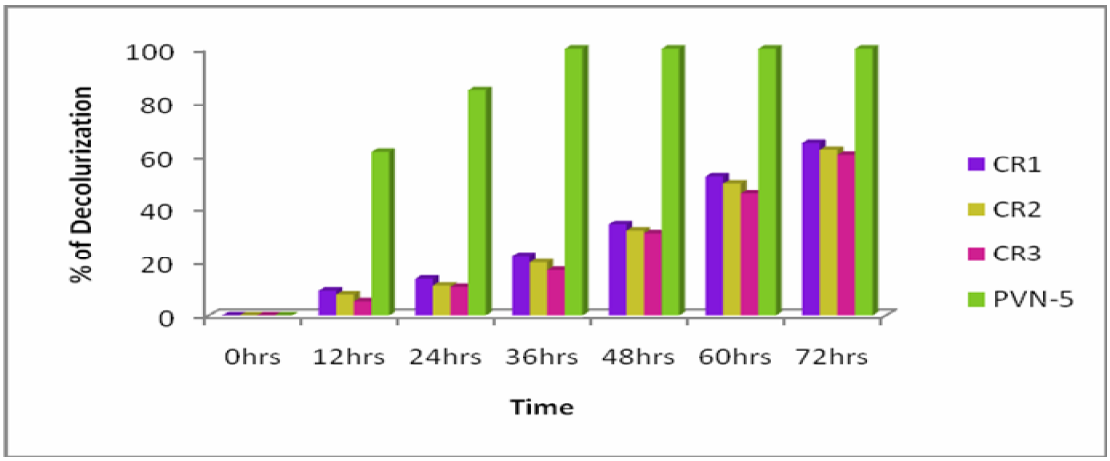


Figure.7 Decolourization profile of Azo dye DB-8(100ppm) by individual isolates & the Consortium PVN-5



individual isolates. Khera *et al*., (2005) reported similar observation while proceeding the comparative studies on potential of consortium & pure bacterial isolates.

Decourization of Direct Blue-8

The consortium PVN-5 showed a maximum decolourization of 95.23% of DB-8 in 12 hrs and complete decolourization in 24 hrs. The individual isolates CR1,CR2 & CR3 showed decolourization of about 78.69%,75.62 & 70.13% respectively (Fig.6).

Decolourization of Malachite Green

The dye Basic Green-4 was not decolourized significantly by the strains CR1,CR2 & CR3 (**Fig.7).** However the consortium PVN-5 achieved complete decolourization of the dye Malachite green (100ppm) in 36 hrs **.**Therefore the consortium PVN-5 showed maximum efficiency in decolourizing the dye Basic Green-4 than the individual isolates.

In this study, the capability of 3 isolates CR1,CR2 & CR3 to decolourize 3 dyes independently and as a mixed culture designated as PVN-5 was evaluated.These strains were isolated from soil samples collected from dye polluted areas and textile mill effluents and consortium PVN-5 based on these 3 isolates was developed and described previously (Saratale *et al*., 2009).

The individual isolates CR1,CR2 & CR3 were able to decolourize DR-28 & DB-8 efficiently. However individually the strains were not able to decolourize BG-4. This variation in decolourization of 3 dyes by pure isolates might be due to the structural diversity of the dyes used in the study (Fig.1). Sani and Banerjee., (1999) reported that dyes with simple structure and low molecular weight show higher rate of decolourization, whereas decolourization of highly substituted, high molecular weight dyes are more difficult. This is also similar to the report given by Chen *et al*., (2003) of varied colour removal capabilities of six bacterial strains for structurally and functionally diverse dyes belonging to azo group, anthraquinone and indigoid groups.

The versatility of consortium PVN-5 was evident from the results as it was able to decolourize all the three tested dyes completely with varying time. The decolourization rate achieved for all the individual dyes were significantly higher than that of axenic cultures (Table.1). The comparision of decolourization potential of PVN-5 with abiotic control and autoclaved culture proved that the removal of Direct Blue-8 by autoclaved cells was mainly due to adsorption of dye by the biomass; whereas the methanol extraction of living cells after complete dye decolourization did not result in any dye recovery which indicates that the decolourization was due to the bacterial activity. The higher decolourization efficiency of consortium PVN-5 might be attributed to the concerted activities of the constituent strains. The higher decolourization potential due to concerted activities of the mixed culture was also reported by Nigam *et al*., (1996).

The concerted activities of the consortium PVN-5 consisting of 3 bacterial strains,viz CR1,CR2 & CR3 resulted in increased decolorization efficiency as compared to that shown by individual strains. The consortium was able to decolourize the dyes Direct Red-28, Direct Blue-8 & Basic Green-4 having diverse structures at a faster rate than individual axenic cultures.

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