

Doi: 10.58414/SCIENTIFICTEMPER.2024.15.spl-2.21

ORIGINAL RESEARCH PAPER

Investigating optimal conditions for direct red 37 biodegradation using *Enterococcus innesii* strain CV10

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Abstract

The study tested the decolorization of Direct Red 37 dye using *Enterococcus innesii* CV10, a potential dye-decolorizing bacterial isolate. The optimal conditions were a temperature of 37°C, an inoculum size of 1-mL, an age of 18 hours, a pH of 8, and a water bath. The decolorization became less pronounced with increasing dye concentration and almost nonexistent at certain values. Decolorization was also studied about certain metals, carbon and nitrogen sources, and dietary factors. Dye degradation is seen in the meta-ring cleavage, also known as Positive Rothera's reaction.

Keywords: Decolorization, Enterococcus innesii CV10, Size of inoculum, Age of inoculum, Rothera's reaction, COD.

Introduction

Textile (azo) dyes pose a challenge to wastewater treatment due to their resistance to degradation, making it difficult to meet regulatory standards for effluent color and degradation using conventional aerobic treatments. Traditional aerobic biological treatment (Carliell, C. M., Barclay, S.J., Naidoo, N., Buckley, C. A., Mulholland, D. A. & E. Senior. 1995) combined with primary, physical, and chemical treatments, is effective but can result in hazardous chemical sludge, (Ministry of Environment & Forests 2003) contributing to increased processing costs. Microbial decolorization of azo dyes begins with the reductive breakdown of the azo link in anaerobic environments. On the other hand, it produces amines associated with dyes, which are not degradable in

anaerobic environments and can accumulate in harmful quantities. Environmental challenges in textile operations include the significant utilization of organic dyes (Peralta-Zamora, P. Kunz, A., de Moraes, S. G., Pelegrini, R, Reyes, J., & N. Duran. 1999), high water consumption and the high water consumption of textile manufacturing facilities (Zollinger, H. 1987). Bioremediation of azo dyes occurs in two distinct phases: anaerobic reduction of the azo bond, producing aromatic amines, and aerobic digestion by a diverse bacterial community, leading to complete mineralization (Nortemann, B.J, Baumgarten, H., Rast, G. & H. Knackmuss. 1986).

Wastewater is a mixture of dissolved and suspended contaminants from various sources. It can release volatile compounds, transport pathogenic microorganisms, and contain nutrients promoting aquatic organisms' growth. Treatment methods include physical, chemical, and biological processes for effluent quality. Physical separation techniques eliminate larger solids and debris, while chemical treatments disinfect wastewater and remove inorganic substances. Biological treatments degrade biodegradable organic materials, converting them into gases and biomass. These processes can be aerobic, anaerobic, or a combination of both. The main purposes of these processes include removing carbonaceous organic matter, nitrification, denitrification, dephosphatation, and stabilization. Direct dyes, developed to form covalent bonds with cellulose fibers like cotton, are widely used in the textile industry

How to cite this article: Kachhela, V., Rank, J., Kothari, C. (2024). Investigating optimal conditions for direct red 37 biodegradation using Enterococcus innesii strain CV10. The Scientific Temper, **15**(spl-2):133-139.

Doi: 10.58414/SCIENTIFICTEMPER.2024.15.spl-2.21

Source of support: Nil
Conflict of interest: None.

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Received: 22/10/2024 **Accepted:** 19/11/2024 **Published**: 30/11/2024

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because of their low energy requirements and wide color spectrum. But even under normal aerobic conditions, they won't break down. This study investigates reductions in COD and Rothera's ring cleavage reaction, decolorization of azo dyes in textiles containing various metals and nutrients, and more.

Materials and Methods

Dyes

Direct Red 37 textile dye was sourced from Ankleshwar, Gujarat, while media components and chemicals were obtained from Hi-Media Labs, Bombay, using analytical grade chemicals.

Sample Collection

Enterococcus innesii CV10 was acquired through primary and secondary screening and was utilized to examine these parameters.

Determination of Growth

Viable count

A standard method was used to assess the viable count at the specified intervals described in the text.

Turbid metric measurement

The centrifugation-generated biomass was washed three times before being re-dissolved in distilled water to measure optical density at 660 nm.

Biomass (dry weight)

To find the biomass's dry weight, the culture was transferred to Eppendorf tubes that had been weighed, centrifuged, cleaned three times, and dried until they achieved a constant weight.

Total protein

Collecting, cleaning, and centrifuging the culture followed its transfer to Eppendorf tubes. Protein concentration in the supernatant was determined after a second centrifugation of the biomass after incubation with 2.5 M NaOH².

Effects of size of inoculum on decolorization of direct Red 37 by E. innesii CV10

A 16-hour culture of *E. innesii* CV10, grown in CMB, served as the inoculum. A set of 25 test tubes was manufactured, with 100 mL of sterile CMB medium and 100 mg/L of Direct Red 37 added. The inoculum was applied to each of the five flasks in the set at a specific quantity (0.2–1%) and then incubated at 37°C. Control flasks were both injected and not inoculated in the experiment. At 12-hour intervals, one flask was examined to ascertain the biomass, represented as total protein in milligrams per milliliter, and the extent of decolorization.

Effects of age of inoculum on decolorization of Direct Red 37 by E. innesii CV10

Culture of *E. innesii* CV10 at various ages, specifically 6, 12, 18, 24, and 36 hours, was utilized as the inoculum. The direct red 37 dye was tested independently. In Erlenmeyer flasks, 100 mL of sterile CMB medium containing dyes at a concentration of 100 mg.l-1 were prepared. After being inoculated with 1%, or approximately 1.137 x 10¹⁰ cells, each of the five flasks in the group was placed in an incubator set at 37°C.per mL. The setup included control flasks that were inoculated and those that were not. At 24, 48, and 72 hours, samples were obtained from the flasks for the decolorization analysis.

Effects of temperature on the decolorization of various textile dyes by E. innesii CV10

About 250 mL Erlenmeyer flasks were filled with 100 mL of complete medium containing the following concentrations (100 mg.l-1): Following a 20-minute autoclaving duration at 15 bar pressure. The flasks were seeded with a seed culture that was 18 hours old (about 1.137 x 10¹⁰ cells.ml-1) and left to incubate at 10, 20, 37, 50, and 60°C. Direct Red 37 was utilized for this purpose. The incorporation of suitable controls was also detailed in earlier works. Samples were collected at 24 and 48 hours to analyze the decolorization.

Effect of pH (buffer) on decolorization of Direct Red 37 E. innesii CV10

A comprehensive medium containing Direct Red 37 was prepared using a range of buffer solutions. Many buffers were chosen in the preparation, including 0.1 M citrate buffer at 4 and 5 pH, 0.1 M sodium phosphate buffer at 6 and 7, and 0.1 M Tris buffer at 8 and 9. All of the other experimental parameters followed the rules that had been set up before. Samples were collected every 24 hours for analysis of decolorization. (Bradford, M.M. 1978)

Influence of Tween-80 on the decolorization of Direct Red 37 E. innesii CV10

The following were added to 250 ml Erlenmeyer flasks: 100 mL of complete medium, 100 mg l-1 of Direct Red 37 dye, and different concentrations of Tween-80 (0.003, 0.006, and 0.009%). The flasks were autoclaved, inoculated, and placed in a 37°C static incubator. The decolorization analysis required samples to be collected every twelve hours.

Effects of concentration of Direct Red 37 on its decolorization by E. innesii CV10

The following concentrations of Direct Red 37 (mg. l-1) were added to the complete medium in Erlenmeyer flasks: 100, 200, 300, 400, 500, 600, 700, and 800. Before being incubated at 37°C under static conditions, the flasks were autoclaved and seeded with about 1.137 x 10¹⁰ cells.mL-1.

The decolorization analysis required samples to be collected every 24 hours.

Effects of carbon sources on the decolorization of Direct red 37 by E. innesii CV-10

Along with 0.25% carbon sources, including glucose, galactose, inositol, arabinose, sorbitol, mannitol, fructose, rhamnose, mannose, and xylose, 100 mg l-1 of Direct Red 37 was added to Mineral Salt Medium. Each component was pressure-sterilized for 10 minutes at 10 psi before being introduced to the medium. Later, the medium was supplemented with 1% of an 18-hour-old culture containing approximately 1.137×10^{10} cells.mL-1.

Effects of nitrogen sources on the decolorization of Direct red 37 by E. innesii CV-10

The effects of several nitrogen sources were tested by substituting $\mathrm{NH_4NO_3}$ in glucose-containing MSM with peptone, yeast extract, casein acid hydrolysate, glycine, $\mathrm{NH_4Cl}$, $(\mathrm{NH_4)_2SO_4}$, $\mathrm{KNO_3}$, or $\mathrm{NaNO_3}$. All other experimental parameters remained consistent with those outlined earlier.

Influence of metals on the decolorization of Direct red 37 by E. innesii CV-10

After mixing 100 mL of CMB with 100 mg.l-1 of Direct Red 37 in 250 mL Erlenmeyer flasks, the mixture was autoclaved for 20 minutes at 121°C. A pre-measured volume of sterile metal stock solution was added to the medium to achieve concentrations of 0.025 and 0.05 mg.mL⁻¹. So, instead of MgSO4 (0.02 g.l-1), the investigated metals were added to the whole medium. Each flask was incubated at 37°C after adding 1% inoculum, about 1.137×10¹⁰ cells.mL-1. Inoculated and non-inoculated control samples were used in the experiment. Samples were collected at predetermined intervals for analysis.

The stocks of various metal salts, including $BaSO_4$, $FeSO_4$.7 H_2O , $ZnSO_4$.7 H_2O , $C4H_6O_4$ Pb.3 H_2O , $MgSO_4$.7 H_2O , MnC_{12} , $CuSO_4$, CoC_{12} .6 H_2O , SnC_{12} .2 H_2O , and $A_{12}O_3P_2O_3$, were sterilised by filtering.

COD removal and decolorization

In 250 mL Erlenmeyer flasks, 100 mL of CMB containing 100 mg.l-1 of Direct Red 37 was inoculated with a 1% inoculum of *E. innesii* CV-10, approximately 1.137×10¹⁰ cells.mL-1, and incubated at 37°C. There were inoculated and noninoculated control samples used in the experiment. The decolorization analysis required samples to be collected at certain intervals. The analysis of chemical oxygen demand (COD) followed the APHA standard (Andrew D.E., Lenore, S.C. & E.G. Arnold. 2005).

Rothera's ring fission reaction

The ortho- and meta-cleavage of two major aromatic chemical metabolism intermediates, protocatechuate and catechol, were evaluated using the techniques originally proposed by Hosokawa. (Stanier, R.Y., Palleroni, N.J., & M. Doudoroff. 1966)

A complete medium containing dye at a concentration of 100 mg/l was inoculated with 1% (approximately 1.137×10^{10} cells/mL) of 18-hour-old cultures. A constant temperature of 30° C was maintained in the culture flasks. There were inoculated and non-inoculated controls in the study. After 72 hours, 10 cc of each flask's contents were separated and then spun in a centrifuge at 4° C for 10 minutes at 10,000 rpm. In order to conduct the Rothera reaction test, the supernatant was discarded, and the pellet was re-suspended in either distilled water or phosphate buffer (100 mM, pH 6.8).

Meta-cleavage of ring

The collected pellets were suspended in 2 mL of tris buffer, which has a concentration of 200 mM and a pH of 8. After adding 0.2 mL of toluene and 0.2 mL of catechol or protocatechuate (1 M), the mixture was rapidly agitated. A few minutes later, a yellowish hue appeared, indicating the presence of meta-cleavage action; hence, the test was deemed positive.

Ortho-cleavage

One gram of (NH4)2SO4 was combined with 2.5 mL of the cell solution. For the last hour of incubation, the assay mixture was kept at 30°C. Shaken to get the pH down to about 10, the mixture was then supplemented with half a milliliter of 5 N ammonia and a drop of 1% sodium nitroprusside once the incubation period had ended. A rich purple color forms when there is ortho-cleavage.

Effects of size of inoculum on decolorization

The ideal inoculum size required for enhanced and expedited decolorization by *E. innesii* CV10 was evaluated by adjusting the inoculum size, ranging from 0.2 to 1-mL per 100 mL.

Figure 1 illustrates the impact of inoculum size on the decolorization of dyes. An inoculum size of 0.2 mL resulted in a 17% decolorization after 24 hours. By the 36 to 48-hour mark, this increased to 78%. With a 0.4 mL inoculum, 24% decolorization was observed within 24 hours, rising to 82% after 48 hours. As the inoculum size was increased to between 0.6 and 0.8 mL, variations in decolorization percentages were noted. The dye underwent rapid

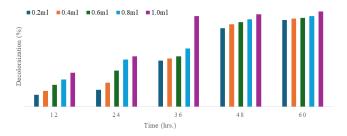


Figure 1: Decolorization of Direct red 37 by E. innesii CV10 culture initiated with inocula of varying size (mL/100 mL)

decolorization in flasks containing 1-mL of inoculum.

The decolorization activity peaked at 90% after 36 hours with a 1% inoculum concentration, and it became better as the inoculum size increased. Nevertheless, there were no noticeable changes in decolorization seen after 48 and 60 hours.

If the amount of cells in the inoculum rises, the dye will decolorize more, as long as the medium and dye are infinitely supplied. While the inoculum size increased, the rise in decolorization did not match when *E. innesii* spp. were introduced to textile effluent. (Sani, R. & U. Banerjee. 1999)

Effect of age of inoculum on decolorization

At various points in their life cycles, microbes develop distinct physicochemical and physiological characteristics that are reflected in the primary and secondary metabolites they produce. Because of azoreductase activity, which occurs in anaerobic environments, bacteria can decolorize pigments. A lot depends on the specific cultural context in which decolorization is carried out. By inoculating reaction flasks with different colored microbial populations collected at different intervals, this study aimed to evaluate the influence of inoculum age.

Bacterial isolates of *E. innesii* CV10 were cultivated for varying durations (6, 12, 18, 24, and 36 hours) using a specific carbon source. The percentage of decolorization was assessed spectrophotometrically at a wavelength of 513 nm. The highest level of decolorization occurred with an 18-hour-old inoculum, followed by the 12-hour culture, while the least decolorization was noted in the 36-hour-old culture.

The most effective decolorization of the dyes occurred when an 18-hour-old culture was utilized as the inoculum.

Effects of temperature on decolorization

Microbial growth is influenced by specific temperature ranges, making temperature a crucial physical factor that directly and indirectly impacts cellular metabolism. Enzyme-

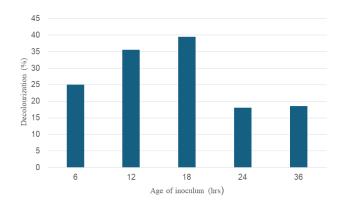


Figure 2: Effects of age of inoculum on decolorization by Direct Red 37 (λ_{max} 513) by *E. innesii CV10*

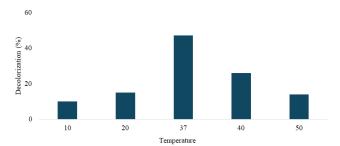


Figure 3: Effects of temperature on decolorization by Direct Red 37 $(\lambda_{max} 513)$ by *E. innesii CV10*

catalyzed processes are particularly sensitive to incubation temperature, as fluctuations can enhance or inhibit enzyme activity. Enzymes drive the decolorization and degradation of dyes, and the extent of these reactions can be measured by the quantity of dye that has been decolorized.

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The decolorization activity of our culture peaked at 37°C after an increase from 10 to 37°C during incubation (Figure 3). However, a further increase in temperature led to a slight decrease in the decolorization activity of *E. innesii* CV10. This reduction at elevated temperatures may be due to a decline in cell viability or the denaturation of the azoreductase enzyme (Pearce, C.I., Lioyd, J.R. & J.T. Guthrie. 2003).

Effects of pH on the decolorization of textile dyes by E. innesii CV10

The medium's ionic strength (pH) plays a crucial role in nearly all physiological processes. Specific pH levels achieve the optimal conditions for degradation or synthesis. Additionally, both decolorization and biodegradation depend on pH. This study assessed the impact of pH on the decolorization of different textile dyes.

Bacterial cultures typically show the highest levels of decolorization at a pH close to 8. Research indicated that *E. innesii* CV10 achieved optimal decolorization of Direct Red 37 (74%) at a pH of 8 (Figure 4). Although some decolorization occurred at pH 4, it was minimal (4%). *E. coli* and *P. luteola* demonstrated the most effective decolorization at pH 7, maintaining a consistent decolorization rate up to pH 9 (Chang, J.C. & Y.C. Lin. 2001) *Pneumoniae* RS-13 was able to completely degrade Methyl Red within a pH range of 6 to 8.15

Furthermore, it has been observed that the reduction of azo dyes by bacterial cultures typically increases the medium's pH by approximately 0.8 to 1.0 units (Pearce, C.I., Lioyd, J.R. & J.T. Guthrie. 2003).

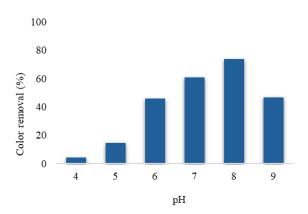


Figure 4: Effects of pH on decolorization by Direct Red 37 (λ_{max} 513) by *E. innesii CV10*

Effects of Tween-80 on decolorization of Direct red 37 by E. innesii CV10

Dye absorption is enhanced by Tween-80 because it enhances cell permeability. An effective decolorization rate of 59% was achieved using a 0.003% Tween-80 concentration. As shown in Figure 5, the decolorization rates rose from 62 to 77% as the concentration of Tween-80 increased from 0.006 to 0.009%, in comparison to 53% decolorization seen without Tween-80.

Effects of dye concentration

The decolorization capability of *E. innesii* CV10 was investigated using Direct Red 37 at various initial concentrations ranging from 100 to 800 mg/l (Figure 6).

As the concentration of dye rises, the decolorization of Direct Red 37 diminishes. This reduced efficiency in decolorization can be attributed to increased inhibition at elevated dye concentrations (Verma, P. & D. Madamwar. 2002).

Our isolate has the capability to decolorize dyes at concentrations significantly higher than those typically found in wastewater, making it an effective solution for treating industrial wastewater that contains dyes.

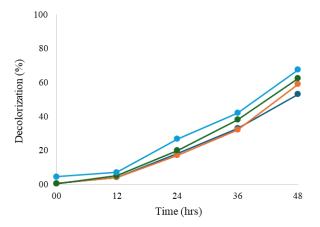


Figure 5: Effects of Tween 80 on decolorization by Direct Red 37 $(\lambda_{max}513)$ by *E. innesii CV10 (%): zero,* (\blacksquare); 0.003(\blacksquare); 0.006, (\blacksquare) and 0.009, (\blacksquare)

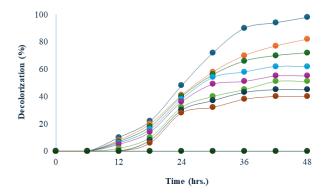


Figure 6: Effects of dye concentration on decolorization by Direct Red 37 (λ_{max} 513) by *E. innesii CV10 100,* (\blacktriangle); 200, (\blacktriangle); 300, (\blacktriangle); 400, (\blacktriangle); 500, (\blacktriangle); 600, (\blacktriangle); 800 (\blacktriangle)

Effects of C source

Cells have fundamental requirements, including nutrients such as carbon (C), nitrogen (N), sulfur (S), and phosphorus (P). Carbon is a crucial nutrient, fulfilling the cell's demands for synthesizing various structural components and acting as a vital energy source.

In the current investigation, we present the impact of various carbon sources on the decolorization of the textile dye Direct Red 37 by *E. innesii*. Despite the fact that the chosen strain, CV-10, was able to thrive on all of the carbon sources examined, the degree to which it decolorized depended on the specific nutritional carbon source employed (Figure 7).

Twelve hours after inositol, arabinose, sorbitol, mannitol, fructose, rhamnose, mannose, and xylose were added to static cultures, the decolorization activity ranged from nine percent to twelve percent. After 24 hours, this activity increased to 22 and 50%, respectively, with the exception of mannitol and glucose. Static cultures of *E. innesii* CV-10 growing on glucose, mannitol, and fructose achieved decolorization rates of 100, 94, and 90% of the initial dye concentration, respectively, within 48 hours. The other carbon sources facilitated decolorization rates between 66 and 72%.

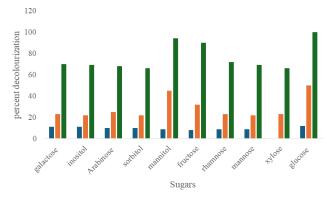


Figure 7: Effects of various carbon sources on decolorization of Direct Red 37 (λ_{max} 513) by *E. innesii CV10 12 h* (\blacksquare); 24 h, (\blacksquare) and 48 h (\blacksquare).

Effects of N source

The capability of *E. innesii* CV-10 to decolorize Direct Red 37 using glucose as a carbon source, along with various nitrogen sources, is illustrated in Figure 8. *E. innesii* CV-10, when cultivated on these media, achieved a decolorization rate of 8 to 11% within the first 12 hours across all nitrogen sources. After 24 hours, the decolorization increased to 20 to 28% of the initial dye concentration. Ammonium sulfate emerged as the most effective nitrogen source, facilitating a remarkable 98% decolorization after 48 hours. Following ammonium sulfate, ammonium chloride, glycine, and ammonium nitrate were identified as effective nitrogen sources, resulting in decolorization rates of 67, 64, and 60%, respectively. For the other nitrogen sources evaluated, the decolorization ranged from 28 to 48%.

Effects of metals

The effects of various metals on the decolorization of Direct red 37 were tested using static cultures of *E. innesii* CV-10. These metals included barium sulfate, ferrous sulfate, zinc sulfate, lead, magnesium sulfate, manganese chloride, copper sulfate, cobalt chloride, stannous chloride, aluminum, and mercury chloride.

At a concentration of 0.025 g.l-1, aluminum chloride achieved the highest level of decolorization at 82%. Following this, stannous chloride lead barium sulfate and manganese chloride demonstrated significant decolorization rates of 76,72,68, and 68%, respectively. At 62 and 55%, respectively, ferrous sulfate and magnesium sulfate produced considerable decolorization. Cobalt chloride, copper sulfate, zinc sulfate, and mercury chloride showed the lowest rates of decolorization (10, 12, 9, and 0.3%, respectively), as shown in Figure 9.

The findings indicated that the presence of aluminum and mercury chloride produced the highest (77%) and lowest (0.7%) decolorization at 0.05 g.l-1 metal concentrations, respectively. Stannous chloride (65%), lead (68%), barium sulfate (72%), manganese chloride (74%), magnesium sulfate (57%), and ferrous sulfate (42%), were the metals that caused the decolorization when other metals were present. Decolorization rates were 7, 8, and 9% for cobalt chloride, zinc sulfate, and copper sulfate, respectively (Figure 10).

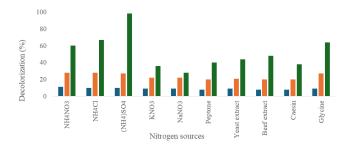


Figure 8: Effects of various nitrogen source sources on decolorization of Direct Red 37 (λ_{max} 513) by *E. innesii CV10 12 h* (\blacksquare); 24 h, (\blacksquare) and 48 h (\blacksquare).

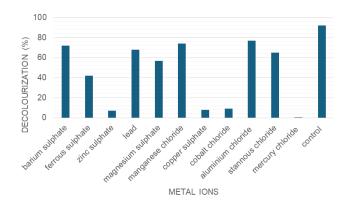


Figure 9: Effects of various metals (0.05 g.l-1) on decolorization of Direct Red 37 (λ_{max} 513) by *E. innesii CV10*

The enhancement of decolorization activity in the presence of specific metals may suggest the participation of metalloproteins. However, in certain instances, such as with aluminum and manganese sulfate, an increase in metal concentration correlates with a decrease in decolorization activity. This reduction in decolorizing capability has been linked to the suppression of growth.

Decolorization versus COD removal

With a starting concentration of 11088 ppm of 100 mg.l-1 Direct Red 37, the COD load in the reaction flasks was 11088 ppm. The decrease in COD when treated with *E. innesii* CV-10 is shown in Figure 10.

The initial COD load of the medium containing Direct Red 37 was 11088 ppm. Following 12 hours of incubation, the application of *E. innesii* CV-10 resulted in a 25% decolorization of the dye and a reduction of 9092 ppm. At the end of the first day, the COD had dropped to 5654 ppm, with 47% dye decolorization; after another day, it had dropped to 2221 ppm, with 80% Direct Red 37 decolorization, marking a total drop in COD.

Biodegradation of dye evidenced by Rothera's ring fission reaction

In order to determine if the organisms that facilitate ring opening activate the critical enzyme system, Rothera's

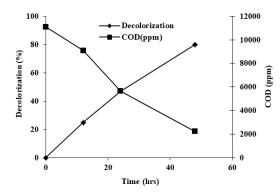


Figure 10: COD reduction during decolorization of Direct red 37 by *E. innesii CV-10*

Table 1: Ring cleavage reaction of various textile dyes by *E. innesii CV-10*

Textile dyes	Ring cleavage	
	ortho	meta
Direct Red 37	-	+

ring fission reaction was performed utilizing direct red 37 textile dyes (see Table 1). The generation of β -ketoadipate (ortho-cleavage) and the yellow molecule (α -hydroxy malonic semialdehyde) (meta-ring cleavage) from catechol were assessed in order to ascertain the meta- or ortho-ring fission methods¹².

The findings demonstrated that all chosen dyes underwent meta-cleavage, while none exhibited a positive reaction for ortho cleavage. Typically, bacteria employ extradiol (meta) or intra-diol (ortho) ring fission to break down the aromatic nucleus. In general, extradiol (meta) ring fission is associated with plasmid encoding (Holloway, B.W. & A.F. Morgan, 1986), whereas intra-diol (ortho) ring fission is linked to chromosomal encoding (Harayama, S., Rekik, M., Wasserfallen, A. & A. Bairoch, 1987).

Controls, which involved growing without dye in the medium, did not exhibit any color change in the assay mixtures. Based on these results, it appears that the aromatic ring breakage enzyme system is dye-dependent.

Decolorization, a positive Rothera reaction, and a decrease in the dyes' total COD are all outcomes that strongly suggest the mineralization and assimilation of the dyes.

Conclusion

The study reveals that *E. innesii* CV10 is highly effective for decolorizing dyes, with an optimal inoculum concentration of 1% and an 18-hour culture. The optimal temperature for decolorization is 37°C, and the ideal pH level is 8. Tween-80 enhances the process. Higher dye concentrations reduce decolorization efficiency. CV-10 effectively decolorizes Direct Red 37 under static culture conditions. Various carbon sources, including glucose, mannitol, and fructose, are effective. Ammonium sulfate is the most effective nitrogen source. Metals like aluminum and other metals influence *E. innesii* CV-10's decolorization activity. The highest COD reduction coincides with the peak decolorization rate.

The textile-processing industry produces a variety of dye-laden wastewater, which presents challenges in treating it due to pH, salt concentrations, and chemical structures. *E. innesii* CV10, an isolate, shows remarkable efficiency in dye decolorization under various conditions, including inoculum ages, sizes, temperature, and pH. It can decolorize high dye concentrations, making it suitable for large volumes of textile waste. The research also shows significant degradation of

dyes, assimilation, and mineralization. The isolate, *E. innesii* CV-10, effectively decolorizes Direct Red 37 and other textile dyes under optimal conditions and metals. This suggests the potential of microbial decolorizing systems for treating dye-laden wastewater.

References

- Andrew D.E., Lenore, S.C. & E.G. Arnold. 2005. Standard Methods for the Examination of Water and Wastewater. 5thedi. centennial edition by APHA, AWWA and WEF. 5.15-5.17.
- Bradford, M.M. 1978. A rapid and sensitive method for the quantification of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
- Carliell, C. M., Barclay, S.J., Naidoo, N., Buckley, C. A, Mulholland, D. A. & E. Senior. 1995. Microbial decolourisation of a reactive azo dye under anaerobic conditions. *Water SA*. 21:61-69.
- Chang, J.C. & Y.C. Lin. 2001. Decolorization kinetics of recombinant *E.coli* strain harboring azo dye decolorization determinants for *Rhodococcus* sp. *Biotechnol. Lett.* 23: 631-636.
- Harayama, S., Rekik, M., Wasserfallen, A. & A. Bairoch. 1987. Evolutionary relationships between catabolic pathways for aromatics: Conservation of gene order and nucleotide sequences of catachol oxidation genes of pWWO and NAH7 plasmid. *Mol. Gen. Genet.* 210: 340-343.
- Holloway, B.W. & A.F. Morgan. 1986. Genome organization in *Pseudomonas. Ann. Rev. Microbiol.* 40: 79.
- Ministry of Environment & Forests 2003. Hazardous Wastes (Management and Handling) Amendment Rules 2003, New Delhi, India.
- Nortemann, B.J, Baumgarten, H., Rast, G. & H. Knackmuss. 1986. Bacterial communities degrading amino and hydroxy naphthalene-2 sulfonates. *Appl. Environ. Microbiol.* 52:1195-1202
- Pearce, C.I., Lioyd, J.R. & J.T. Guthrie. 2003. The removal of color from textile wastewater using whole bacterial cells: a review. *Dyes & Pigments*. 58: 179-196.
- Peralta-Zamora, P. Kunz, A, de Moraes, S. G., Pelegrini, R, Reyes, J., & N. Duran. 1999. Degradation of reactive dyes: a comparative study of ozonation, enzymatic and photochemical processes. *Chemosphere*. 38:835-852.
- Sani, R. & U. Banerjee. 1999. Decolorization of triphenylmethanes dyes and textile and dyestuff effluent by Kurthia sp. Enz Microbial Technol. 24: 433-437.
- Stanier, R.Y., Palleroni, N.J., & M. Doudoroff. 1966. The aerobic *Pseudomonas:* A taxonomic study. *J. Gen. Microbiol.* 43:159-271.
- Stanier, R.Y., Palleroni, N.J., & M. Doudoroff. 1966. The aerobic *Pseudomonas:* A taxonomic study. *J. Gen. Microbiol.* 43:159-271
- Verma, P. & D. Madamwar. 2002. Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescens*. *World J. Microbiol. Biotechnol*. 1: 393-396.
- Wong, Y. & J. Yu. 1999. Laccase-catalyzed decolorization of synthetic dyes. Water Res. 33: 3512-3520.
- Zollinger, H. 1987. In: Color Chemistry-Synthesis, Properties and Applications of Organic Dyes and Pigments. VCH Publishers, New York. 92-102.