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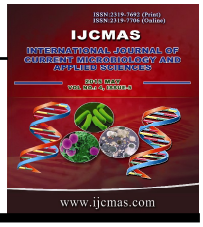

Original Research Article

Decolorization of Acid Blue 25 dye by individual and mixed bacterial consortium isolated from textile effluents

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A B S T R A C T

The dye decolorizing bacterial isolates *Bacillus sp1*, *Bacillus sp2*, *Acinetobacter sp.*, *Citrobacter sp.* and *klebsiella sp.* were isolated from the textile effluents. Different parameters such as carbon source, nitrogen source, temperature, pH and dye concentrations were optimized for decolorization of acid blue 25 dye by using selected bacterial isolates. The aim of the study was the decolorization of acid blue 25 dye showed maximum 70% decolorization under optimum conditions. Among all isolates the *klebsiella sp.* was found to be most efficient in dye decolorization. All the selected isolates showed maximum decolorization in static condition compared to shaking condition. The optimum pH obtained for decolorization of acid blue 25 by bacterial isolates was 7-8 but *Citrobacter sp*. showed maximum decolorization at optimum pH of 6-7 at 37^0C and in *Citrobacter sp.* it was at 25^0C. Bacterial consortium i.e. combination of five bacterial isolates showed highest decolourization potential activity when compared to the individual isolates at different parameters. Maximum decolorization potential was observed in the presence of glucose as carbon source and yeast extract as a nitrogen source.

K e y w o r d s

Anthraquinone dye, Bio-decolorization, Bacterial isolates, Acid blue dye 25, static condition

Introduction

Synthetic dyes have variety of colors and a high stability to light, temperature and microbial attack. Furthermore, some synthetic dyes such as Anthraquinone dyes are carcinogenic or mutagenic (Spadaro et al., 1992). (Poonam Dayaram et al., 2008,) reported that the synthetic and reactive dyes showed decolorization of textile effluents of dye contaminated soil.

Synthetic dyes are widely used in the textile industries and their effluents are discharging. Insufficient treatment of dyestuff wastes of the industries leads to contamination of soil and natural water bodies. Physical-chemical treatment processes have disadvantages where the contaminant is not destroyed, while biological treatment methods are cheap and offers the best alternative with proper analysis and control the environmental pollution (Banat et al., 1996). One promising strategy is the use of microbes that possess the ability to decolourize

synthetic dyes. Microbial decolourization and degradation is an environment friendly, cost-competitive and alternative to physico-chemical decomposition processes for the treatment of industrial effluents (Verma and Madamwar, 2003). Many bacterial and fungal species have the ability to absorb or degrade the textile dyes. Bacterial decolourization of Anthraquinone dyes is either aerobic or anaerobic (Pandey et al., 2007). Nature offers wide microbial community for decolorizing the textile dyes, anthraquinone dye decolourizing bacteria can be isolated from soil and effluent water, however their potential ecological niches were targeted.

Anthraquinone dyes represent the second largest class of textile dyes extensively used in the industries due to their wide array of color shades. They are resistant to degradation due to their fused aromatic structure which remains for long time causing considerable concern to the environmental pollution and also dye retention of the soils or effluents. Bioremediation techniques have gained much attention, microbial decolorization and degradation is cost- effective and eco-friendly compared to different conventional methods. Some potential bacteria have the ability to decolorize synthetic / commercial dyes used for textile dyeing. Effluents from textile and dyeing industries cause serious pollution to the environment.

In the present study, we record to isolate potential dye degrading bacteria and the development of bacterial consortium from individual isolates. We tried with different combinations by mixing of five bacterial isolates (*Bacillus sp.*1, *Bacillus sp*.2, *Acinetobacter sp., Citrobacter sp*. and *Klebsiella sp*.) four bacterial isolates (*Bacillus sp.*1, *Bacillus sp*.2, *Acinetobacter sp., Citrobacter sp*.) and three bacterial isolates (*Bacillus sp.*1, *Acinetobacter sp., Citrobacter sp*.). But combination of five bacterial isolates showed highest decolourization capability when compared to other combinations like four and three bacterial isolates.

Materials and Methods

Sample collection

Waste water along with soil samples contaminated with textile effluents and dyeing industry were collected from the discharges of Siera Silk Mills from Bangalore. The water samples were collected sterilized screw cap bottles and soil samples were collected by polythene bags.

Dye

Acid blue 25 an Anthraquinone based dye supplied by sigma Aldrich chemicals limited, water soluble and widely used in textile industries. Its maximum absorption was 600-630 nm with the following chemical structure.

Medium

The Bacterial cultures grown at 37^0C in the basal medium viz Zhou and Zimmermann medium (ZZ) containing the following in g/l, Glucose 0.5%,Yeast extract 0.5%, $(NH_4)_2SO_4$- 0.5g/l, KH_2PO_4 - 2.66g/l, Na_2HPO_4 - 4.32 g/l, dye - 100 mg/l.

Isolation and Screening of potential strains

For isolation the textile effluents were collected as sources of microorganisms Cultures were obtained through serial dilution by pour plate method. Isolated colonies were then obtained was preserved

for the further use. Fresh culture was then inoculated in to nutrient broth and incubated for 24 hrs at 37^0C.In order to screen potential strain 2% 24 hrs fresh inoculums was transferred in to the 100 ml of ZZ medium containing dye and incubated at 37^0C.Potential strains utilizing dye as a nutrient source which were placed on ZZ agar plates and incubated. From these enriched cultures isolated colonies were repeatedly & subsequently inoculated on nutrient agar to obtain potential cultures. Therefore strains that showed high decolourization potential were chosen to be for decolorization and degradation studies at different concentration, pH temperature & other nutrient factors etc..

Screening and identification of dye degrading bacterial isolates

The organisms isolated on nutrient agar was further sub cultured on modified ZZ medium containing 100mg of acid blue 25 dye and incubated at 37^oC under static conditions. After six days of incubation the decolourization potential was calculated. The bacterial isolates utilizing the dyes as a source of nutrient, showing high efficiency of decolourization were selected for the study.

Decolorization was studied on various carbon sources such as (Glucose, Fructose, Maltose, Sucrose & Mannitol), Nitrogen sources (Yeast extract, Beef extract & Peptone), different dye concentration (100-600mg/l) pH values (5, 6, 7,8and 9) and temperature (25^0,37^0,45^0C).Growth was monitored by using spectrophotometrically.

After decolorization, the decolourized medium was centrifuged at 10000 rpm for 20 minutes to separate the cell lysate and supernatant. Decolourization potential was determined by measuring the absorbance of culture supernatants at 600 nm using UV-Vis spectrophotometer. Percentage of decolourization calculated by the following formula:

$D = [D_0\text{-}D_1/D_0] \times 100$

Where, D=Decolorization in percentage, D_0=Initial absorbance, D_1=Final absorbance.

Results and Discussion

Total Fourteen bacterial cultures were isolated, identified and screened for the decolorization degradation of Anthraquinone dyes from textile effluent. All the strains tested were further selected & screened for dye degradation on morphologically and biochemically characterized for identification. Based on biochemical test they were identified as *Bacillus sp.*1, *Bacillus sp*.2, *Acinetobacter sp., Citrobacter sp*. and *Klebsiella sp*. were selected for Acid blue 25 dye degradation. Dye degradation experiments were tested on selective medium i.e. ZZ medium and decolorization potential was observed by five bacterial isolates individually and by the development of bacterial consortium (Nachiyar et al .2012 & Maulin p.Shah 2014). Bacterial consortium with combinations of five bacterial isolates (*Bacillus sp.*1, *Bacillus sp*.2, *Acinetobacter sp., Citrobacter sp*. and *Klebsiella sp*.), four bacterial isolates (*Bacillus sp.*1, *Bacillus sp*.2, *Acinetobacter sp., and Citrobacter sp*.) and three bacterial isolates (*Bacillus sp.*1, *Acinetobacter sp., and Citrobacter sp*.) were tested and the percentage was recorded (Table 1). Among three combinations the most potential decolourization activity was observed. With combination of five bacterial isolates (Bacterial consortium) showed high decolourization percentage and further study was carried.

Effect of carbon source on dye decolorization

Optimization of dye decolorization with five bacterial isolates was analysed by the incorporation of Glucose, Sucrose, Fructose, Maltose, Mannitol) in Zimmermann's medium. Decolorization potential of five bacterial isolates tested has been enhanced in which showed maximum percentage of decolorization by adding glucose content to the medium likewise showed decolorization potential of *Acineto bacter sp.* 65%, *Bacillus sp.1* 55%, *Bacillus sp.2* 18.9%, *Citrobacter sp.* 23.5% respectively (Fig 1) Supplementing the glucose component as a carbon source gave the highest decolorization rate when compared to other carbon sources. Glucose alone showed 85% decolorization but in combination of nitrogen source it showed 95% decolorization. The combination of five bacterial isolates (Bacterial consortium) showed the decolourization activity of Acid blue 25 in presence of content alone was about 89%, Mannitol was about 76.34%, Sucrose 79.77%, Maltose 78.64% and fructose 81.58% under static conditions. Addition of glucose content revealed that all the strains could utilize the dyes with high % of decolourization in which it has helped in the bacterial growth rate and consequently increases the percentage of decolorization rate. (Mohan et al.2012)

Effect of nitrogen source on dye decolorization

Acid blue 25 an Anthraquinone based dye was studied for decolorization activity in liquid medium supplemented separately with nitrogen sources viz. Yeast extract, Beef extract and Peptone. In the presence of yeast extract as a nitrogen source showed maximum decolorization in all bacterial isolates. *Klebsiella sp.* showed 81% of decolorization in the presence of yeast extract. In combination of five bacterial isolates showed the decolorization activity of Acid blue 25 with yeast extract was about 86%, beef extract 56% and peptone 59% under static conditions. Among all strains investigated maximum percentage of decolorization showed *klebsiella sp.*The bacterial consortium was found to degrade 86% of the acid blue dye in the presence of yeast extract, 56.27% of Beef extract and 59.92% of peptone (Fig 2).

Effect of pH on dye decolourization

The hydrogen ion concentration showed profound effect on the biological activities of the organisms. The effect of pH on dye decolourization was resulted that increase of pH at 5 to 9 and maximum percentage decolourization was noticed at pH 8 and decrease in decolourization was observed at pH 5.High efficacy of decolourization was observed at 7 to 8 pH within 48 hrs of incubation. *Bacillus sp.1, Citrobacter sp., Acinetobacter sp.* isolated from textile effluents exhibit decolorization 74%, 67% and 75% at pH 7.*klebsiella sp* showed decolorization 92% at pH 8.By bacterial consortium (1,2,3,4 & 5) the range of decolorization activity was showed 96% at pH 8 with in 48 hrs under static conditions respectively.(Fig 3)

Effect of Temperature on dye decolourization

In order to optimize the physical factors conducted with different temperatures and it is the most important parameter for decolorization & degradation process. Decolourization activity of bacterial culture was found increased with increase of incubation temperature from 25^0C to 45^0 C and maximum activity was observed at 37˚C and decrease in decolourization potential

was recorded with increase in temperature. *Klebsiella sp.* showed highest decolorization potential activity 92% of Acid blue 25 at 37^0C.*Citrobacter sp.* showed decolorization rate (80%) at 25^0C among all isolates. Bacterial consortium i.e. in combination of five bacterial isolates (1, 2, 3, 4 &5) showed the maximum decolorization of acid blue 25 (96%) at 37^0C, 88% at 25^0C and 29% at 45^0C under static conditions. (Fig 4)

Effect of dye concentration on decolourization

Decolorization rate was increased with increase in initial concentration of dye. Optimizations studies carried out by using different concentrations of dye i.e. 100 mg to 500 mg/l, the rate of decolorization of all five bacterial isolates showed maximum decolorization at initial concentration of 200mg/l of acid blue 25 dye. If further dye concentration increases resulted decrease in decolourization activity. Decolourization activity of bacterial consortium was tabulated with Acid blue 25 at different concentrations varying from 100 mg to 500 mg (Fig.5). Maximum decolourization was observed up to (50-300mg/l). Similar results were mentioned by (Khalid et al 2008) dye concentration can influence the efficiency of decolorization through combination of factors including the toxicity imposed by dye (Saranraj et al 2011) at higher concentration.

Effect of Static and Shaking condition on decolourization

The results indicate that decolorization was not dependent on biomass concentration and not correlated with the dissolved oxygen levels. Maximum decolorization was observed under static conditions by *klebsiella sp., Citrobacter sp., Acinetobacter sp., and Bacillus sps.* 1&2 when compared to shaking conditions. In combination of five bacterial isolates, maximum decolorization 96%was observed under static condition with in 48 hrs of incubation (Fig 6). Similar results reported by (Madamwar et al.2005) under static conditions decolourization was high and in agitation decolourization was neglizable.

Table.1 % of dye decolorization by bacterial isolates and mixed culture from textile effluent

Bacterial isolates	Dye Decolorization
Bacillus sp.1	74.17%
*Bacillus sp.*2	58.3%
Acinetobacter sp.	75.58%
Citrobacter sp.	61.44%
Klebsiella sp.	90.13
Bacterial consortium *(Bacillus sp.1, Bacillus sp.2, Acinetobacter sp., Citrobacter sp., Klebsiella sp.)*	98%
Bacterial consortium *(Bacillus sp.1, Bacillus sp.2, Acinetobacter sp., Citrobacter sp.,*	77.49%
Bacterial consortium *(Bacillus sp.1, Acinetobacter sp., Citrobacter sp.)*	78.92%

Figure.1 Dye decolorization at different carbon sources by individual isolates and bacterial consortium

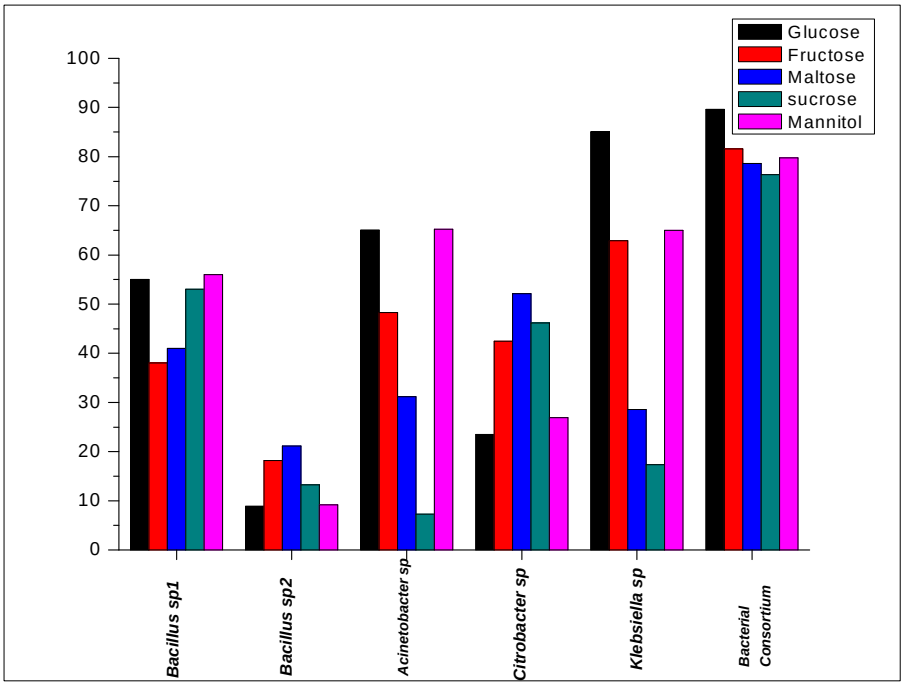


Figure.2 Dye decolorization at different Nitrogen sources by individual isolates and bacterial consortium

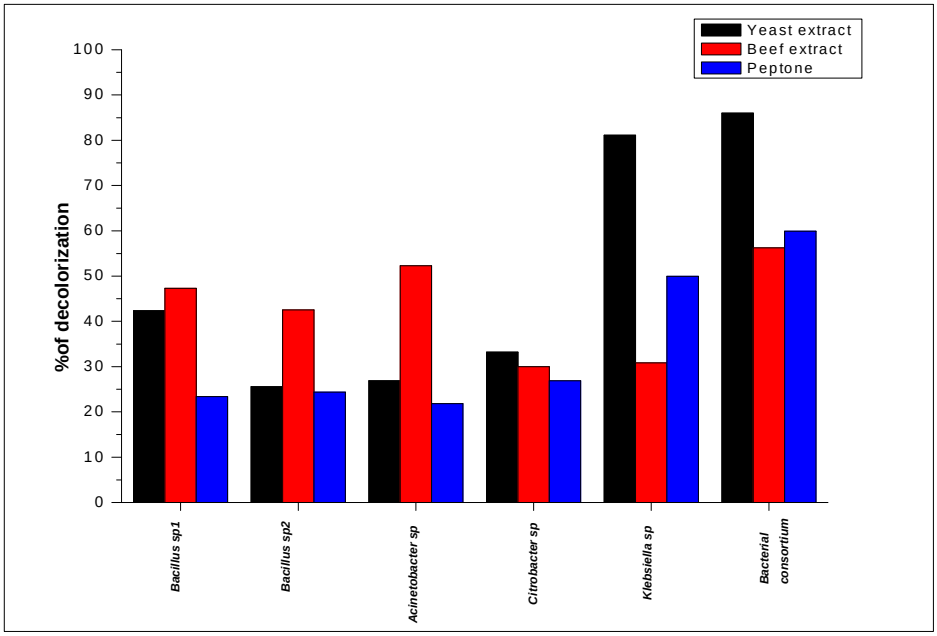


Figure.3 Dye decolorization at different pH by individual isolates and bacterial consortium

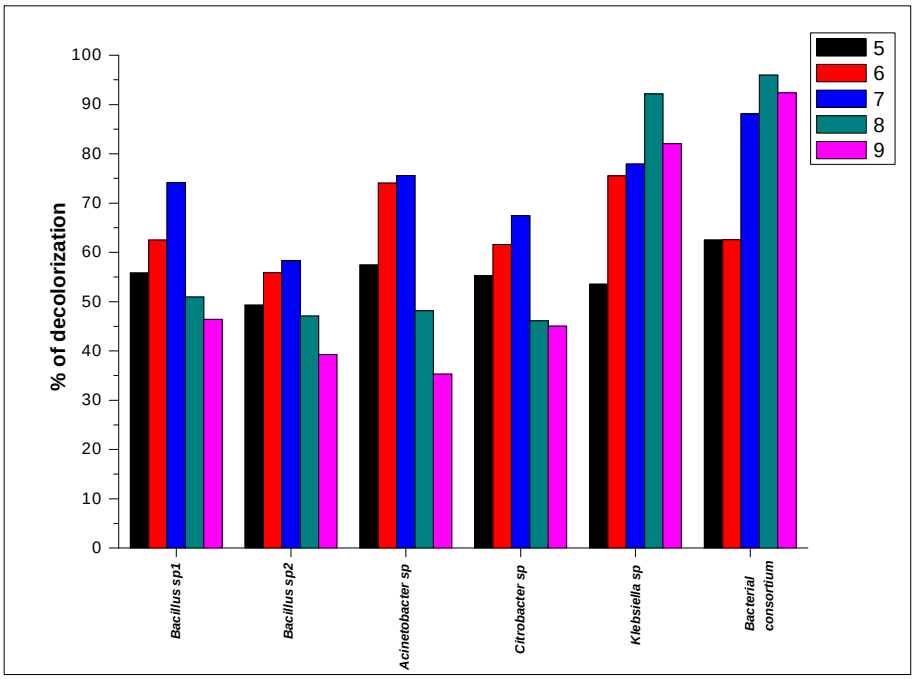


Figure.4 Dye decolorization at different temperatures by individual isolates and bacterial consortium

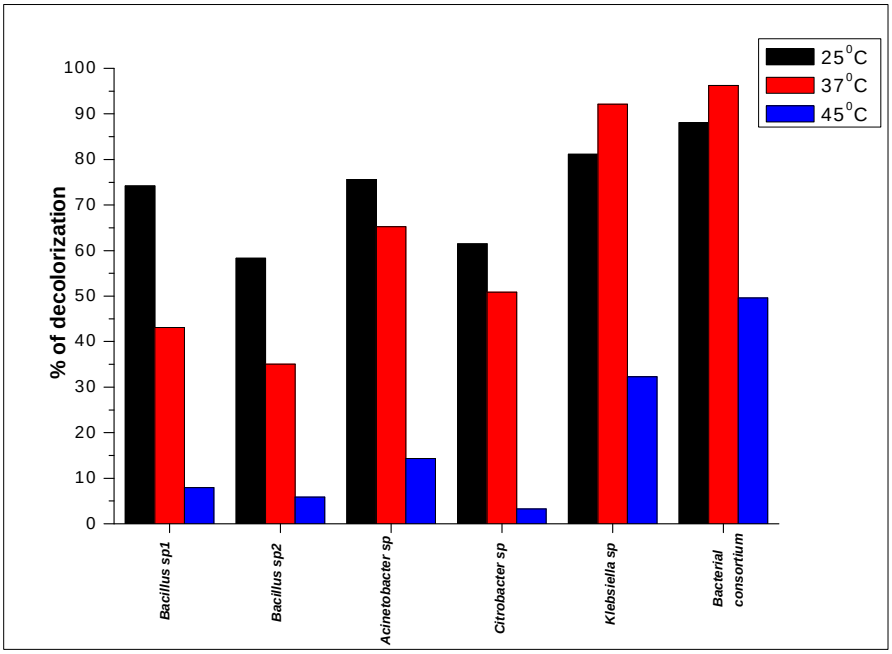


Figure.5 Dye decolorization at different concentrations by individual isolates and bacterial consortium

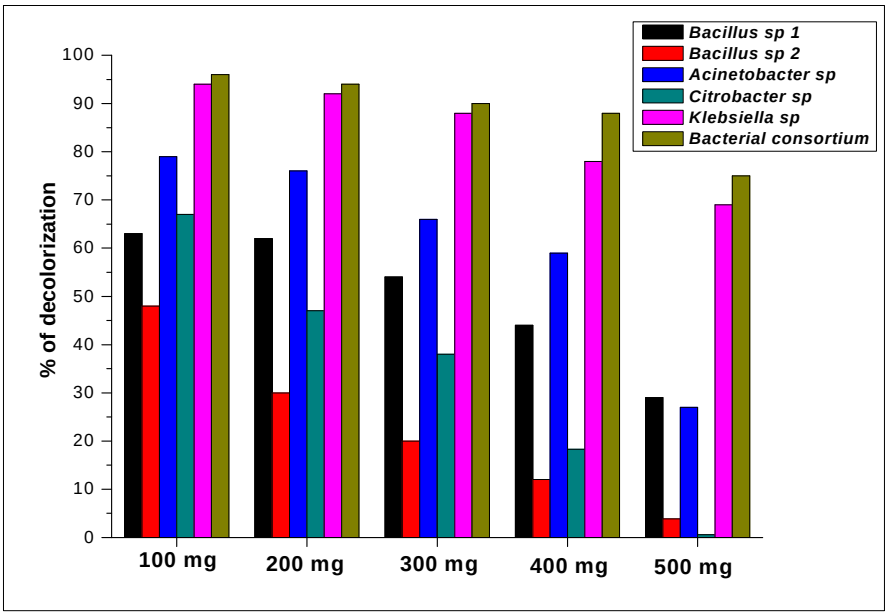
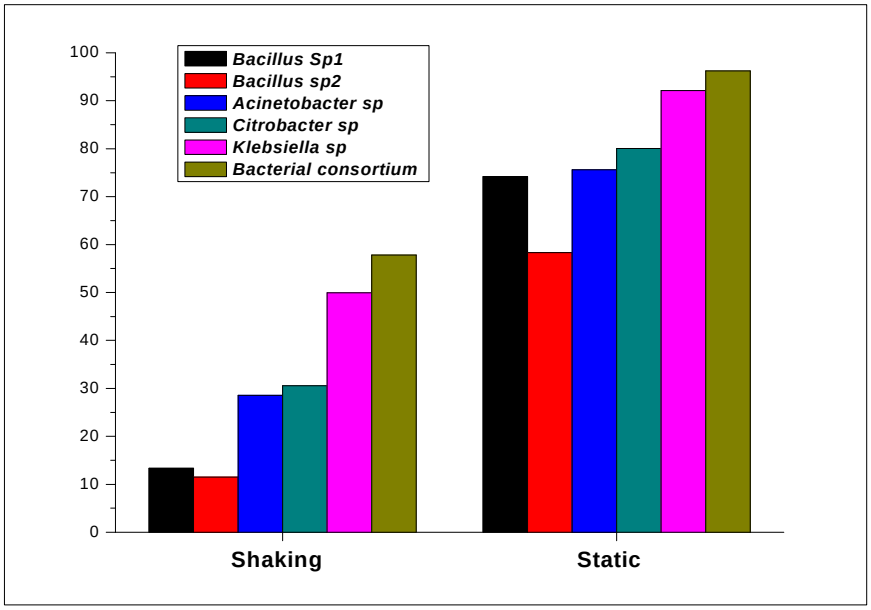


Figure.6 Dye decolorization under agitation and static conditions by individual isolates and bacterial consortium



The present study mainly focused on textile effluent and sludge produced by efficient treatment of plant is rich source of decolourization of dye by bacterial isolates. Among the bacterial sps isolated from textile effluents such as *Citrobacter sp., Acinetobacter sp., Bacillus sp. 1& Bacillus sp. 2.Klebsiella sp.* showing 70-90% of decolorization of Acid blue 25 at maximum pH 8, temperature at 37^0C, dye concentration 300 mg, glucose and nitrogen concentration 0.5%. In the same lines the specific combinations of bacterial consortium tested for decolorization showed maximum percentage of decolourization 96%.These indigenous bacterial strains could be utilized for treatment of dye present in waste water with high degrading and decolourizing activity against various reactive dyes commonly used in textile industries. In comparison with individuals consortium works more effective in terms of decolorization and degradation of acid blue 25.It is proposed that these bacterial sps has practical potential application in the bioremediation & biodegradation of various dye effluents.

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