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DECOLORIZATION OF REACTIVE ORANGE – 16 BY *SERRATIA MARCESCENS*

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ABSTRACT:

In this study *Serratia marcescens* was used to decolorize reactive orange – 16. *Serratia marcescens* could decolorize reactive orange – 16 up to 92.52% within 10 days at pH – 7.4 - 8, temp. 26 ˚C - 30 ˚C, inoculum size 2ml in static condition. Glucose is suitable carbon source for dye decolourization. Nitrogen sources and Metal compounds had inhibitory effect on decolourization of reactive orange – 16.

KEY WORD: *Decolorization, Reactive orange – 16, Serratia marcescens.*

INTRODUCTION:

Textile processing operations are considered an important part of the industrial sector in both developing and undeveloped countries. However the textile industry is one of the most complex manufacturing industries. Textile industry is most water polluting industry in world wide. Most of the textile manufacturers are located on the river banks and coastal areas because of low cost transportation and available source of water. They also release waste water in river. The waste water generated from the textile processing industries contains high amounts of suspended soils, dissolved solids, un-reacted dye stuffs (colour) and other auxiliary chemicals that are used in the various stages of dyeing and other processes.

The textile industry produces large volumes of wastewater in their dyeing and finishing processes. Synthetic dyes and pigments are extensively used in the textile industries. So, synthetic dyes are release in textile effluent. Dye effluents are among the major pollutants discharged into the environment. Coloured wastewater from textile industry is rated as the most polluted in all industrial sectors. The colour of synthetic dyes in effluent is one of the most obvious indicators of water pollution. The discharge of highly coloured effluents containing dyes can be damaging to the receiving bodies [Nigam P, Banat I.M, Singh D, Marchant R, (1996b)] and can result in serious environmental pollution problems. These effluents have common characteristics as their high colouration since a small amount of residual dye (of the order of mg l/1) can be sufficient to cause a significant visual effect and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies. While coloured organic compounds generally impart only a minor fraction of the organic load to wastewater, their colour renders them aesthetically unacceptable. Synthetic textile dyes belong to the most dangerous pollutants which, as part of the industrial effluents, contaminate steadily higher amounts of waste water. Presence of colour and its causative compounds has always been undesirable in water used for either industrial or domestic needs. Colour is a visible pollutant. Common man may not object to the discharge of colourless effluents laden with toxic and hazardous pollutants. On the other hand the discharge of coloured effluents, though less toxic, are often objected by the public on the assumption that colour is an indicator of pollution. It is therefore, not surprising to note that colour in wastewater.

Different methods are available for the remediation of dye wastewaters. These include physicochemical methods, like adsorption, chemical oxidation, precipitation, coagulation, filtration, electrolysis, photo degradation, and biological, and microbiological methods. Adsorption on activated carbon is an effective method for the removal of colour, but it is too expensive (Fu and Viraraghavan, 2001). The major disadvantage of physicochemical methods has been largely due to the high cost, low efficiency, limited versatility, interference by other wastewater constituents, and the handling of the 252 Water Air Soil Pollut (2010) waste generated (van der Zee and Villaverde, 2005; Kaushik and Malik, 2009). Traditional wastewater treatment technologies have proven to be markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of these pollutants (Forgacs *et al*; 2004). The development of efficient and environmentally friendly technologies to decrease dye content in wastewater to acceptable levels at affordable cost is of utmost importance (Couto, 2009). Biological methods are generally considered environmentally friendly as they can lead to complete mineralization of organic pollutants at low cost (Pandey *et al*; 2007). It is now known that several microorganisms, including fungi, bacteria, yeasts, and algae, can decolorize and even completely mineralize many azo dyes under certain environmental conditions (Pandey *et al;* 2007). The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages; the process is relatively inexpensive, the running costs are low, and the end products of complete mineralization are not toxic (Forgacs *et al*; 2004). Thus, biodegradation is a promising approach for the remediation of synthetic dyes wastewater because of its cost effectiveness, efficiency, and environmentally friendly nature (Verma and Madamwar 2003; Jirasripongpun *et al;* 2007; Shedbalkar *et al;* 2008; Gopinath *et al*; 2009). As a best alternative, much interest is now focused on biodegradation of dyes (McMullan *et al;* 2001; An *et al;.* 2002). Bioremediation may be the most effective method of treating industrial dyes wastewater (Nozaki *et al;* 2008).

This study deals with biological decolourization of dye by the *Serratia marcescens.* An attempt has been made to optimize conditions for the efficient decolourization and the exploitation of the microorganism for dye removal.

MATERIALS AND METHODS:

Dye:

Reactive orange – 16 (λ_{max} = 494nm), a commercial dye used in textile industries, was provided by Jaysynth Impex Ltd., Ahmedabad, Gujarat. Dye structure is depicted in fig. – 1.

Culture maintenance and inoculum preparation:

Commercial strain of *Serratia marcescens* was used for experiments, maintained on nutrient agar slant. Culture was obtained from Microbiology laboratory of S. M. Panchal Science Collage, Talod, Gujarat.

The flask containing 50ml nutrient broth inoculated with culture of *Serratia marcescens* preserved on NA slant; incubate at 26 ˚C for 24 h, on Rotary shaker. This actively growing, young, fresh culture was used as inoculum for next decolorizing assay.

Decolourization assay:

The decolourization experiments were carried out using spectrophotometric method.(Meiyin Xu *et al;* 2006; Hala *et al;* 2008, Sheth N. T& S. R. Dave, 2009, Jadhav *et al;* 2010). The absorbance spectra of dye before and after decolourization were scanned by SL- 160 double beam UV – Vis spectrophotometer (Elico), at maximum absorbance of each individual dye. All the decolourization experiments were performed in quadruplicate.

Decolourization study of Reactive orange – 16 was carried out in 250ml Erlenmeyer conical flasks containing 100ml of N – broth medium having individual dye (100 mg/l final concentration) were sterilized by autoclave at 15 pound pressure and 121 ˚C (Prescott *et al,* 2002). After autoclaving these three different flasks containing three different dyes were inoculated with 2ml of young fresh inoculum, incubated at 26 ˚C, at pH – 7.4, for 10 days in static condition.

All the studies were carried out with 2ml inoculum, at 26 ˚C, at pH – 7.4, for 10 days in static condition, unless stated otherwise. At regular interval (5, 7 and 10 days) 3ml samples were withdrawn from the flask to monitor decolourization rate, centrifuge in micro centrifuge (RM -12C REMI) at 6000rpm for 15 min. supernatant was analysed for remaining dye content.

The degree of decolourization of the tested dye was measured at its maximum absorbance wavelength (λ_{494} for reactive orange – 16,), with the supernatants using SL- 160 double beam UV- VIS spectrophotometer (Elico). Results were corrected according to blank (dye free) samples. Non inoculated sterile control flasks, which contained only the dye – free N – broth, were incubated at 26˚C, at pH – 7.4, in static condition.

Decolourization rate was expressed as percentage decolourization and calculated using the formula (Jadhav *et al,* 2010) –

$$\%\ \text{decolourization} = \frac{A-B}{A} \times 100$$ (Where, A – initial absorbance, B – observed absorbance)

Above mentioned protocol was followed during the study of effect of dye concentration, effect of static and shaking condition, effect of inoculum size, effect of carbon source, effect of nitrogen source, effect of initial pH, effect of incubation temperature and effect of metal compound.

Effect of inoculum size, dye concentration, incubation condition, initial pH, incubation temperature on dye decolourization:

To determine effect of inoculum size four different inoculum size were studied such as 1ml – 4ml (1ml = approx. 2.5 – 3 × 10^7 cell/ml). 50mg/l – 200mg/l dye concentration were studied, the initial pH range was prepared from 2 – 10 with step size of 2 for each individual set. The effect of temperature was studied during the incubation at 10 ˚C – 60 ˚C. Static verses Shaking condition was also studied.

Effect of Carbon sources, Nitrogen sources, Metal compound on decolourization:

During this study organism was supplemented with additional carbon sources, nitrogen sources and metal compounds along with nutrient broth. Different carbon sources like glucose, sucrose, maltose and dextrose were added as supplement individually to the nutrient broth at 1g/l concentration. Similarly, in the next two sets different nitrogen sources like peptone, yeast extract, urea and ammonium nitrate, different metal compound such as $Mgcl_2$, $Fecl_3$, $Zncl_4$, $Mncl_2$ and $Cocl_2$ were added at a concentration of 1g/l to the medium.

RESULTS AND DISCUSSION:

Decolourization of reactive orange – 16 by *Serratia marcescens*

Decolourization of reactive orange – 16 was observed on dyes 5, 7 and 10, respectively under static condition, at pH 7.4 & temp. – 26 ˚C .After 10 days 92.52% decolourization was obtained.

Effect of inoculum size, dye concentration, incubation condition, initial pH, incubation temperature on dye decolourization:

The percentage decolourization obtained with different inoculum size was different in dye. The percentage decolourization obtained in Reactive Orange – 16 with different inoculum size were 78.67%, 92.38 ± 0.53%. Graph: 1 show percentage decolourization for different inoculum size.

Inoculum size up to 2ml is appropriate because the further rise in inoculum size has no benefit in decolourization. Further increase of 2ml gives only 0.40% to 0.60% higher decolourization. N. T. Sheth and S. R. Dave, (2009) reported 91.1% decolourization of Reactive Red BS. C. I–III by *Pseudomonas aeruginosa.* Guo *et al;* (2008) have also reported maximum azo acid dye removal rate of 5.3 mg dye g $cell^{-1}$ h^{-1}. Further rise in inoculum size show no beneficial effects. Similar observations have been recorded by Moosvi *et al;* (2005).

The decolourization efficiency of Reactive Orange – 16 varies from 94.54% - 54.76% for dye concentration of 50 – 200 mg/l respectively, as shown in Graph: 2.

This study indicates that increase in dye concentration ultimately results in decease in percentage decolourization. Similar studies were reported earlier for Rapid Biodegradation and decolourization of Direct Orange – 39 by isolating bacteria *Pseudomonas aeruginosa* strain BCH could decolorize the dye maximum up to 1500 mg/l with 66% decolourization efficiency and minimum 50 mg/l with 93.06% decolorization efficiency (Jadhav *et al,* 2010). Dhanve *et al,* (2008) also reported that *Exiguobacterium* spp. RD_3 could decolorize Reactive Yellow – 84 A maximum up to 1 g/l with 21.05% decolourization efficiency.

Reactive Orange – 16 shows 92.52% and 78.67% decolourization at static and shaking condition respectively after 10 days. Pradeep Verma and Datta Madamwar (2003) also reported in Decolourization of synthetic dyes by a newly isolated strain of *Serratia marcescens* that decolourization was drastically reduced using agitated conditions.

The Hydrogen ion concentration shows profound effect on the biological activities of the organism. It was found that change in initial pH significantly affect the decolourization rate.

Serratia marcescens was able to decolorize Reactive Orange – 16 at pH 8 up to 90.38 % and at pH 10 up to 90.46% The pH range for decolourization activity for dye was found 7.4 – 10.00. While pH 2 – pH 6 inhibits dye decolorization(Graph: 3). Optimum pH for decolourization by *Serratia marcscens* was 7.4. Pradeep Varma and Datta Madamwar, (2003) reported same observations with *Serratia marcescens.*

Reactive Orange – 16 was decolorized up to 92.52 % at 26 ˚C and up to 90.16% at 30 ˚C(Graph: 4). Optimum temperature for dye decolourization was 26 ˚C.

Effect of Carbon sources, Nitrogen sources, Metal compound on decolourization:

Some carbon source (Glucose, Sucrose, Maltose & Dextrose) were chosen to determine their effects on decolourization of Reactive Orange – 16 by *Serratia marcescens.* The strain was able to grow on all tested carbon sources, but decolourization activities observed among them are different.

The decolourization activity increased after 10 days, static condition with glucose, sucrose, maltose & dextrose in Reactive Orange – 16.

The highest percentage decolourization obtained in Reactive Orange – 16 was 97.04% with additional supplement of glucose (1 g/l) in Nutrient broth (Graph: 5).

These studies shows that additional supplement of carbon source give rise in percentage of decolourization but among them addition of glucose give highest percentage decolourization in Reactive Orange – 16. Here *Serratia marcescens* with carbon source supplements gives increase percentage decolourization in Nutrient broth, but % decolourization by *Pseudomonas aeruginos* and *Pseudomonas* strain BCH were reduced (Jadhav *et al,* 2010, Sheth N. T. & Dave S. R., 2009). *Shewaness decolorationis* SR also reduce percentage decolourization with additional carbon sources (Meiying *et al,* 2006).

Graph: 5 Effect of carbon source on decolourization of Reactive Orange – 16.

To study the effect of various nitrogen sources such as peptone, yeast extract, urea and ammonium nitrate (1g/l) were supplemented individually in Nutrient broth medium.

The percentage decolourization was decreased in Reactive Orange – 16. The highest percentage of decolourization was shown up to 88.68% (Graph: 6). Supplemented individual nitrogen sources were found to be less effective as compared with incorporation of individual carbon sources in Nutrient broth medium.

The decolourization efficiency was found to be decreased up to 10% than that of carbon sources. These observations show that nitrogen sources were inhibiting the decolourization activity of *Serratia marcescens.*

Some metal compound ($MgCl_2$, $FeCl_3$, $ZnCl_2$, $MnCl_2$, $CoCl_2$,) were chosen to determine their effect on decolourization efficiency. These metal compounds (1g/l) were additional supplements in Nutrient broth.

The percentage decolourization decreased in Reactive Orange – 16 with additional supplements of metal compounds. Metal compounds inhibit decolourization efficiency of *Serratia marcescens.*

Comparative study of these all metal compound is presented in graph: 7. Meiying*at el al;* (2006) also found that $HgCl_2$ decrease the decolourization efficiency of *Shewanella decolorationis* S12 for anthraquinone.

CONCLUSION:

The present study confirms that Reactive Orange – 16 was decolorized by *Serratia marcescens.* Optimum pH and incubation temperature for decolourization was 7.4 and 26 ° C respectively. The highest decolourization was shown with additional supplements of carbon source such as glucose, nitrogen sources and metal compounds were inhibiting decolourization activity of *Serratia marcescens.*

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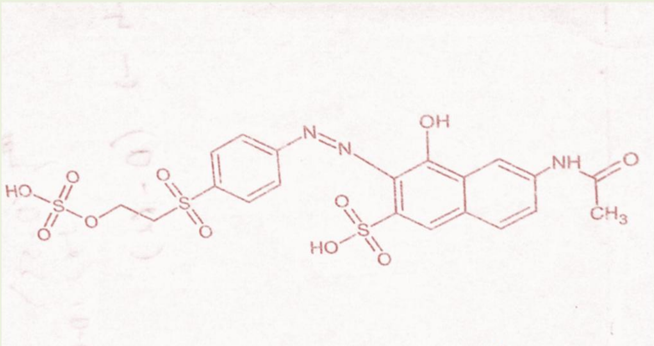
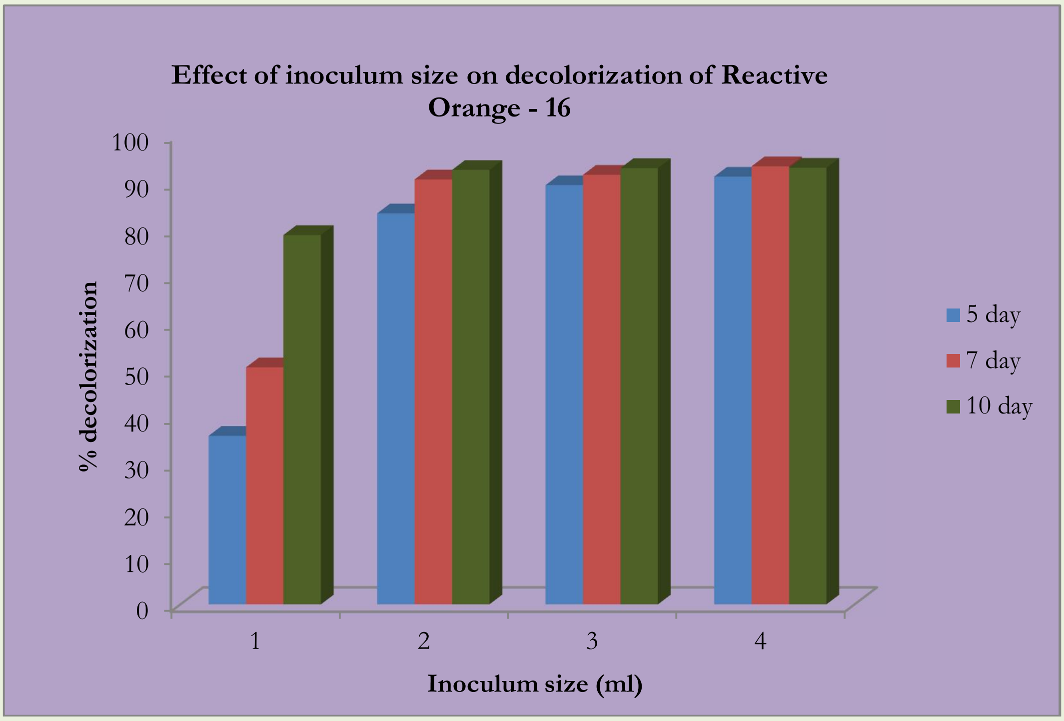
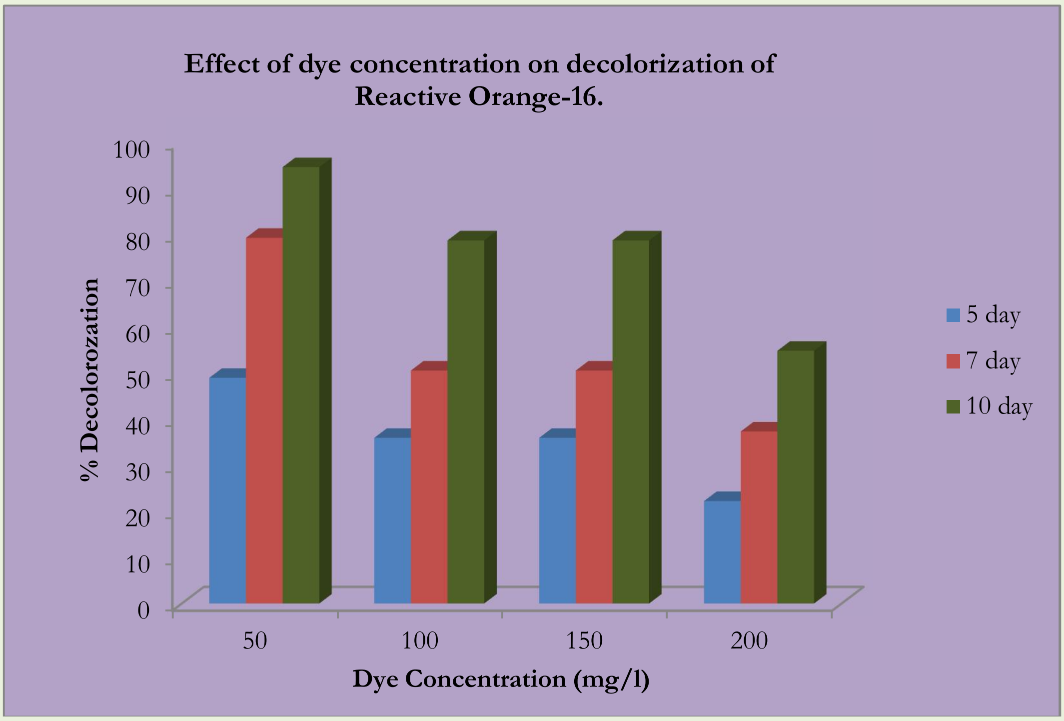


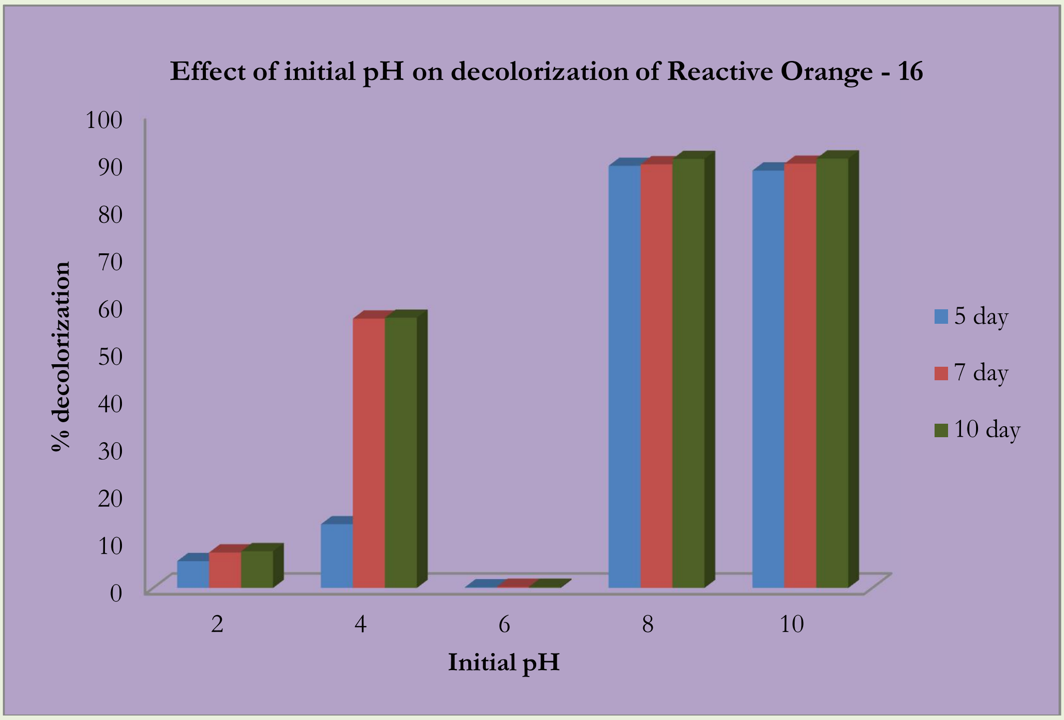
Fig: 1 Chemical structure of Reactive orange – 16



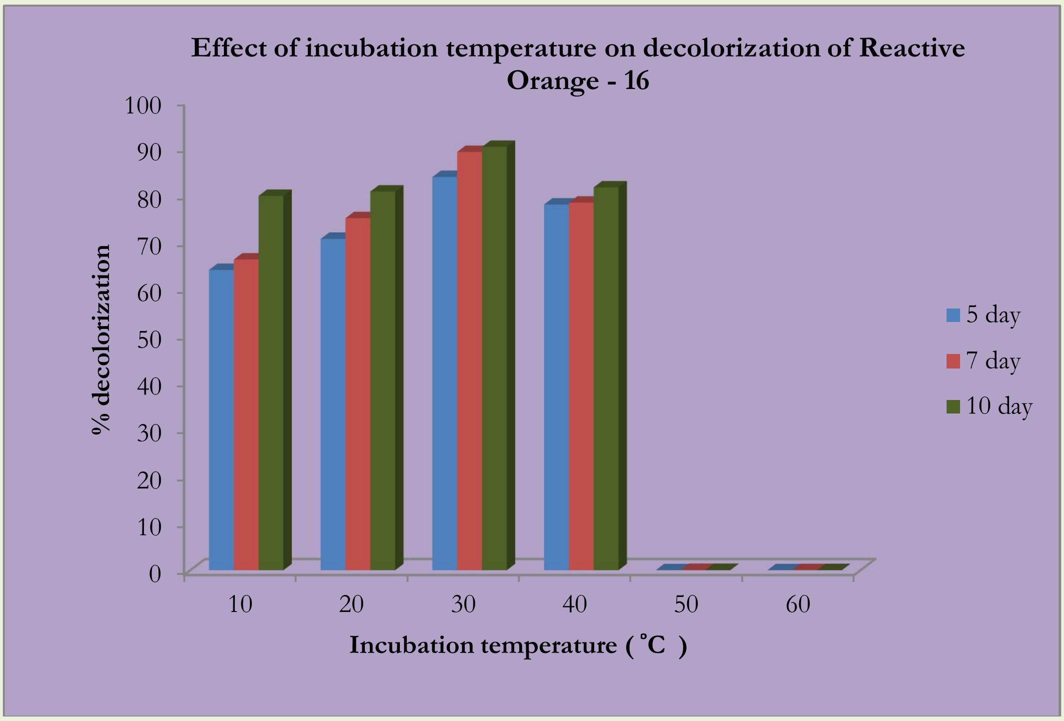
Graph: 1 Effect of inoculum size on decolourization of Reactive Orange – 16



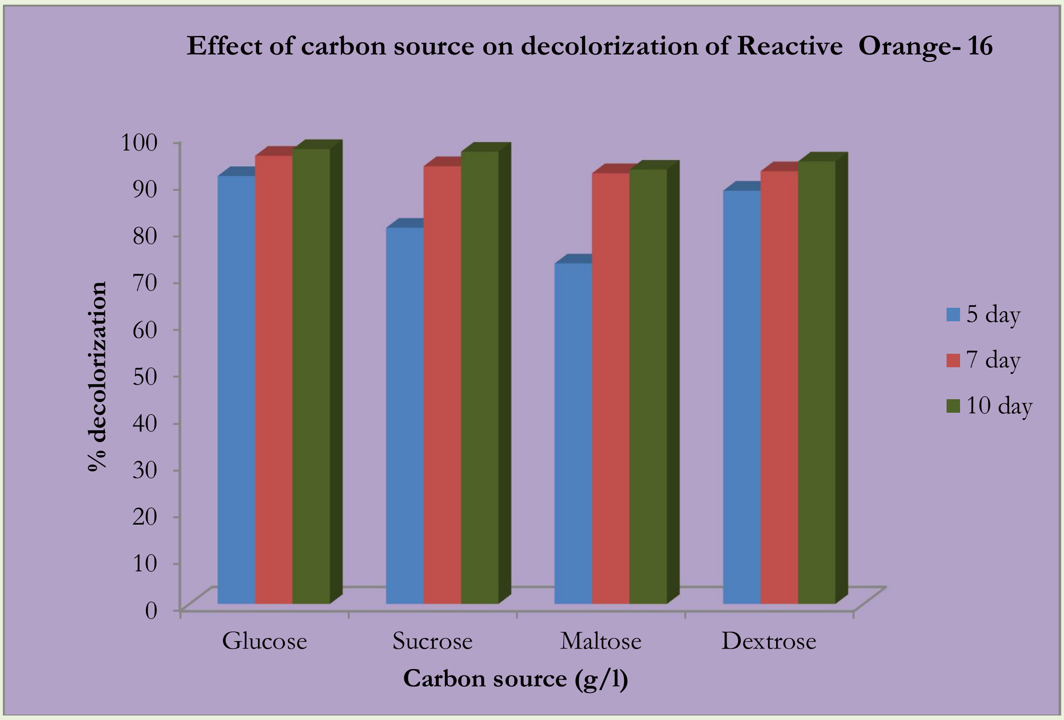
Graph: 2 Effect of dye concentration on decolorization of Reactive Orange-16



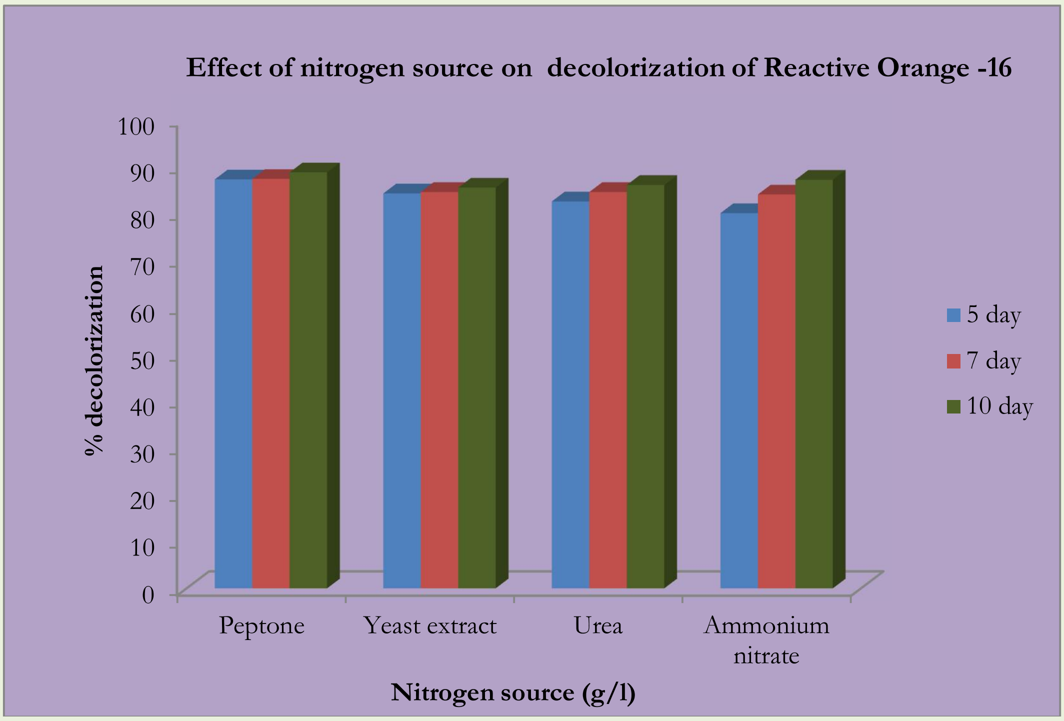
Graph: 3 Effect of initial pH on decolourization of Reactive Orange– 16



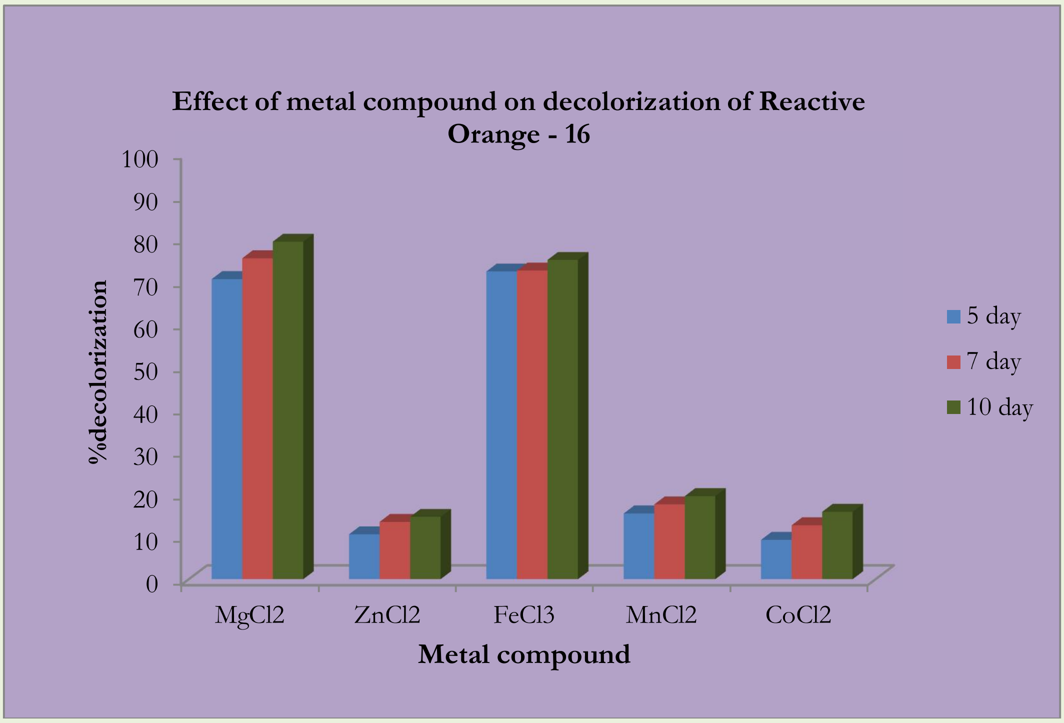
Graph: 4 Effect of incubation temperature on decolourization of Reactive Orange – 16



Graph: 5 Effect of carbon source on decolourization of Reactive Orange – 16



Graph: 6 Effect of nitrogen source on decolourization of Reactive Orange-16



Graph: 7 Effect of metal compound on decolourization of Reactive Orange-16