ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bio decolorization of simulated dye waste water using *Saccharomyces cerevisiae*.

Brindha Kannan[2], Pamela Sinha[1], Sathesh Kumar Annamalai[1], and Kantha Deivi Arunachalam[1]*.

[1]Centre for Environmental Nuclear Research, SRM University, Kattankulathur, Tamil Nadu. 603 203 India
[2]Department of Civil Engineering, Tagore Engineering College, Chennai, Anna University Tamil nadu. 600 025 India.

ABSTRACT

Textiles Industrial effluents became the significant source of water pollution due to its coloring chemicals. Large numbers of dyes used in textile industries are discharged into the environment as effluents. Colored waste water is treated by physical and chemical process however, *Saccharomyces cerevisiae* were used for dye decolorization under simulated conditions. The pH, dye concentrations and microbial cells concentration affect the biodegradation of dyes. Simulated dye water was decolorized using yeast concentrations at 5%, 7%, 10%, 12% and 15% (v/v) and 79.31%, 71.26%, 74.23%, 77.89% and 73.67% degradation was observed. Various dye concentrations on the decolorization at 1ppm, 2ppm, 4ppm and 8ppm was 89.96%, 85.14 %, 77.11% and 68.69% respectively. At optimum pH 7 the degradation was 95.06%. The maximum decolorization at 1ppm and 2 ppm was 89.96% and 85.14% respectively using 5% inoculum. The FTIR analysis of control and samples showed the production of primary and secondary amines and after decolorization showed peaks at 1615 cm− 1 and 1371 cm− 1 indicating synthesis of secondary aliphatic amines and dimethyl groups. These results concluded that S*accharomyces cerevisiae* can be an alternative species for treatment of textile effluents to reduce the environmental impact by their pollutants.

Keywords: Reactive dye, Waste water, *Saccharomyces cerevisiae,* pH and FTIR.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Large numbers of different chemical dyes were used for various industrial applications especially in textile industries, and significant proportion appeared in the form of waste water and spilled into the environment. Dyes are composed of a group of atoms responsible dye color called chromophore or substituents that intensify the color of the chromophores called auxopore. The main chromophores are azo (-N=N-, carboxyl (-C=O), methane (-CH=), nitro (-NO2) and auxochromes are amine (-NH3), carboxyl (-COOH), sulfonate(-SO3H) and hydroxyl (-OH). The auxochromes belong to classes of reactive, azo, direct, basic, pigment, vat, sulfur, solvent and disperse dye [1].

Among all the textile dyes, the reactive dyes are the prominent class of colorants used in tattooing, cosmetics, printing, and consumer's products because of their chemical versatility and stability. The durability of dyes causes pollution once these are released into the environment because, up to 50% of the initial dye mass used in dyeing process remains in the spent dye bath in its hydrolyzed form which no longer has an affinity for fabric and cannot be recycled in dyeing process [2]. Reactive dye industrially has a world market share of 60–70%. Approximately, 10-15% of the dyes used in the textile industries remain unutilized, and 50% unutilized had been released with effluents. Dyes are difficult to biodegrade because of its synthetic and complex aromatic molecular structures which make them steady [3, 4]. Bioremediation is the microbial cleanup approach where microbes can acclimatize themselves to toxic wastes and new resistant strains develop, which can transform various toxic chemicals into less harmful forms. Many reports have suggested that the degradation of complex organic substances can be brought about by microbial enzymes [5, 6]

In recent years, numerous studies have been focused on various microorganisms which can biodegrade the dyes in waste water, which include many bacteria, fungi, and algae capable of decolorizing a broad range of dyes with high efficiency [7, 8]. The removal of reactive dyes is problematic because they can easily pass through conventional treatment systems without much change. Various physical, chemical methods such as absorption, oxidation, chemical coagulation and precipitation, ozonationare usually employed to remove the colors before discharging into the environment [1]. However, these methods have some limitations as high cost and disposal ofalarge quantity of sludge with some toxic waste produced during these processes. De-colorizations can be an effective tool where indigenous microorganism like yeast (*Saccharomyces cerevisiae*) can be used for the treatment of industrial dye effluent because of their limited studies was carried out, and it is economical and eco-friendly [9].

Saccharomyces is a genus in the kingdom of fungi that includes many species of yeast. It is known as the brewer's yeast or baker's yeast. Other members of this genus contain the wild yeast *Saccharomyces paradoxes* is closest to *Saccharomyces cerevisiae, S.bayanus* which are used in wine making and *Saccharomyces boulardii* used in medicine. As yeast cells of the genus, *Saccharomyces* are non-pathogenic, easily available and can grow in simple, in expensive medium. This economical biomass can be utilized for removal of dyes from textile industry effluent [9]. Reactive dye selected for this research was on several factors including relatively slow biodecolorization kinetics, commercial use, and previous research conducted. The present study has been proposed to decolorize the dye using yeast strain.

MATERIALS AND METHODS

Chemicals

The textile dye Reactive Navy blue was obtained from Dye industry in Chennai, Tamil Nadu. A stock solution of the dye (1g/L) was prepared using de-ionized water and used through out the studies [1] (Figure: 1).

Organism and mother culture condition

Saccharomyces cerevisiae MTCC463 strain was obtained from Institute of Microbial Technology Chandigarh, India. The pure strain was maintained at 4^{o}C on Malt-yeast medium, under static conditions. The growth curve was done by taking sample at every 1 hr interval of time till the decline phase which was at the 10th hr. The log phase was observed at the 3rd hr which showed the good growth and the rapid multiplication of yeast cells. The dye was added in the log phase. at the 3rd hr of the growth for further experiments.

ISSN: 0975-8585

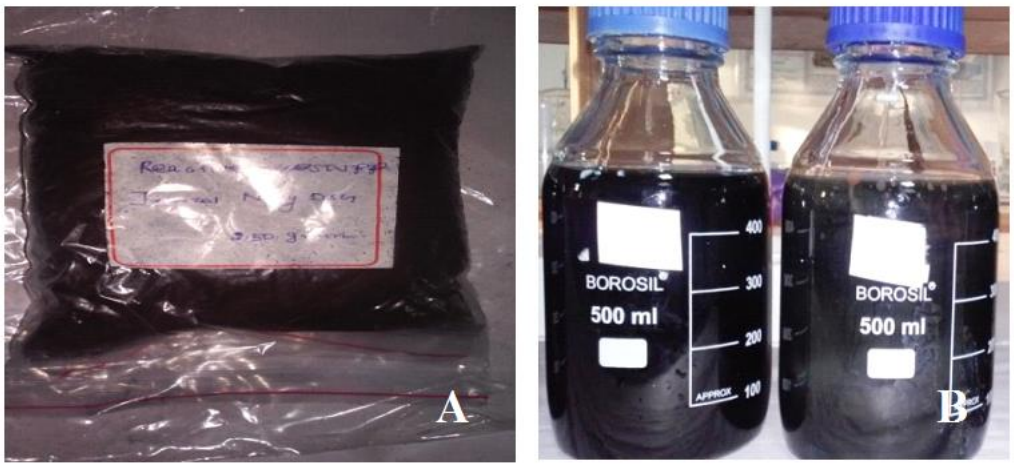

Figure 1: A) Reactive Navy Blue dye powder, B) Reactive navy blue dye stock solution.

Decolorization of dye at different concentration

5% of the inoculum was inoculated from the mother culture in to the Malt-Yeast extract broth (MYB) and incubated at 30°C in a rotatory shaker at 130 rpm over-night [10]. From (1g/L) of the dye stock solution, various dye concentrations such as 1ppm, 2ppm, 4ppm, and 8ppm were prepared and added into the medium containing inoculam. The liquid medium containing dye without the inoculam was used as the control. The aliquat of samples (3mL) were taken at every 24 hrs. duration, centrifuged at 10000 rpm for 15min and analysed the supernatant for decolorization by using a UV-visible spectrophotometer at 615nm.

Effect of Yeast cell concentration on Dye decolorization

To examine the degradation of dye by *S. cerevisiae* culture, various concentration of living yeast cells of 5%, 7%, 10%, 12% and 15% (v/v) were added to the conical flasks containing 100 mL sterilized MYB medium with 1ppm dye concentration. 3mL samples were taken at evry 24 hrs. duration, centrifuged at 10,000 rpm for 15 min to remove suspended biomass. The dye concentration in the supernatant was analyzed spectrophotometrically at 615nm over 0^{th} hrs, 24^{th} hrs, 48^{th} hrs, 72^{nd} hrs, and 96^{th} hrs.

Dye degradation at different pH

Dye degradation experiments were done using 5% of the inoculum in MYB medium at different pH ranging between 5, 6, 7, 8 and 9. The pH of the medium was adjusted to desired value using 0.1 N NaOH and 0.1 N HCL and autoclaved at 121°C for 15 min. The dye solution at a concentration of 1ppm was added to the liquid growth medium at 3^{rd} hr. of log phase maintained temp 30°C and kept for over night incubation at rotatory shaker. 3mL of the sample were taken at every 24^{th} hrs. duration, centrifuged at 10000 rpm for 15 min and the dye decolorized in the supernatant was determined using UV-Visible spectrophotometry at 615nm.

Analytical methods

The percentage removal of dye was calculated using the following equation:

$$\textbf{Dye decolourisation (\%)} = (C_i - C_f) / C_i \times 100$$

where: C_i represents the initial dye concentrations (mg/L)
C_f represents the final dye concentrations (mg/L)

FTIR Analysis

100 ml culture samples were collected from the control, 1ppm and 8ppm flasks at 24^{th} hr and 48^{th} hrs. The cell bio mass was extracted by vaccum filtration and the cells were dried pelleticed with KBr. The

ISSN: 0975-8585

samples were then ground, desorbed at 60°C for 24 hrs. and pressed to obtain IR transparent pellets. The absorbance for FT-IR spectra of the samples using an FT-IR Spectrum 2000 Perkin-Elmer spectrometer was determined within a scanning range of 500-4000 cm- 1. The FT-IR first calibrated for background signal scanning using control sample of pure KBr, and scanned the experimental sample. The FT-IR spectrum of dyes and the degraded dye samples of 1ppm and 8ppm concentrations for 24th hrs and 48th hrs were analyzed [11].

RESULTS AND DISCUSSION

Effect of Yeast culture concentration on decolorization

The effect of concentrations of initial iniculam of *S.cerevisiae* was determined under shaking incubation. It was observed that the concentration of the initial inoculum was directly proportional to the dye degradation that is higher rate of decolorization of reactive dye concentration under static conditions such as 79.31%, 71.26%, 74.23%, 77.89%, 73.67% were observed with 5%, 7%, 10%, 12% and 15%(v/v) size of inoculum respectively. However under control conditions i.e. with 0% inoculum, there was no decolorization. The highest decolourization was achieved by 79.3% for 5% inoculum within 96th hrs. So looking at the pattern of dye decolourization and the growth of the yeast in the liquid medium, the optimum value of concentration was found to be 5%. (Figure: 2)

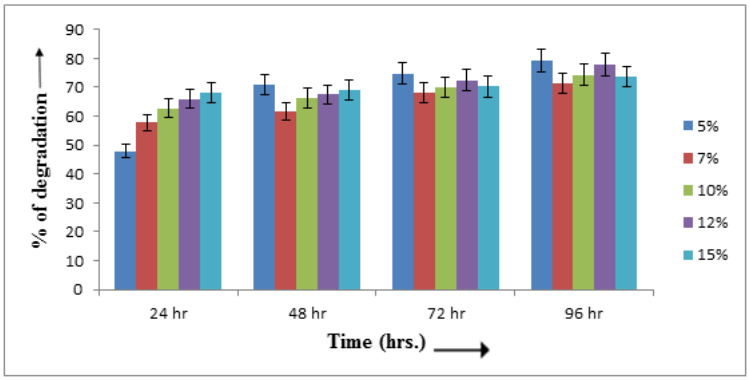

Figure 2: Effect of *S. cerevisiae* concentration on the reactive navy blue dye decolorization.

Effect of Dye Concentrationondecolorization

The effect of dye concentration on decolourization was done at 1ppm, 2ppm, 4ppm and 8ppm prepared from the stock solution(1g/L) keeping other parameters constant such as 30°C temp, 5% inoculum and pH of medium as 6.2. Where as the dye-containing liquid medium was used as the control. All of the experiments were carried out in duplicate. The concentration of dye was determined by reading at the absorption maximum peak at 615 nm for Reactive Navy Blue. The high percentage 89.96% of decolourisation was observed in 1ppm at 96 hrs; whereas 2ppm, 4ppm and 8ppm the decolourisation obtained at 96 hrs were 85.14%, 77.11% and 68.69% respectively (Figures: 3 & 4).

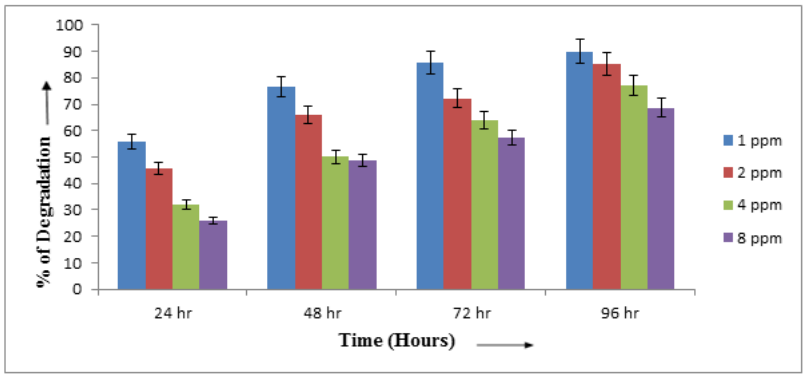

Figure 3: Effect of reactive navy blue dye concentration on decolorization by *S. cerevisiae*

ISSN: 0975-8585

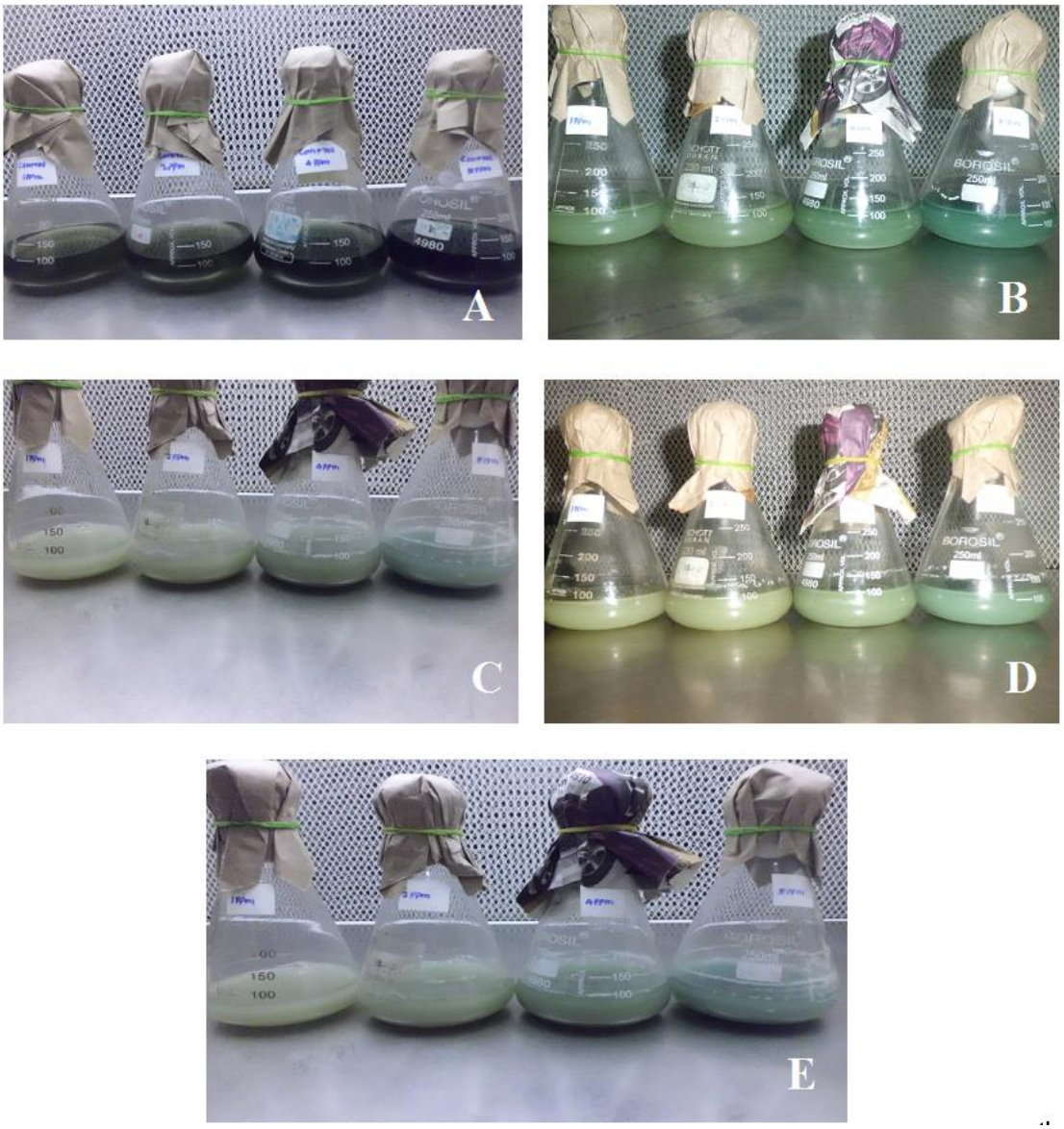

Figure 4: Different concentration of dye degradation: A) Control , B) 24th hrs. Degradation of dye, C) 48th hrs. Degradation of dye , D) 72th hrs. Degradation of dye, E) 96thhrs. Degradation of dye

Effect of pH on decolorization

The effect of pH on decolorization of dye was done with 5% growing yeast using liquid MYB medium at various pH ranges between 5, 6, 7, 8 and 9 while keeping other parameters constant such as 30°C temperature, 5% inoculam, and 1 ppm dye concentration. The pH was adjusted to the desired value using 0.1 N of NaOHandor 0.1 N HCL. The maximum decolourisation of 95.06%was obtained at pH 7 whereas in pH 5, 6, 8 and 9 the decolourization was 72.75%,, 92.78, 80.37% and 83.42% respectively.The decolourisation rates was very muchsimilar for pH 8 and 9 at 72 and 96 hrs of incubation. It can be explained that positive charges of the biomass lead to the attraction between the anionic species of the dye and the surface of the bio sorbent at a lower pH. The electrostatic attraction force of the dye compound with the sorbent surface is likely to be reduced when the pH value increased [12]. In other words at a lower pH(6 and 7), the biomass of cell is positively charged leading to decrease in free negatively charged adsorbent sites which are favorable to the adsorption of negatively charged dye (Figure: 5).

ISSN: 0975-8585

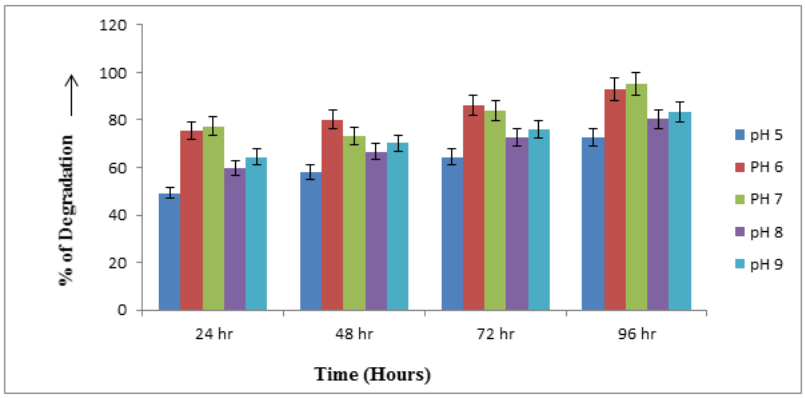

Figure 5: Effect of pH on the decolorization of reactive navy blue dye by *S. cerevisiae*.

FTIR Analysis

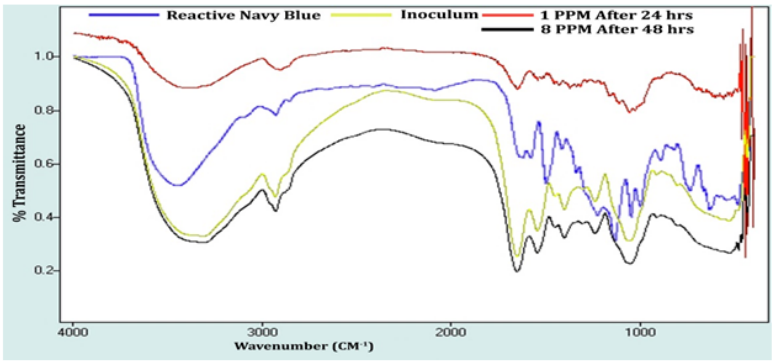

Figure 6: FTIR Spectrum of dye, inoculum, 1ppm and 8ppm concentration of samples.

Table 1: FTIR Spectrum of dye, inoculum, 1ppm and 8ppm concentration of samples and its functional groups.

Functional Groups	Details of the Peaks of Samples			
	Dye	Inoculum	1ppm	8ppm
O-H stretch alcohols and/or phenols	3445.59 cm^{-1}	3980 cm^{-1}	3414.11 cm^{-1},	3302.98 cm^{-1}
C-H stretch alkane	2927.81 cm^{-1}	2997 cm^{-1}	2903.67 cm^{-1}	2927.41cm^{-1}
primary amines,	1621.89cm^{-1} 1579.73 cm^{-1}	_	_	_
C-C stretch aromatics ,	1414.21cm^{-1}	at1587 cm^{-1}	_	_
N-O symmetric stretch.	1339.20 cm^{-1}	1482 cm^{-1}	1546.85cm^{-1}	1545.14 cm^{-1}
C-N stretch aliphatic amines	1226.48cm^{-1}	_	1240.90 cm^{-1}	1241.17 cm^{-1}
C-N stretch aromatic amines	1136.52 cm^{-1}	1329 cm^{-1} 1284 cm^{-1}	_	_
=C-H bend alkenes	1049.71 cm^{-1}	_	_	_
C-Cl stretch for alkylhalides,	823.24 cm^{-1}	_	_	_
-C ≡ C- H:C-H bend alkynes	635.66 cm^{-1}	_	_	_
C-Br stretch alkyl halides.	547.65 cm^{-1}	_	_	_
C-H wag (-CH_2X) alkyl halides	_	1187 cm^{-1}	1160.85 cm^{-1}	_

As shown in figure 6 and table 1, the FTIR spectrum of the samples observed were as follows; the inoculum alone is in pink color, the reactive dye alone is green in colour, the culture filtrate of the decolourized dye at 1ppm is blue and the decolourized dye at 8ppm is black in colour. The different functional groups present in the various samples analysed are represented by the peaks of the FTIR spectrum. The spectrum of the dye alone displayed peaks at 3445.59 cm^{-1} for O-H stretch alcohols and/or phenols, 2927.81 cm^{-1} for C-H stretch alkane, 1621.89 and 1579.73 cm^{-1} for primary amines, 1414.21cm^{-1} C-C stretch for aromatics, 1339.20 cm^{-1} for N-O symmetric stretch,1226.48 $cm^{-1}$and 1136.52 cm^{-1} for C-N stretch for aliphatic amines and C-N stretch aromatic amines respectively, 1049.71 cm^{-1} =C-H bend alkenes, 823.24 cm^{-1} for C-Cl stretch for alkylhalides,635.66 cm^{-1} for -C ≡ C- H:C-H bend alkynes and 547.65 cm^{-1} for C-Br stretch alkyl halides.

The spectrum of the inoculum and the culture filtrate of decolourized dye at1ppm and 8ppm displayed peaks at 3980 cm^{-1}, 3414.11 $cm^{-1,}$ and 3302.98 cm^{-1} respectively representing the functional group of O-H stretch (free hydroxyl) alcohols and/or phenols. The peak at 2997 cm^{-1} of inoculum, 2903.67 cm^{-1} of 1ppm and 2927.41cm^{-1} of 8ppm represented the C-H stretch alkenes. Thepeak at1587 cm^{-1} of inoculum represented C-C stretch aromatics. The peaks observed at1482 cm^{-1} of inoculum, 1546.85cm^{-1} of 1ppm and 1545.14 cm^{-1} of 8ppm represented the functional group of N-O asymmetric stretch of the nitro compound. The peak at 1329 cm^{-1} and 1284 cm^{-1} of inoculums represented C-N stretch aromatic amines similarly peaks at 1240.90 $cm^{-1}$of 1ppm and 1241.17 cm^{-1} of 8ppm represented C-N stretch aliphatic amines. The peaks at 1187 $cm^{-1}$and 1160.85 cm^{-1} of inoculum and 1ppm represented C-H wag (-CH_2X) alkyl halides. In dye and inoculum, O-H stretch alcohols and/or phenols, C-H stretch aliphatic amines are same in compounds and for dye, inoculum some of the complex are different. The over results indicated that functional groups on the surface of yeast affected the adsorption process.

CONCLUSION

The parameters such as pH of the medium, different initial dye concentrations and initial yeast iniculam concentration influences the decolorization process. The maximum decolorization was observed at pH 7 with 5% of inoculum for 1ppm of the dye concentration at 96thhrs incubation at 30^{o}C. The FTIR analysis of the inoculum, the dye and the cuture filtrate at 1 and 8ppm of dye concentration helped us to inferthe mechanism of decolorization process. The biodegradation abilities of microorganisms can be enhanced gradually by exposing them to higher concentrations of synthetic organic chemicals. Microorganisms exposed to higher levels of pollutants evolve mechanisms and pathways for degrading them.

ACKNOWLEDGEMENT

Authors wish to thank the management of SRM University, Kattankulathur, Tamil Nadu, India, for providing necessary facilities and providing support for the completion of this work.

REFERENCES

[1] Arun Prasad A.S., BhaskaraRao K.V. Global Jr. of Biotechnology & Biochemistry 2010; 5(2): 80-86.
[2] Zollinger, H. Color Chemistry. VCH publishers, New York. 1987; 3: 92-100
[3] Fu, Y., and Viraraghavan, T. Bioresource Technology 2001; 79(3): 251-262.
[4] Pearce, C.I., Lloyd, J.T., and Guthrie, J.T. Dyes and pigments 2003; 58: 179-196.
[5] Aksu, Z. Process Biochem 2003; 38: 1437-1444.
[6] Banat, I.M., Nigam. P., Singh, D. and Marchant, R. Bioresource Technol 1996; 58: 217-227.
[7] Fu Y. and Viraraghvan T. Biores. Technol. 2002; 82: 139-145.
[8] Ramakrishna K. R., Viraraghavan T. Wat. Sci. Technol. 1997; 36: 189–196
[9] JadhavJ. P. and Govindwar S. P. Yeast 2006; 23: 315–323.
[10] Sambrook, J., E.F. Fritsch and T. Maniatis: Molecular cloning: Andlaboratory manual. 2 Edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 1999.
[11] Dhanve R.S., Shedbalkar U.U., Jadhav J.P. Biotechnol and Bioprocess Eng. 2008; 13: 53-60.
[12] Mahmoud, M.S. HBRC Journal 2016; 12: 88–98.