JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

# Isolation of Azo dyes degrading microorganisms

**Amruta uttamrao Jadhav, Chaitanya Vijay Joshi**
Assistant professor, Student
K.T.H.M College Nashik.

**Abstract-**
Azo dyes square measure used widely within the manufacture of varied goods like animal skin, textile, plastics, paper, hair care merchandise and cosmetics. chemical (group) dyes square measure the most important group of dyes utilised in trade representing over half the annual production. it's been calculated that 100% of the dye stuff throughout this dying method doesn't bind to the fibres and is so discharged into the waste product or setting. Chemical group dyes have structural properties that aren't simply degradable beneath natural conditions..Over the past year many bacterial strains have been studied for their abilities to decolorization and detoxify dyes. Considerable attention has been given in evaluating the capability of microorganisms in decolorizing and degrading azo dyes. The effectiveness of microbial decolorization depends on the adaptability and activity of selected microorganisms.this study work is to isolate some microbial strains, which possessed the ability to decolorize simulated effluent containing dyes covering various classes like mono-azo /diazo/triazo, acidic, reactive, sulphonated/ non sulfonated dyes, direct dyes, Reactive dyes and triphenylmethane dyes. The isolates showing significant decolorization were identified to ascertain their taxonomic position.
From textile effluents nineteen (5 Actinomycetes & fourteen Bacteria) microbic species were isolated. Once screening the foremost economical 3 cultures square measure elite for additional study out of them one was microorganism and 2 square measure actinomycetes. All 3 species will decolour and detoxify 'Acid dyes'(Methyl red), 'Reactive dyes' (Reactive red-195, Reactive black five. Reactive yellow-145), Triphenylmethane dye (Malachite green) and simulated effluent (Mixture of ten dyes).
In order to check physiological and metabolic aspects of the decolorization and detoxification method Reactive yellow- 145was elite as a model dyestuff beneath static and shaking conditions. Once optimised for chemical science parameters, microorganism pure cultures show 94% decolorization among 3 days beneath stationary conditions and Actinomycetes pure cultures show 91 % look after decolorization among five days beneath shaking conditions.

**Keywords-** Detoxification, Azo dyes, Reactive yellow, Consortia, Decolorization.

## Introduction-

Colourants have been used by man since prehistoric times and square measure defined by their ability to soak up or emit lightweight within the visible range(400-700 nm). Colourants could also be either inorganic or organic compounds. Each team will be divided into natural and artificial representatives. However, today, several natural colourant square measures are made synthetically.Colorants square measure either dyes or pigments.[1]

### 1.1 Dyes:

Dyes square measure colouring agents. associate aromatic ring structure plus a facet chain is sometimes needed for resonance and so to impart colour. a range of dyes square measure utilised in the textile trade. At

the present regarding 100,000 dyes for that 5000 square measure business merchandise. Although dye molecules vary in composition and behaviour, each dye molecule has distinctive chemical teams, liable for a selected property of the dye.2

**1.2 Classification of dyes:**

All aromatic compounds absorb magnetic attraction energy however solely those who absorb light light with wavelengths within the visible vary (-350-700 nm) square measure colored. Dyes contain chromophores, delocalized negatron systems with conjugated double bonds, and auxochromes, negatron-withdrawing or electron- donating substituents that cause or intensify the colour of the chemical group by neutering the energy of the electron system. Usual chromophores square measure -C-C-, -C-N-, -C-O, -N-N-, -NO2 and quinoid rings, usual auxochromes square measure

-NH3, -COOH, -SO3H and -OH.

Based on chemical structure or chemical group, 20-30 completely different teams of dyes will be discerned. chemical group (monoazo, disazo, triazo, polyazo), anthraquinone, will of phthalocyanine and triarylmethane dyes square measure quantitatively the foremost necessary teams. alternative teams square measure diarylmethane, indigoid, azine, oxazine, thiazine, xanthene, nitro, nitroso, methine, thiazole, indamine, indophenol, lactone, aminoketone and hydroxyketone dyes and dyes of undetermined structure (stilbene and sulphur dyes).

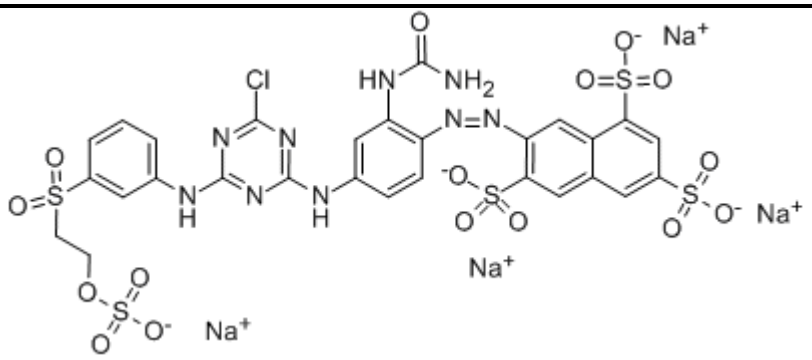
**1.3Azo dyes:**

Azo dyes measure the most important cluster of artificial dyes and pigments with industrial application because of their comparatively straightforward synthesis and nearly unlimited variety and kinds of substituent. chemical group dyes contain a minimum of one -N-N- covalent bond and plenty of completely different structures square measure doable. Monoazo dyes have just one -N-N- covalent bond, whereas cation, triazo and polyazo dyes contain 2, 3 or additional N-N- double bonds, severally. The chemical group teams square measure typically connected to aromatic hydrocarbon and hydrocarbon rings, however may also be hooked up to aromatic heterocyclic or enolizable acyclic teams. Chemical group colourants place shade from yellowness to orange, red, violet and brown. The colours rely for the most part on the chemical structure, whereas completely different shades rather rely upon physical properties.12

• **Golden yellow (Reactive Yellow 145):**

The most important characteristics of reactive dyes is the formation of covalent bonds with the substrate to be colored. Reactive dyes are a class of extremely colored organic substances, mainly used for textiles that attach themselves to their substrates by a chemical reaction that forms a covalent bond between the molecule of the dye and that of the fiber. The dyestuff thus becomes a part of the fiber and is much less likely to be removed by washing than are dyestuffs that cleaves by adsorption.

Reactive Yellow 145 is a mono azo dye containing one azo bond and sulfonic group within its structure. Reactive group of these bonds are difficult to break and responsible for imparting color to the compound. (The sulfonic group not present in Methyl red azo dye hence its degradation is comparatively easier than Reactive yellow.) Conventional techniques remove dye only by adsorption or chemical precipitation methods and hence the azo bonds are left unbroken in nature leading to their longer persistence in the environment.



20

Biodegradation of azo dyes process may be aerobic or anaerobic or involve a combination of both environments. Several bacterial systems have been observed to degrade dyes and participate in the degradation process.

Biodegradation of pollutants in natural ecosystems is influenced by various environmental factors including pH. Temperature, pollutants concentration and oxygen, and hence optimization of these physical and environmental parameters is important in order to increase the dye degradation efficiency of the organisms.

**1.3.1 Azo dye properties:**

The different, mainly aromatic, side groups around the azo bond help to stabilise the -N-N- group by making it part of an extended delocalized systeme. This also has the effect of making many azo compounds colored. As delocalized or conjugated systems often absorb visible frequencies of light. Aromatic azo compounds (R-R aromatic) are usually stable and tend to produce strong vivid colours.

The general formula for making an azo dye requires organic compounds, a coupling component and a diazo component. Since these can be altered considerably, an enormous range of possible dyes are available especially as the starting molecules are readily available and cheap. Furthermore, the simplicity of the reactions mean that the process can be scaled up or down very easily. Energy requirements for the reaction are low, since most of the chemistry occurs at or below room temperature. The environmental impact is reduced by the fact that all reactions are carried out in water, which is easy and cheap to obtain, clean and dispose of. All these factors make azo dyes very cheap to produce. Azo dyes are much more stable than most of the natural food dyes. Azo dyes are stable in the whole pH range of foods, are heat stable and do not fade when exposed to light or oxygen. This makes azo dyes applicable in nearly all foods. The only disadvantage is that azo dyes are insoluble in oil or fat. Only when azo dyes are coupled to a fat soluble molecule, or when they are dispersed as very fine particles, oils can be colored.

**Determination of □max of dyes**

The max of the dyes were determined using UV/VIS spectrophotometer (Shimadzu make). The sterilised dyes were dissolved in distilled water to obtain light colour tinge of respective dyes and simulated effluent. The dye solutions were scanned for maximum absorbance in wavelength range 200-800 nm.19

**Table: 1.3.1 □max for the dyes:**

| Name of the dye | □max in nm |
|---|---|
| Reactive red | 540 |
| Reactive yellow-145 | 418 |
| Orange- II | 483 |
| Malachite green | 617 |
| Reactive black | 573 |

**1.4. Diazotization:**

Synthesis of most azo dyes involves diazotization of a primary aromatic amine to give a diazonium salt. The diazonium compound is then coupled with one or more nucleophiles. Amino- and hydroxyl- groups are commonly used coupling components. The coupling reaction is generally in para position in respect to the amino- or hydroxyl- groups. The foundation of the production of azo dyes was laid in 1858 when P. Gries discovered the reaction mechanism, diazotization, for the production of azo compounds. The general scheme of azo dye synthesis may be divided into two stages.

**Stage 1- Diazotization**

This involves a primary aromatic amine, called the diazo component. It is treated in low temperature, acid conditions with sodium nitrite to form an unstable diazonium salt.

**Stage 2- Azo coupling**

The diazonium salt is reacted with a coupling component (for example a phenol or an aromatic amine). This forms the stable azo dye.

**1.5 Dyes Discharge:**

It is estimated that 15% of the total production of colourants is lost during synthesis and processing. During textile processing, inefficiencies in dying result in large amounts of dyestuff being lost to the wastewater. The amount of dye lost is dependent upon the dye application class Particularly Reactive dyes are most inefficient with respect to fixation on fibre and also they are produced highest among all dyes. Hence the dye pollution problem is severely associated with reactive acid dyes.6

**1.6 Water Pollution:**

The first contaminant observed in water is the colour . Many dyes are visible in water at concentrations as low as 1 ppm. The dye manufacturing and dyeing industry wastewater typically contain dye concentration in the range 10-200 ppm . Textile industries consume large volumes of water and chemicals for wet processing of textiles. The chemical reagents used are very diverse in chemical composition ranging from inorganic compounds to polymers and organic products . Textile dye effluents are complex, containing a wide variety of dyes, natural impurities extracted from the fibres and other products such as dispersants, levelling agents, acids, alkalis, salts and sometimes heavy metals. In general, the effluent is highly colored with high biological oxygen demand (BOD) and chemical oxygen demand (COD), it has a high conductivity and is alkaline in nature. The presence of very low concentration of dyes in effluent is highly visible and undesirable.8

**1.6.1 Recalcitrant nature of dyes :**

Due to complex chemical structure, dyes are resistant to fading on exposure to light, water and many chemicals and are highly persistent in the natural environment. Many dyes are difficult to decolorize due to their complex structure and synthetic origin. Without adequate treatment these dyes remain stable in the natural environment, for instance half life of hydrolyzed Reactive Blue 19 is about 46 years at pll 7 and $250^0$C . The conventional wastewater treatment, which relies on aerobic biodegradation, has low removal efficiency for reactive and other anionic soluble dyes. Due to low biodegradation of dyes, a conventional biological treatment process is not very effective in treating a dyes wastewater.

**2. Materials:**

**2.1: collection of samples:**

Three samples were taken for isolation of microorganisms on nutrient agar plates containing congo red and methyl red dyes . First was water samples from fish tanks, second was soil from the Rhizosphere and third was soil samples mixed with dyes used for colouring of statues.

**2.2 Enrichment and Isolation:**

Three different suspensions were prepared from all three samples which were mentioned above,
Majority of earlier reports suggested that microorganism require co substrate for the azo dyes degradation, hence enrichment of sample collected was performed in medium containing yeast extract, peptone and dye also added. So that enrichment of only microorganisms takes place which can grow in presence of dye, and in later steps can degrade dye.

For isolation of different microbial species present in samples spread plate technique was used.
.The nutrient agar plates each containing different azo dye (100 ppm) prepared. From an enriched sample 0.1 ml broth was spreaded on dye containing nutrient agar plates (100 ppm).
Plates were incubated at 30°C for 24 hrs. And observe for decolorization.

**2.2: Screening:**

Screening of isolates degrading azo dyes was done using nutrient agar plate containing respective dyes( Reactive yellow -145, Reactive red -195, Reactive black-5, Malachite green, Methyl red, Acid orange-II, mixture of all dyes).

**2.2.3:Preparation of different dye concentration**

50ml distilled water + 0.5gm dye powder → 10mg/ml (stock solution)

1ml of stock + 99ml of distilled water → 10mg/100ml(100 mg/1000ml=100ppm)
(10mg/ml)

**Table no.2.2.3 Preparation of different dye concentration a),b)**

**a)For 100 PPM**

| PPM (Concentration of dye) | Medium broth(ml) | Stock(ml) |
|---|---|---|
| 100 | 500 | 5 |
| 100 | 400 | 4 |
| 100 | 300 | 3 |
| 100 | 200 | 2 |
| 100 | 100 | 1 |
| 100 | 50 | 0.5 |
| 100 | 10 | 0.1 |

**b) For 50 PPM**

| PPM (Concentration of dye) | Medium broth(ml) | Stock(ml) |
|---|---|---|
| 50 | 500 | 2.5 |
| 50 | 400 | 2 |
| 50 | 300 | 1.5 |
| 50 | 200 | 1 |
| 50 | 100 | 0.5 |
| 50 | 50 | 0.25 |
| 50 | 10 | 0.05 |

Selected isolated colonies were picked up by using straight Nichrome wire and spot inoculated on dye containing nutrient agar plates, and again incubated as above.
After incubation the bacterial colony and actinomycetes colony showing good zone of clearance were maintained on nutrient agar and Actinomycetes isolation agar slants respectively.
The bacterial isolates were inoculated in sterile glucose broth containing different azo dyes (50 ppm) in different tubes and incubated at 30°C under static conditions.
The actinomycetes isolates were inoculated in different 50 ml flasks containing actinomycetes isolation broth with various azo dyes (50 ppm) and incubated at 30°C under shaking conditions.
Actinomycetes required more incubation period than that of bacteria. Then at equal intervals of time the broth inoculated with each isolate was centrifuged and absorbance was taken at the respective max of each dye.
Same procedure was carried out by using a mixture of all dyes.

**2.3 Observations:**

**2.3.1 Isolation:**

After incubation isolated colonies which were showing zones of clearance on the dye containing plates were observed. Zone of clearance around the colony on nutrient agar containing congo red was shown in fig 2.1

**Fig 2.1 Zone of clearance around the colony on nutrient agar containing congo red.**

**2.3.2: Screening:**

We observed that the isolates SSP.Act-1. Act-2 grew very rapidly. Also, the dye concentration in each of the tube and flask inoculated with SSP. Act-1, Act-2 decreased considerably within 24hr, 72hr.72hr respectively. After incubation, observation was done. Table 2.3 illustrates visual decolorization profile before 48 hrs, with respect to simulated effluent of dyes such as, reactive yellow, reactive red, orange-11, congo red, methyl red reactive black etc. The difference between control and degraded dye was shown in fig.2.2 and Visual Decolorization profile of Methyl red was summarised in table 2.4

Decolorization profile with respect to different dyes showed in Fig. 2.3 and summarised in table 2.4

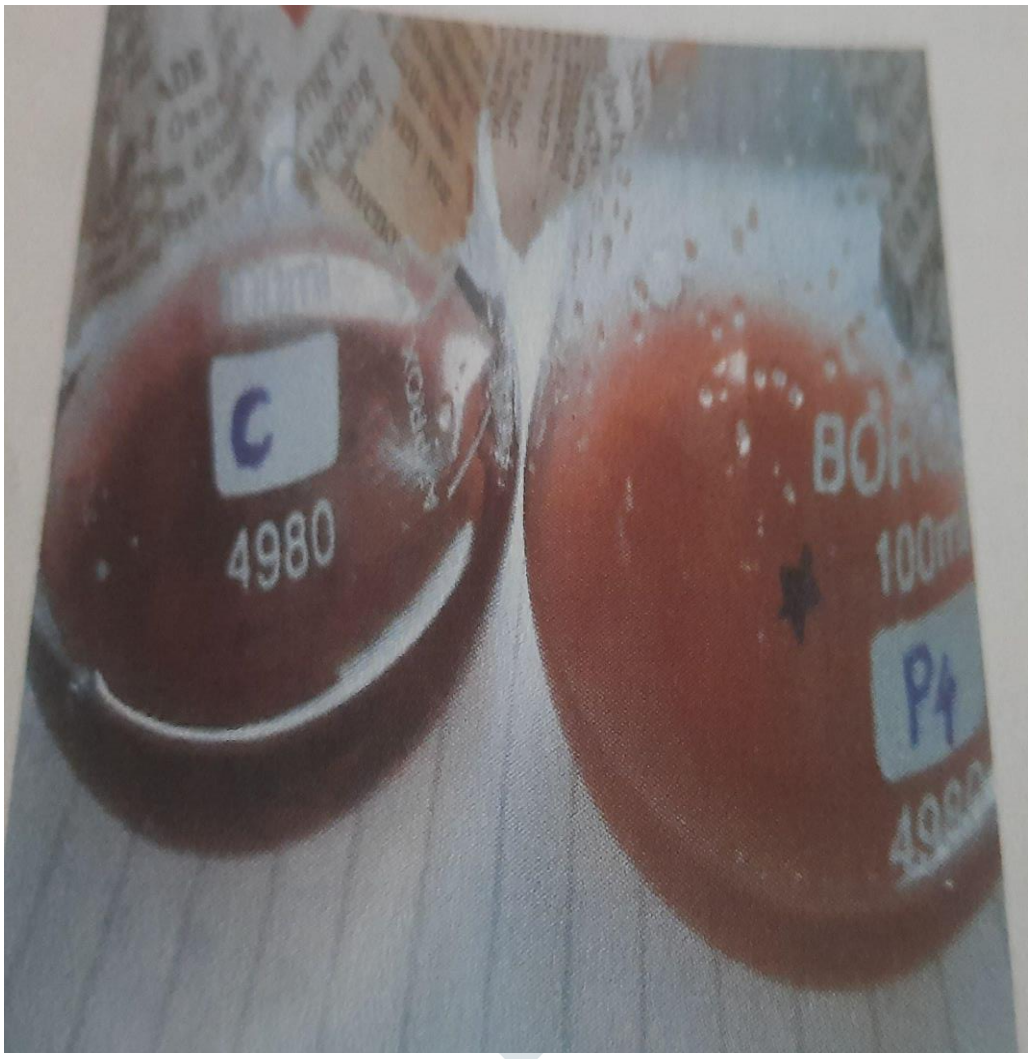



**Fig- The difference showed between control (C) and test (p4) after incubation.**

**Table 2.4 Illustrate decolorization profile with respect to 6 different dyes and of dye mixture.**

| Sr. | Isolates | Type | Source | Visual decolourization profile of methyl red |
|---|---|---|---|---|
| 1 | C2 | Bacteria | Rhizosphere soil | +++ |
| 2 | G2 | Bacteria | Fish tank water | +++ |
| 3 | H1 | Bacteria | Fish tank water | ++ |
| 4 | F1 | Bacteria | Fish tank water | ++ |
| 5 | B1 | Bacteria | Soil containing waste dyes(SCWD) | ++ |
| 6 | H6 | Bacteria | Fish tank water | + |
| 7 | I2 | Bacteria | Fish tank water | + |
| 8 | T | Bacteria | Fish tank water | +++ |
| 9 | S | Bacteria | Fish tank water | ++ |
| 10 | SSP | Bacteria | SCWD | ++++ |
| 11 | ACT-1 | Actinomycetes | SCWD | +++ |
| 12 | ACT-2 | Actinomycetes | Fish tank water | +++ |
| 13 | ACT-X | Actinomycetes | Fish tank water | + |
| 14 | P1 | Bacteria | SCWD | ++ |
| 15 | P3 | Bacteria | SCWD | ++ |
| 16 | P5 | Actinomycetes | SCWD | + |
| 17 | P6 | Bacteria | SCWD | + |
| 18 | P7 | Bacteria | SCWD | + |
| 19 | P10 | Actinomycetes | SCWD | ++ |

**Before 48hours, +- poor decolorization, ++-moderate decolourization, +++-good decolourization, ++++-excellent decolourization.**

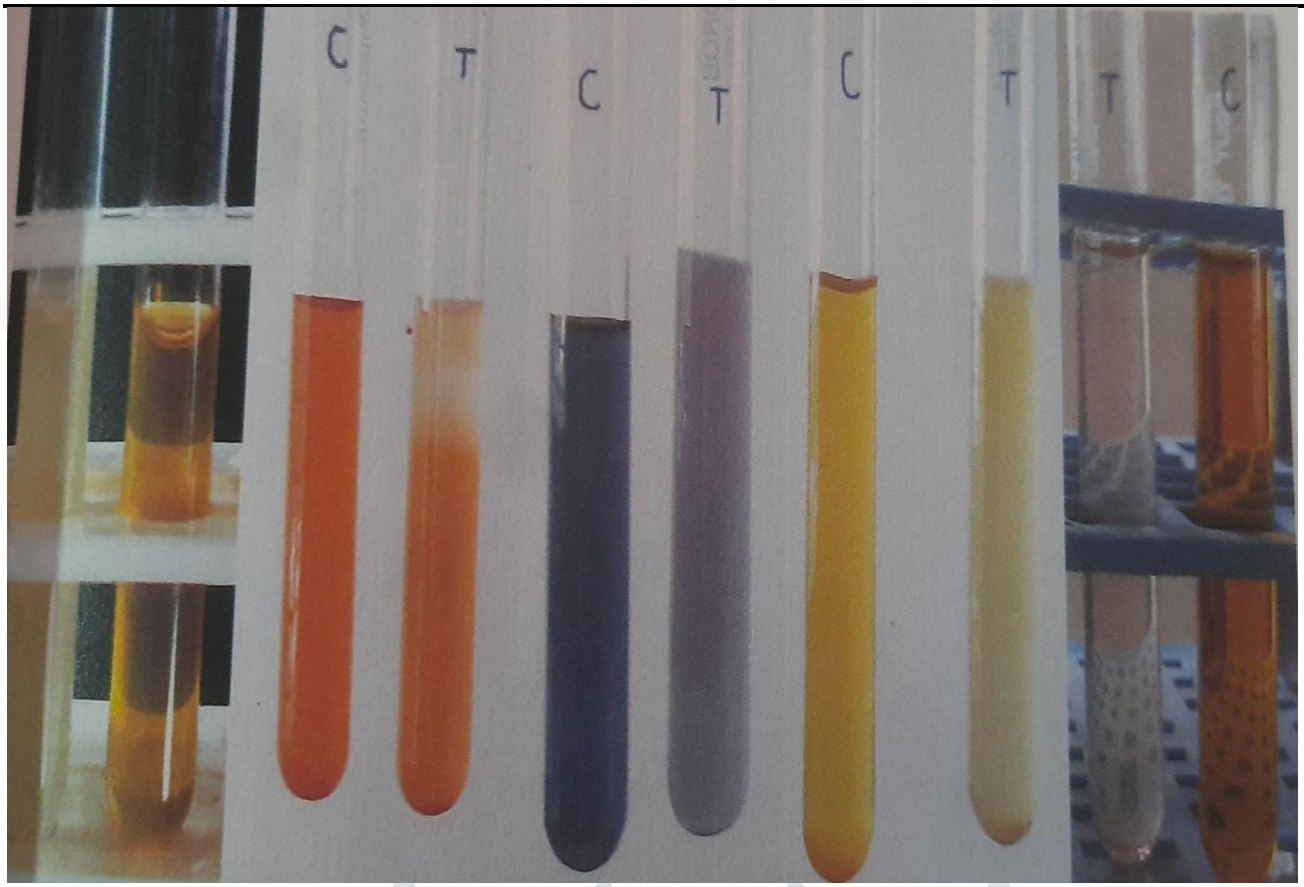



**Fig- Showed decolourization profile with respect to different dyes (C-Control, T-Test).**

**2.4 Results:**

**2.4.1 Isolation:**
After isolation 19 isolates were obtained out of them 5 where Actinomycetes 14 were bacteria.

**2.4.2 Screening:**
1. In screening a total three isolates were selected, two actinomycetes (Act-1, Act-2) and one bacteria (SSP) which were efficient azo dye degraders.

2. Pure cultures of strains SSP, Act-1. Act-2 was obtained as a result of screening.

3.The percent decolorization by pure culture of strain SSP, Act-1, Act-2 was higher than the pure culture of other isolates, that is the dyes were not degraded more rapidly by others. Therefore the isolates SSP, Act-1, Act-2 were best decolorizers among all the tested.

**2.5 Conclusions:**
After isolation and screening following conclusions were made.

1. This study revealed that there is significant decolorization by isolates SSP.Act-1,Act-2 of many dyes within 24-72 hrs.

2. Also,a mono azo dye reactive yellow-145 is decolorized efficiently by these isolates.
Thus, reactive yellow-145 was chosen as the model dye for further study.

3. For development of practical bioprocess for decolorization, isolates having high decolorization capacity is very important and hence considering the decolorization of dyes, the strain SSP, Act-1, Act-2 was selected for further studies.

**REFERENCES**

1. Abd El-Rahim WM, El-Ardy, Mohammad H (2008) Aeration as a factor in textile dye bioremoval by Aspergillus niger African J of Biochem Res 2(1):30-39.

2. An H., Qian Y., Gu X. and Tang W. Z. (1996) Biological treatment of dye wastewaters using an anaerobic-oxic system: Chemosphere 33, 2533+2542

3. Angenent L. T. and Dague R. R. (1995) A laboratory-scale comparison of the UASD and ASBR processes. In 50th Purdue Industrial Waste Conference Proceedings. Ann Arbour Press Inc, Chelsea, Michigan, pp. 365-377. 4. APHA (American Public Health Association) (1989) Standard Methods for the Examination of Water and Waste-water, 17th ed. APHA, Washington.

5. Basibuyuk M. and Forster C. F. (1997) The use of sequential anaerobic/aerobic processes for the biotreatment of a simulated dyeing wastewater. Environmental Technology 18, 843+848.

6. Boe R. W., Boardman G. D., Dietrich A. M., Michelsen D. L. and Padaki M. (1993) Pilot scale study on anaerobic treatment of a textile wastewater. In Hazardous and Industrial Wastes. Proceedings of the Mid-Atlantic Waste Conference.

7. British Standard 6068 (1995) Water QualityDExamination and Determination of Colour.

8. Carliell C. M., Barclay S. J. and Buckley C. A. (1996) Treatment of exhausted reactive dye bath events using anaerobic digestion laboratory and full-scale trials. Water SA 22. 225-233

9. Carliell C. M., Barclay S. J., Naidoo N., Buckley C. A., Mulholland D. A. and Senior

E. (1994) Anaerobic decolorization of reactive dyes in conventional sewage treatment processes. Water SA 20, 3414344.

10. Churchley J. H. (1994) Removal of dye waste colour from sewage eventS the use of a full scale ozone plant. Water Science and Technology 30(3), 275+284,

11. DeleÁ e W., O'Neill C., Hawkes F. R. and Pinheiro H. M. (1998) Anaerobic treatment of textile events: a review. Journal of Chemical Technology And Biotechnology 73, 3234335.

12. ETAD (1998) German ban of use of certain azo compounds in some consumer goods. http://www.etad.com/ information.

13. Field J. A., Stams A. J. M., Kato M. and Schraa G. (1995) Enhanced biodegradation of aromatic pollutants in co-cultures of anaerobic and aerobic bacterial consortia. Antonie van Leeuwenhoek. 67, 47+77.

14. Fitzgerald S. W. and Bishop P. L. (1995) Two-stage anaerobic/aerobic treatment of sulfonated azo dyes. Journal of Environmental Science and Health Part ADEnvironmental Science and Engineering and Toxic and Hazardous Substance Control 30, 1251+1276.

15. Geisberger A. (1997) Azo dyes and the lawDan open debate. Journal of the Society of Dyers and Colourists 113, 197-200. Grey N. F. (1999) Water Technology. Arnold, London, p. 304.

16. Van der Zee FP, Bisschops IAL, Lettings G, Field JA (2003) Activated carbon an electron acceptor and redox mediator during the anaerobic biotransformation of a dyes. Environ Sci Technol 37(2) 402-408

17. Venkata Mohan S. Vijay Bhaskar Y. Karthikeyan J (2003) Biological decolorization of simulated azo dye in aqueous phase by algae Spirogyra species. Int J Environ Poll 21(3):211-222.

18. Wahrmann K, Mechsner KL, Kappeler T (1980) Investigation on rate determining factors in the Microbial reduction of azo dyes. European Journal of Applied Microbiology 9:325-338.

19. Yang PY Nitisoravut S, Wu JYS (1995) Nitrate removal using a mixed culture entrapped microbial cell immobilisation process under high salt conditions. Water Res 29(6):1525-1532.

20. Van der Zee FP (2001) Anaerobic azo dye reduction. Ph.D. Thesis, Wageningen University.