DE-COLORIZATION OF SYNTHETIC DYE EFFLUENT ACID BLUE 9 USING *SACCHROMYCES CEREVICEA* AND *ASPERGILLUS NIGER*

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Abstract

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life. The objective of this present investigation was to study the comparison of de-colorization to the isolated yeast strain from soil. Those isolated strains are decolorizing the synthetic dye effluent Acid blue 9. The isolated strains were more or less equal to the decolorized as respectfully in *Sacchromyces cerevicea* and *Aspergillus niger* 88% and 83%.

Key words: Microbial oxidation, Synthetic dye effluent, assay technique, *Scchromyces cervicea* and *Aspergillus niger*.

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Introduction

Increasing industrialization and urbanization leads to environmental pollution. Among the various textiles dyeing industries discharge large volume of waste water after the dyeing process. Waste waters from textile industries pose a threat to the environment. Approximately 10,000 different dyes and pigments are industrially and over 0.7 million tons of synthetic dyes are produced annually worldwide. Environmental pollution due to textile industry effluent has increased during the recent years. Dyes are widely used in textiles, rubber, paper, plastic and cosmetic industries for coloring process. Moreover it is textile dye waste water is commonly high BOD, COD, heat, color, pH and the presence of organic content then low in biodegradable potential. The textile finishing generates a large amount of waste water contain dyes and represents one of the largest causes of water pollution. As10-15% of dyes are lost in the effluent during the dyeing process (1,2,3,4). Azo dyes have been used increasing in industries because of their ease and cost effectiveness in synthetic dyes compared to natural dyes. However the azo dyes are toxic in nature for due to the carcinogenic and mutagenic effect. Azo bonds present in these compound are resistant to breakdown with the potential for the persistence and accumulation in the environment (5). Several physico chemical techniques have been proposed for treatment of colored textile effluent such as Adsorption, flocculation, coagulation and precipitation, chemical oxidation and reduction electrochemical treatment for the removal of dye from effluent. There are several types of dyes in Acid dyes, Basic dyes, Reactive dyes, Direct dyes etc.., there are classified in to two types 1.the chromophore and an auxochrome. The chromophore is –NO, -NO2, -N=N, -C=C-, or C=O it is applied to a completely conjugated system. The auxochrome is –OH, -NH2, -NHe, it is the interaction of the auxochrome with the chromophore that develops the color intensity (6). The present study deals with the comparison of isolated yeast strains were decolorize the synthetic dye effluent. The dis advantages of the other physico chemical treatment techniques are effective for color removal but use more energy of chemical. Chemical treatment cause secondary pollution during dispose off. For the flocculation is high amount of flocculating agents are need. For the membrane filtration is much cost. The advantages of the biological method are very low cost effective for compared with other conventional methods. And lower sludge production eco friendly.

Materials and Methods

Isolation of organisms

10 ml of dye effluent was taken in 90 ml sterile water blanks and serially diluted to 10^{-6} times using sterile water. By thorough shaking, 1ml of aliquot from 10^{-6} dilution was drawn and plated in mineral source medium and 1ml of aliquot from 10^{-4} dilution was drawn, poured and plated in potato – Dextrose agar (PDA) medium. The plates were incubated at 37°C in an incubator for 48 hrs for yeast growth and 5 days for fungal growth. The isolated micro organisms were further purified by subsequent sub culturing and maintained in slant culture at 4°C. Yeast and fungal isolates used for decolorizing the synthetic dyes effluent were characterized both morphologically and biochemically.

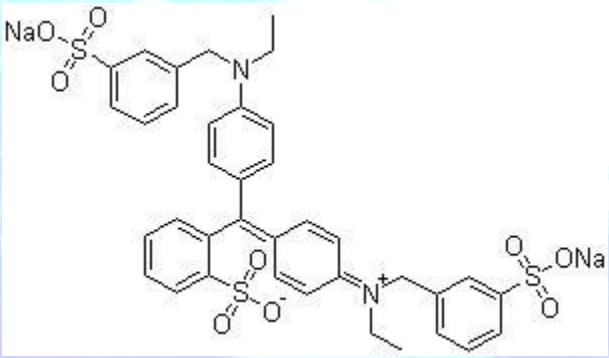


Fig 1 Molecular structure of acid blue 9

Preparation of dye effluent

Synthetic dye effluent was prepared by dissolving 1 g/ L of Acid blue 9 with 5 g/l of Sodium chloride and 3 g/l of sodium carbonate. The prepared effluent is Brilliant blue in colour and has a high alkaline pH of 9. The synthetic effluent characteristics give the nature of effluent which is of very harmful to human kinds and aquatic organisms when fed into the aquatic environment

De-colorization of effluent using isolated yeast

The yeast isolates were maintained on the YPD agar slants. This culture were inoculated in YPD broth and kept under shaking condition (120 rpm) for 24 hrs. This culture was used under

optimized conditions to decolorize the synthetic dye effluent. The pH was maintained at 7.0. Dye effluent with 10ml of yeast isolates culture with the addition of carbon (glucose) and nitrogen source (sodium nitrate) OD values were measured for 3 days at 12 to 72 hrs of time interval at 628nm in UV- spectrophotometer.

Biochemical test for *Sacchromyces cerevicea* and *Aspergillus niger*

The surface of the colonies of *A.niger* is covered by a dense of jet black conidia. The underside of the colony is buff and yellow-grey, *A.niger* from the dematiaceous fungi. Microscopically, the vesicles are globes and measure up to 75mm in diameter. The spherical, black conidia are presented.

De-colorization of dye effluent using isolated fungi

The fungal isolates were maintained on the PDA agar slants. This culture were inoculated in PDA broth and kept under shaking condition (120 rpm) for 5 days. This culture was used under optimized conditions to decolorize the synthetic dye effluent. The pH was maintained at 7.0. Dye effluent with 10ml of fungal isolates culture with the addition of carbon (glucose) and nitrogen source (sodium nitrate) OD values were measured for 5 days at 628nm in UV-spectrophotometer. The percentage of colour reduction was measured.

Characterization and identification of decolorizing yeast and Fungal cultures

The strains *Sacchromyces cerevicea* and *Aspergillus niger* isolated from waste fruits samples. These strains were evaluated for its ability to decolorize synthetic dye effluent.

The growth of *Sacchromyces cerevicea* and *Aspergillus niger* biomass in dye containing media with supplementary carbon and nitrogen source was applied as a possible approach to enhance dye bio-sorption by the yeast and fungal biomass. Based on the morphological and biochemical tests, the isolated yeast culture was identified as *Sacchromyces cerevicea* and fungal culture was identified as *Aspergillus niger*.

De-colorization of the dye effluent by yeast and fungal cultures

The de-colorization of synthetic dye effluent with inoculums was studied on both static and shaking condition. The isolated Sacchromyces cerevicea and *Aspergillus niger* was inoculated at

37^0C. In dye effluent, the shaking condition showed considerable reduction in colour compared with static culture. The de-colorization of the dye effluent by fungal culture was significantly increased with incubation period from 3rd day to 4th day.

De-colorization of synthetic dye effluent using yeast culture and fungi culture

Fig. 2 showed the growth of the yeast and fungal strains and the de-colorization percentage of the dye with yeast and fungal strains in the course of incubation till in 3 to 5 days. *Sacchromyces cerevicea* and *Aspergillus niger* culture were incubated in static and shaking conditions. After 72 hrs of incubation period the OD values were measure at 628 nm in UV-spectrophotometer. The results of experiments conducted on de-colorization of dye effluent by yeast culture *Sacchromyces cerevicea* showed 45% of colour removal on shaking condition after 72 hrs. But on static condition the *Sacchromyces cerevicea* only 30% colour removal on dye effluent after 72 hrs. On both of these studies, 10% inoculums were used on dye effluent for colour removal. The *Aspergillus niger* exhibited a colour reduction of 40% and 28% at static and shaking condition respectively after 168 hrs. With the culture time increased, the concentration of extracellular enzymes was enhanced, resulting in the complete de-colorization of the effluent in 168 hrs. The present study indicate that the isolate *Sacchromyces cerevicea* is an excellent strain for the de-colorization of Acid blue 9 synthetic dye effluents, and that it might be a practical alternative for wastewater treatment of dyes in textile industry.

Estimation of Chemical Oxygen Demand (COD):

Standardization of Ferrous ammonium sulphate solution:

Ferrous ammonium sulphate solution was taken in the burette. 20cc standard potassium dichromate solution was pipette out into a conical flask. 20cc concentrated sulphuric acid and 20cc distilled water were added. Then cooled and 0.5cc ferroin indicator was added. The mixture was titrated against ferrous ammonium sulphate solution. The end point was a sharp change of blue to red color.

COD solution:

A known quantity of waste water sample was pipette out in to a 500ml standard flask. Then 20cc standard dichromate solution was pipette out into the same distilling flask. Followed by 20cc concentrated sulphuric acid was added and mixed gently. Then 20cc distilled water and 0.2g silver sulphate were added. The solution was refluxed for 2hrs with glass beads over heating

mantle. Then the flask was cooled. Inside of the condenser was rinsed with small amount of distilled water and collected the washing into the distilling flask. Then 0.5cc ferroin indicator was added. The excess dichromate present in the solution was titrated with standard ferrous ammonium sulphate. The appeared end point was blue to red.

$$COD\ mg/l = \frac{(A-B)\times N\times Equi.\ Wt.\ of\ O2\times 1000}{Volume\ of\ sample}$$

A Volume of ferrous Ammonium sulphate used for blank

B Volume of ferrous Ammonium sulphate used for sample

Equivalent weight of Oxygen-8

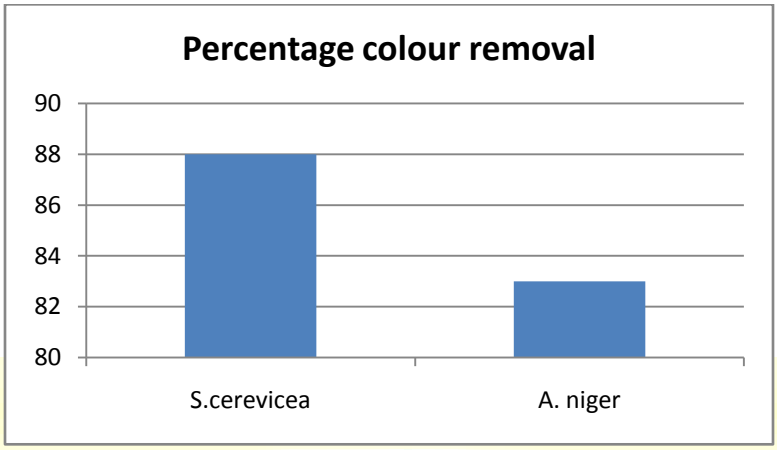
N-Normality of FAS-0.1

COD values were compared between the initial medium containing dye solution and decolorized medium.

The blank was also performed with 25cc distilled water instead of sample of waste water was titrated against standard ferrous ammonium sulphate.

Results and Discussion

The strains yeast and fungi isolates were from contaminated soil were evaluated for its ability to decolorize industrial dye effluent. The growth of yeast and fungi biomass in dye containing media amended with supplementary carbon and nitrogen source was applied as a possible approach to enhance dye bio-sorption by the biomass. The COD of the given sample is found to be 780mg/lit.



De-colorization of the dye effluent by both isolates, the de-colorization of textile dye effluent with inoculums was studied in shaking condition. The isolated bacterial strain was inoculated at 37 ^{0} C in dye effluent, the shaking condition showed considerable reduction in colour. The de-colorization of the dye effluent by culture was significantly increased with incubation period from 3rd day.

Conclusion

Acid blue dye is degradable under aerobic conditions with a concerted effort of isolated yeast and fungi. Yeast de-colorized with 88% followed by fungi 83% color removal was done. We concluded that the isolated yeast strain which can able to decolorize azo dye effluent approximately equal to bacterial strain potentially.

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