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Microbial De-colorization of Acid blue 9: An Experimental Study

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Abstract: De-colorization of synthetic dye with microbes is environmental friendly and very low cost effective method. Microbial de-colorization is the ultimate way for controlling of textile pollution in the environment. Isolation and identification of dye de-colourizing isolates from natural environment as soil and food were carried out. Biochemical tests, growth curve and Plate assays were also conducted. Synthetic dye effluent preparation and its characteristics were also studied. Yeast strains can able to decolorize the synthetic dye approximately equal to isolated Bacterial strains as 83% and 85% is respectively. It is clearly evident from the results that yeast strains showed high performance as bacterial strains. This study was to investigate the comparison of de-colorization capacity of isolated Bacterial strains with isolated Yeast strains.
Key words: De-colorization, Synthetic dye, Acid blue 9, plate assay, Growth curve, Bacteria, Yeast.

Introduction

Many microorganisms including bacteria, yeast, algae and aquatic fungi, are found to be capable of degrading dyes at various concentration. Since 1985, the catabolism of aromatic hydrocarbons by microbes has been widely discussed. The typical pathway of acid dye degradation in microorganisms occurs via the formation of catechol derivative following by ring cleavage through the ortho-fission or meta-fission pathway. In this work, we characterized the capacity of an isolate strains to degrade not only acid dyes but also formaldehyde, another toxic pollutant. Although many microorganisms exhibit a catabolic operation on formaldehyde, a strain with effective operation on both toxic ingredients has been rarely reported. Increase in the world's population, placed pressure for the industries to reclaim and reuse some of its waste or face the prospect of being shutdown. This was due to the combined pressure of increasing water and waste costs and increasing regulatory requirements of discharged waste. The textile industry is one of that demands large quantities of water, produce large amounts of waste. Dye compounds are synthetic organic compounds with complex molecular structures and larger molecular weights. These properties augment treatment difficulties of dye waste. Dyeing wastewater causes serious environmental problems due to its high color, large amount of suspended solids and high chemical oxygen demand. The increase of public concern as well as tighter regulations has challenged the environmental research community to explore new lines in reducing environmental problems associated with such waste. At present, there are various technologies available in the treatment of textile waste. Among them are adsorption, ion-exchange, fenton oxidation, ozonation, coagulation and precipitation, aerobic and anaerobic process, membrane filtration. The negative aspects of these technologies are that either they lack the efficiency in reducing the many diverse pollutants in the dye waste or when the treatment technology appears to be promising, the cost of operation is very high thus prohibiting the

particular technology to be applied to the large scale waste common to this industry. Dyes are exclusively organic compounds. Most of the organic pigments are chemically and structurally related to dyes, confusion between dyes and pigments exist where, the terms dye and dyeing are much better known by the lay public that the more technical expressions pigments. Dyes are applied to various substrates as textiles, leather, paper, hair etc., from a liquid in which they are completely or at least partially soluble. In contrast to pigments, dyes possess a specific affinity to a given substrate. Synthetic dye solutions are used by most researchers in their investigation of treatment technologies since synthetic solutions was useful in obtaining information on how individual dyes react to different types of treatment. Apart from, constant composition of a synthetic solution enables the specific study of treatment efficiency on a particular treatment technology (1,2,3).

In general, there are three classifications of wastewater treatment methods. These are physical, chemical and biological treatment. The main drawback with these technologies is that they generally lack the broad scope of treatment efficiency required the capital cost, or operating cost become prohibitive when applied to the large scale water needs common to this industry. Biological methods are generally cheaper to apply in the removal of organics and colour from dyeing and textile waste water. However, dyeing wastewater cannot be readily degraded by the conventional biological processes such as trickling filters and activated sludge process, because the structures of most commercial dye compounds are very complex and many dyes are non-biodegradable in nature due to their chemical nature and molecular size.

Materials and Methods

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.

Dye effluent and soil samples

Soil samples and dye effluents for the isolation of micro-organisms were collected in and around dyeing factories, in Pallipalayam Erode district, Tamil Nadu, India, and samples were collected in sterilized bags, containers and stored for further analysis.

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 Nutrient agar medium for Bacteria. Yeast extract- Peptone- Dextrose YPD for yeast. The plates were incubated at 37°C in an incubator for 24 h for bacterial growth and 3 days for Yeast growth. The isolated micro organisms were further purified by subsequent sub culturing and maintained in slant culture at 4°C. Bacterial isolates used for decolorizing the textile dyes effluent were characterized both morphologically and biochemically (4,5).

Biochemical tests for isolated strains

Bacterial Strain

Gram straining- Gram positive; Motility test- Motile; Starch hydrolysis test- Positive;

Yeast Strain

Germ tube test- Positive

Decolourization of effluent using isolated bacteria

The bacterial isolates were maintained on the nutrient agar slants. This culture were inoculated in nutrient broth and kept under shaking condition (120 rpm) for 24 h. The 24h old cultures were used under

optimized conditions to decolorize the dye effluent. The pH was maintained at 7.0. Dye effluent with 10ml of Bacterial isolates culture with the addition of glucose and sodium nitrate OD values were measured for 5 days at 24, 72 and 120 h of interval at 628 nm in spectrophotometer.

Decolourization of dye effluent using isolated yeast

In order to test the potentiality of various known microbial agents on decolourization of textile dye effluent, an experiment was measured and conducted with yeast culture using a combination of carbon and nitrogen sources. Treatment was conducted with control. 10ml of sample were drawn from the inoculated treatment and the OD values were measured for 5 days at 24, 72 and 120 h intervals at 628 nm in the spectrophotometer. The percentage of colours reduction was measured (6).

Acid Blue 9

Synthetically Acid Blue 9 was collected for laboratory experiments from Himedia Laboratories pvt ltd, Vadhani, Mumbai.

Fig 1. Acid Blue 9 solution.

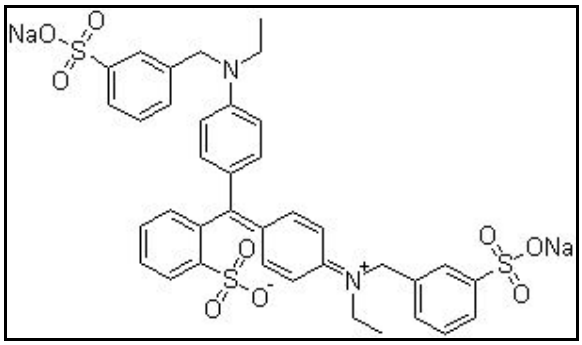


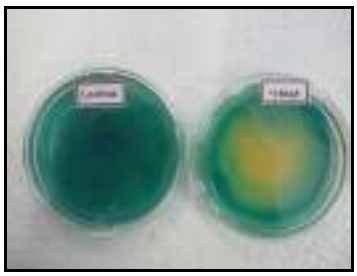
Fig 2. Molecular structure of Acid

Because of Benzene ring the electron de-colorization, then the colures are changed or decolorized. Functional group are transformed the electron such as R and N=N groups. IUPAC name-Di-azonium is soluble in water or ethanol molecular weight 792.85 molecular formula $C_{37}H_{34}N_2Na_2O_9S_3$ wavelength 628nm.

Plate assay of isolated Strains

Plate assay was performed for the detection of decolorizing activity of isolated strains. The Nutrient agar medium and acid blue 9 dye were autoclaved at 121^0C for 15 minutes. Isolated strains were nutrient agar medium plates containing Acid blue 9 (1g/l). The plates were wrapped with Para film and were incubated at 37^0C for 3 days. The plates were observed for clearance of the surrounding the colonies.

Bacterial Strain



Yeast strain

The growth of the bacterial and yeast strains and the decolourization percentage of the dye with bacterial and yeast strains in the course of incubation till 3 days. This culture was incubated in static and shaking conditions. After 24 hrs of incubation period the OD values were measure at 628 nm in spectrophotometer. The results of experiments conducted on decolourization of dye effluent by bacterial culture

showed 85% of colour removal on shaking condition after 168 hrs. The yeast strain exhibited a colour reduction of 83% for shaking condition after 168 h. With the culture time increased, the concentration of extracellular enzymes as enhanced, resulting in the complete de-colorization of the effluent in 168 h. The present study indicate that the isolates were approximately equal potential for the de-colorization of reactive synthetic dyes effluents, and that it might be a practical alternative for wastewater treatment of dyes in textile industry.

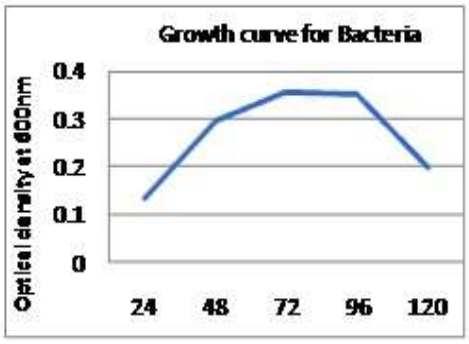
Analytical Methods

Cell growth was determined spectrophotometrically by measuring absorbance at 600 nm using a UV-spectrophotometer for growth and 628nm for de-colorization. Prepare control without inoculums can measure with the same absorbance.

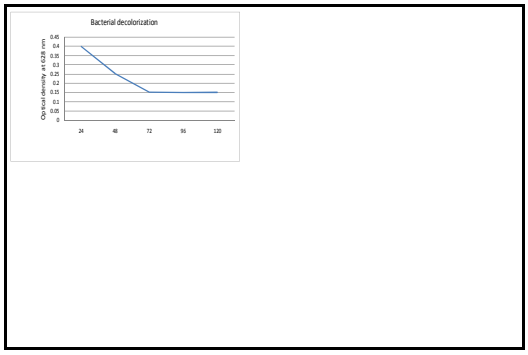
Growth Curve

The microorganisms' growth over time can be graphed as cell number versus time. This is called a growth curve. This curve typically has four distinct phases, and death phase. First, the lag phase was observed. It is characterized by increase in cell number; however, the cells are actively metabolizing, in preparation for cell division depending on the growth medium. The second phase of growth called the exponential (or) log phase. Rapid cell multiplication was takes place. The third stationary phase belongs to the cell growth as such the second phase but not in increase, constantly maintained. The final death phase belongs to the starting of cell death and the productions also decreased.

The above statements were observed on the all isolated strains as bacteria and yeast.

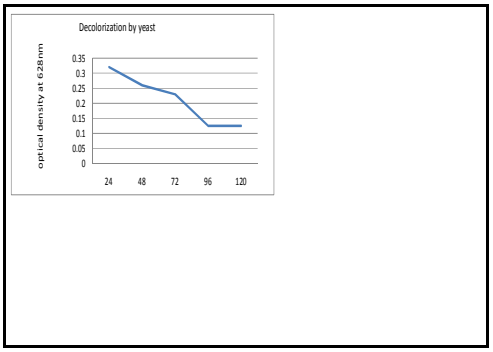


Growth curve for Bacteria.

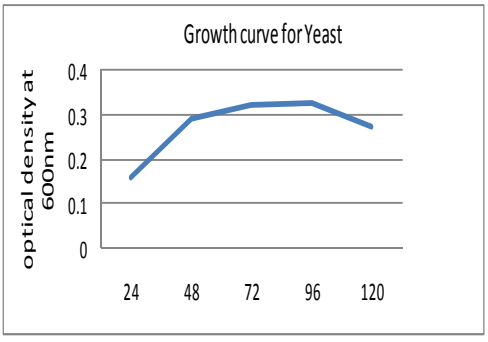


De-colorization using Bacterial strain

The isolated bacterial strain was maintained at Nutrient agar medium. During 120hrs incubation the maximum growth observed at 72hr and the optical density also maximum at 600nm also.



De-colorization using Yeast.



Growth curve for yeast strain

Isolated Yeast strain was maintained at Yeast Peptone Dextrose medium for preparing growth curve, the maximum growth was observed at 96hr and the optical density was noted at 600nm.

Results and Discussion

The strains Bacterial and yeast isolates were from contaminated soil were evaluated for its ability to decolorize industrial dye effluent. The growth of Bacteria and yeast 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 bacterial and yeast biomass. 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 yeast 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 bacteria and yeast. Bacteria should highest de-colourization with 85% followed by Yeast 83% colour 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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