International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.12, pp 5114-5117, October 2014

CBSE-2014 [2nd and 3rd April 2014]

Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Experimental Studies on Decolourization of Azo Dye in Textile Effluent by using Bio Remediation Technique

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Abstract: Studies were carried out on the decolorization of azo dyes by Bacillus species from effluent of textile industries. About 10 bacterial strains were isolated from the effluent of textile industries, Bacillus sp. showed remarkable ability in decolorizing the widely utilized azo dyes. Phenotypic characterization and phylogenetic analysis based on 16S rDNA sequence comparisons indicate that these strains belonged to the genus Bacillus subtilis. It showed nearly 90% decolorization ability within 3 days of incubation. Textile wastewater having diverse characteristics could be decolorized effectively using *Bacillus subtilis*. The Bacillus subtilis could decolorize azo dyes in a wide range of salt concentration (up to 20% w/v), temperature (25–40°C), and pH (5–11) after 4 days of incubation in static culture. Bacillus subtilis readily grew in and decolorized the high concentrations of dye (5000 ppm). UV–Vis analyses before and after decolorization and the colorless bacterial biomass after decolorization suggested that decolorization was due to biodegradation, rather than surface adsorption.

Keywords: Biodegradation; Bioremediation; *bacillus subtilis*; Textile effluents.

Introduction

The textile industry is one of the most important industries in Tamilnadu that generate a high volume of waste water. The water consumption in textile industry, especially in dyeing and washing processes, is too high. Therefore, large amount of wastewater is produced. Strong colour of the textile waste water is the most serious problem of the textile waste effluent. The disposal of these wastes into receiving water causes damage to the environment. Textile effluents are characterized with high COD (400-3,000 mg/L), BOD5 (200-2,000 mg/L), Total Solids (1000-10000 mg/L), Suspended Solids (100-1000 mg/L), TKN (10-100 mg/L), Total Phosphorus (5-70 mg/L), Conductivity (1000-15000 µS/cm) pH (5-10 usually basic) and possibly heavy metals and strong color.[(1)]

Classification of Dyes

Dyes are coloring pigment that imparts color to the substrate. All aromatic compounds absorb electromagnetic energy but only those that absorb light with wavelengths in the visible range (400-800 nm) are colored. Azo dyes are characterized by presence of nitrogen-nitrogen (N=N) in its chemical structure. The azo groups are generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocyclic groups.

Microbial Process

Various physicochemical processes have been found to be high cost, limited versatility, greatly interfered by other waste water constituents, and/or generate waste product that pose additional serious disposal problem. Microorganisms can breakdown most compounds for their growth and/or energy need. Complete degradation of any compound ultimately yields water and either carbon dioxide or methane. Incomplete degradation will yield breakdown products which may or may not be less toxic than the native pollutant.

Bioremediation can be defined as "a treatability technology that uses biological activity to reduce the concentration or toxicity of a pollutant. The biological techniques include bacterial and fungal bio-sorption and biodegradation in aerobic, anaerobic, anoxic or combined treatment process. The treatment of textile effluent containing dye has been carried out by various physical and chemical methods over the last two decades for the removal of color from waste water. These methods have limited applicability as they are expensive and lead to the production of solid waste. [(2)]

Effective Microorganism is the consortia of valuable and naturally occurring microorganisms which secretes organic acids and enzymes for utilization and degradation of anthropogenic compounds. These days, microbes are collected from the waste water and sludge which are believed to have the resistance against the hazardous compounds. This is particularly due to their tolerance capacity even at the higher concentrations of xenobionts.[(3, 4)]

Material and methods

Sample collection

The textile effluent was collected from a CETP unit from Ayyampet Kanchipuram, Tamil Nadu. The sample was collected in sterile glass-screw cap tubes and preserved at 4 ̊C in refrigerator.

Dyes and Chemicals

The dye Direct Orange 102 and chemicals used throughout the study was obtained from Sigma Aldrich, India. All the chemicals were of highest purity available and were of analytical grade.

Figure 1 Dye solution before & after Treatment

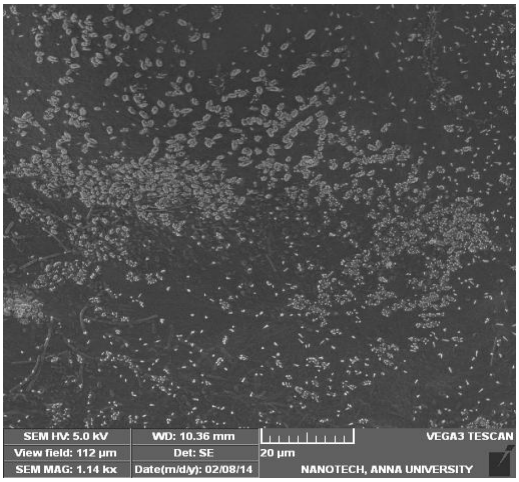

Figure 2. Sem Image of bacteria

Identification of the Bacterial Isolates

Identification of the bacterial isolates was carried out by the routine bacteriological methods i.e., by the colony morphology, preliminary tests like Gram staining, capsule staining, endospore staining, motility, catalase and oxidase, plating on selective medias and performing biochemical tests. The 16S rDNA sequences were initially analyzed at NCBI server using BLAST tool and sequences were down loaded. [(5)]

Experiments

Dye decolourization experiments were carried out in 100 ml flasks containing 50 ml of Direct azo dyes (500 mg/l), traces of yeast extract, sucrose and glucose. The pH was adjusted to 7±0.2 using sodium hydroxide

and hydrochloric acid solution. Then, the flasks were autoclaved at 121°C for 15 minutes. The autoclaved flasks were inoculated with 5ml of bacterial inoculum of each isolates. The flasks were kept in mechanical shaker and incubated at 37°C for 4 days. Samples were drawn at intervals for observation. 10 ml of the dye solution was filtered and centrifuged to separate the bacterial cell mass. Decolorization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wavelength maxima (λm = 480 nm) of dye. The percentage of decolorization was calculated. All experiments were done in triplicates. [6, 7]

Results and Discussions

1) **Effect of pH:** 100 ml of nutrient broth inoculated with *Bacillus subtilis* was amended with 250 mg/l of dye with varying pH from 5–9 were experimented. pH was adjusted using either HCl (0.1M) or Na_2CO_3 (0.1M). Results clearly indicate that the optimum pH for color removal is at slightly alkaline and the rate of color removal tends to decrease rapidly at strongly acid or strongly alkaline pH values.

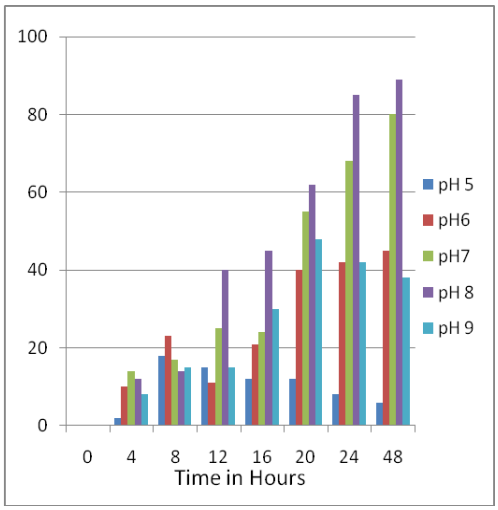

Figure 3 Dye decolorization at different pH

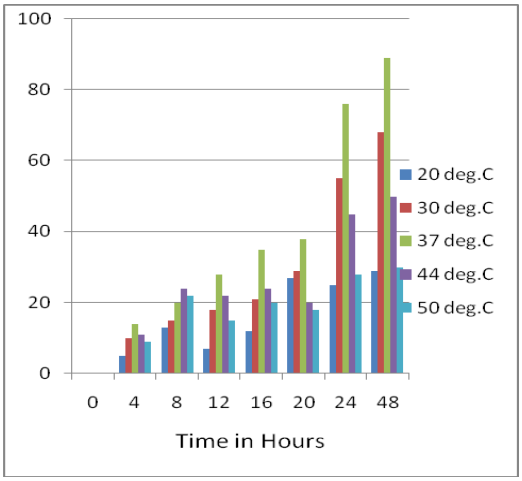

Figure 4 Dye decolorization at different Temperatures

2) **Effect of Temperature:** 100 ml of bacterial culture amended with 250 mg/l of dye under different temperature were determined. The rate of color removal increases with increasing temperature, within a range of 35-37^{o}C. The temperature required to produce the maximum rate of color removal tends to correspond with the optimum cell culture growth temperature of 30–37 ^{o}C. The decline in color removal activity at higher temperatures is attributed to the loss of cell viability or to the denaturation of the azo reductase enzyme.

3) **Effect of Agitation:** 1ml of bacterial cultures were transferred into separate 100 ml conical flask containing fresh nutrient medium containing Direct Orange (250 mg/l) and were incubated at 30 ^{o}C, under static condition. One set of flask was incubated under agitation at 180 rpm and temperature of 30^{o}C while the second set was incubated under stationary condition at 30^{o}C for a period of 48 hours. The uninoculated dye Medium supplemented with respective dye served as the control. There was a higher decolorization in shaking condition than in the static condition due to the better oxygen transfer and even nutrient distribution.

4) **Effect of Concentration***:* Various concentrations of dye (50 to 500mg/l), and inoculum sizes of were used to examine the effect of initial dye concentration and inoculums size on the decolorization rate. *Bacillus subtilis* was cultivated for 48 h in conical flask containing 100 ml nutrient broth. Incubation was done at 30^{o}C. The UV and visible spectra of the samples were measured in ethanol with a UV-Vis double beam Spectrophotometer. Hydrogen discharge tungsten filament lamp was used as a source of light and maximum absorbance was recorded. The optical densities (OD) measured were then converted to the dye concentrations using the respective standard curves.

Conclusion

The biodecolorization process studied presents a feasible and economical method of treating colour effluents. Results show that textile wastewater having diverse characteristics could be decolorized effectively using *Bacillus subtilis*. The dye is degradable under aerobic conditions with a concerted effort of bacteria

isolated from textile dye effluent. Nutrients (carbon &nitrogen sources) and parameters (pH, temperature and inoculum size) had significant effect on dye decolorization. *Bacillus subtilis* showed highest decolorization of dye effectively during optimization and *Bacillus subtilis* showed consistent decolorization of the dye throughout the study. Interestingly, the bacterial species *Bacillus subtilis* used in carrying out the decolorization of Direct Orange dye in this study showed promising results. The ability of the strain to tolerate, decolorize dyes at high concentration gives it an advantage for treatment of textile wastewaters.

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