

Isolation and Screening of Water Microbes For Decolourisation of Textile Dye Waste

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ABSTRACT

Azo dyes are widely used in textile industry. Unused dyes, consisting mainly non biodegradable released along with waste water streams without any proper pre-treatment which cause nuisance for environment and accumulate in flora as well as fauna. These also exhibit allergic, carcinogenic and mutagenic properties for human beings. Isolation and screening of azo dye degrading bacteria are economic in biodegradation and detoxification. In the present study, 200 waste water samples were collected from dye-contaminated sites of textile industries and bacterial species such as *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Psuedomonas putida* were isolated and identified. Evaluation of decolorizing properties of these bacteriae were done by UV-Vis spectroscopy (A_{\max} 596 nm) in different concentrations using different carbon sources such as Hans's medium and GYP medium. Maximum decolourisation of 0.1% azo dyes were recorded to be 89.0%, 91% and 86% in Hans medium containing charcoal source by *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Psuedomonas putida* respectively at 24 hrs. These bacterial isolates may be utilized in large scale for pre-treatment for ecological balance by avoiding water pollution.

Key words: Azo dyes, *Pseudomonas aeruginosa*, Biodegradation, Detoxification, Hans's Medium, Ecological Balance.

INTRODUCTION

In present days, Azo dyes also cause a serious environmental pollution by release of waste water through textile industrial¹. The annual production of azo dyes is estimated to be around one million tons, and more than 2000 different azo dyes are currently in use². Azo dyes are used in a number of industries such as textile dyeing, food, cosmetics, paper printing, with the textile industry as the largest consumer³. In Textile industry, large amount of water and variety of chemicals are used⁴. The huge amount of water is used in many processes such as cleaning, rinsing, dyeing and washing⁵. Due to use of huge amount of water in textile industries, water layer fall near textile industrial areas. Farmer's have only option to use polluted water for agriculture and also used in day to day work. Therefore, by using

of this polluted water, different types of disease occur in humans as well as in animals⁶. Not only that agriculture crop also affected by this waste water⁷. So, treatment of such waste water could help to meet irrigation requirement of the crops. The discharge of these highly colored industrial effluents is very dangerous when it fall in water resources, as these dyes in the water absorb sunlight, which decreases the intensity of light absorbed by aquatic plants, ultimately reducing photosynthesis and the oxygenation of water reservoirs⁸⁻⁹. Physical appearance of the colored water gives bad impact on aesthetic value. These dyes are xenobiotic in nature and in some cases, these are mutagenic and carcinogenic¹⁰⁻¹¹. Allergic effects and health problem in human due to these dyes have also been reported by several scientists¹²⁻¹⁵ so, Azo dyes contamination has emerged as a serious issue

due to negative impact on water ecosystems and human. Different factors *i.e.*, high water solubility, high molecular weight and fused aromatic ring structures, which reduced degradation of dyes and inhibit penetration in biological cell membranes¹⁶⁻¹⁸. Various physiochemical methods can be used for the removal of azo dyes from the wastewater¹⁹. Some of these methods are effective but are quite expensive because they generate significant amounts of chemical sludge waste. This chemicals waste causes disposal problem and also limit the use of these methods²⁰⁻²². Therefore, in such situations, biological treatment may be a beneficial and being eco-friendly. Microorganisms have developed enzyme system for the decolorization and neutralization of azo dyes under certain conditions²³⁻²⁵. So, present study was designed to isolate efficient azo dye decolorizing bacterial strains from the textile effluents. Since the bacterial isolates were originated from the dye contaminated textile wastewater of local industry, so they can easily adapt to the prevailing local environment. Therefore, such bacteria can be used to develop an effective biological treatment system for the waste waters contaminated with azo dyes.

MATERIALS AND METHODS

Water Sampling

Sampling sites were selected on the basis of outlet drain from textile dyeing units. 200 samples of Textile waste water (TWW) were collected from areas

of Nathnagar of Bhagalpur, Bihar, India nearby many small textile dyeing units are actively operating.

Isolation and Screening of Bacterial Strains

Bacterial strains were cultured from TWW by using MSM agar medium. The culture medium containing 50 ml MSM agar medium was inoculated with 10 ml of TWW then incubated at 35°C for 7 days till the appearance of microbial colonies. 150 actively growing colonies were randomly selected for screening of azo dye decolorizing bacteria and stored in refrigerator for further studies²⁵.

Screening of Azo dye Decolorizing Bacteria

Microbial colonies that appeared on the agar medium were washed gently with sterile water and re-suspended into the flasks containing fresh glucose peptone yeast (GPY) and Hans's broth media with azo dye at a concentration of 0.1%. The culture media containing 200 ml of GPY and Hans's broth in 500ml in Erlenmeyer flasks were inoculated with selected bacteria and incubated at 35°C for 36 hrs under static conditions. The respective cells were harvested by centrifugation at 10000 rpm for 10 min then decolorization was determined using spectrophotometer at 596 nm.

Optimization of Different Carbon Source for Enhancement of Decolorization

Carbon sources (charcoal, glucose, fructose, sucrose, maltose, xylose, manitol,

Table 1: Decolorization of azo dye (0.1%) in different carbon source in Hans media

Carbon Source (5gm /L)	% Decolourazation		
	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Psuedomonas putida</i>
Glucose	71.06	89.5	85.5
Fructose	64.84	80.0	78.7
Sucrose	55.30	60.0	65.8
Charcoal	89.00	91.0	86.0
Maltose	50.96	55.4	60.2
Xylose	35.73	50.0	50.4
Manitol	34.67	65.0	59.0
Cellulose	43.47	40.0	45.0
Starch	40.15	54.0	40.2
Lactose	33.56	30.8	30.1

cellulose, starch and lactose) at the concentration of 5 gm/L were used for microbial isolates for enhanced decolorization of dyes. Optimization analysis was carried out using broth medium while culture conditions were used in screening of azo dye decolorizing bacteria.

RESULTS AND DISCUSSION

Three different bacterial colonies (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*) were isolated from TWW. Primary screening of dye decolorizing bacteria was assessed on Han's medium using different carbon source at the concentration of 5gm/L on 0.1% azo dye. In charcoal carbon source maximum decolorization i.e., 89.0%, 91% and 86% by different bacteria i.e., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas putida* were observed respectively. In Lactose

carbon source minimum decolorization by different bacteria 33.56%, 30.8% and 30.1% were observed respectively in lactose carbon source (Table 1). In GYP medium by using different carbon source (5gm/L) on 0.1% azo dye were used and maximum decolorization by different bacteria 70.23%, 75.0% and 65.0% were observed respectively in charcoal carbon source. Minimum decolorization by different bacteria 32.00%, 30.8% and 34.1% were observed respectively in lactose carbon source (Table 2).

Second screening was performed by using Hans medium with Charcoal (5gm/L) carbon source at different time interval incubations to different bacteria i.e., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*. It was observed that maximum decolorization was at 24 hrs time interval by all three bacteria but all of them *Pseudomonas aeruginosa* has maximum decolorization capacity (Table-3).

Table 2: Decolorization of azo dye (0.1%) in different carbon source in GYP media

Carbon Source (5gm /L)	% Decolorization <i>Bacillus subtilis</i>	% Decolourazation by <i>Pseudomonas aeruginosa</i>	% Decolourazation by <i>Pseudomonas putida</i>
Glucose	69.21	79.5	60.5
Fructose	65.60	80.0	75.7
Sucrose	60.46	61.0	60.8
Maltose	54.39	52.4	59.3
Charcoal	70.23	75.0	65.0
Xylose	48.92	49.0	52.4
Manitol	39.22	61.0	55.0
Cellulose	38.11	37.0	40.0
Starch	34.96	50.0	39.5
Lactose	32.00	30.8	34.1

Table 3: Different percentage of decolorization by different bacteria at different time intervals on Han's medium

Time (hr.)	% Decolourazation by <i>Bacillus subtilis</i>	% Decolourazation by <i>Pseudomonas aeruginosa</i>	% Decolourazation by <i>Pseudomonas putida</i>
0	0	0	0
6	35	40	38
12	55	63	58
18	81	82	80
24	89	91	86
30	80	85	82
36	75	77	73

Earlier it was also reported that decolorization of Azo dye occurs by photosynthetic bacteria²⁶. Further the impact of slik dye waste and distillery effluent have also been evaluated on different test animals²⁷⁻²⁸.

CONCLUSION

On the basis of above study, it concludes that *Pseudomonas aeruginosa* is a best decolorizer of Azo dye in Hans's medium of charcoal carbon source as compare with *Basillus subtilis* and *Psuedomonas putida* in GYP medium at 24hrs.

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