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DEGRADATION OF AZO DYE BY DYE-DEGRADING BACTERIA

Chaturwedi Shashi Bhushan, Maharjan Rubi and Chaudhary Richa*

Department of Microbiology, D.A.V. College, Lalitpur, Nepal

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Synthetic aromatic compounds consisting of various functional groups are known as dyes. These colored compounds are often discharged in effluents, and they are very dangerous to aquatic life. The study was carried out to study potential azo dye-degrading bacteria. Bacteria were isolated from 9 soil and 7 effluent samples collected from different parts of Kathmandu Valley. Isolated bacteria were tested for dye tolerance and dye-tolerant bacteria were subjected to decolorization of three acid dyes: acid yellow 25, acid green 23 and acid orange 7. A change in optical density was observed to study the impact of inoculum concentration. All 19 isolated potential bacteria were identified by morphological and biochemical tests. Among these, 6 (60%) of Gram-positive and 4 (50%) of Gram-negative bacteria were dye tolerant. Three bacteria SO2-3W, SO3-4W and S16-4P were able to decolorize acid green 23. The impact of the increase in inoculum concentration was increased in decolorization percentage. SO2-3W, SO3-4W and S16-4P were identified as *Bacillus* spp. and *Pseudomonas aeruginosa,* respectively. The ability of *Bacillus* spp. and *Pseudomonas* spp. to tolerate and decolorize textile dyes at high concentrations encourages their use as a potential bioinoculant in the treatment of textile wastewater.

Keywords: Azo dye, *Bacillus* spp., *Pseudomonas aeruginosa*, Textile, Wastewater

INTRODUCTION

Dyes are compounds that contain chromophore and auxochrome groups. Chromophore groups of dye are saturated and are responsible for colour. Auxochrome groups are responsible for the reaction of dye with the surface to be dyed (1). Industries like textile, tannery, paper, pulp, paint, electroplating and leather, used different dyes. Direct discharge of industrial effluent is detrimental to both aquatic and terrestrial ecosystems. These colored effluents discharged into rivers and lakes changes the physicochemical properties of water, including pH, light penetration, electric conductivity, COD, and BOD (2). Dye bioaccumulation at optimal tropic levels of the food chain may lead to their transport and distribution over some time. Due to the chemical and photolytic stability, synthetic dyes are found to be highly persistent in the natural environment. Where these chemicals play the role of making healthy human cells turn into cancer cells in the lab. Human studies on azo dye-based hair dyes and cancer risks are mixed (3). Some studies have reported an increased risk of certain cancers in people, who work with dye. Human studies on azo dye-based hair dyes and cancer risks are mixed (3). However, various studies about bioremediation of wastewaters have been constantly conducted over years (4). Degradation of azo dyes by Bacteria, begins with reductive cleavage of the –N=N-bond as the initial step. Generally, decolorization of azo dyes occurs under anoxic and aerobic conditions by different types of bacteria. The problems related to physicochemical methods of textile waste management cover production of sludge, and toxic byproducts, huge infrastructure requirements, high budget requirements and limited applicability (5). Biotic methods are an attractive alternative method to control colored wastes. They are relatively easy to handle, low cost, environmentally friendly and the by-products that are produced during metabolic activity are less toxic in nature The study was performed with the objective to assess the potential azo dye degrading bacteria.

MATERIALS AND METHODS

Collection of samples: All 7 effluent and 9 soil samples were collected from different industries in clean collection bottles. Three azo dyes were used in this study, consisting of acid dyes acid orange, acid yellow and acid green. The work was done in the Microbiology Lab of D.A.V. College from February 2022 to April 2022.

Isolation of organisms: Bacteria were isolated from effluent and soil samples on fresh nutrient agar media plate, following standard bacteriological culture method by serial dilution and pour plate technique. The single colonies were streaked on each of three sterile nutrient agar medium, containing 0.5g/L (weight/volume) acid orange, acid yellow and acid green respectively followed by 24 hours incubation at 37°C. Bacterial strains which were able to grow in dye containing agar plates which were inoculated in 20 ml of freshly prepared nutrient broth medium amended with individual acid dyes (50 mg/ L) (6).

Screening of dye degrading activity: The inoculated test tubes were incubated at room temperature (23°C–25°C) under static conditions along with an un-inoculated control. Aliquots from each tube were taken 7 days after incubation, then subjected to centrifugation at 4000 rpm for 15 minutes. After centrifugation, supernatants absorbance was measured at wavelength of 520 nm using a colorimeter. The percentage decolorization of initially added acid dye was calculated using the formula given below.

$$\text{Percentage Decolourization} = (A-B)/A \times 100$$

Where A is the absorbance of un-inoculated control and B is the absorbance of inoculated sample (7). On the basis of the potential to decolorize, three different acid dyes, the most efficient dye decolorizing bacterial isolate that is 3 bacterial isolates were selected for studying the impact of different concentrations of inoculums for further screening. Inoculum concentrations of 1/50 (volume/volume), 1/100 (volume/volume), and 1/200 (volume/volume) of bacterial isolates were inoculated to each test tube. Aliquots from each tube were taken after different time intervals and subjected to centrifugation (4000 rpm for 15 minutes). After centrifugation, the absorbance of supernatants was measured.

**Corresponding Author: Richa Chaudhary, Microbiology Department, D.A.V. College, Lalitpur, Nepal; E-mail: san143ric@yahoo.com*

For identification of the potential bacteria, morphological characteristics of bacteria such as margin, size, shapes, type of colony, nature of colony (mucous, rough, smooth, transparent, etc.) of degrading bacteria were observed. Gram's staining and biochemical tests were done (8).

RESULTS

Eighteen morphologically different bacteria were isolated. The Gram staining of all bacterial isolates indicated growth of 10 Gram-positive bacteria and 8 Gram-negative bacteria (Figure 1).

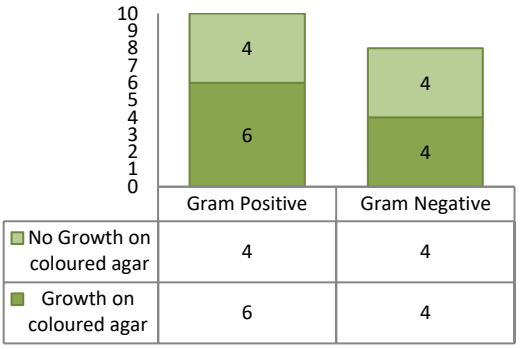

Figure 1: Growth of Bacterial isolates on 0.5g/l dye-amended Nutrient Agar plates.

All isolates decolorized acid green dye having different potential, which ranges from 2.94 % to 58.82% (Table 2). All isolates changed acid yellow dye to darker colours (Table 3). The optical density of the acid yellow dye solution increased which showed negative decolorization.

The three most effective bacteria were selected to assess the impact of inoculum concentration. The isolate SO2-3W decolourized acid green with decolourization percentage of the highest of 63.72% at 1/50 inoculum concentration (Figure 2).

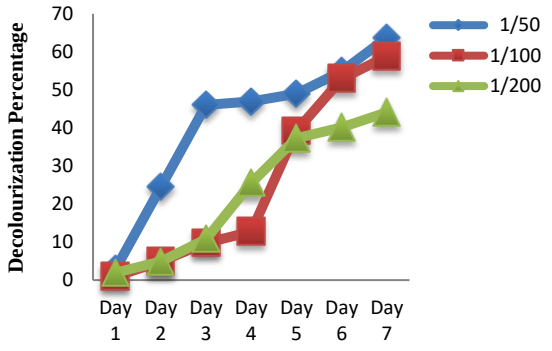

Figure 2: Decolourization % of SO2-3W at different concentration.

The isolate SO3-4W decolourized acid green with decolourization percentage of highest of 46% at 1/50 inoculum concentration. The decolourization percentage by this isolate was comparatively less than other two isolates (Figure 3).

These results showed the most efficient dye decolorizing isolate was S16-4P exhibiting the highest potential of decolorization with 69.1% decolorization at an inoculum concentration of 1/50. Decolourization percentage was relatively lower at 1/100 and 1/50 concentrations for all three isolates S16-4P, SO2-3W and SO3-4W (Figure 4).

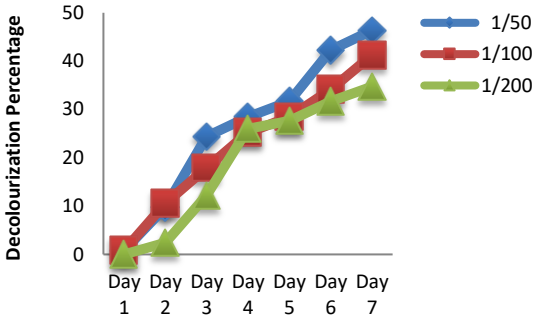

Figure 3: Decolourization % of SO3-4W at different concentrations.

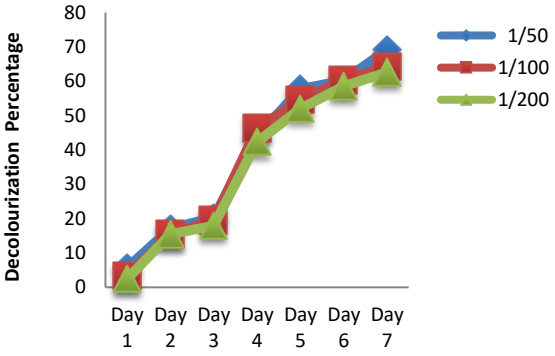

Figure 4: Decolourization % of S16-4P at different concentration.

The decolourization rate of S16-4P was not much altered with the change in concentration of inoculums. In SO2-3W at 1/50 concentrations, the decolourization percentage escalated highly up to 3 days. However, the growth in other bacterial isolates was almost parallel regardless the concentration of inoculums.

The three most efficient bacteria capable to degrade acid green 25 were identified with Gram's staining, colony morphology, and biochemical tests as represented in appendix IV. Both SO2-3W and SO3-4W showed similar morphological characteristics on nutrient agar plates and identical biochemical reactions. SO2-3W and SO3-4W both were Bacillus sp. isolated from two different samples. Bacterial isolate S16-4P was a Gram- negative, bacilli shaped, catalase positive, oxidase positive, indole negative, MR negative, VP negative, Simmon Citrate test positive, and Motility Test positive and identified to be

Table 1: Percentage Decolourization of 0.5g/l Acid Green.

Bacterial isolates	Optical Density		Percentage of Decolourization
	Initial (A)	Final (B)	
SO2-3W	1.02	0.42	58.82%
SO2-4W	1.02	0.92	11.8%
SO3-4W	1.02	0.70	31.37%
SO5-3W	1.02	0.99	2.94%
SO6-3W	1.02	0.98	3.92%
SO7-3W	1.02	0.79	22.55%
S10-3W	1.02	0.99	2.94%
S12-3W	1.02	0.85	16.67%
S14-4C	1.02	0.80	21.57%
S16-4P	1.02	0.53	48.1%

Table 2: Antibiotic-resistant pattern of presumptive identified Enterobacteriaceae from poultry wastes.

Bacterial isolates	Optical Density		Percentage of Decolourization
	Initial (A)	Final (B)	
SO2-3W	0.91	1.43	-57.14 %
SO2-4W	0.91	0.92	-1.09 %
SO3-4W	0.91	1.33	-46.15 %
SO5-3W	0.91	0.92	-1.09 %
SO6-3W	0.91	0.93	-2.19 %
SO7-3W	0.91	1.79	-96.70 %
S10-3W	0.91	0.90	1.09 %
S12-3W	0.91	1.03	-0.13 %
S14-4C	0.91	0.99	-8.79%
S16-4P	0.91	1.80	-97.80 %

Table 3: Percentage Decolourization of Acid Yellow.

Bacterial isolates	Optical Density		Percentage of Decolourization
	Initial (A)	Final (B)	
SO2-3W	0.19	0.26	-36.84 %
SO2-4W	0.19	0.2	-5.26 %
SO3-4W	0.19	0.12	36.84 %
SO5-3W	0.19	0.19	0
SO6-3W	0.19	0.19	0
SO7-3W	0.19	0.28	-47.36 %
S10-3W	0.19	0.2	-5.26 %
S12-3W	0.19	0.2	-5.26 %
S14-4C	0.19	0.23	-21.05 %
S16-4P	0.19	0.86	-352.6 %

Pseudomonas aeruginosa.

DISCUSSION

SO2-3Wwas isolated from dumping site and it might be degrading the azo dye present in waste materials of dumping site. Similarly, SO3-4W was isolated from soil from Taudaha; place was polluted with different textile and plastic materials. waste of textile industry to degrade the textile dyes methyl red and navy blue. In aerobic biodegradation of azo dye utilizing bacteria, the dye molecule is separated into peripheral and central pathways. The peripheral pathways, a huge variety of molecules are converted into few central intermediates. Aerobic mechanism involves the replacement of other functional group of the aromatic ring with hydroxyl group, followed by cleavage and incorporating 2 oxygen atoms. The reduction reaction causes catalytic cracking of conjugated dye bonds by reductase.

The degradation of acid yellow and acid orange was not attained by the aerobic reduction by the isolated bacteria. However, acid green effectively decolorized with decolorization percentage ranging from 69.1% at inoculums concentration of 1/50 (v/v) by bacterial strain S16-4P to 2.94% at inoculums concentration of 1/50 by SO5-3W. The effect of decolorization depended upon the inoculums concentration because inoculum level of decreased the decolorization level also decreased.

This study correlates with the result of Kumar et al. (9) where percentage decolorization increased with increased inoculums concentration. Bhimani (7) identified 5 dye degrading bacteria as *Pseudomonas* sp. among 37 dyes decolorizing bacterial isolates. Zissi (10), stated that *B. subtilis* co-metabolizes p-aminoazobenzene in the presence of glucose as carbon source, producing aniline and p-phenylenediamine, as the nitrogen-nitrogen double bond is broken. *Bacillus subtilis* HM, able to decolorize aerobically eight different sulfonated azo dyes.

Decolorization of Fast Red was achieved through microbial degradation rather than biosorption or adsorption as indicated by the uncolored biomass or its methanol extracts (11). Degradation percentage of reactive blue and reactive black by *Bacillus subtilis* was 90% and 70 % in the study carried out by Bhimani (7). The optimization of the metabolites, carbon substrates and other enzymes helped attain higher percentage of decolorization. The higher temperature causes thermal inactivation of proteins in the bacterial cell structures such as the cell membrane. In a study conducted by Affrin & Affrin (12), the optimum temperature for decolorization of reactive green was 37°C in 9 days with 67.3% by *Pseudomonas aeruginosa*. Factors as incubation temperature is important to produce the maximum rate of color removal as it tends to correspond with the optimum cell growth temperature which is in between 35-45°C. The decolorization percentage of 69.1% was obtained in 7 days by *Pseudomonas aeruginosa.*

The specific ability of *Pseudomonas aeruginosa* of bio-decolorization of even at lower inoculum concentrations makes this strain a worthwhile bio-resource for the treatment of textile wastewater. The existing problems of wastewater treatment in Nepal can be solved using bacterial dye degradation techniques inappropriate and viable ways (13). The isolated and identified bacterial strains were found to be more effective and have potential of textile degradation under versatile conditions.

CONFLICTS OF INTERESTS

The authors have declared that no competing interests exist.

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