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BIODEGRADATION OF DISPERSED HETEROCYCLIC TEXTILE AZO DYE BY BACTERIA ISOLATED FROM DUMPING GROUND NEAR BOISAR

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Abstract

Azo dyes are the most important synthetic colorants which have been widely used in textile, printing, paper manufacturing, etc. One of the class of azo dyes that finds a wide application in the textile industry for its intense colouration and excellent dyeing properties is Dispersed heterocyclic azo dyes. The extensive use of these dyes has added to one of the major environmental pollutants causing harm to flora and fauna of the ecosystem in which it is released as effluent from the textile industry. In the present study the disperse heterocyclic azo dye was decolourized by the bacteria isolated and selected from the dumping grounds of Khaira Phata, Boisar by Replica plate technique. The growth of bacteria and decolourization of the dispersed heterocyclic azo dye was observed around the colonies obtained on replica plates embedded with 400 ppm of Disperse Blue. Optimization of the conditions (Temperature and Static and shaker conditions) was done to efficiently decolourize the Disperse Blue. The % efficiency of decolourization was determined spectrophotometrically at 580 nm. Culture No. 3 decolourized the dye efficiently to 53.75% under Static conditions at room temperature.

Introduction

Azo dyes are the most important synthetic colorants which have been widely used in textile, printing, paper manufacturing, etc. Chemically they are prepared by simple methods of diazotization and coupling. These dyes consisted of one or many -N=N- (azo) groups. Different modifications are made to obtain the desired color properties, yield and particle size of the dye for improved dispersibility.(4)

One of the class of azo dyes that finds a wide application in the textile industry for its intense colouration and excellent dyeing properties is Dispersed heterocyclic azo dyes.
(5).

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Due to its excellent light fastness, resistance to heat, these dyes are mainly used for dyeing polyester, cotton and nylon fabrics.

The extensive use of these dyes has added to one of the major environmental pollutants causing harm to flora and fauna of the ecosystem in which it is released as effluent from the textile industry. It has proved carcinogenic and allergic to the people working with these dyes in the textile industry. It has led to eutrophication of the water bodies leading to disturbing the pond ecosystem and death of the fishes as its solubility in water is very less.

Due to its hazardous effects on the ecosystem, measures are taken to detoxify the dispersed azo dyes by physical, chemical and biological methods. Amongst the three methods and techniques used to detoxify the dispersed azo dyes, the biological method for detoxifying the dyes proves to be useful.(7)

The bioremediation of azo dyes is a reliable approach to remove or degrade these recalcitrant chemicals. Microbial detoxification using bacteria and fungi is preferred as it is less costly and the microbes can be reused in the detoxification process. Also minimum space is required for the treatment of the effluent contaminated by the dispersed azo dyes.(8)

The present study aimed to test the biodegradation efficiency of Dispersed heterocyclic azo dyes by the bacteria isolated from the dumping ground near Boisar. The degradation of dye is indicated by the decolourization of the dye by the selected bacterial isolates. As the dumping site in Khaira Phata, Boisar is dumped with the textile and other xenobiotic waste it serves as a source of the dye degrading bacteria.

Materials and Methods

Sample collection

Surface soil was collected from the dumping site loaded with xenobiotic wastes like textile waste near Khaira Phata, Boisar. Soil samples from 4 different areas from the same dump yards were collected in order to have varied pollution of the bacteria.

Dye Preparation

Dispersed heterocyclic Azo dye i.e, Disperse Blue used in textile was obtained from m/s Rangwala in Crawford market. λ max of Disperse Blue was detected by using UV Spectroscopy by determining the for selection of the dye.

400 ppm of the dispersed blue dye was prepared in distilled water and autoclaved.

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Isolation And Selection Of The Dye Degrading Organisms

The soil sample collected was serially diluted using sterile saline. 10 fold dilutions were prepared and 10^{-7},10^{-8}, 10^{-9} were plated on sterile nutrient agar plates and incubated at room temperature for 24 hrs. After 24 hours of incubation the colonies obtained were transferred on the sterile medium containing Nutrient agar and 400 ppm of the dispersed Blue dye by replica plate technique. The replica plates were incubated at room temperature for 24 hours.

After 24 hours of incubation the pure colonies obtained on replica plates containing 400 ppm of the dispersed blue heterocyclic dye were selected and studied for their microscopic, cultural (using simple and selective media) and biochemical (like sugar fermentation, Enzyme activity, IMViC and T.S.I) characteristics. The selected colonies were transferred in sterile nutrient broth containing 400 ppm of the dispersed blue heterocyclic azo dye for scaling up the culture.

Optimization and Dye Degradation Efficiency Assay

The efficiency of the dispersed blue heterocyclic azo dye was determined by carrying out Dye Degradation Efficiency Assay.

Concentration of 400 ppm dye was prepared using 500 ppm of the stock dye in sterile nutrient broth. The cultures selected were optimized for determining the dye degradation efficiency of the isolates under parameters like temperature - (room temperature and 37^{o}C) and shaker and static condition. 1%, 3% and 5 % cultures were added to the dye. Dye degradation was noted by determining the decolourization at 580 nm using UV-Visible spectrophotometer.

Observations

The bacterial isolates were selected by replica plate technique as they showed the zone of decolourization around the colonies obtained on the selective plate containing 400 ppm of dispersed blue heterocyclic azo dye.

The microscopic, cultural and biochemical characteristics were studied by carrying the Gram staining for microscopic determination, use of simple and selective media for cultural characteristics and different biochemical characteristics including sugar fermentation, enzyme activity and IMViC and T.S.I,respectively.The isolates were named by their numbers. All the sets were incubated at room temperature for 24 hours.

Gram staining and morphology of the isolates obtained on Nutrient Agar containing 400 ppm of the dye system incubated at room temperature for 24 hours.

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Microscopic observations

Culture Nos.	Gram nature	Morphology	Arrangement
1	Gram negative	Short thick rods	In duplets
2	Gram positive	Short rods	In singles
3	Gram positive	cocci	In chains

Cultural characteristics were determined by isolating the selected organisms on a simple medium and the selective media.

Culture Nos.	Name of the Media					
	Nutrient agar	**Mac Conkey's agar**	**Starch agar**	**CLED agar**	**EMB agar**	**Salt mannitol agar**
1	1 mm; Pale yellow	1 mm; Pink color	Luxuriant growth; Decolorization observed around the colonies (Starch hydrolysis)	1 mm; Pink color	Black centered colonies after 24 hours	No growth
2	Pinpoint; Pale yellow	No growth	Luxuriant growth; Decolorization observed around the colonies (Starch hydrolysis)	No growth	No growth	Pinpoint; yellow
3	Pinpoint; white	Pinpoint; pale yellow	No decolourization (starch not hydrolysed)	Pinpoint; Pink	No growth	No growth

Biochemical Characteristics

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Culture Nos.	Sugar utilization						Enzymatic		MR	VP	Indole	Citrate	T.S.I			
	Glucose		Mannitol		Sucrose		Catalase	Urease					Slant	Butt	H_2S	Gas
	Aerobic	Anaerob ic	Aerobic	Anaerob ic	Aerobic	Anaerobic										
1	Acid gas	Acid gas	Acid gas	Acid gas	Acid gas	Acid gas	+	-	+	-	-	+	Acidic	Acidic	-	+
2	Acid	Acid	-	-	Acid	Acid	+	-	-	-	-	-	Acidic	Acidic	+	-
3	Acid	Acid	-	Acid	Acid	Acid	+	+	+	-	-	-	Acidic	Acidic	+	-

Optimization and Dye Degradation Efficiency Assay

%Efficiency of dye degradation=C0-C1/C1x 100

Where C0= initial absorbance before decolourization by bacterial isolates

C1= final absorbance after decolourization by bacterial isolates.

(2)

Culture No.	Efficiency of dye degradation (%)											
	Room Temperature						37°C					
	1% static	1% shaker	3% static	3% shaker	5% static	5% shaker	1% static	1% shaker	3% static	3% shaker	5% static	5% shaker
1	46.6	16.1	44.6	24.2	35.5	1.61	1.07	24.19	25.81	49. 46	44.46	25.81
2	39.34	34.01	48.48	36.65	39.44	31.47	42.39	34.82	25.38	40.76	7.61	45.6

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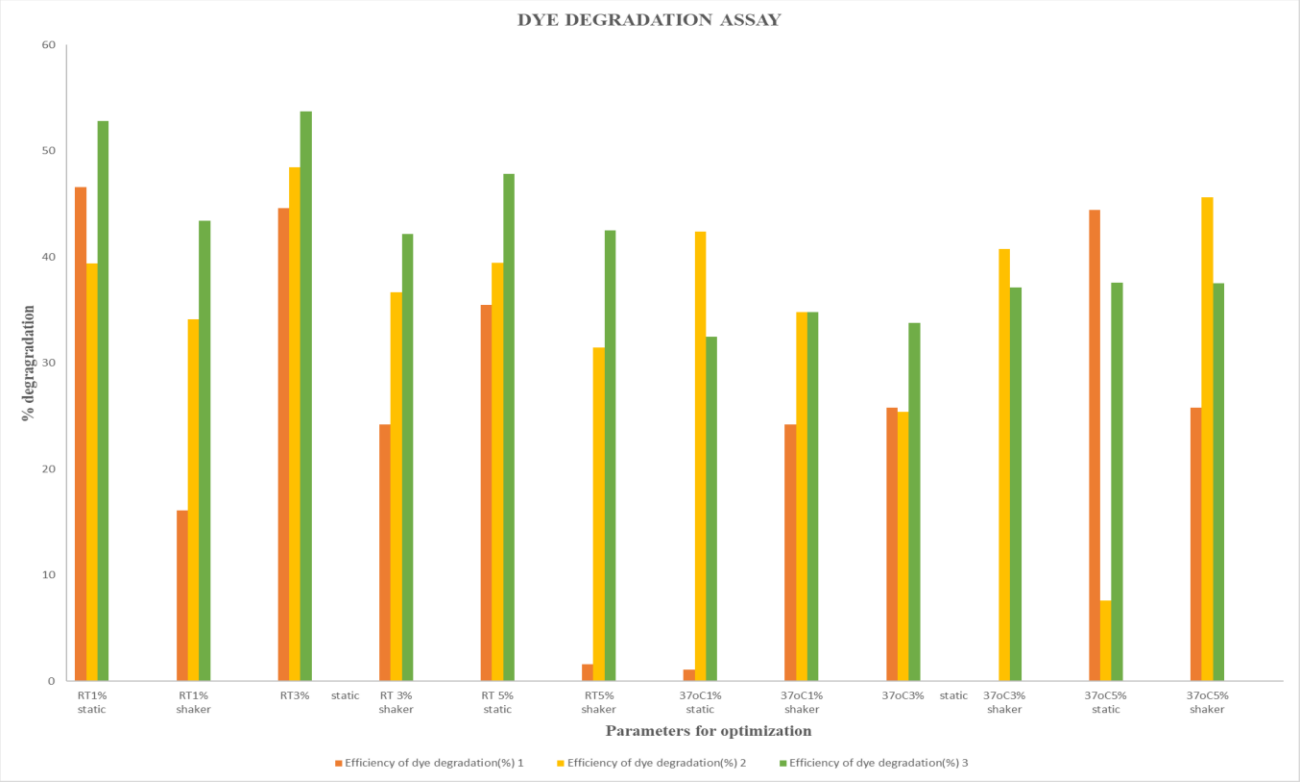
3	52.84	43.42	53.75	42.16	47.85	42.51	32.46	34.78	33.80	37.10	37.59	37.52

Graph:



Results

In the present study the three bacterial isolates isolated from the soil collected from the dumping yards of Khaira Phata, Boisar and selected by replica plate technique were found to be Culture 1- Gram negative short thick rods in duplets, Culture 2 - Gram positive short rods and Culture 3- Gram positive cocci in chains to decolourize Disperse Blue heterocyclic azo dye efficiently which was determined by appearance of the zone of decolourization around the colonies. The results obtained in the present studies were similar Sadia Zafar *et al*,(3) where *Pseudomonas spps. Bacillus substilis* and *Staphylococcus aureus* efficiently degraded the textile heterocyclic azo dyes. The growth on selective media and biochemicals in the present study helped to distinguish the bacteria on the basis of their cultural and biochemical characteristics. These results of the present study were similar to those obtained by Sadia Afrin (3)
Further identification can be confirmed by using PCR amplification technique.

In the present study the efficiency of the decolourization of the disperse heterocyclic azo dye, i.e, Disperse Blue was determined by UV-visible spectrophotometrically at 580 nm. Culture No. 3

efficiently decolourized (53.75%) Disperse Blue at room temperature and under static conditions where the culture density of 3% proved to be helpful in decolourization of the dye. 3% Culture No. 2 under static condition efficiently degraded Disperse Blue dye to 48.48 %. Whereas 3% of Culture No.1 decolourized the dye to 49.46% at 37^0C under shaker conditions.

These results were found to be consistent with the research work performed by Sadia Afrin et.al, where *Pseudomonas spps*. and *Enterococcus spps*. decolourized the dispersed heterocyclic azo dyes at 37^0C under shaker conditions.(1)

Conclusions

From the results obtained from the present study it can be concluded that the cultures isolated from the dumping grounds of Khaira Phata, Boisar were Gram negative short thick rods in duplets, Gram positive short rods in singles and Gram positive cocci in chains. Further these cultures are named as - Culture No.1, Culture No. 2 and Culture No.3 on the basis of the distinguishing cultural and biochemical characteristics. The bacterial isolates selected by Replica plate technique where the growth and decolourization of the dye was observed around the colonies. Culture No. 3 was found to be more efficient amongst the 3 isolates in decolourization of Disperse blue (Disperse heterocyclic azo dye) textile dye. The dye degradation efficiency by 3% Culture No.3 was found to be 53.75% under static conditions at room temperature.

In future study to be carried out the bacterial isolates can be identified and the other parameters like nutrient requirements can be optimized for increasing the efficiency of the dye decolourization.

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Conflict of Interest

The authors declare no conflict of interest.

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