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Isolation, Screening and Identification of Acid Black Degrading Bacterial Isolates

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Abstract:- Dyes are colored organic compounds that are used in various places like paper, leather, hair, drugs and cosmetics, waxes greases, plastic and textile materials. Textile mills discharge millions of gallons of the effluent as hazardous toxic waste, full of Color and organic chemicals from dyeing. Acid black is azo dye which is widely used in textile industries, there are many degradation way to degrade this type of dye but microorganism having its own advantages over other method, so here we are tried to isolate and identify bacterial isolates which having a potential to degrade acid black dye.

Keywords:- *Acid Black; Bacterial Isolates; Degradation***;** *Isolation.*

I. INTRODUCTION

Azo dyes are synthetic organic dyes that contain nitrogen as the azo group -N=N- as primary chromophore their molecular structure, more than half the commercial dyes belong to this class. [1,2]. Textile dyes Cause respiratory diseases, irritation to the mucous membrane, and the upper respiratory tract. Many dyes and their breakdown products are carcinogenic, mutagenic, and are toxic to life [1, 4]. Acid black dye is one of the types of azo dye. Particularly in the case of azo dyes, Effluent treatment becomes a serious issue because of their Negative impact on water ecosystems and human health, especially that thousands of azo dyes have been developed for Use on every type of fiber [5]. 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 [7].In recent years, several studies have focused on the use of microorganisms that are capable or potent to biodegrade and/or bio-accumulate toxic compounds [9]. The bioremediation technology offers several advantages; it can be performed on site; generally has lower cost and minimum inconvenience in the process; eliminates the waste permanently; Can be used in conjunction with methods of physical and chemical treatments; hasMinimal environmental impact and, therefore, has wide public acceptance and also Encouraged by regulatory authorities [10]. In present study, we Isolated bacterial isolate check it's degradation potential and identified bacterial isolate by biochemical testing.

II. MATERIAL AND METHOD

A. Sample Collection

Sample collection was done from Jetpur (Gujarat, India.) and Rajkot (Gujarat, India.)CETP. Samples were in the form of untreated liquid effluent, sludge, and soil. Samples were stored in refrigerator and various tests were performed within 24 hours of collections.

B. Media

- *Dye*

The textiles dye Acid black (λ_{max} 573nm,) was produced from dye manufacturing industries located in Ankleshwar (Gujarat, India). The media, its components, and other chemicals used in this study were of analytical grade.

C. *physiochemical characteristics of sample*

The effluent sample before treatment were tested for its physiochemical characteristics like Colour, pH, BOD, COD, TSS, TDS etc.

D. Isolation of dye degrading bacterial isolates:

Erlenmeyer flask(250 ml containing) 50 ml of BH medium(autoclaved at 120°C,15 psi, for 15 min.)with supplement with acid black dye(100 PPM) with 1ml of sewage water and it put on shaker for a period of a week at room temperature. 1ml enriched Culture was spread on BH agar consisting acid black dye (100 ppm) and sub culturing was performed until pure culture were obtained.

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➢ *Culture Preservation and Maintenance :*

For preservation of pure bacterial culture was done in 40 % glycerol. for future use. Working culture were maintained by subculturing on nutrient agar slant and stored at 4° c at intervals of 15 days.

➢ *Gram's Staining and Cell Morphology*

Gram's staining of each of the isolated colony were carried out to check the whether the isolated Bacterial isolates are gram's positive or negative.

E. Screening of Dye Degrading Bacterial Isolates

This isolates were streaked on BHA was supplemented with varied concentration of dye (100ppm, 200ppm, 500ppm, 600ppm, 800ppm, and 1000ppm).Growth response of all bacterial isolates were observed.

F. Identification By Biochemical Testes

Identification was done using VITEK®2 GN Card which is based on established biochemical method.

F. Decolorization assay

Spectral analysis of dyes before and after decolorization was carried out by UV-vis spectrophotometer (Elico BL 198).Color measurements were performed in centrifuged samples (control and experimental). The decolorized dyes were monitored at the interval of 24h incubation. O.D of test samples (decolorized) and controls were taken at λ 573. The decolorizing activity was expressed in terms of percentage decolorization and determined by monitoring the decrease in absorbance at λmax of acid black dye. The un-inoculated complete medium supplemented with dye was used as control. All experiments were carried out in triplicate. The average of three values was taken, and the standard deviation was carried out. The value plotted in all graphs represents the average value of the data taken during every experiment.

Degradation (%) was calculated according to the formula:
Degradation (%) = [(AC – AI)/ AC] x100
Where, AC, Absorbance of control (uninoculated medium)AI, Absorbance of the inoculated medium.
Turbidity = OD (before centrifugation) -OD (after centrifugation)

III. RESULT

A. Physio-chemical characterization

The sample were collected in a sterilized container from site. The color, temperature and pH and other physiochemical characteristics like BOD,COD ,TSS,TDS etc. were measured on the same day of collection of the sample. Results are as shown in table.

Table:1 characteristics of sample collected from different stages of CETP jetpur

Properties	Effluent(J)
Color	Bluish green
Temperature	26
pH	6.28
TS	3000mg/l
TSS	2000mg/l
TDS	1000mg/l
BOD	0.6

B. Isolation of dye degrading bacterial isolate

Sewage sample contain large array of diverse microbes.These microbes were isolated by enrichment culture process. All microbes which are presented in soil may not be actively involved in degradation process. Hence, enrichment of bacteria having dye degrading potential becomes necessary in order screen dye degrading isolates and tolerance level of isolates. The dye containing enrichment medium repress the growth of bacterial population subsequent transfer to fresh medium consisting acid black dye resulted in growth of some selected bacteria. A total nine isolated were obtained at the end of enrichment process during further screening (based on its growth rate). Nine isolates were selected denoted as is-1, is -2, is-3, is-4 , is-5, is-6 , is-7 , is-8 and is-9.

➢ *Colony Characteristics*

Out of Nine isolates 5 were Gram negative and 4 were Gram positive. Among them there arrangement like single, double and in chain. A characteristic pigmentation of colony is no pigmentation and brownish color pigment along with entire undulate margin and circular, irregular forms were observed. Size of colonies varied from small to large having smooth or rough surface and elevation like flat and raised

Isolates	Size	Shape	Color	Margin	Elevation	Opacity	Consistancy	Gram's nature
IS 1	Small	Round	Cream pigment	Entire	Flat	Opaque	Moist	Gr-ve
IS 2	Modrate	Round	Cream pigment	Entire	Flat	Opaque	Moist	Gr-ve
IS 3	Punctiforn	Round	Cream pigment	Entire	Flat	Opaque	Moist	Gr+ve
IS 4	Small	Round	No pigment	Entire	Flat	Opaque	Moist	Gr-ve
IS 5	Punctiforn	Round	No pigment	Entire	Raised	Translucent	Moist	Gr-ve
IS 6	Small	Irregular	No pigment	Entire	Flat	Translucent	Moist	Gr+ve
IS 7	Punctiforn	Round	No pigment	Entire	Raised	Translucent	Moist	Gr-ve
cIS 8	Punctiforn	Round	Brownish	Entire	Flat	Tracnslucent	Dry	Gr+ve
IS 9	Small	Irregular	No pigment	Entire	Flat	Opaque	Moist	Gr+ve

Table 2.Colony Characteristic and Gram's Nature of Bacterial Isolates

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C. Screening of Dye Degrading Bacterial Isolates

This isolates were streaked on BHA was supplemented with varied concentration of dye (100ppm, 200ppm, 500ppm, 600ppm,

800ppm, and 1000ppm). According to these test isolates is 7 is-8 and is-9 were grow at low concentration of dye (100ppm). Is-3,is -4,is -5 and is -6 were tolerate moderate concentration of dye (500ppm, 600ppm, and 800ppm) but is-1 and is-2 were tolerate up to 100 to 1000ppm concentration of dye.

Here, it was noted that when concentration of dye was increased at that time growth of the isolates was decreased.

Table 3 :Growth response bacterial isolates on BHA with different concentration dye

Isolates	100ppm	200ppm	500ppm	600ppm	800ppm	1000ppm
IS 1	+++	+++	+++	++	++	++
IS 2	++	++	++	++	++	+
IS 3	++	++	+	++	+	-
IS 4	++	++	+	++	+	-
IS 5	++	++	++	++	+	-
IS 6	++	++	++	++	+	-
IS 7	+	+	+	+	-	-
IS 8	+	+	+	-	-	-
IS 9	+	+	+	-	-	-

- = no growth, + = low growth, ++ = moderate growth, +++ = high growth

D. Biochemical Characterization and Identification of Bacterial Isolates

Biochemical characterization of one potential bacterial isolate was carried out using VITEK®2 systems (table 4.). *(*Note: In Table 4 column title "No." represents the well number on VITEK®2 card; it does not mean serial number).* VITEK®2 system identify organisms based on reaction being analyzed. It is required to have sufficient information to analyze the typical reaction of the claimed species to a set of discriminating biochemistry. Based on positive and negative observations the identification of an unknown bacterial isolates is carried out from a given list of identified strains. If a unique identification pattern is not recognizing, then strain is recognized to be a member of outside the scope of database.

➢ *Percent probabilty*

As part of the identification process, the software compares the test set of reactions to the expected set of reactions of each organisms or organisms group that can be identified by the product. A quantitative value, the percent probability is calculated and relates to how well the observed reaction of each organism carried out. A perfect match between the test reaction pattern of single organisms, or organisms group. According to biochemical tests Isolate 1 having a 97 percent probability with *Pseudomonas aeruginosa*

No.	Test	Code	Isolate
			IS 1
2	Ala-Phe-Pro-ARYLAMIDASE	APPA	-
3	ADONITOL	ADO	-
4	L-Pyrrolydonyl-ARYLAMIDASE	PyrA	-
5	L-ARABITOL	lARL	-
7	D-CELLOBIOSE	dCEL	-
9	BETA-GALACTOSIDASE	BGAL	-
10	H2S PRODUCTION	H2S	-
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	-
12	GlutamylArylamidasepNA	AGLTp	+
13	D-GLUCOSE	dGLU	+
14	GAMMA-GLUTAMYL-TRANSFERASE	GGT	+
15	FERMENTATION/ GLUCOSE	OFF	-
17	BETA-GLUCOSIDASE	BGLU	-
18	D-MALTOSE	dMAL	-

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19	D-MANNITOL	dMAN	+
20	D-MANNOSE	dMNE	+
21	BETA-XYLOSIDASE	BXYL	-
22	BETA-Alanine arylamidasepNA	BAlap	+
23	L-Proline ARYLAMIDASE	ProA	+
26	LIPASE	LIP	+
27	PALATINOSE	PLE	-
29	Tyrosine ARYLAMIDASE	TyrA	-
31	UREASE	URE	-
32	D-SORBITOL	dSOR	-
33	SACCHAROSE/SUCROSE	SAC	-
34	D-TAGATOSE	dTAG	-
35	D-TREHALOSE	dTRE	+
36	CITRATE(SODIUM)	CIT	+
37	MALANATE	MNT	+
39	5-KETO-D-GLUCONATE	5KG	-
40	L-LACTATE Alkalinization	lLATk	+
41	ALPHA-GLUCOSIDASE	AGLU	-
42	SUCCINATE alkalinization	SUCT	+
43	Beta-N-ACETYL-GALACTOSAMINIDASE	NAGA	-
44	ALPHA-GALACTOSIDASE	AGAL	-
45	PHOSPHATASE	PHOS	-
46	Glycine ARYLAMIDASE	GlyA	-
47	ORNITHINE DECARBOXYLASE	ODC	-
48	LYSINE DECARBOXYLASE	LDC	-
53	L-HISTIDINE Assimilation	lHISa	-
56	COURMARATE	CMT	+
57	BETA-GLUCURONIDASE	BGUR	-
58	O/129 RESISTANCE (comp.vibrio.)	O129R	-
59	Glu-Gly-Arg-ARYLAMIDASE	GGAA	-
62	ELLMAN	ELLM	-
64	L-LACTATE assimilation	lLATa	+

Table 4:- Biochemical Characterization of bacterial isolates

E. Degradation by spectrophotometry

Dye degradation by spectrophotometery was carried out. Degradation activities (%) was calculated, **isolate -1** degrade **acid black** dye 31.07%, 43.28%, 71.66 % , 94.69 %, and 98.37 % from day 1 to day 5 respectively.

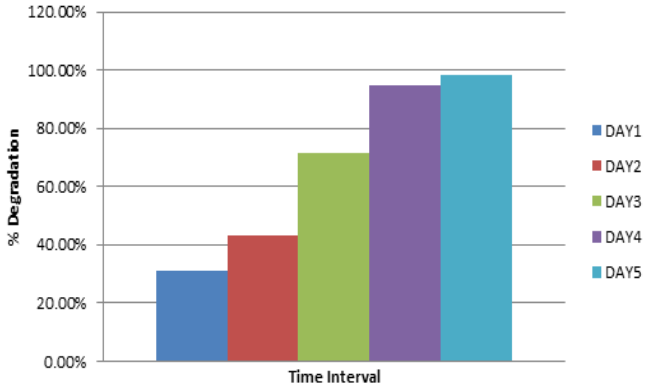

Fig 1: Degradation of acid black by isolate-1

IV. DISCUSSION & CONCLUSION

Textile dyeing and printing industries widely use various structurally diverse azo dyes, and therefore, effluents from the textile industry are extremely variable in composition [11]. The dye concentration in the industrial wastes stream typically varies from 10-50 mg.l-1[12].However, changes in operating conditions may lead to still higher concentrations of dye, and therefore, it is important to know if the native dye decolorizing microbial community can handle higher concentrations. Bioremediation is cost-effective and eco-friendly, as it is an enhanced way of biodegradation of various organpollutants. Many researchers have demonstrated the potential use of microorganisms for biological treatment of textile effluents[13,14].

According to the concept of combined anaerobic-aerobic treatment, azo dyes should be removed from the water phase by (anaerobic) reduction followed by (aerobic) oxidation of the dye's constituent aromatic amines. Combined anaerobic-aerobic treatment, therefore, holds promise as a method to effectively remove azo dyes from wastewater. Under aerobic conditions, the azo dyes are non-degradable by most of the bacteria, and the isolation of bacteria, which use dye as a sole source of carbon, has been quite difficult [15].

There are reports on the decrease in decolorization activity with an increase in dye concentration. O'Neil et al. (1999)reported that the dye concentration in the reactive dye bath effluent was observed within a narrow range of 100-200 g.l-1. Kothari (2005) reported a decrease in decolorization activity with an increase in the concentration of Kemifix Red F6B. Verma & Madmwar (2002) reported that lower decolorization efficiency was due to higher inhibition of azo enzyme reductase at high dyestuff concentration.

In present study we, isolated bacterial isolates by enrichment culture techniques, study it's gram staining and colony morphology, check its tolerance for acid black dye. By performing tolerance test we get one potential bacterial isolate, which is identified by biochemical testing and its degradation was check by calculation percent degradation**.**

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