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RESEARCH ARTICLE

DECOLORIZATION OF DARK RED 2B AZO DYE BY *SPHINGOMONAS PAUCIMOBILIS* ISOLATED FROM TEXTILE EFFLUENT.

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Abstract

Synthetic azo dyes, present in textile effluent, are chemically Synthetic azo dyes found in textile effluent are chemically stable and persist in environment if discharged untreated. They cause many environmental issues and are toxic to aquatic life as well as carcinogenic and mutagenic to humans. There exist many physico-chemical methods for the removal of dyestuff form textile effluent, but all are having some disadvantages. Use of microorganisms for decolorization and degradation of such azo dyes in textile effluent is one of the thrust area of research. Many bacterial strains have been shown to degrade and mineralize azo dyes in waste water. In this study, indigenous bacterial strains were isolated from textile effluent capable for decolorization of Dark Red 2B azo dye. *Sphingomonas paucimobilis*, was isolated showing highest capacity of dye decolorization of 98.46% among 16 isolates obtained after screening. The optimum temperature and pH for dye decolorization was found to be 37 °C and 7, respectively. 1% of glucose supplementation was found to be optimum for maximum dye decolorization by the bacterium. Cell free extracts of dye decolorization flask of *Sphingomonas paucimobilis* no adverse effect on *Phaseolus mungo* seed germination and radical and Plumule development was also enhance as compared to dye alone.

Introduction:-

Synthetic dyes are complex aromatic chemicals, designed to resist the effect of detergent, sun light and harsh temperatures[23]. They are chemically and photochemically very much stable and are extremely persistent in natural ecosystems. The estimated annual production of synthetic dyes has been estimated over 10^6 tons worldwide[25]. Synthetic dyes are widely used in industries like textile, paper, food, cosmetics and pharmaceuticals with textile industry being the largest consumer[2]. Amongst all synthetic dyes, azo dyes are the largest group of dyes used in textile industries and are characterized by the presence of azo groups – N = N – [30]. In textile industry, during improper wet processing operations and dyeing procedures nearly 30-70 % of the azo dyes used remain unfixed on to the textile substrate and thus finds its way into textile effluent[18]. This concentration reaches as high as up to 10-200 mg/l in textile effluent[15]. If discharged untreated into the natural ecosystem, azo dyes result in conversion of azo groups into and aromatic amines. Bioaccumulation of such products could also result in toxic effects on aquatic life and shows carcinogenic and mutagenic effects on humans[1, 29].

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Dyes can be removed from wastewater by a variety of physico-chemical methods such as coagulation, flocculation, reverse osmosis, oxidation and electrochemical methods[12, 19, 20]. These methods can only transfer dyes from one phase to another as well as are having many disadvantages like high-energy costs, high sludge production and generation of by-products[31]. In contrast to that, genetic diversity and metabolic versatility makes microorganisms more favorable as a biological option for the treatment of textile effluent. Fungi[3] and algae[9] uses adsorptive strategy rather than degrading dyestuff present in textile effluent. It is well known that many bacteria can degrade and completely mineralize azo dyes under certain condition[8, 10, 17, 28]. Additionally, the intermediate metabolites of decolorization process, like aromatic amines, can also be completely degraded by bacterial enzymes[25]. Thus, in this study a bacterium *Sphingomonas paucimobilis,* capable of decolorizing Dark Red 2B azo dye was isolated and the effect of various physico-chemical parameters on dye decolorization by bacterium was investigated.

Materials and Methods:-

Soil and effluent samples were collected form chemically contaminated sites near the vicinity of Surat city. Samples were collected in sterile plastic jar and stored at 4 °C till further use. Enrichment of the collected samples was done by inoculating 1 ml of effluent (1 mg of soil) sample in 100 ml sterile Bushnell Hass (BH) medium (HI Media Pvt. laboratories, Mumbai) supplemented with 100 ppm of Dark Red 2B dye purchased from local textile dyes suppliers of Surat city in 250 ml Erlenmeyer flask. Flasks were incubated at 30 °C at 100 RPM for 2 days.

Screening of Dye Decolorizing Bacterial Isolates:-

For isolation of dye decolorizing bacteria, 0.1 ml of enriched suspension was spreaded on BH agar plates supplemented with 200 ppm dye and incubated at 30°C for 24 hours. Bacterial isolates having a clear zone around their colonies were taken as dye decolorizing bacteria and subjected to secondary screening. During secondary screening isolates were inoculated in 100 ml BH medium supplemented with 200 ppm of dye. The flasks were incubated at 30 °C at 100 RPM. 5 ml of sample was removed aseptically and centrifuged at 10,000 RPM for 10 minutes at 4 °C. Decolorization efficiency was analyzed by measuring the absorbance of culture supernatant at 530 nm using UV-VIS Spectrophotometer (Shimadzu-UV-3600 Plus). The decolorizing efficiency was expressed as percentage of decolorization, which is calculated by the following formula:

$$\text{Percentage of Decolorization}^{6} = \frac{\text{Initial absorbance} - \text{Final Absorbance}}{\text{Initial absorbance}} \times 100$$

Selected bacterial isolate was characterized on the basis of its biochemical characteristic. Biochemical tests were carried out on Phoenix Instrument, version: 6.01 A and Epi Center, version: V6.20A.

Dye Decolorization Studies:-

All the decolorization experiments were performed in triplicates. For dye decolorization, selected bacterial isolate was inoculated in 100 ml BH medium (pH 7) supplemented with 200 ppm of Dark Red 2B. The flasks were incubated at 30 °C on rotary shaker at 100 RPM. Samples were removed aseptically and dye decolorization was measured by the method described earlier.

Effect of pH and Incubation Temperature:-

For pH optimization, initial pH of 100 ml BH medium (with 200 ppm of dye) was adjusted to 5, 6, 7, 8 and 9 and decolorization assay was carried out. A loop full of bacterial suspension was inoculated and incubated at 30 °C at 100 RPM. The optimal temperature for dye decolorization differs greatly from one bacterium to another. Bacterial suspension was inoculated in BH medium (with 200 ppm of dye) and incubated at different temperatures like 30 °C, 37 °C, 40 °C and 50 °C at 100 RPM. Samples were removed and checked for dye decolorization.

Effect of Co-Substrates:-

Additional co-substrates were added individually to BH medium like additional carbon supplementation (glucose, sucrose and maltose) at various concentrations of 0.2 %, 0.5 % and 1.0 % on w/v basis. For this, 100 ml of BH medium (with 200 ppm of dye) were supplemented with different carbon sources in different concentrations, inoculated with bacterial suspension and checked for dye decolorization regularly.

Effect of Dye Concentration:-

Various dye concentrations ranging from 100 ppm to 1000 ppm were added to 100 ml of BH medium at pH 7 in order to examine the effect of varying initial dye concentration on decolorization.

Phytotoxicity Studies[26]:-
Phytotoxicity was performed in order to assess the toxicity of the untreated and treated dye to common agricultural crops. Decolorization metabolites were extracted using ethyl acetate, air dried and dissolved in sterile distilled water. Ten seeds of *Phaseolus mungo* (mung) plants were sowed into a plastic petri dish with daily watering of (5 ml) of 300 ppm dye solution (positive control) and extracted metabolites (test sample). Negative control set was carried out using distilled water (daily 5-ml watering) at the same time. Germination and length of shoot and root were recorded after 7 days.

Results and Discussion:-

Isolation and Identification of Dye Degrading Bacteria:-
The main aim of this study was to screen bacterial strains capable of decolorization and degradation of Reactive Red 2B Azo dye. Sixteen different bacterial isolates showing zone of decolorization were isolated and were subjected to secondary screening. After secondary screening, maximum dye decolorization up to 86.32 % was obtained by bacterial isolate, which was identified to be *Sphingomonas paucimobilis* on the basis of cultural and biochemical characteristics.

Dye Decolorization Studies:-
Dye decolorization studies resulted in maximum dye decolorization up to 92.38 % by *Sphingomonaspaucimobilis* as showed in the Figure 1.

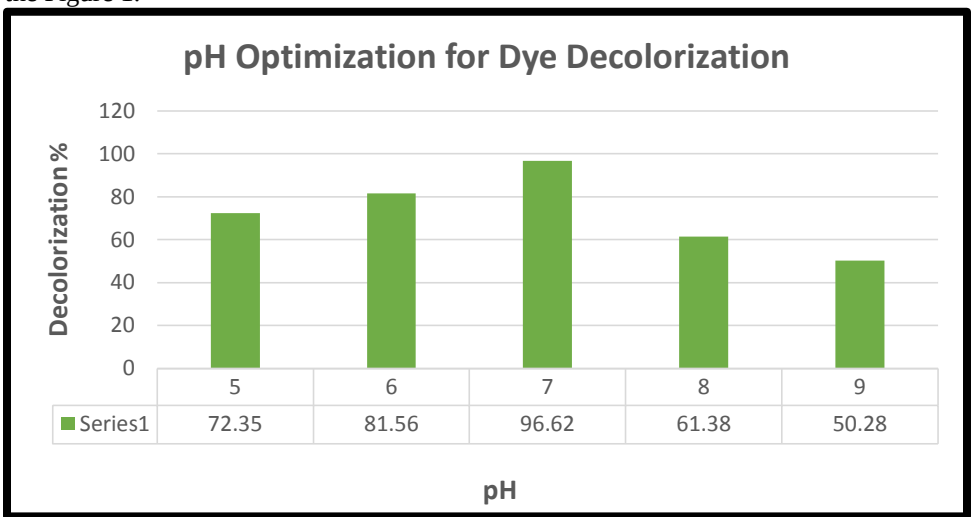

Figure 1:-Decolorization of Dark Red 2B by *Sphingomonas paucimobilis.*

Effect of pH on Dye Decolorization:-
It was found that change in pH significantly affected the rate of dye decolorization. The isolated bacterium showed maximum dye decolorization of 96.62 % was obtained at pH 7 (Figure 2) whereas the decolorization was decreased at acidic or basic pH. Our findings were supported by other workers who also obtained maximum decolorization of Methyl Red dye at neutral pH by *Bacillus* species and observed Remazol Black B dye decolorization of 100% at pH 7 by bacteria isolated from contaminated sites[6, 7, 16, 21].

Effect of Temperature on Dye Decolorization:-
The dye decolorizing potential of isolated bacterium was tested at different temperatures. Form the results it was found that the decolorization ability decreased with increase in temperature. Maximum dye decolorization of 97.35 % was obtained at 37 °C of incubation temperature (Figure 3). These findings were supported by other researchers as they observed 93 % decolorization of Reactive Violet 5R at 37 °C by bacterial consortium JW-2[22]. Decrease in the dye decolorization activity at higher temperature might be due to loss of cell viability or due to denaturation of enzymes responsible for decolorization[27].

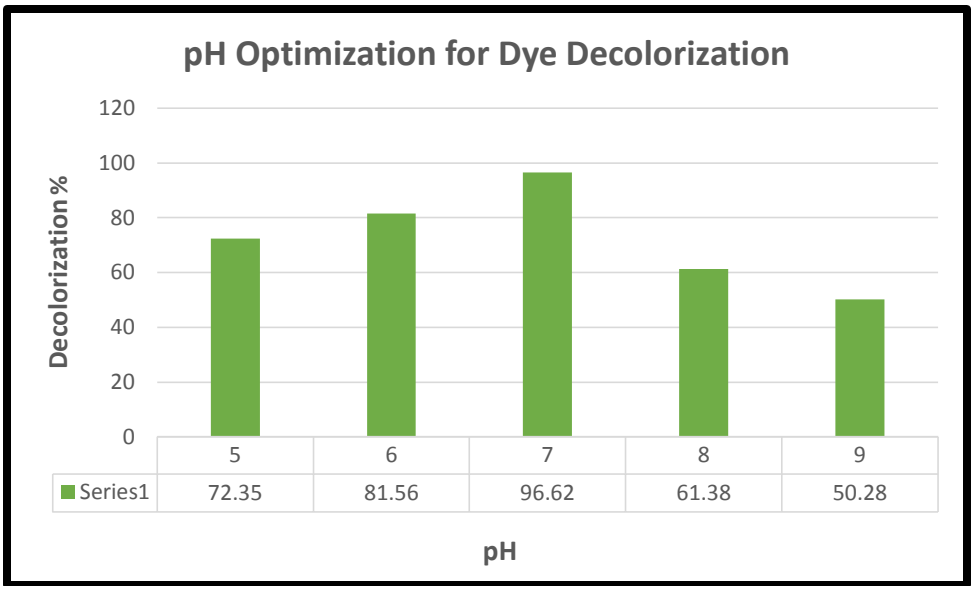

Figure 2:- pH optimization for decolorization of Dark Red 2B by *Sphingomonas paucimobilis.*

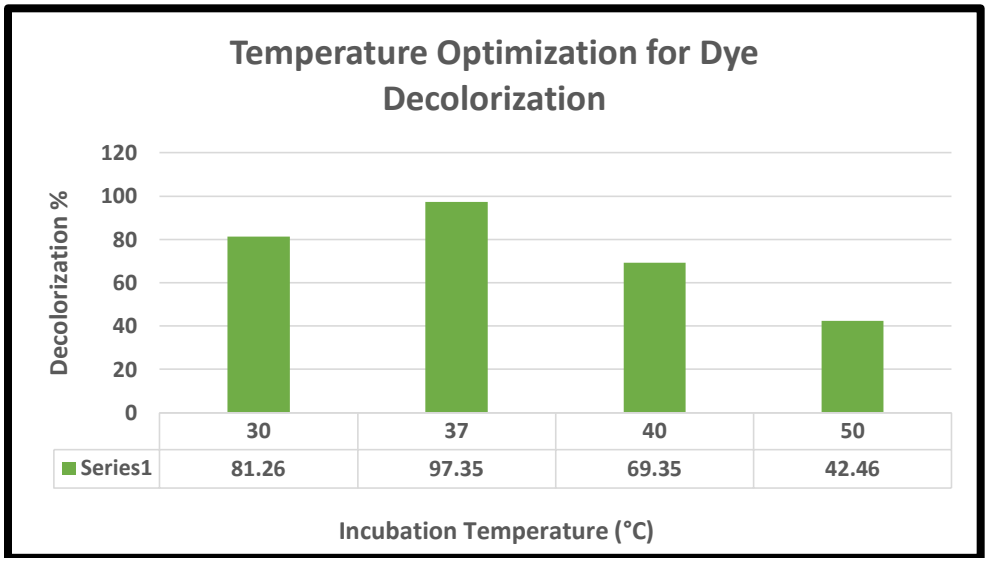

Figure 3:- Temperature optimization for decolorization of Dark Red 2B by *Sphingomonas paucimobili*s.

Effect of Co-Substrates on Dye Decolorization:-

Different carbon supplementation (glucose, sucrose and maltose) were used to assess their effect on decolorization. The bacterium exhibited efficient decolorization in presence of 1 % glucose, whereas sucrose and maltose did not show promising effect on dye decolorization. The result of carbon supplementation study showed that maximum dye decolorization of 94.60% was obtained at 1 % Glucose (Figure 4). Several reports are available for dye decolorization in presence of additional carbon source. Similar results were obtained by researchers as they obtained 90 % of dye decolorization in presence of glucose as additional carbon source for decolorization of Reactive Red 180 dye by *Citrobacter* species CK3[32]. In contrast other researchers found that maltose also shows stimulatory effect on dye decolorization observed that bacterial strains grew well and completely decolorized K-2BP where either yeast extract or peptone was present in the medium[11, 14].

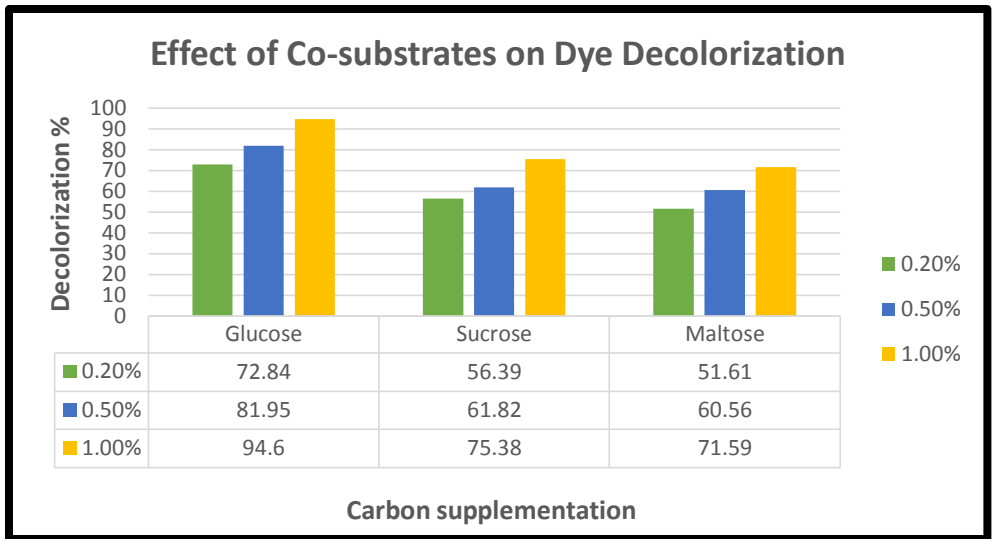

Figure 4:- Effect of co-substrates on decolorization of Dark Red 2B by *Sphingomonas paucimobilis.*

Effect of Dye Concentration on Decolorization:-

For this study, various dye concentrations ranging from 100 to 1000 ppm were used along with BH medium. The bacterium showed decolorization at various dye concentrations but at higher dye concentrations ability of decolorization decreased as the toxicity increased (Figure 5). It has been reported that generally dye decolorization efficiency of various bacterial isolates decreases with increase in dye concentration[26].

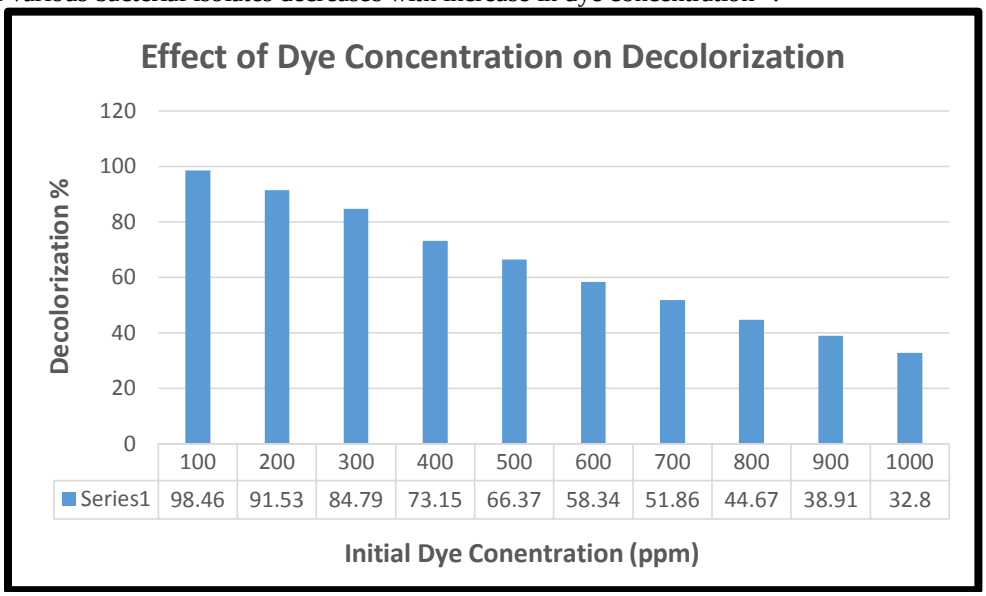

Figure 5:- Effect of initial dye concentration on decolorization of Dark Red 2B by *Sphingomonaspaucimobilis*

Phytotoxicity:-

Most common methods used to study phytotoxicity are monitoring of Plumule and radicle growth and assessing seed germination. The studies showed that shoot and root lengths were affected in the presence of pure dye but it was less affected when tested with extracted dye metabolites (Table1.). This indicates the detoxification of dark Red 2B dye with bacterial isolate. [13]observed similar result indicating % of germination was unaffected by degraded metabolites of Dark orange 39 dye on *Triticumaestivum* and *Phaseolus mungo* seeds.

Table 1:- Phytotoxicity study of decolorization product of Dark Red 2B on *Phaseolusmungo*

Sphingomonas paucimobilis	*Phaseolus mungo* seeds		
	Water	**300 ppm Pure Dye**	**Extracted Metabolites**

Germination (%)	100	40	100
Plumule (cm)	15.8	6.9	13.9
Radicle (cm)	5.6	2.0	4.6

From this study it is clearly observed that the isolated bacteria *Sphingomonas paucimobilis* has great potential of azo dye degradation. Previously this bacterium has been investigated by many scholars for its capability of decolorization and degradation of toxic azo dyes which was observed to be 99.63% in case of Methyl Red Azo dye by this bacterium under shaking condition at 30 °C in minimal salt medium at 750 ppm dye concentration[5]. Also, other researchers used *Sphingomonas paucimobilis* for decolorization of toxic textile dyes like Malachite green and Methylene blue[4, 24].

Conclusion:-

From this study it is concluded that bacterial isolate *Sphingomonaspaucimobilis* showed 98.46 % of dye decolorization at 100 ppm dye concentration. The best temperature and pH for decolorization of Reactive Red 2B azo dye was found to be 37 °C and 7.0 respectively. The most effective co-substrate for dye decolorization was found to be 1% glucose. The reactive Red 2B dye showed phytotoxicity against *Phaseolus mungo* seeds, after efficient decolorization of the dye, the degraded products obtained show less toxicity. As a conclusion, this study reports that the dye decolorization potential of *Sphingomonaspaucimobilis* can be exploited for bioremediation treatment of azo dyes contaminated in the industrial effluent. Thus, degraded dyes can be safely discharged into the ecosystem without harming the natural flora.

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