Original Research Article

EFFECT OF VARIOUS CARBON AND NITROGEN SOURCES ON DECOLORIZATION OF TEXTILE DYES BY *PSEUDOMONAS TAIWANENSIS* STRAIN TNZ3

Abstract:

The co-substrate is a crucial component in the bio-decolorization process that helps bacteria to grow and carry out their fundamental functions. Different types of textile dyes with diverse chemical structures are frequently employed in the textile industry, and the most of them are resistant to biodegradation because of their xenobiotic nature. Due to the low carbon content of textile dyes, particularly of azo dyes, co-metabolic conditions are required for decolorization process. Hence, the current study was aimed to determine which striking carbon and nitrogen sources are preferred for generating co-metabolic conditions to decolorize the following dyes: Disperse Yellow-3, Malachite Green, Basic Red-18, and Reactive Blue-4. Different carbon and nitrogen sources were used to isolate, screen, and evaluate different bacterial strains for dye degradation. A potential bacterial strain *Pseudomonas taiwanensis* strain TNZ3 was identified based on 16s rDNA sequence and selected for further study. It was found that the presence of yeast, peptone, NH4Cl, sucrose, glucose, fructose, maltose, galactose, lactose, and starch in dye contaminated media effectively enhanced azo dye degradation rate by *Pseudomonas taiwanensis* strain TNZ3. In the medium inoculated with *Pseudomonas taiwanensis* strain TNZ3, sucrose and yeast with peptone extracts induced the greatest decolorization among diverse carbon and nitrogen sources. In all dyes, the maximum decolorization occurs at 1.5% yeast extract supplementation. The results made it abundantly clear that various carbon and nitrogen sources used as co-substrates played effective role on dye decolorization process.

Key Words: Carbon sources, nitrogen sources, co-substrate, decolorization, textile dye, *pseudomonas taiwanensis* strain tnz3

Introduction

Recent years have seen a significant increase in scientific interest in color removal in particular.

Dye-bearing effluents have been treated using physico-chemical techniques, such as activated carbon adsorption, advanced oxidation, electrolysis, coagulation, flocculation, etc. any of these techniques may result in secondary contamination and are frequently economically unviable [1-3]. “They have one or more azo groups (R1-N=N-R2) and aromatic rings mostly substituted by sulfonate groups” [4].

Due to their chemical resistance, dyes are extremely difficult to remove from the environment using standard techniques. Thus, it can be seen that up to 90% of reactive dyes are released into the environment untreated. The fact that these colors cannot be removed from the body through chemical or physical means gave rise to the notion that biological treatment techniques may be used to remove environmental pollutants. Numerous studies have demonstrated that bacteria may remove a range of natural and synthetic colors from anaerobic and aerobic environments by biosorption, bioaccumulation, and biodegradation [5–11].

Supplements Azo dyes lack of carbon and nitrogen sources, making it difficult for them to biodegrade without these elements being supplemented [12]. Most often, complex organic supplies like yeast extract, peptone, or a combination of complex organic sources and carbohydrates are needed for azo dye decolorization by mixed as well as pure cultures [13]. “Reducing equivalents from diverse carbon sources are transferred to the dye during the decolorization of azo dyes by reduction of the azo bonds. A few experiments used extra carbon and nitrogen sources to conduct the azo dye decolorization. Effective decolorization was recorded with the addition of organic nitrogen sources as peptone, beef extract, urea, yeast extract, and so on because they replenish NADH, which serves as an electron donor for the reduction of azo dyes by microorganisms” [14]. It has been claimed that bacteria can decrease the azo bond aerobically by co-metabolizing azo dyes as their only source of carbon and energy [15].

Wastes from the textile industry have recently attracted the attention of environmentalists since they pose a severe threat to the environment [16]. A significant number of toxic, non-biodegradable, and auxiliary chemicals, such as

dyes, surfactants, salts, softeners, and auxiliary additives, are also present in these textile effluents. Commonly, the dyes prevent photosynthesis, quickly deplete oxygen, make soil unsuitable for irrigation, increase BOD, and affect the permeability and texture of the soil.

The textile industry has access to about 10,000 commercially available dyes and pigments worldwide [17]. Reactive dyes make up the majority of the largest class of colorants with diverse chromophoric groups, including azo, anthraquinone, triarylmethane, etc. More than half of the world's companies that produce dyes use azo dyes (-N N-) group extensively due to their simple dyeing process and broadest color spectrum. Mainly because their presence is evident and has a substantial impact on water quality and the ecosystem, synthetic dyes from waste water are more significant than other colorless products. The optimal method for wastewater treatment must be found in order to eliminate colors and other dangerous elements from textile effluents [18]. But using microorganisms to clean dye effluents is a versatile and practical approach. [19]

Although breaking down refractory contaminants using microorganisms to biologically process textile wastes is a useful and suitable method, some synthetic colors are physically resistant to the biological processes [20]. The degradation of azo dyes requires the presence of microorganisms [21]. Determining dye-degrading microorganisms is therefore essential, as is maximizing the rate of microbial degradation in respect to environmental parameters, such as the effect of the co-substrate, the pH, the temperature, the size of the inoculums, the salt content, and the concentration of the dye [22]. Prior studies have shown that bacteria require additional sources of carbon and nitrogen for both growth and biodegradation [23]. It was said that since dye contains toxic elements, bacteria cannot utilize it as a co-substrate. However, only a small number of researchers have identified bacterial cultures that may utilize colors as carbon or nitrogen sources [24]. Microorganisms require organic sources of carbon and nitrogen to break down indigo because dye cannot be used as a growth substrate. The current study set out to determine how various nitrogen and carbon sources impacted the efficiency of the decolorization process for textile colors using several bacterial cultures.

Materials & Methods

- **Sample collection**

Wastewater is collected from different textile dyeing industries in Sathia, Sirajgonj, Gazipur, Madhapdi, Narshingdhi, Bangladesh.

- **Analysis the physico-chemical characteristics of textile effluent**

Measurement of Total solid (TS), Total Dissolved Solid (TDS), pH, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD)

- **Isolation of bacteria from collected sample**

Liquid Culture ⟶ Platting ⟶ Streaking ⟶ Sub- Culture ⟶ Pure Culture

- **Biochemical characterization and Antibiotic sensitivity test**

Methyl Red test, Simmons Citrate test, Catalase test, KOH test, Oxidase test, Voges-proskauer test, TSI test, MacConkey test, SIM test & Different kind of antibiotic test have been done.

- **Effect of pH & Temperature on bacterial growth**

Bacterial Growth measurement in pH-6, pH-7 & pH-8 & Temperature-20˚C, 37˚C & 42˚C.

- **Identification of Bacteria by16S rDNA Primer**

Genomic DNA isolation ⟶ PCR & PCR product purification ⟶ Sequencing of bacteria Blast of sequence

Collection and storage of the sample

Wastewater was collected from different textile dyeing industries in Sathia, Sirajgonj, Gazipur, Madhapdi and Narshingdhi, Bangladesh. Samples were taken from a variety of locations, such as drainage channels for stagnant textile dyeing wastewater. Samples were in the form of untreated liquid wastewater and untreated sludge. All samples were collected in sterile plastic bottles and polyethylene bags and stored at 4°C in the refrigerator for 24 h to avoid changes in their physicochemical properties.

Dyes

Four azo dyes namely Malachite green, Basic Red 18 and Disperse Yellow-3, Reactive Blue-4 were procured from Dysin-Chem limited, Dhaka. Molecular structure

of Malachite green, Basic Red 18, Disperse Yellow-3 & Reactive Blue-4 dyes are shown in Figure 1-4.

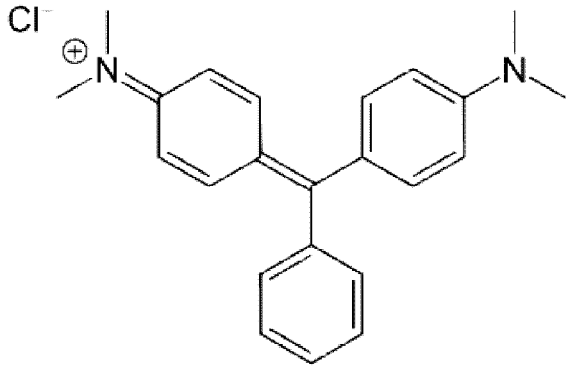

Fig 1: Malachite Green

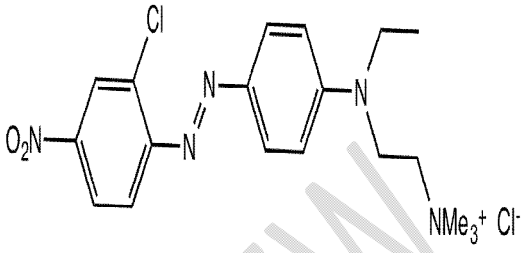

Fig 2: Basic Red-18

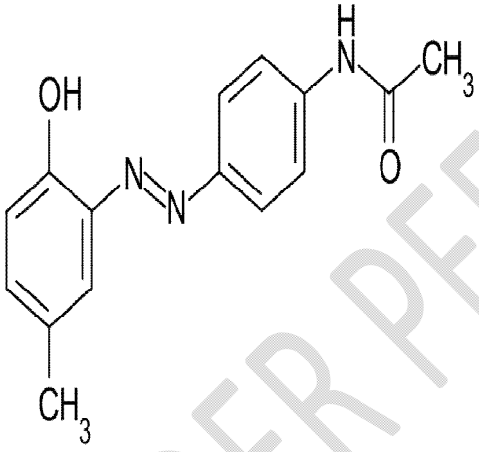

Fig 3: Disperse Yellow-3

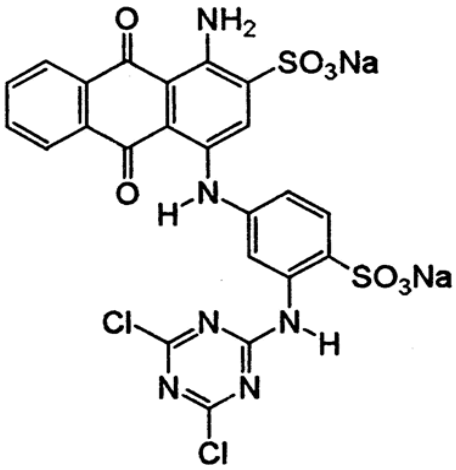

Fig 4: Reactive Blue-4

Source: Wikipedia

Fig 1-4 : Chemical structures of Four azo dyes

Isolation and Screening of Dye decolorizing bacteria

From the wastewater produced by the textile industry, bacteria were identified. At Luria bertani broth media containing textile dyes at 100 ppm concentration in pH 7 under static circumstances for 24 hours, morphologically diverse bacterial isolates were tested for decolorization. Additionally, the bacterial isolates were identified using gene sequences and a conventional biochemical technique.

Genomic DNA Extraction & 16S rDNA Gene Amplification

Genomic DNA was extracted from dye decolorizing bacteria using CTAB method. The PCR primers used to amplify 16S rDNA fragments were the bacteria-specific primers a forward primer 8F (5'-AGA GTT TGA TCC TGG CTC AG-3'; Tm: 61°C); and a reverse primer 806R(5'-GGA CTA CVS GGG TAT CTA AT-3'; Tm: 67.4°C). A total of 25 µl of reaction mixture consisted of – water 15µl, MgCl2 2.5µl, buffer 2.5, dNTPs 0.5µl, template 1µl, primer (forward 2 µl and reverse 2 µl). The PCR amplification was performed by Swift™ Minipro Thermal Cycler (Model: SWT-MIP-0.2-2, Singapore) using the following program: Denaturing at 95°C for 5 minutes, followed by 40 cycles of 40 seconds of denaturing at 95°C, 60 seconds of annealing at 65°C and 2 minutes of elongation at 72°C with a final extension at 72°C for 10 minutes. Then, the PCR products were subjected to 1% agarose gel electrophoresis, stained with ethidium bromide and visualized on a UV transilluminator for the presence of about 1500 bp PCR products.

The amplified PCR product was purified using AccuPrep® Gel Purification Kit (Bioneer Company, Korea) according to the manufacturer's protocol. PCR amplified 16s rDNA of the isolates screened as submitted for automated sequencing (Applied Biosystems 3130) at the Center for Advanced Scientific Research (CARS) of the University of Dhaka, Bangladesh. The sequence generated from the automatic sequence of PCR amplified DNA analyzed by NCBI BLAST Program (http://www.ncbi.nlm.nih.gov) for discover a similar organism possible through association of similar sequences. Finally, the isolates were determined based on partial sequence alignment of 16S rDNA with sequences available in database.

Sequencing of 16S rDNA & BLAST Analysis

The nucleotide sequence of the 16S rDNA was sequenced on both sides through the Big Dye chain termination cycle sequencer (ABI) and the sequence is decoded on Dideoxy Sanger 3130XL String Genetic Analyzer (ABI). The final method is then assembled by the Cap3 program for genetic sequencing. Gene sequence was determined by looking for similarities in the database via BLASTn for 16S rDNA.

Measurement of Decolorization Efficiency

The decrease in absorbance at absorption maxima (λmax) was monitored using a UV-visible spectrophotometer to evaluate decolorization activity in terms of %

decolorization. Uninoculated MS medium supplemented with corresponding dyes were used as a reference. At different time interval, 2 ml of sample was taken from reaction mixture and centrifuged at 10000 rpm for 10 min for biomass separation. The concentration of dye in the supernatant was determined by monitoring the absorbance at maximum absorption wavelength (λmax) at 660 nm by Spectrophotometer for all dyes. Bleaching dosage was calculated according to the following formula

$$Dye\ Decolourization\ (\%) = \frac{Initial\ absorbance - Final\ absorbance}{Initial\ absorbance} \times 100$$

Effect of various carbon and nitrogen sources on dye degradation

To determine the effectiveness of different carbon sources such as glucose, sucrose, fructose, maltose, galactose, lactose, and starch as well as nitrogen sources yeast extract, peptone, and ammonium chloride, Use *Pseudomonas taiwanensis* strain TNZ3 to Disperse Yellow-3, Malachite Green, Basic Red-18, and Reactive Blue-4. These dyes were used directly to the media.

Results

Physico-Chemical Characteristics of Textile Effluent

In the present study, physico-chemical characteristics of the collected textile effluent was analyzed and the results were showed in Table-1

Table-1 Physico-chemical characteristics of the collected textile effluent

Sr. No:	Sample	Nature of Sample	TS (mg/l)	TDS (mg/l)	pH	COD (mg/l)	BOD (mg/l)	Temp (℃)	Color	Odor
1	Sample 1	Water	3700	2300	6.3	710	270	35	Black	Foul
2	Sample 2	Sludge	4800	2100	6.8	750	280	35	Black	Foul
3	Sample 3	Water	4300	2500	7.4	620	250	35	Black	Foul
4	Sample 4	Sludge	4100	2700	6.0	785	310	35	Black	Foul

Isolation and Identification of Dye Decolorizing Bacteria:

Isolated bacterial strains identified by morphological and biochemical tests were subjected to 16S rRNA gene sequence analysis.

Table 2: Morphological characteristics of bacterial isolates (*Pseudomonas taiwanensis* strain TNZ3)

Characteristics	*Pseudomonas taiwanensis* strain TNZ3
Size	Large
Shape	Circular
Color	Cream
Margin	Irregular
Surface	Rough
Elevation	Elevated
Opacity	Opaque
Consistency	Sticky
Gram's Character	Gram – v e
Morphology	Rod
Motility	Motile

Table 3: Biochemical characteristics of bacterial isolates (*Pseudomonas taiwanensis* strain TNZ3)

Test Name		**Isolates Name**
		Pseudomonas taiwanensis strain TNZ3
Macconkey Agar Test		+
Catalase Test		+
Methyl Red (MR) Test		-
Voges-Proskauer (VP) Test		-
Sulfide indole	**H_2S formation**	-

motility test (SIM)	**Indole Production**	-
	Motility	+
Citrate Utilization Test		+
Triple Sugar Iron Test	**H_2S production**	-
	Lactose/sucrose fermentation	+
	Glucose fermentation	+
	Gas production	+
Oxidase Test		+
KOH Test		Viscous (Sticky)

+ (Growth) - (No Growth)

Analysis of 16S rRNA gene sequence revealed that the isolate was *Pseudomonas taiwanensis* strain TNZ3 (Accession Number: OM345146).

Effect of pH & Temperature:

Optimum pH for dye decolorizing bacteria was determined at 28°C temperature in liquid broth medium. Decolorizing bacterial isolate was exhibited maximum growth at pH 7 and the minimum growth at pH 8.

Optimum temperature for dye decolorizing bacteria was determined at pH 7 in nutrient broth medium. The optimum temperature for dye decolorizing bacteria was found to be 28°C temperature. The maximum growth rate was observed at 28°C temperature and the minimum growth rate was observed at 37°C temperature.

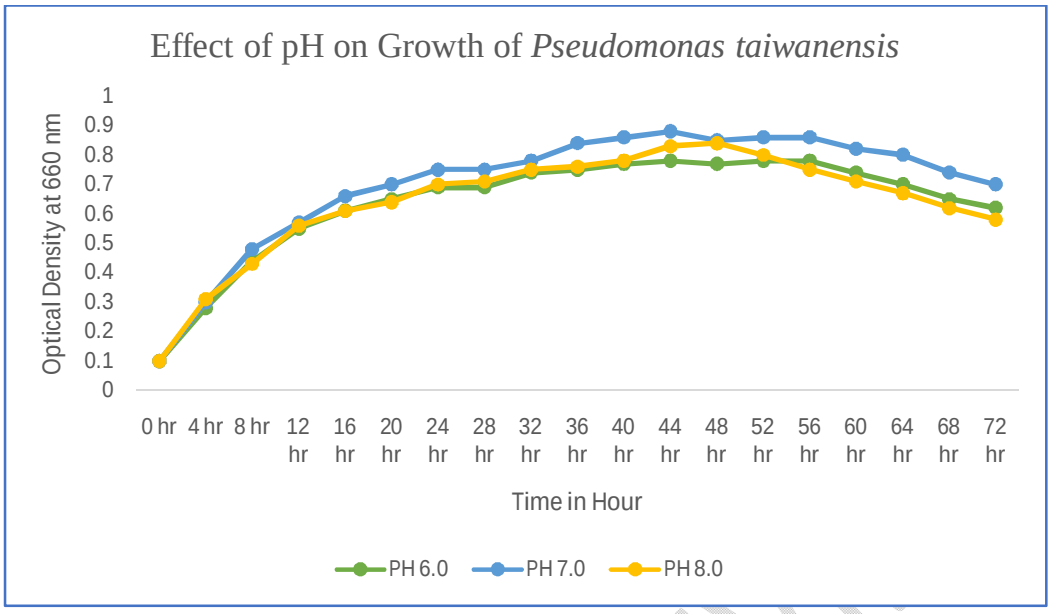

Fig. 5: Effect of pH on growth of bacteria *Pseudomonas taiwanensis*

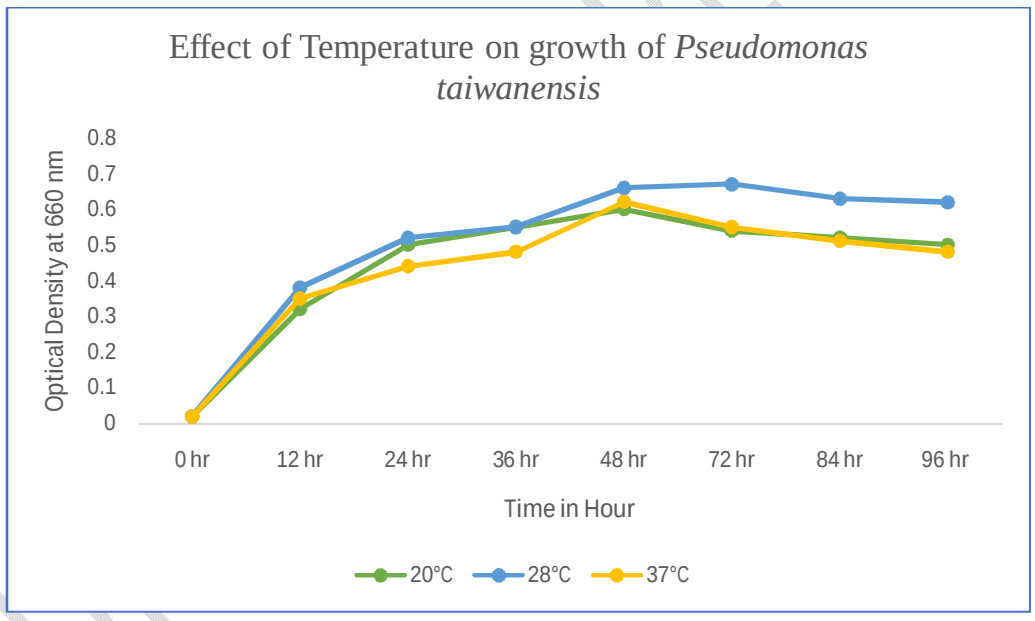

Fig. 6: Effect of temperature on growth of bacteria *Pseudomonas taiwanensis*

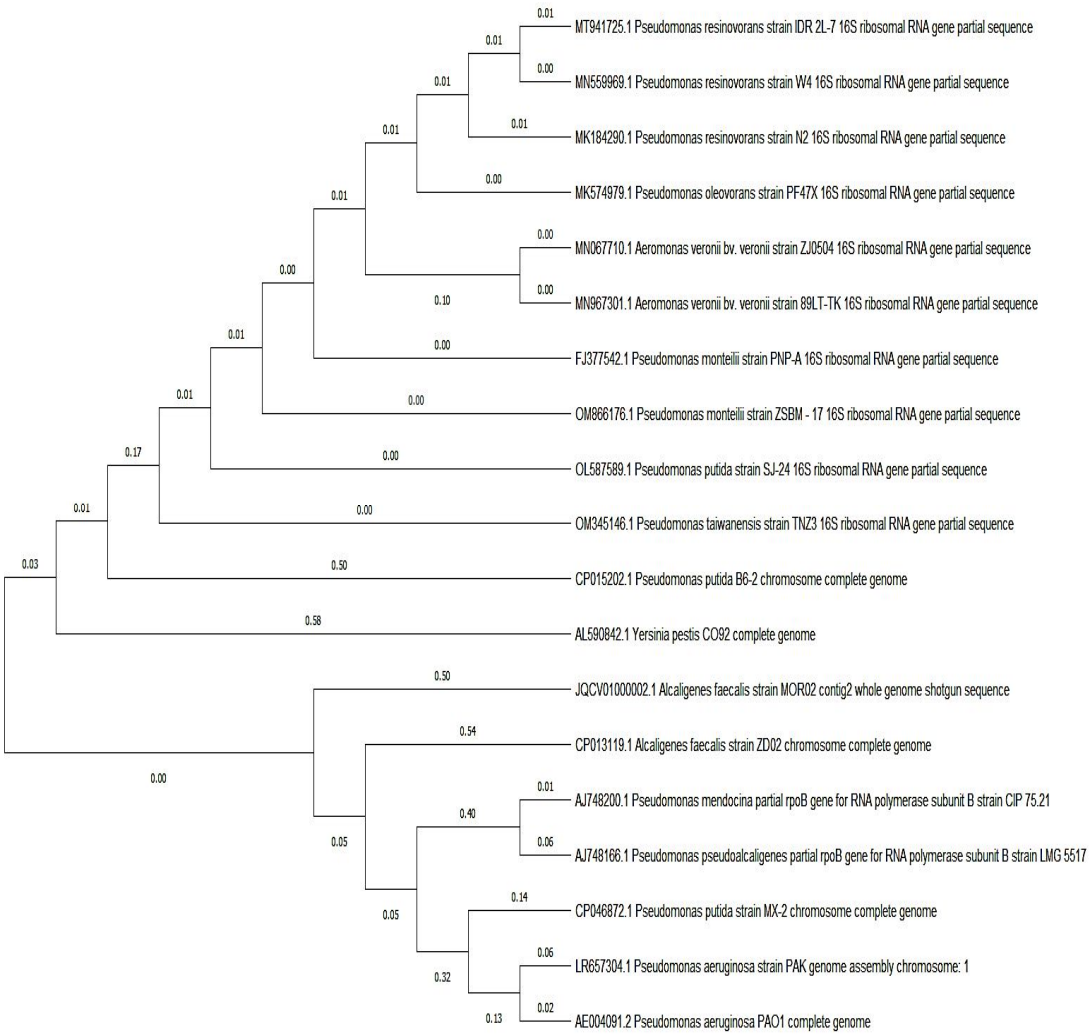

Fig 7: Phylogenetic Tree showing the genetic relationship among *Pseudomonas taiwanensis* strain TNZ3 and reference 16S rDNA sequences from the GenBank based on partial 16S ribosomal RNA gene sequences.

Decolorization of Azo dyes in presence of Carbon Sources:

Biological methods are easy to employ and operate at a minimal cost. *Pseudomonas taiwanensis* strain TNZ3 has been named as potential bacteria. Due to the low carbon concentration of the dyes, biodegradation was particularly challenging without an additional carbon source. As a result, various co-substrates were added to the medium, and each dye's decolorization was examined separately (Table-4). *Pseudomonas taiwanensis* strain TNZ3 effectively decolored 100 ppm dyes of

Disperse Yellow-3, Malachite Green, Basic Red-18, and Reactive Blue-4 at rates of 85.01%, 80.09%, 77.05%, and 72.42%, respectively, in the presence of sucrose.

Figure (8-11) demonstrates that the culture effectively decolored the range of co-substrates studied, including glucose, fructose, maltose, galactose, lactose, and starch. Compared to other substances, glucose supported a higher rate of decolorization. At glucose concentrations of 0.5 g, the pace and amount of decolorization were observed in order to determine the optimal concentration of glucose in the medium for maximum decolorization. The *Pseudomonas taiwanensis* strain TNZ3 culture shown decolorization of these four colors in amounts of 83.58%, 75.58%, 75.52%, and 71.18%, respectively. When fructose, maltose, galactose, lactose, and starch were compared, the decolorization potential was found to be lower.

However, the co-substrate concentration used in this investigation was only adequate to enable early growth. Sucrose appeared to be a superior co-substrate when all the co-substrates were examined, both in terms of decolorization and biodegradation.

Given that textile industry wastewater included dye in the range near the experimental setup, the dye concentrations utilized in the current study were 100 ppm and 200 ppm. An effective way to examine the part played by a single factor in an occurrence was to determine the impact of the carbon and nitrogen sources by changing the experimental design of one factor at a time while keeping the other factors constant.

The impact of carbon sources on dye decolorization shows unequivocally that growth was not necessary for color removal. However, between the development of bacterial isolates and specific carbon sources, dye removal was more significant.

Table: 4 Decolorization of Azo dyes in presence of Carbon Sources at 100 ppm

Azo Dyes	Decolorization (%)						
	Sucrose	Glucose	Fructose	Maltose	Galactose	Lactose	Starch
Disperse Yellow-3	85.01±1.13	83.58±1.25	82.32±1.10	79.95±1.15	77.12±1.11	72.83±1.05	70.45±1.18

Malachite Green	80.09±1.20	75.58±1.28	74.91±1.15	72.82±1.18	70.58±1.21	67.63±1.08	64.89±1.12
Basic Red-18	77.05±1.15	75.52±1.17	72.06±1.17	70.41±1.12	68.43±1.08	64.47±1.12	61.64±1.05
Reactive Blue-4	72.42±1.21	71.18±1.11	67.52±1.24	63.95±1.22	60.15±1.14	57.25±1.10	55.35±1.20

Decolorization of Azo dyes in presence of Nitrogen Sources:

On the eighth day of incubation, all strains demonstrated indications of maximum color elimination. *Pseudomonas taiwanensis* strain TNZ3 employed yeast extract, peptone, and ammonium chloride as nitrogen sources to decolorize these four colors. In yeast with peptone extract, the maximum decolorization of these four colors was 95.51%, 93.56%, 85.98%, and 70.35% (Table-5). Better results were obtained using yeast and peptone combined with ammonium chloride than when the yeast and peptone were used individually. When peptone extract was utilized in combination with ammonium chloride, the percentage of decolorization in Disperse Yellow-3 increased from 79.5% to 85.9% (Fig:12-15). Other dyes yielded findings that were comparable.

Additionally, the chemical makeup of the dyes had an impact on how quickly colors faded. The medium became acidic as dye concentration rose. Dyes with low molecular weight and simple structures deteriorated rapidly in large quantities, whereas those with complicated structures were more difficult to break down, which required a laborious procedure.

The findings made it abundantly evident that the effects of the individual and co-substrates on the use of the dye and on the development of bacterial isolates in the appropriate fermentation medium were distinct. After one week of incubation, the decolorization rate naturally reduced as degraded metabolites accumulated.

Table: 5 Decolorization of Azo dyes in presence of Nitrogen Sources in 192 Hours

Azo Dyes	Decolorization (%)				
	Peptone	Yeast	Peptone+NH4Cl	Yeast+NH4Cl	Yeast+ Peptone
Disperse Yellow-3	79.5	80.3	84.9	89.19	95.51
Malachite Green	84.71	84.83	86.49	89.55	93.56
Basic Red-18	55.29	77.07	81.98	84.11	85.98
Reactive Blue-4	42.35	63.64	67.63	69.28	70.35

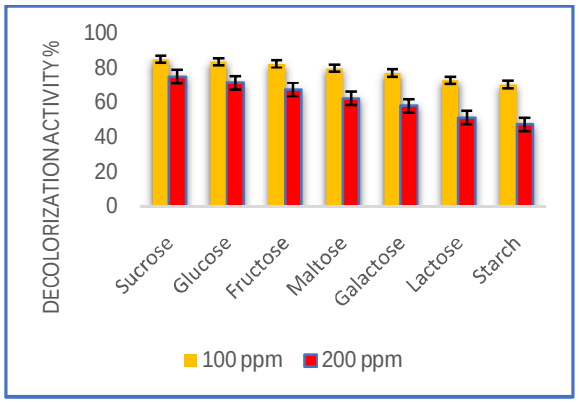

Fig 8: Decolorization of Disperse Yellow-3 by using different carbon sources

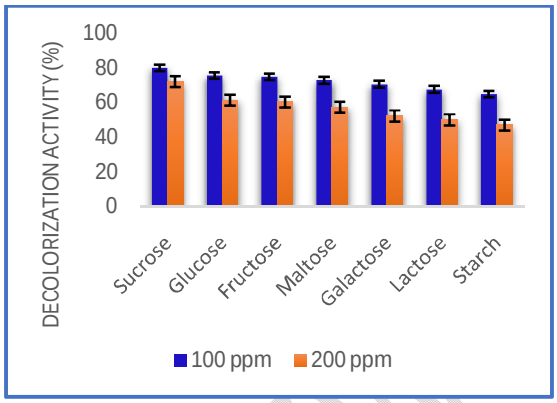

Fig 9: Decolorization of Malachite Green by using different carbon sources

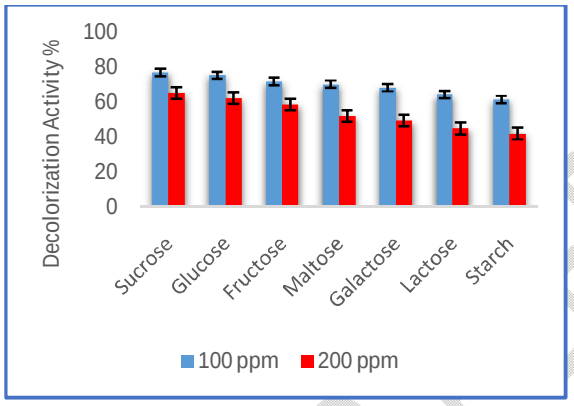

Fig 10: Decolorization of Basic Red-18 by using different carbon sources

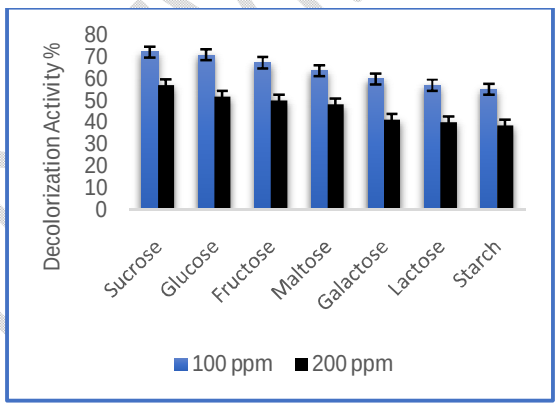

Fig 11: Decolorization of Reactive Blue-4 by using different carbon sources

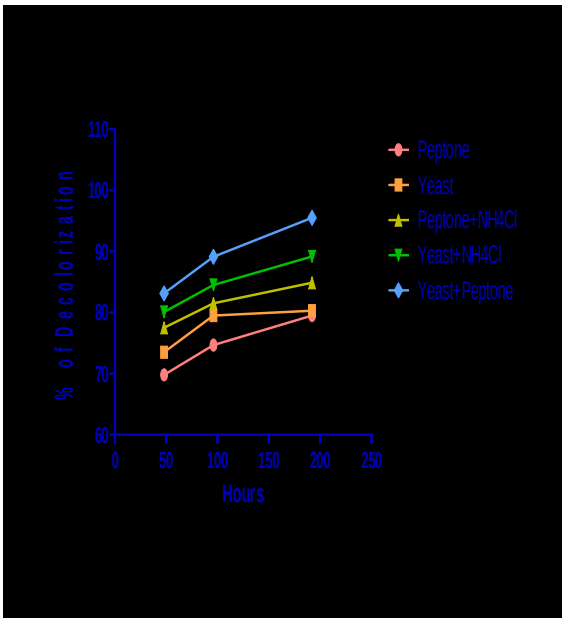

Fig 12: Effect of Various Nitrogen Sources on Decolorization of 100 ppm Disperse Yellow-3 dye by *Pseudomonas taiwanensis*

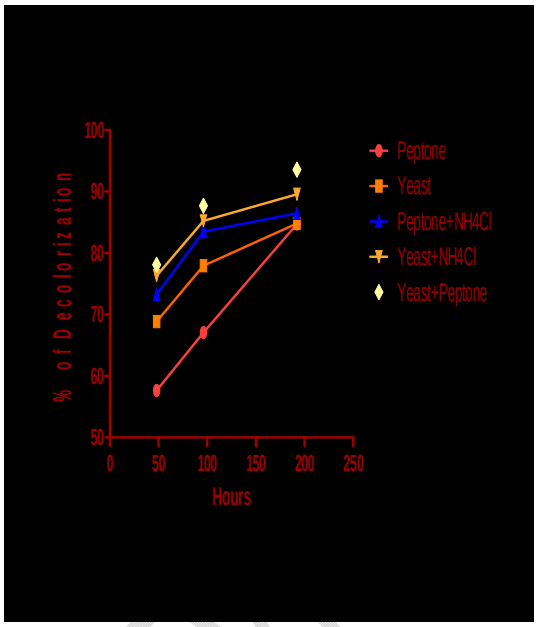

Fig 13: Effect of Various Nitrogen Sources on Decolorization of 100 ppm Malachite Green dye by *Pseudomonas taiwanensis*

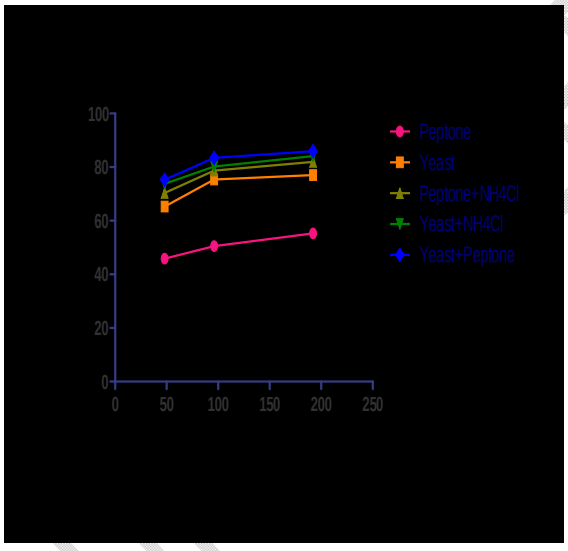

Fig 14: Effect of Various Nitrogen Sources on Decolorization of 100 ppm Basic Red-18 dye by *Pseudomonas taiwanensis*

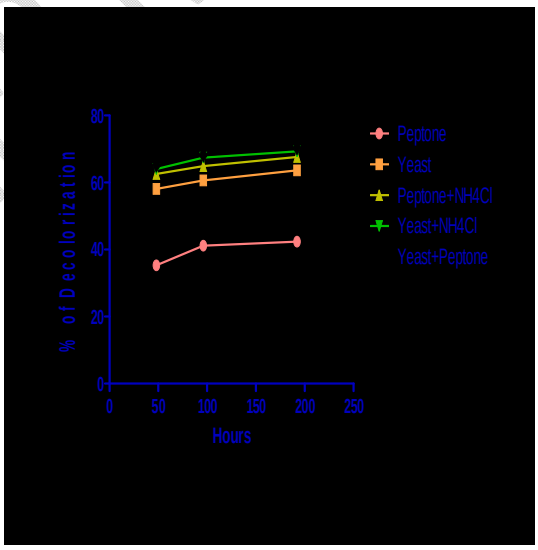

Fig 15: Effect of Various Nitrogen Sources on Decolorization of 100 ppm Reactive Blue-4 dye by *Pseudomonas taiwanensis*

Discussion:

The effectiveness of different carbon and nitrogen sources to support dye decolorization was investigated since the findings of the experiment showed the need for co-substrates to produce better decolorization.

Sucrose, fructose, glucose, and starch worked well as co-substrates with decolorization percentages ranging from 70% to 85%. The culture's ability to decolorize grew with growth and depended more on the structure of the dyes, increasing decolorization from 55.3% to 85.01%. Additional starch concentration increases did not enhance decolorization. According to the findings, sucrose is the best sugar to use for the most decolorization.

However, the combination of yeast and peptone acted as better co-substrates for nitrogen sources, demonstrating 70% to 95% azo dye decolorization. Both peptone and ammonium chloride, as well as yeast and ammonium chloride, produce outcomes that are comparable.

Once more, the amount of yeast extract is crucial to the decolorization of azo dyes. By raising the concentration of yeast extract up to 1.5%, the decolorization rate was raised to 98.8%. Following that, the decolorization rate dropped.

According to a study by Singh *et al.* [25] *Staphylococcus hominis* RMLRT03 decolored Acid Orange dye when the environment had the most carbon and nitrogen sources. These outcomes matched the information discovered in the current investigation. Another study revealed that decolorization rate of Remazol Golden Yellow dye was increased significantly by employing starch and lactose [26].

There are numerous accounts on dye decolorizing when there are extra carbon sources present. According to Joe *et al.* [27], adding glucose to the medium increases the pace at which Clostridium bifermentans strains decolorize Reactive Red 3B-A and Reactive Black 5 [28]. When maltose was the carbon supply, the strain's dye-decolorization efficiency was 80.66%. Compared to other carbon sources in culture medium, it is significantly greater. [29]. 95% of the reactive violet-5 dye is decolored by isolate GSM-3 when a nitrogen supply is present. [30].

“The sequence of the carbon sources' effects on the RR 239 decolorization efficiency for the entire incubation period of strain CH12 was glucose (95-100%) > maltose (91-

100%) > trisodium citrate (72-100%) > starch (69-99%) > carbon-free (27-51%)" [31] "Starch is frequently used in the textile industry for sizing purposes, and because it is washed during the subsequent processing steps, there is an excessive concentration of starch in the textile effluent". [32]. "Urea, peptone, starch, and ammonium chloride showed less decolorization and were quite similar to the control, however the medium supplemented with glucose had 66% decolorization". [33] Yeast extract and peptone both increased B. laterosporus' ability to decolorize the dye by 100% and 98%, respectively. [34]

Recent studies indicated that starch decolorization was delayed compared to glucose due to low glycoside hydrolase enzyme activity, but the combination of starch and glucose achieved good color removal yield [35]. According to Khehra et al. [13], glucose acts as a co-substrate to accelerate the decolorization of RV5R when the dye concentration is increased by up to 2.5 g/l. This was primarily caused by the glucose metabolism's synthesis of NADH and FADH.

Due to dyes' toxicity to microorganisms, previous research found that low concentrations provided higher color removal and that decolorization % declined at high dye concentrations [36] .

Additionally, studies have demonstrated that media containing yeast extract worked best for decolorizing Rancid Fast Blue dye [37]. The current investigation confirms the findings of Gomare et al [36] earlier work in that peptone and other inorganic nitrogen sources did not improve color removal by bacterial species (2009). With the addition of yeast extract, a wide spectrum of dyes had a higher percentage of their color removed [38]. Nevertheless, a review of the literature demonstrates that yeast extract is the best co-inducer for bacterial isolates [39-40].

Because the bacterial culture was unable to decolorize the dye in the absence of a co-substrate, it appears that the presence of an additional carbon source is required for both bacterial growth and dye decolorization [41].

A strain of *Bacillus subtilis* may aerobically reductively cleave p-amino azobenzene to corresponding aromatic amines in the presence of glucose as a co-substrate. Similar to this, under aerobic circumstances when growing on nutritive broth or glucose-containing medium, strains of *Pseudomonas stutzeri*, *Acetobacter*

liquefaciens, *Citrobacter* sp., and *Klebsiella pneumoniae* were able to reductively decolorize Acid Red 2 dye [41].

“The most readily available and efficient carbon source for microbial metabolism is glucose, and it has frequently been shown that adding it increases the effectiveness of azo dye breakdown” [42]

This can be explained by the fact that while glucose is a source of reducing equivalents, the amount produced at lower glucose concentrations would not be sufficient to support effective decolorization [43-45]. Alternately, glucose may accelerate the decolorization process by promoting the development of bacteria that are rapidly respiring, which quickly exhausts the oxygen in the medium and favors the anaerobic reduction of azo dyes [44].

The metabolism of carbohydrates was necessary for *Pseudomonas aeruginosa* to decolorize azo dye [46]. Hu demonstrated that yeast extract is a more effective nitrogen source for azo dye decolorization [47].

Methyl red was equally efficiently broken down by *Klebsiella pneumoniae* RS13 in conditions with different glucose concentrations of 0.5 to 5 g. [39]. Yeast extract concentration was directly connected with *Pseudomonas luteola* growth and azo dye decolorization effectiveness; the highest decolorization efficiency of 94% was noted in medium with 0.3 g yeast extract [48]. The regeneration of NADH, which serves as an electron donor for the reduction of azo bonds, is thought to depend on the metabolism of yeast extract.

Accordingly, it was deduced from the aforementioned data that an electron donor source and redox mediators are necessary for the effective reduction of azo dyes. Maltose was broken down catalytically, and the produced electrons were transferred to azo bonds by redox mediators, leading to the formation of the matching aromatic amines. Maltose can be viewed here as an electron donor in this mechanism, whereas azo dyes can be viewed as an electron acceptor. Due to oxygen's high redox potential, it preferentially uses released electrons in environments where there is an abundance of oxygen. But in oxygen-poor environments, electrons are directed toward azo dyes to weaken azo bonds via a redox mediator. Additionally, additional nitrogen source (proteose peptone) was added to the minimum medium to refill the redox mediator.

In general, co-substrate modified media had the best percentage decolorization. This outcome was consistent with Moosvi *et al.* (2005) [49], who found that the presence of yeast extract and starch in the medium accelerated the biodegradation process. The inability of the bacterial culture to decolorize dye in the absence of additional carbon and nitrogen sources highlights the necessity of co-substrates for growth and decolorization [41].

Conclusion:

Environmental protection is seriously threatened by the careless discharge of industrial effluents into water bodies. The largest and most useful class of dyes, azo dyes account for more than 50% of all dyes manufactured annually. The textile dyeing facilities have a problem with the detoxification and disposal of sludge. The ideal method for detoxification of sludge is microbiological treatment. When the decolorization is done with carbon sources, the decolorization rate is better for sucrose as compared with other carbon sources for all dyes. In case of decolorization with nitrogen sources, combination of Yeast and Peptone extract shows better rate of decolorization for all dyes. Among four dyes Disperse Yellow-3 exhibit better result because of its simple chemical structure. Altogether, it can be concluded that the utilization of different co-substrates in bacterial culture play vital role on dye decolorization process.

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