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ORIGINAL ARTICLE

Optimization of Environmental Parameters on Decolourization of Indigo Dye by the Bacterial isolates

Devika P. Vala*[1], Devyani R. Tipre[1], Shailesh R. Dave[2], Darshna K. Patel[1], Shivranjani B. Gajjar[3]
[1]Department of Microbiology and Biotechnology, School of Science, Gujarat University Ahmedabad 380009, India.
[2]Xavier's Research Foundation, Loyola Centre for Research and Development, St. Xavier College Campus, Navrangpura, Ahmedabad 380009, India.
[3]Department of Physics, C.U. Shah. University, Surendranagar- 363030, India.
***Correspondent Author:** Email: vala.devika@gmail.com,

ABSTRACT
The textile industry uses multiple types of dyes like reactive, azo, anthraquinone, and triphenylmethane. When dyes are not completely used up during the colouring process, they frequently wind up in water bodies as waste, which pollutes the water. Due of this, the industry is one of the main causes of global water contamination. The release of coloured wastewater from industries into the environment creates undesirable consequences for the ecosystem and human beings owing to its pernicious effect. The potential of bacterial agents isolated from various sources, including dye-contaminated soil and textile effluent, to successfully decolourize and breakdown these dye pollutants, resulting in enhanced water quality, has been demonstrated. One of the most significant and widely used types of synthetic dyes used in the denim dyeing process for textiles are indigo dyes, which are aromatic compounds with a complicated structure. The present study was conducted to investigate the optimum conditions of inoculum size, pH, temperature, and carbon and nitrogen source for the efficient decolourization of Indigo dye. The study involved the selection of a best and efficient bacterial consortium possessing a high potential to decolourize indigo blue dye in a liquid medium. Consortia were selected from the natural samples which were collected from the dye contaminant site of the denim plant. Bacterial consortia showing high decolourization rates were used for the optimization of the decolourization of indigo dye. Decolourization was studied with 10 mg/L to 100 mg/L Indigo dye concentration. The optimum conditions determined for the selected bacterial consortia for decolourization were, inoculum size 1 ± 0.5% containing ~10^8 cells/mL, pH 7.5±0.5, temperature 34±2 ℃, Glucose 1 ± 0.5% of NH_4Cl 0.5-1%, with the mineral salt medium. After providing optimized condition, the rate of decolourization was efficiently increased. Bacterial isolates of consortia were tentatively identified based on the classical approach of cultural, physiological, and biochemical analysis.
***Keywords*;** *Textile industry, water contamination, Textile effluent, Pollutants, Consortium.*

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INTRODUCTION

One of the oldest and most significant manufacturing sectors in the world is the textile industry which employs some 35 million people globally and has made a significant contribution to the growth of many economies [6]. But the textile sector is regrettably one of the most polluting despite its evident necessity. Desizing, scouring, bleaching, dyeing, and finishing of textiles are wet processing sector of the textile industry. These procedures use a water, alkalis, salts, surfactants, dyes, and pigments [13].When textile dyes are dumped into water bodies, they not only change the water's look but also the stability of aquatic ecosystems because they block light from accessing the water. Because of this, the oxygen levels in the aquatic environment are reduced, and the toxicity of dyes has an adverse effect on the aquatic species that live there [11]. The disposal of untreated textile dye wastewater has therefore become a major global concern in many countries due to the dangers that these dye pollutants pose both to human and aquatic

life. The effluent from the textile and dyeing industries is primarily contaminated by textile dyes. 5%–10% of the dyes used are discharged into the environment with wastewater, and the coloured wastewater has a detrimental impact on the photosynthetic activity and dissolved oxygen level in the water bodies it is released into. As a result, treating this particular form of wastewater for colour is typically more crucial than treating wastewater containing other colourless organic substances [1]. Generally speaking; cloth dyes are very resistant to biological deterioration. The wastewater from the textile and dyeing industries can't be successfully decoloured using traditional biological treatment methods like activated sludge systems. Decolourizing wastewater holding dyes has been the subject of numerous studies using a variety of techniques and biological systems [12],[14],[17]. Various biological systems, including fungi, bacteria, enzymes, can be used to decolourize dye-containing wastewater [4],[19],[16],[18],[9],[16]. Denim cloth is coloured using indigo dye (*C.I. 73015 Acid Blue 74*)[14]. Moreover, its harm has been documented [3]. High amount of indigo dye are discharged into rivers and lakes with wastewater because it is resistant to decolourization by activated sludge systems. It needs to be decoloured using environmentally favourable techniques due to its detrimental effects. The decolourization of indigo by bacteria, fungi, and enzymes has been documented [3], [14], [18]. Due to their great efficacy and eco-friendliness, biological treatment approaches utilizing aerobic or anaerobic bacteria have garnered significant interest as an effective substitute [5]. The microbial clean up method known as bioremediation uses microorganisms that can adapt to hazardous wastes and spontaneously evolve new resistant strains that can change a variety of toxic compounds into less damaging forms [10]. Actual textile waste water may be effectively and consistently degraded by microbial processes [7]. Because it uses environmentally friendly remediation techniques, the use of microorganisms for remediation is thus a potential remedy for environmental contamination. Laccase enzymes and bacteria perform dye decolourization differently and require various decolourization conditions to achieve optimal levels of decolourization. The application of bacterial organisms is that they can breakdown and mineralize dyes in addition to having a shorter growing period. The effectiveness of bioremediation methods is constrained by the need for specific decolourization conditions for laccase enzymes and bacteria, and the potential for the emergence of new resistant microorganism strains. This study has looked at finding bacterial strains that can be utilized to break down and remove colour from dye wastewater as well as trying to understand how they work. The primary goals of this study is to investigate the employment of bacteria in pure or mixed cultures in the biodegradation of the dyes used in the textile and dyeing industry, namely azo, anthraquinone, and triphenylmethane colours. Therefore, the comparative investigation of the effects of different cultural circumstances on a chosen consortium's ability to decolorize indigo dye was done in this research.

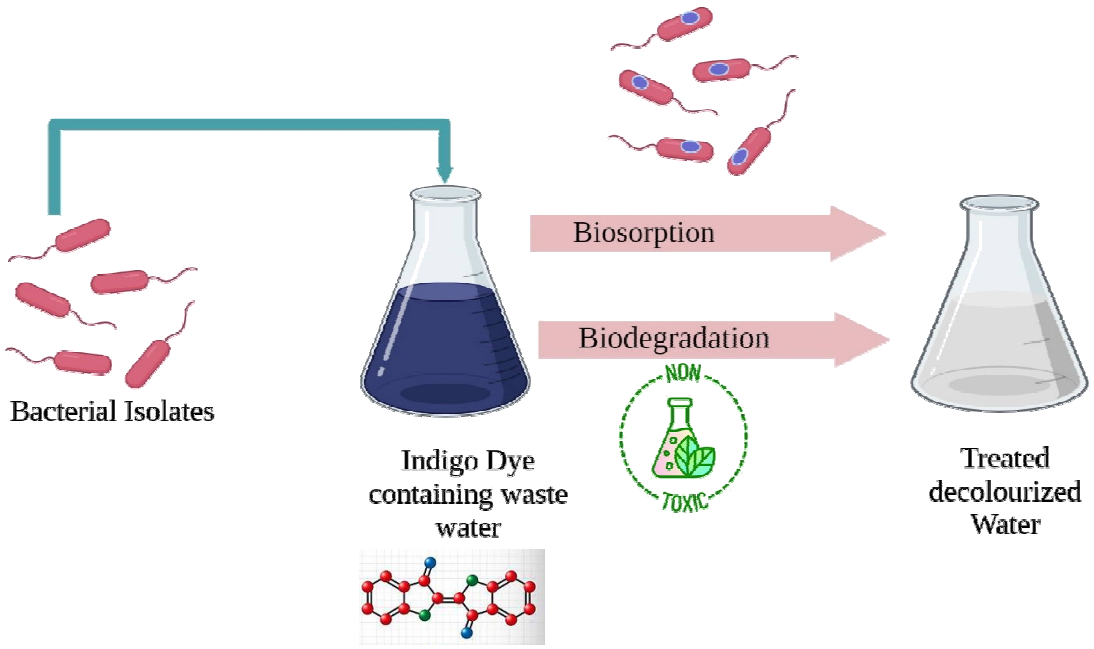

Figure 1 Decolourization of effluent water

MECHANISM OF DYE DEGRADATION:

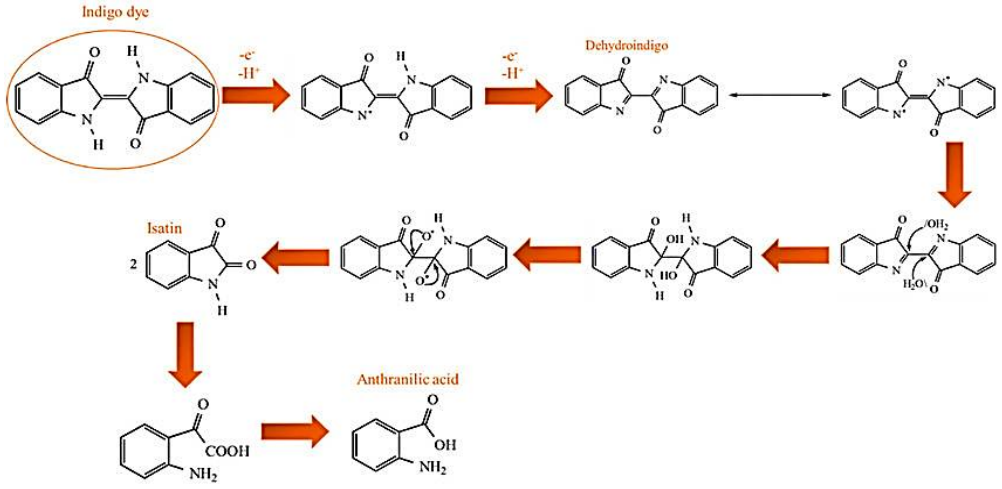

Figure 2: A possible mechanism for degradation of indigo dye

MATERIAL AND METHODS

Dye

The synthetic liquid indigo-blue dye, 96% pure, chemical formula $C_{12}H_8O_2N_2$, collected from denim Industry. Indigo was prepared as a stock solution of 1000 mg/L by dissolving in concentrated H_2SO_4 and utilized at various concentrations (100 mg/L). All the microbiological media and medium ingredients used in the present study were purchased from HI media (Mumbai, MH. India).

Isolation, screening, and identification of dye decolourizing bacteria

The dye decolourizing bacteria were isolated from the waste water of local dying houses in Ahmedabad-India. Sample is named as 20-B during experiment. 10 ml of Coloured collected water sample was added in 100 ml of nutrient broth for enrichment Purpose. The selected sample showing substantial decolourization of the dye were selected for further studies. Isolation of dye decolourizing bacteria were carried out from the consortium via serial dilution and plating them relevant dilutions on Nutrient agar medium containing, Peptone 5 g/L, Beef extract 3 g/L, Sodium chloride 5 g/L, Agar 15 g/L, Dye (Indigo blue) 100mg/L, Agar 20 g/L (pH 7.0). All the isolated bacteria were further studied by inoculating them in nutrient broth containing dye and incubated at 37 ◦ C under static conditions for 48 hrs. The effect of decolourization was observed visually. Dye decolourizing bacteria were identified on the basis of morphological and biochemical tests according to Bergeys Manual of Systematic Bacteriology [15].

Dye decolourization evaluation

Decolourization activity was performed in 100 ml of BSH medium containing 100 mg/L Indigo dye and 10% (v/v) inoculum of selected Enriched sample. Uninoculated dye containing medium served as control. Inoculated medium and control was incubated at 37±2 ◦ C for six days under static culture condition. About 4 ml samples were withdrawn aseptically and centrifuged at 10,000 rpm (g value: 10,375) for 20 minutes. The supernatant was used for measuring absorption at 620 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). The percent decolourization of effluent was determined by using the formula:

$$D = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

Where, D-decolourization in %; A0-initial absorbance; A1-final absorbance

Dye decolourization optimization

Decolourization of Indigo textile dye by selected consortium was optimized with respect to the effect of 1%, carbon sources (glucose, fructose, starch, sucrose, mannitol , maltose), 1% nitrogen sources (NH_4Cl,$(NH_4)_2SO_4$,yeast extract, meat extract, peptone), temperature (25,35,45,55°C), pH (4,5,6,7,8,9,10,11), and inoculum % (1,5,10,15,20,25), aeration optimization carried out by providing two conditions(static and shaking at 150 rpm). Initial experiments were carried out with 1%, (v/v) inoculum of each selected consortium in BSH medium, without culture was served as control. All the flasks were

incubated at 37 ±2 ◦ C under static conditions for seven days. After interval of 24hrs the samples were withdrawn and analysed for percent decolourization.

RESULT AND DISCUSSION

Isolation, screening, and identification of dye decolourizing bacteria

The sample was collected from Denim textile industry, Ahmedabad, Gujarat, India. The production of yarn, installed spindle age, and fabric and garment output have all increased in the textile industry. These sectors contribute favourably to India's economic transformation. Samples of textile dye wastewater were taken from the effluent disposal site to screen for and isolate microorganisms that degrade dye. The likelihood of bacteria being able to decolourize dye wastewater is quite high. Industries that are responsible for soil and water contamination include the textiles and dyeing sector. Large amounts of water and chemicals, including caustic soda, bleaching solutions, soda ash, sulfuric acid, and sodium peroxide, are consumed by them. Certain enterprises that cause pollution release a lot of dye-containing effluents into water bodies. Today, the government and people are quite concerned about this pollution issue, which is mandated by law. In order to comply with regulations, industrial units are currently searching for affordable ways to reduce their pollutant loads. The bacterial cultures from consortium were identified by microscopic, biochemical characters were identified observations as shown in figure 3 and identified as Bacillus sp., Proteus sp., Pseudomonas sp. Though its consortium, metagenomics-based analysis will be performed for confirmative results.

Table 1. Identification of dye decolourizing bacteria from the selected consortium.

Kit for - Gram Positive		Kit for Gram-negative		
Test	**S1**	**Test**	**S2**	**S3**
Gram's nature	**+**	**Gram's nature**	**-**	**-**
Shape	Rod	**Shape**	Rod	Rod
Motility	Non-Motile	**Motility**	Motile	Motile
Glucose	A &G	**Glucose**	A&G	
Lactose	A	**Adonitol**	-	-
Xylose	A	**Lactose**	-	-
Mannitol	A	**Xylose**	-	-
Maltose	A	**Mannitol**	-	-
Sucrose	A	**Maltose**	-	-
Indole	-	**Sucrose**	+	-
Methyl Red	+	**Sorbitol**	-	-
Voges-Prausker	-	**Rhamnose**	-	-
Citrate Utilization	+	**Indole**	+	-
H_2S Production	+	**Methyl Red**	+	-
Gelatinase	+	**Voges-Prausker**	-	-
Catalase	+	**Citrate Utilization**	V	-
Oxidase	-	**H_2S Production**	$^2A/A_5$, $4H_2S$+	-
TSI	$^3A/A_5$, $4H_2S$+	**Gelatinase**	+	+
Starch	+	**Catalase**	+	+
Casein	+	**Oxidase**	-	+
TBA	**+**	**Urea**	**+**	**+**

V- Variable, TSI-Triple Sugar Iron, 2-Acid, 3- Acid-Gas, 4- Hydrogen Sulphide, 5- Negative, 6-Positive

Dye decolourization optimization

Effect of carbon source on dye decolourization

Six carbons sources such as glucose, fructose, starch, sucrose, mannitol & maltose were used. Figure 3 shows the range of activity on decolourization of indigo dye with glucose was,35%, 25%, 78%, 96%, 98%, 99%,The range of activity on decolourization of indigo dye with fructose was, 4.5%, 6%, 10%, 24%, 35%, The range of activity on decolourization of indigo dye with starch was, was 23%, 56%, 58%, The range of activity on decolourization of indigo dye with sucrose 29%, 66.44%, 75.16%, 83.89%, The range of activity on decolourization of indigo dye with mannitol was16%, 24.73%, 10.52%, 30%, 36%, The range of activity on decolourization of indigo dye with maltose was 12%, 18%, 27%, 36%, 39%, 45%. Fructose shows lowest decolourization activity with selected consortium and glucose found to suitable carbon source for the efficient decolourization by selected consortium.

Effect of nitrogen source on dye decolourization

Five nitrogen sources such as (NH_4Cl, $(NH_4)_2SO_4$, yeast extract, meat extract, peptone was used. Figure 4 shows the range of activity on decolourization of indigo dye with NH_4Cl was ,45%,90.47%,100%,The range of activity on decolourization of indigo dye with $(NH_4)_2SO_4$was 28.6%,48%,61%,85.6%, The range of activity on decolourization of indigo dye with yeast extract was o%,12%, The range of activity on decolourization of indigo dye with Meat extract was 55%,67%, The range of activity on decolourization of indigo dye with peptone was 11%,11%,33%,39%,50%, The range of activity on decolourization of indigo dye with beef extract. Yeast extract is gives least decolourization and NH_4Cl is most suitable nitrogen source for efficient decolourization.

Effect of temperature on dye decolourization

Decolourization of indigo dye was carried out at various temperatures (25,35,45,55°C). Figure 5 Shows that selected consortium decolourized this dye efficiently at 35, 45, 55°C. This dye decolourization capacity of this selected consortium under a broad range of temperatures shows that these strains could be effectively used in dye decolourization.

Effect of pH on dye decolourization

The pH of the medium may affect the dye decolourization activity of bacteria [8]. Therefore, the effect of pH on the dye decolourization activity of this bacterium was also investigated at different initial pH values (4 to11). As shown in figure 6, this result shows selected consortium shows decolourization at all selected pH range but maximum decolourization is observed at 7-8 pH range. Highly alkaline PH shows decolurization on same day which indicated chemical reduction of dye, here that value is not selected because at that PH range organisms are not showing growth.

Effect of inoculum size on dye decolourization

Effect of percent inoculum containing ~10^8 cells/mL on dye decolourization with % (1,5,10,15,20,25) was studied. Figure 7 shows decolourization 1% and 5% inoculum size gives efficient decolourization.1% inoculum size gives better results than 5% inoculum size.

Effect of aeration on dye decolourization

The dye decolourization activity of this bacterium was compared under static and agitated conditions over the course of 7 days. The dye decolourization activity was studied at pH 6.0, dye concentration of 100 mg/L, at temperatures (37°C). The observed dye decolourization activity of this consortium under static and agitated conditions was quite high. Figure 8 shows, compares to shaking condition static condition gives higher range of decolourization in lesser time.

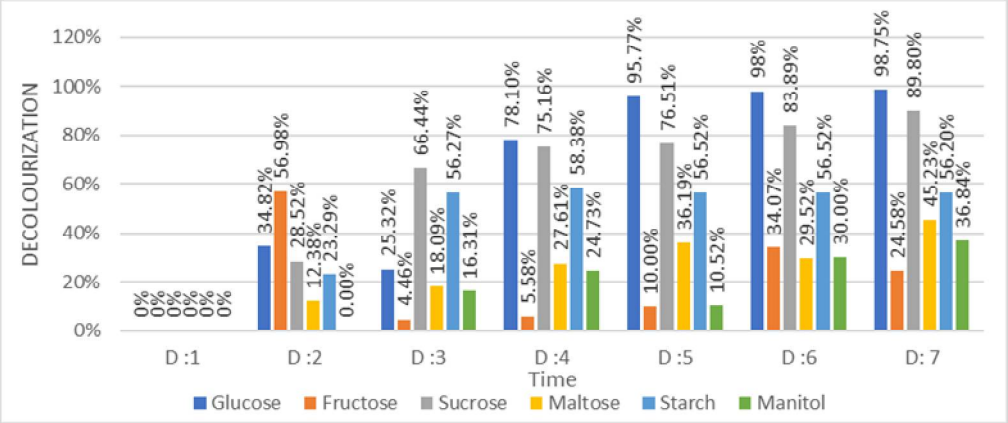

Figure 3. Carbon Source Optimization 20 – B

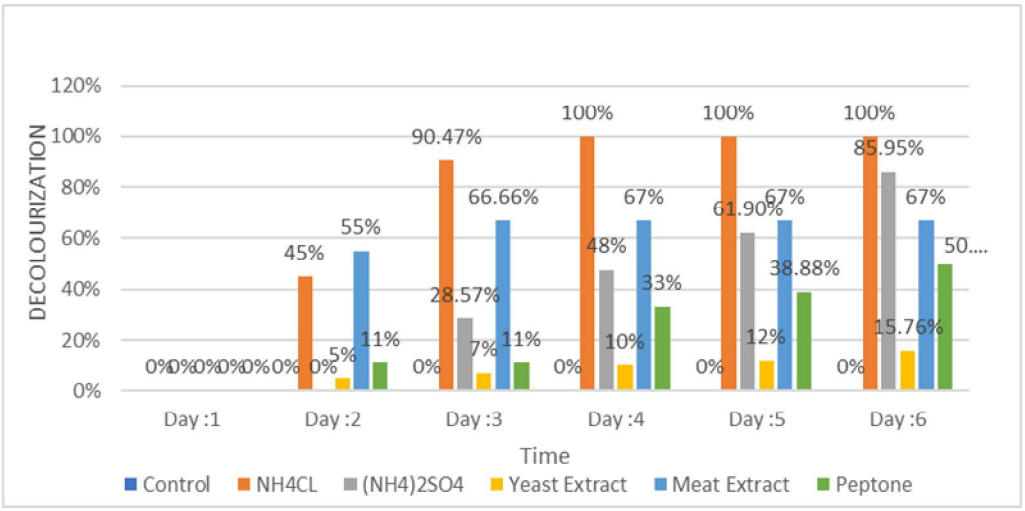

Figure 4. Nitrogen Source optimization 20 – B

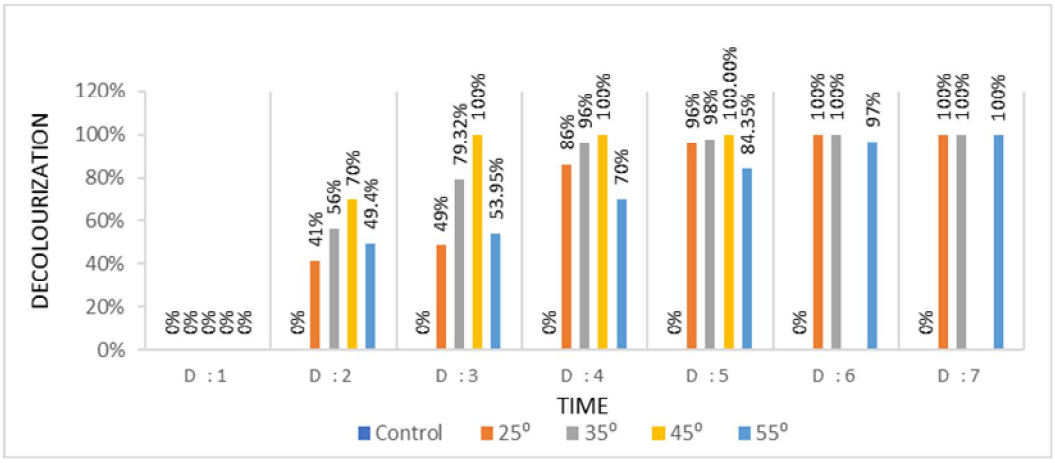

Figure 5. Temperature optimization of 20-B

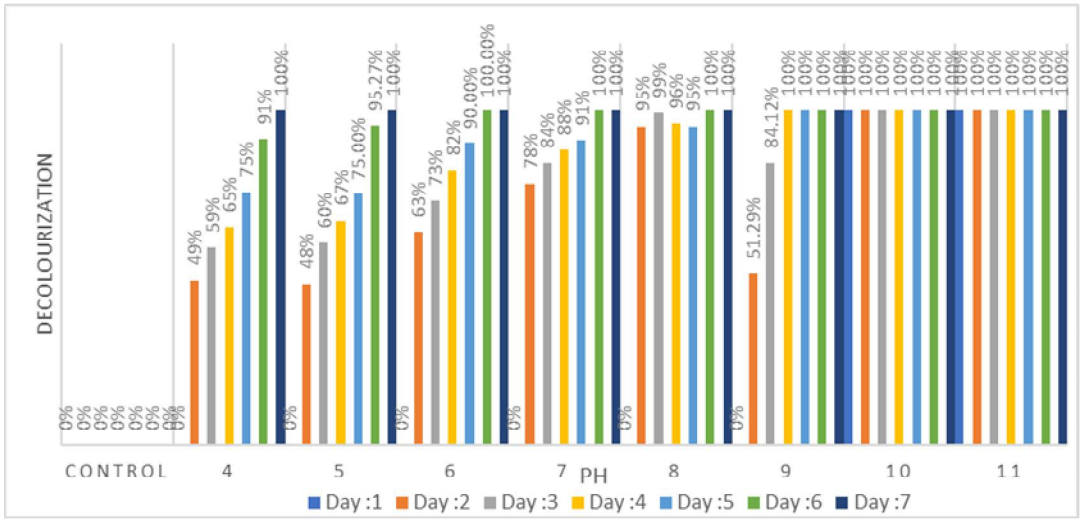

Figure 6. pH optimization of 20 – B

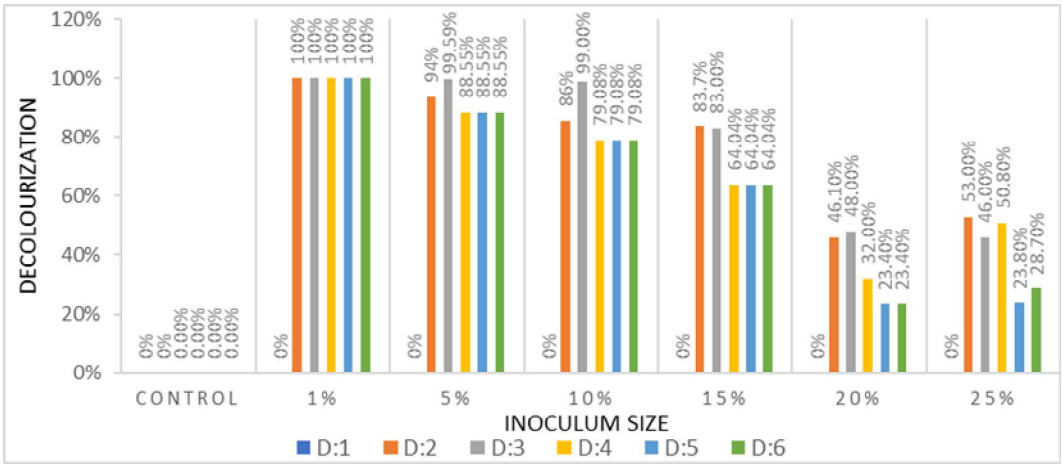

Figure 7. Inoculum size optimization of 20 – B

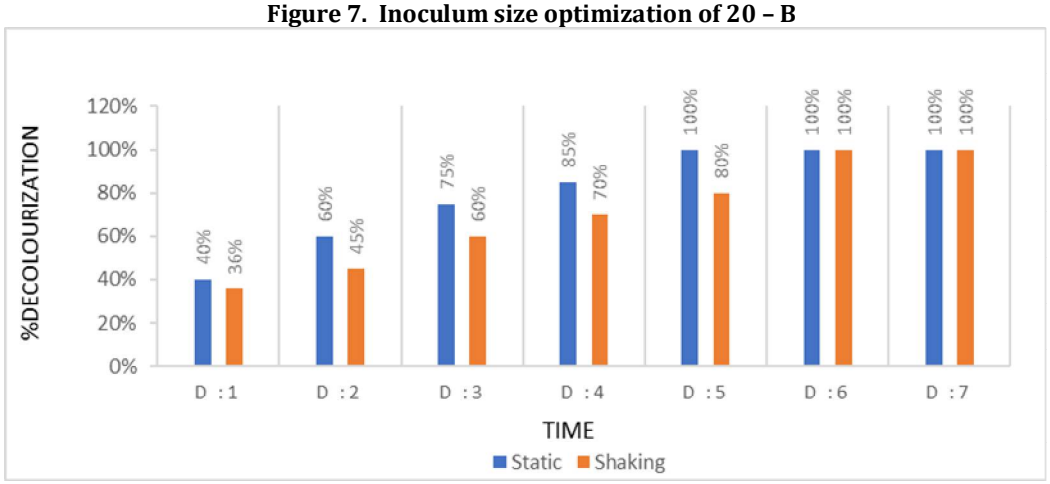

Figure 8. Aeration condition optimization of 20 – B

CONCLUSION

The study investigated the effects of various environmental and nutritional factors on the decolourization of indigo dye by a selected bacterial consortium. Key findings include the identification of glucose as the most effective carbon source, achieving up to 99% decolourization, while fructose was the least effective. Among nitrogen sources, NH_4Cl was the most suitable, with decolourization reaching 100%, whereas yeast extract was the least effective. Optimal temperature for decolourization was identified between 35°C and 55°C, demonstrating the consortium's efficiency across a broad temperature range. The pH range of 7-8 was optimal for maximum decolourization, with highly alkaline conditions showing chemical reduction of the dye but not supporting bacterial growth. An inoculum size of 1% (~10^8 cells/mL) provided the best decolourization efficiency. Additionally, static conditions were found to be more effective than agitated ones for dye decolourization. These findings suggest that the selected consortium is highly effective in decolourizing indigo dye under specific conditions, making it a potential candidate for bioremediation applications.

Authors' Contribution: All authors contributed equally.

Conflict of interest: Author declared no conflict of interest

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