IJBPAS, July, 2024, 13(7): 3215-3226

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIODEGRADATION OF AZO DYE BY BACTERIA ISOLATED FROM TEXTILE INDUSTRY EFFLUENTS

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Received 15th March 2023; Revised 8th July 2023; Accepted 5th Oct. 2023; Available online 1st July 2024

https://doi.org/10.31032/IJBPAS/2024/13.7.8138

ABSTRACT

Since the beginning of time, people have used colour, and as population and industrial expansion have expanded, synthetic dyes have become more popular. Bacteria isolated from a variety of sources, such as soil contaminated with dye and textile effluent, have demonstrated the capacity to decolourise and decompose these dye pollutants, improving the quality of the water. Numerous microorganisms, including fungi and actinomycetes as well as bacteria specially *Bacillus Cereus, Aeromonas hydrophila* and *Bacillus subtilis,* have dye-decolorizing ability. These traits synchronize with the capacity of different strains to function under a variety of a wide range of pH, salinity, and temperature, as well as good performance under conditions that are significant to industry and the environment. The utilization of bacteria in the bioremediation of azo dye-

contaminated environments using the widest range of methodologies and conditions is covered in this research with remarkable results. To highlight the significance of bacterial bioremediation, this study was carried out. According to the results, it was found that the highest percentage of the degradation of congo red dye was 74% at 37°C temperature and pH 6 after 7 days of incubation. The different pH values (5 to 7) and temperature range (25°C to 55°C) were tested to check the effective range of degradation and it was observed that the temp 25°C and pH 5 was found to be most suitable for biodegradation. The percentage of decolorization was affected by the bacterial strain and different concentration. 16S rRNA sequencing analysis revealed that isolate belongs to *Acinetobacter indicus*. This study indicates that this bacterium can be utilized for dye degradation due to high ability of biodegradation.

Keywords: Textile effluent, degradation, dye decolorizing, bioremediation, azo dye

INTRODUCTION

After China, India is the country that exports the most dyestuffs and intermediates. The major consumer of dyestuffs, at about 80%, is the textile sector **[1]**. Industries release sewage and waste water, which allows waste to enter the lake. Consequently, it is a significant source of environmental toxins, and it also has an impact on the aquatic ecology and soil microflora **[2, 3]**. A total of 70% of the dye materials used in textile industries are azo dyes **[4, 5]**. The synthetic dyes are commonly utilized in petroleum products, paper printing, leather dyeing, colour photography, and textile dyeing **[6]**. The sector of textiles consume more water and more than 90% of which releases wastewater directly in environment **[7]**. Synthetic dyes and azo dye themselves and the metabolites generated upon their breakdown or degradation, such as aromatic amines, are responsible for the harmful effects of azo dyes, in particular their capacity to cause mutations. One of the criteria taken into consideration when categorizing the dyes as potentially dangerous to health is the risk of the dye degrading and releasing these carcinogenic amines **[8]**. For the azo dye degradation under anoxic conditions, the use of pure bacterial cultures such as *Proteus mirabilis, Pseudomonas luteola,* and *Pseudomonas sp.* has shown very encouraging results **[9]**. The removal of colours from wastewater effluent has been accomplished using a variety of physicochemical techniques. The physical/chemical methods can be implemented, however, carrying out this technique has the inherent drawbacks of being economically impracticable (as it takes more

energy and chemicals), being unable to entirely remove the resistant azo dyes and/or their organic metabolites. Additionally, this produces a significant amount of sludge that may cause secondary pollution problems, and more requiring labor-intensive procedure **[10]**. In the field of environmental science, "bioremediation" has emerged as a crucial microbiological technique for dealing with various toxins **[11]**. A variety of yeasts, actinomycetes, fungi, bacteria and algae have the ability to decolorize azo dyes. Although bacteria are typically quick to multiply under facultative anaerobic, aerobic, and harsh environmental factors including broad ranges of salinity in pH and temperature. Therefore, bacteria are the most frequently used microorganisms for the degradation of azo dyes **[12]**. Comprehensive sulfonated azo dye removal methods are still a long way off, which needs experimental studies exploring different organisms and technologies **[13]**. The use of bacteria for azo dye degradation is cost effective and most potential process in the current scenario to protect the environment from the toxic effects of components present in azo dye. In the present research the azo dye degrading bacteria is isolated from the highly sensitive zone and tested for its ability to degrade the dye. The physiological, morphological and molecular characterization of bacteria was carried out to unfolde the important properties of organism. The azo degradation studies revealed that the bacterium is highly efficient for degradation and may be used for commercial purposes in future.

MATERIALS AND METHOD

Sample Collection

Collection of soil sample was done from Kumar Cotton Mills Pvt. Limited, Opposite Old Narol Court, Ahmedabad, Gujarat, 382405 (22.9642° N, 72.5903° E). Dye degrading bacteria are present in more concentration in the waste material of the textile industries such as contaminated soil and water. Therefore, the soil sample was collected from the selected site.

Isolation and Screening of Dye degrading bacteria

The dye degrading bacteria were isolated on Nutrient agar medium supplemented with congo red dye. The soil sample was inoculated in the Nutrient broth containing congo red dye and incubated at 37°C for 7 days to observe the decolorization. The collected soil sample was further inoculated on nutrient agar plate having congo red dye for the isolation of dye degrading bacterium. The plates were incubated at 37°C for 24-48 hrs and the colonies producing halo zone against the

reddish pink background of congo red dye were isolated.

Identification of Dye degrading bacteria

The isolates were identified using morphological, biochemical and molecular tests according to the Bergey's Manual of Systematic Bacteriology **[14]**. The Gram staining and carbohydrate fermentation test were performed to identify the metabolic properties of isolates.

Dye Degradation Assay

The degradation assay was performed in 100 ml of nutrient broth with 3 ml of inoculum (or around 2 × 108 cells/ml) and 1.0% of a commercial dye (Congo red) with their respective control. Each flask was incubated for 7 days at static condition. After the complete evaporation process, the residue was weighed and used for the gravimetric value for additional calculations **[15]**.

Phylogenetic Characterization

The identification of bacterial culture was carried out by 16S rRNA gene sequencing analysis at Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, India. Briefly the genomic DNA was isolated from overnight grown cultures **[16]**. Isolated DNA was amplified with primers 27F and 1492R. Polymerase chain reaction was performed using the following thermal conditions: 25 cycles of initial denaturation at 95 °C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 2 minutes, final extension for 72°C for 10 min. The Polymer chain reaction product sequencing was completed using a Big Dye Terminator v3.1 cycle sequencing kit (Thermo fisher) with Taq DNA polymerase. The 16S rRNA sequence was searched against non-redundant database using NCBI BLAST similarity search tool. The 16S rRNA gene sequences were used as a query in the Identify tool of EzBio Cloud server **[17]** and the sequences of related species with validly published names and downloaded and used for phylogenetic analysis using Neighboring joining method of MEGA 11 software **[18]**. The evolutionary distances were computed using Kimura two parameter method **[19]**. The bootstrap test with 1000 replicates were performed in order to infer reliability of the tree generated **[20]**.

Optimization of media conditions for dye degradation

Effect of temperature and pH

To explore the effects of temperatures on degradation, the isolated bacterial colonies were inoculated at different temperature range of 25°-55°C. For the study of the different pH on the degradation of dye, the pH of medium was set in the range of 5-11using various buffers such as Sodium acetate, Tris-HCl and Tris Base. In both the assays the inoculated

medium were incubated for 24 hours and thereafter, the optical density was measured at 600nm.

Effect of different inoculum size

To determine the effect of inoculum size, the medium was inoculated with various concentration of bacterial culture such as 1%, 2%, 3% followed by measuring optical density at 600nm.

RESULT AND DISCUSSION

Sample Collection Site

The soil sample collection was done from Kumar Cotton Mills Pvt. Limited, Opposite Old Narol Court, Ahmedabad, Gujarat. The soil sample was taken 20 to 30 cm below the soil's surface, sterilized polythene container used to collect a soil sample **(Figure 1)**.

Isolation and screening of dye degrading bacteria

The decolorization of dye was observed after 8-10 days of inoculation of sample **(Figure 2A)**. The bacteria grown in the flask were streaked on to the nutrient agar plate containing Congo red dye and the plates were incubated at 37° C for 3–4 days. The bacterial isolates observed on the media after breaking down the medium matrix within 24 hours signifies their ability of dye degradation **(Figure 2B)**. These organisms were potent to degrade the chemical dyes due to their different genetic makeup. Total 13 bacteria were isolated from the collected sample however, the bacterium produced bigger halo zone was selected for further studies.

Morphological Characteristics of Dye Degrading Bacterial Isolate

Colony Characteristics

The morphological characteristic features of bacterial isolate were identified by growing it on nutrient agar plate. As shown in **Table 1**. The bacterial isolate had the medium sized colony with circular shape. The colour of colony was fade white and margin was entire. It was observed that the isolate had the flat elevated colonies. The consistency of colony was soft and it was non pigmented colony.

Gram Staining

The bacteria isolated from the soil sample which were able to degrade the dye were Gram positive, small in size, rod shape and single in arrangement **(Figure 3)**.

Biochemical Analysis

The biochemical and metabolic properties of isolate was identified using the various biochemical test. According to the results of biochemical assays it was observed that the isolate was positive for Indole Test, Methyl Red (MR) Test, Voges-Proskauer (VP) Test, Simon Citrate, Catalase Test, Sucrose Fermentation, Lactose Fermentation and Oxidase Test (+ve) while negative for Dextrose Fermentation **(Figure 4)**.

Molecular characterization of selected isolates

The phylogenetic analysis of AJK using 16S rRNA gene sequence revealed that the isolate belongs to the *Acinetobacter sp*. The isolate was found to have a high similarity with *Acinetobacter indicus* strain with 100% bootstrap support **(Figure 5)**. The 16S rRNA gene sequencing of isolate GenBank has received AJK with the accession number **OQ509712**.

Physical condition optimization of Dye degrading bacteria

Effect of Temperature and pH on potential isolate

The maximum growth of bacterial isolate was observed at 25°C temperature. In light of this, we can say that the bacteria isolated from the textile effluent had the maximum decolorization rate at 25°C. The textile effluent's bacterial isolate had the highest rate of decolorization at pH 7. Therefore, we may state that pH 7 is the ideal pH for dye decolorization. However, the bacterial isolate from the textile effluent had minimum decolorization rate at 55°C and the lowest decolorization at pH 5 **(Figure 6)**.

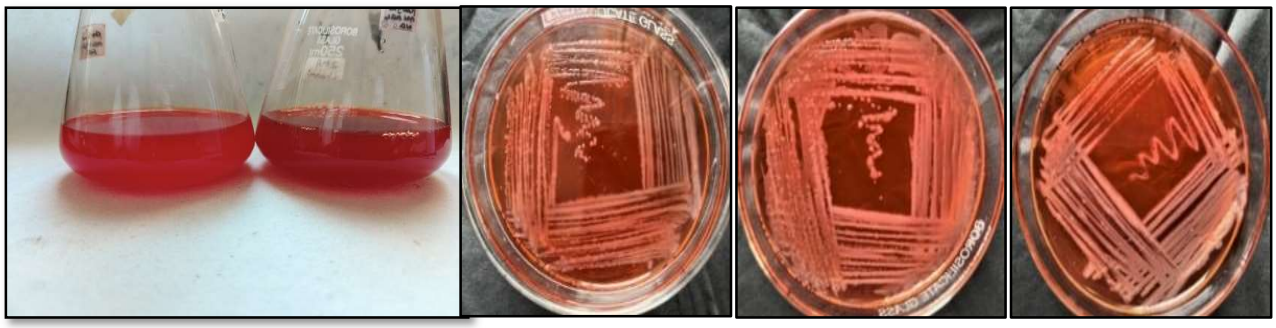
Inoculum Size

The textile effluent's bacterial isolate had the highest growth rate at 1% inoculum. However, the bacterial isolate from the textile effluent had the lowest growth when inoculum size was set at 4% **(Figure 7)**. In light of this, it was observed that the isolate has very high replication power in less time and less concentration which make this isolate more potent for the commercial use of azo dye degradation.

Percentage of decolorization by isolate *Acinetobacter indicus* AJK

The maximum decolorization of the dye was obtain at 74% after 7 days incubation at 37°C and 180 rpm/min **(Figure 8)**. After the seventh day of incubation, the dye colour was found to be decreasing. It has also been observed that under aerobic conditions and with additional carbon sources, *Acinetobacter indicus* AJK was more effectively reducing the colour of congo red dye. *Acinetobacter indicus* AJK was shown to be more effective in dye decolorization among other isolates, and so it is suggested that this bacterial strain might be used in the biological treatment of dyeing mill effluents.

Figure 1: Sample collection



A **B**

Figure 2: Decolorization of dye: A. Decolorization in NB supplemented with Congo red dye B. Isolation of azo degrading bacterial colonies

Table 1: Colony Characteristics of Bacterial Colony

Cellular Morphology	Results
Colony size	Medium
Colony shape	Circular
Colony colour	Off-White
Margin	Entire
Elevation	Flat
Consistency	Soft
Pigmented	Non-pigmented

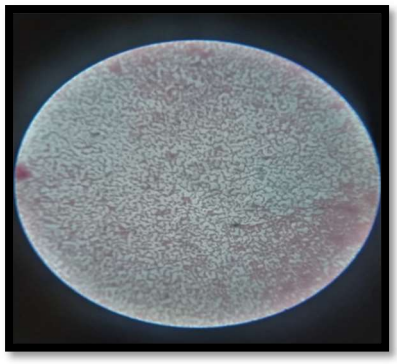


Figure 3: Gram Staining of Isolated Bacteria

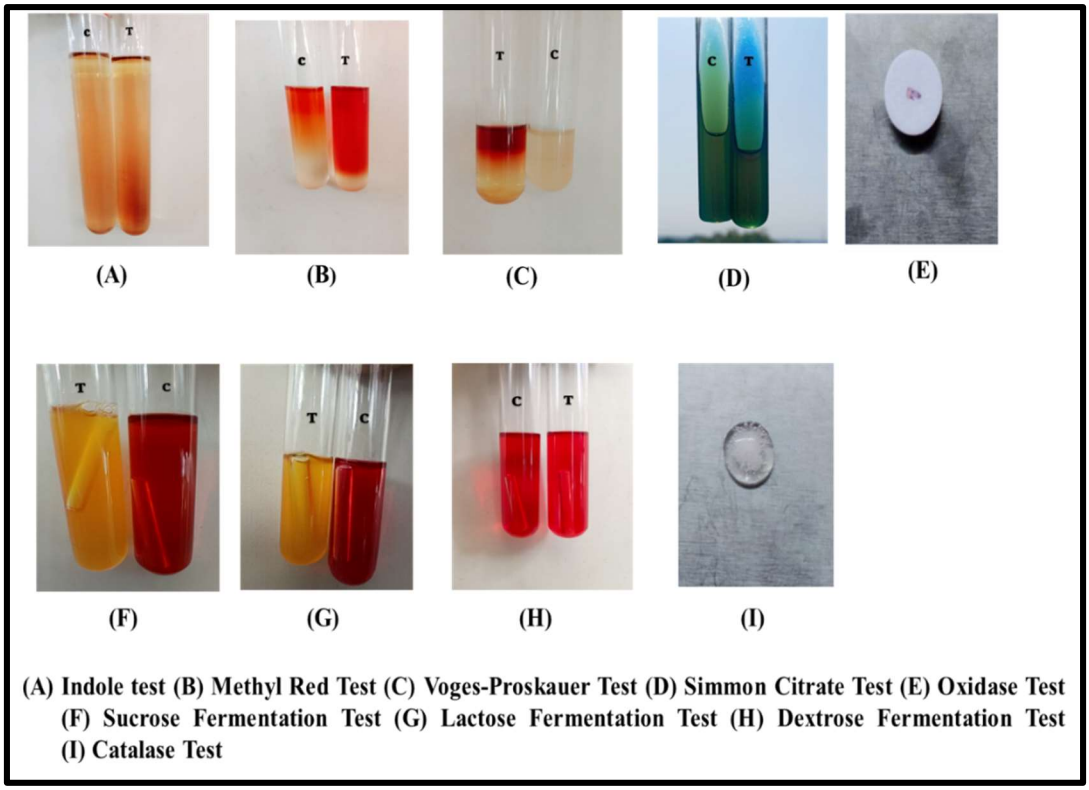


Figure 4: Biochemical Analysis of isolate AJK

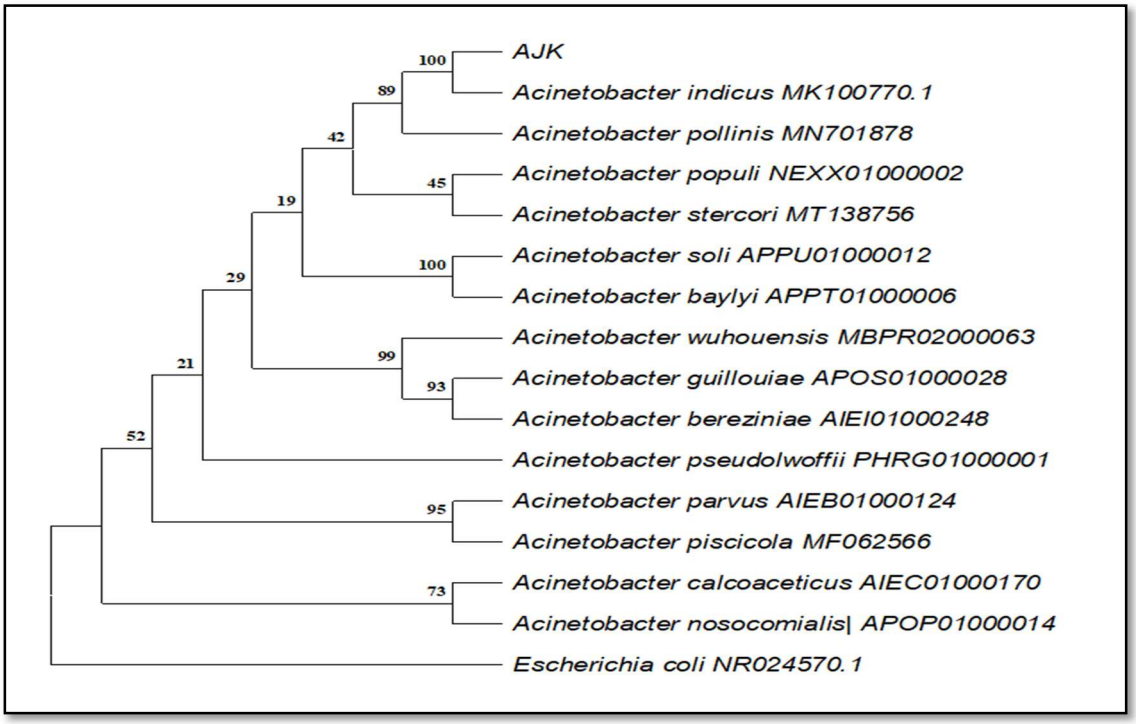
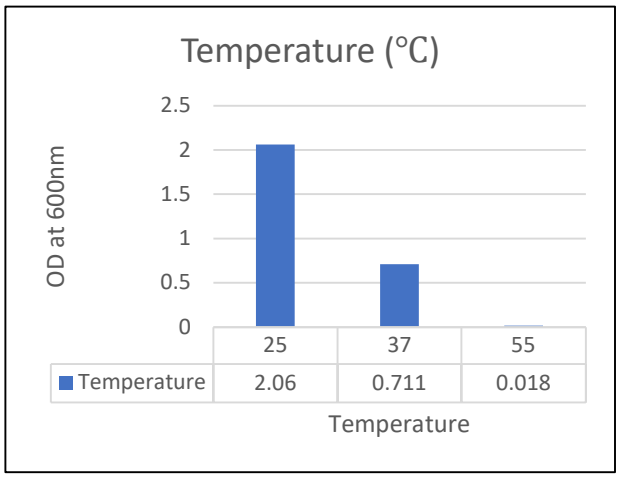


Figure 5: Phylogenetic analysis of strain AJK with the defined species of genus *Acinetobacter*. Bootstrap values of 1000 replicates are indicated at branch points. *Escherichia coli* NR024570.1 served as an outgroup



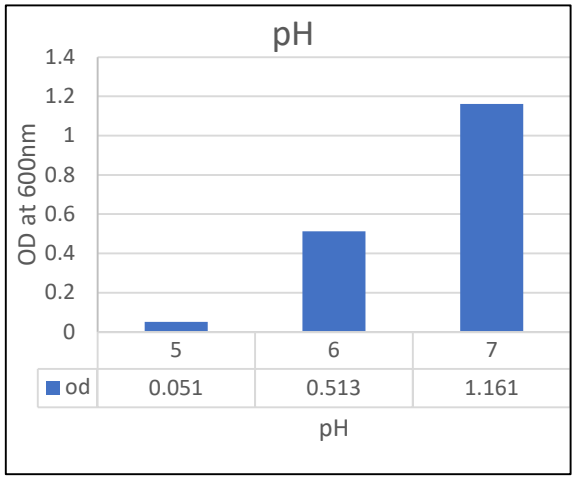


Figure 6: Effect of Temperature and pH on potential Isolate

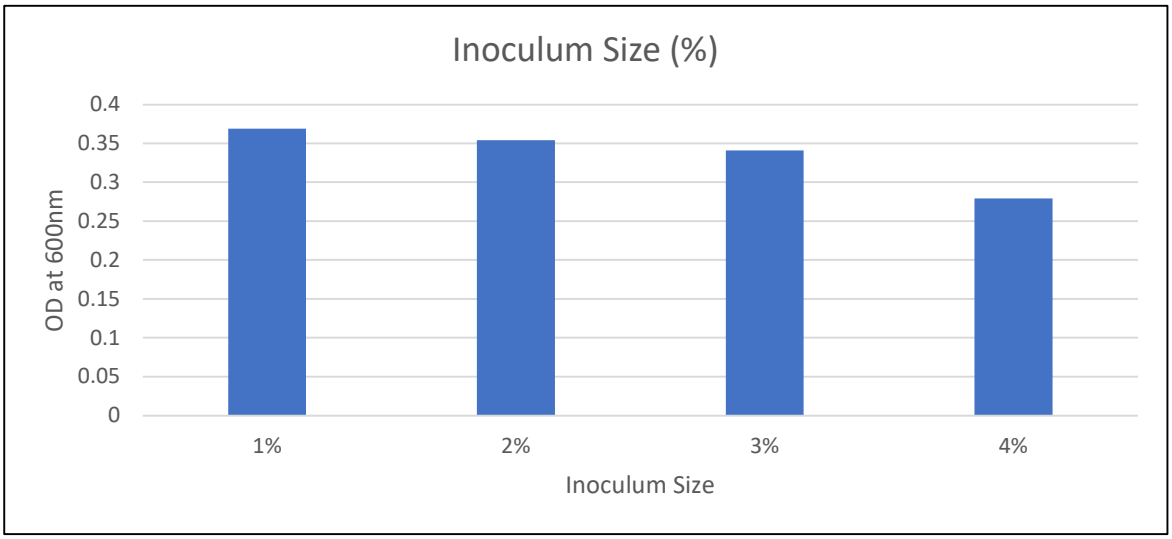


Figure 7: Inoculum Size of potential Isolate

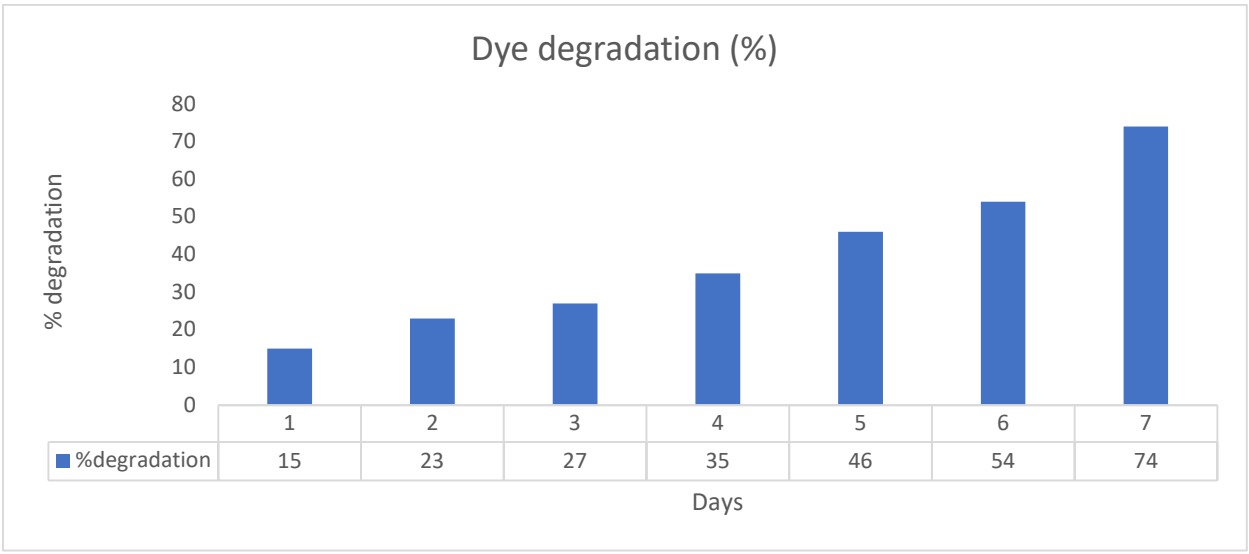


Figure 8: Percentage of decolorization

CONCLUSION

Because of the issues with the environment, humans, and other populations, no procedure is used to discharge textile effluent into the natural environment. Therefore, we must treat the wastewater before releasing it. For it, several forms of time-consuming, expensive, and sludge-producing physical and chemical treatment are used. Therefore, we must choose the biological course of action. In this study, we discovered microorganisms that can be used to decompose the environmentally harmful Congo Red dye. The species of bacteria, the makeup of the media, and the physical factors all affect degradation. According to the optimization's results, the isolate *Acinetobacter indicus* AJK had 74% rate of dye degradation at 37°C and pH – 6 which suggests that the isolate may be used for dye degradation at large scale to prevent the environment.

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