# Degradation of Azo-dye (Disperse Red) Using Rhizosphere Bacterial Consortium

Aigere Sandra Patrick, and Ogbugbue Chimezie Jason

Department of Microbiology, University of Port Harcourt, PMB 5323, Choba, Rivers State. Nigeria.
email: aigeresandra@yahoo.com

**Abstract:** This study investigates the degradation of the azo dye (Disperse Red) using a rhizosphere bacterial consortium. Standard microbiological and molecular techniques were employed to isolate and identify organisms from rhizosphere soil. Degradation of azodye was carried out in a fabricated anoxic and oxic chambers with hydraulic retention time of 40hrs. Initial identification of the bacterial isolates through Gram's reaction and biochemical tests revealed the presence of organisms belonging to the genera *Pseudomonas*, *Lysinibacillus*, and Citrobacter. Molecular and phylogenetic analyses confirmed the isolates as *Pseudomonas aeruginosa*, *Lysinibacillus sphaericus*, *Pseudomonas chengduensis*, and C*itrobacter freundii*. During the preliminary testing, the degradation efficiency was assessed under varying glucose concentrations. Higher decolorization rate of 56.17% was observed in the medium with 10% glucose after 72 hours, while the medium with 5% glucose achieved a 44.17% colour reduction. Notably, lower degradation rates recorded were 11.96% and 12.85% for the 5% and 10% dye enhance glucose mineral salt media, respectively. However, During the actual degradation testing in a double-chamber system enhanced with biochar, the first anaerobic cycle achieved a maximum decolorization of 71.95% after 94 hours, with the first aerobic cycle further enhancing degradation to 90.51%. The second anaerobic cycle increased degradation to 94.78%, and the final aerobic cycle achieved a decolorization of 98.47%. These results show that the rate of Disperse Red degradation is highly dependent on glucose levels and alternating anaerobic-aerobic conditions. This study demonstrates the potential of using rhizosphere bacterial consortia to bioremediate wastewater contaminated with azo dyes, offering an efficient and sustainable method of environmental management. The results underline the need of optimizing ambient conditions to increase microbial degradation processes.
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## 1.0 Introduction

Industrial development is the vehicle that fuels the growth of any nation thereby increasing and enriching the quality of livelihoods. However, development through industrialization has its own negative impact on the environment which can be minimized if the cleaner production technology concept is embraced (Ogunkunle et al., 2016; Ali et al., 2018). One of such industries that have driven development is the textile industry where dyes as major player in coloration is an asset.

Currently there are over 100,000 synthetic commercial dyes known worldwide, and their yearly outcome has been assessed to be over one million tons of dyes (Ajaz et al., 2019). These dyes are different in their chemical makeup characteristics and can be classified into azo, reactive, tri-phenyl methane, heterocyclic and polymeric dyes. It is estimated that 20–50% of these dyes remain unfixed during the dyeing processes and ultimately end up in the dye effluents (Giovanella et al., 2020). Among the different groups of synthetic dyes, azo dyes are the most used in industries and constitute 70% of the total dye production worldwide. This is because of their favorable characteristics such as superior fastness when used on fabric, high stability to light, bright colour, and resistance to microbial attack, water-fastness and simple application techniques. They also have low degradability due to their high chemical, biological and photocatalytic stability. They are characterized by the presence of one or more (-N=N-) azo bonds and can be grouped as mono, di, tri azo dyes (Benkhaya, et al., 2020). After the dying or coloration process in the industries, the by-product of these dyes are discharged into the environment as dye effluent. These effluents poses significant environmental challenges due to their persistence and potential toxicity especially as they are always discharged into water bodies without adequate treatment.

Some of these azo-dyes have been reported to remain in the environment for a very long time because of their structural complexity and resistance to degradation (Ajaz et al., 2019) thereby contributing to extensive water pollution affecting the aquatic ecosystem. Chaudhari et al (2020), reported that as low as 1mg/L of azo dyes discharged or detected in water bodies can lead to the ruin of water merit and

other essential roles of aquatic biological processes. However, when these dye effluents are discharged into land, they tend to affect the growth of plants and influence the ecological diversity and and species evenness. Apparently, given the widespread usage of azo dyes and the documented toxicity on effluent discharge containing azo dyes and their biotransformation by-products from various industries, especially the textile industry, into the environment which in turn negatively affects aquatic organisms in water bodies, soil fertility, plant, animals and the ecosystem balance without any consideration to the impact they may have on the environment. The untreated wastewater consists of highly toxic, mutagenic and carcinogenic properties that are difficult to degrade due to their structural complexity. However, the implication of microorganisms especially bacteria consortium for the complete decolonization and mineralization of azo dye compounds have been widely investigated as an economically feasible and environmentally friendly approach.

These investigations are done In order to proffer solution to this environmental crises using different strategic remediation techniques such as advanced oxidation process, coagulation/flocculation, precipitation, adsorption by activated charcoal, oxidation by ozone, ionizing radiation and ultrafiltration were developed and employed but with limited success. These processes encountered some drawbacks such as high toxicity by-products, high treatment costs, time-consuming, labor-intensive and low efficiency (Albadarin et al., 2017; Pattanaik et al., 2020). These limitations became a priority research concern which led to the need of developing bioremediation tools to decolourize and degrade toxic dye chemicals.

However, biological approaches, including microorganisms and/or their lignin-modifying enzymes (LMEs) have received greater attention in recent years as a way of treating azo dyeing effluents, being a natural recycler. Also, microbial communities are of primary importance in degradation of dye contaminated soils and water bodies as microorganisms alter dye chemistry and mobility through reduction, accumulation, mobilization, and immobilization (Mani et al., 2019; Ajaz et al., 2019). On the contrary, and as an alternate means of improving on the former, microbial system (specialized organisms with reducing enzymes, such as purely anaerobic organisms, several yeasts, and even tissues from higher organisms), genetically modified microorganisms, microbial consortia presented a more attainable and relatively inexpensive and ecofriendly way to degrade azodyes because of their genetic diversity, metabolic versatility and ubiquity. The mechanisms responsible for the degradation of these complex compounds by microorganisms are based on enzymatic conversion due to their ability to attack the dye molecules (Giovanella et al., 2020). To this end, our current study investigated the decolourization of azo dye (Disperse Red) using rhizosphere bacterial consortium.

## 2.0 Materials and Method

### 2.1 Source/Collection of Rhizosphere Soil

The rhizosphere soil of Maize plants (*zea may*) was collected from three different maize grown areas at Rumuekini in Obio-Akpor Local Government Area of Rivers State, located on Lactitude N4º54'17'' Longitude E6º56'36" following the method outlined by Gupta et al. (2022). Each soil sample was thoroughly mixed and homogenized. From a particular location about 50 g of soil were taken as representative sample. Soil samples were collected in polyethene bags, transferred to the Emmadavistic Microbiology Research Laboratory, where these samples were cultured and rhizobacteria isolated.

### 2.2 Source of Azo Dyes (Disperse Red)

Disperse Red 1 (C. I. 11110) was used for this research study. The disperse red synthetic dye is a product of CIBA –GEIGY LTD and was was purchased from Aris Chemical store located at No 32 Wabai Road Sabon Gari in Kano state Nigeria.

### 2.3 Azodye Degradation Medium

Mineral salt medium supplemented with the test dye was used for this study. The medium contained 50mg/L of the Disperse Red 1 (C. I. 11110); Dipotassium hydrogen phosphate ($K_2HPO4$) 1.73g/L; Potassium dihydrogen phosphate ($KH_2PO_4$) 0.68g/L; Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) 0.1g/L; Sodium chloride (NaCl) 4g/L; Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) 0.03g/L; Ammonium nitrate ($NH_4NO_3$) 1g/L; Calcium chloride ($CaCl_2.2H_2O$) 0.02g/L; Glucose ($C_6H_{12}O_6$) 5g/L served as the screening medium to evaluate the degradation potential of bacterial isolates. (Sudharshan et al., 2011; Shafqat et al., 2017; Lebrazi et al., 2020).

### 2.4 Isolation and Characterization of Isolates

The cultural heterotrophic bacterial count was carried out on nutrient agar (NA) using the spread plate method. Soil slurry and wastewater were prepared by adding 1g/1ml of soil/waste water to 9 ml sterile physiological saline (0.85 % w/v; v/v) as diluents. The same procedure previously described by Ogugbue *et al.* (2015) was adapted and an amount of 0.1 ml of each dilution was spread-plated on already prepared

nutrient agar (NA) culture plates in triplicates and incubated using an incubator at 28±2ºC for 24 to 48 h. Pure isolates were identified using standard Gram's reaction, Biochemical and molecular techniques (. (Aneja, 2001; Cheesebrough, 2005; Barisisia et al., 2021).

**2.5 Primary Screening for Dye Decolourization Potential**

All heterotrophic bacteria isolated from rhizosphere soil were subjected to primary screening on plates using mineral salt agar medium supplemented with 50mg/L of Disperse Red. Overnight pure culture of each isolate was inoculated at the center of already prepared dye mineral salt agar plate and incubated inverted for 3 days to detect zones of decolorization. Isolates with zones of decolorization were selected

**2.6 Preliminary Screening for Dye Decolourization under Aerobic and Anaerobic Conditions**

The bacterial consortium was subjected to preliminary screening in tubes containing dye Mineral salt medium. 10mls of Standard inoculum of the consortium was introduce into 90mls of Mineral salt medium containing 50mg/L of disperse red in two set. Each set contained 5% and 10% glucose respectively and incubated in a shaker for 3 to 5 days. Decolorization was determined by taking 2mL aliquots periodically from different test tubes and centrifuging the solutions at 8,000 rpm for 15 min to remove the cells. The absorbance of the supernatants was measured at the 597 nm, for Disperse red by using spectrophotometer as described by Shah et al., (2013).

**2.7 Degradation in Anoxic and Oxic Chambers**

Actual Degradation was done in a fabricated aerobic and anaerobic chamber designed in a way that the anaerobic chamber was attached to the anaerobic chamber as degradation was allowed in a continuous batch process. Cells were immobilized within the chamber using biochar made of sterile chippings and activated carbon (Charcoal). It is worthy to note that indicate that the biochar level is higher in the anoxic chamber than the anaerobic chamber. The percentage degradation of disperse red by microbial consortium was estimate by the equation:

$$\%\ Degradation = \frac{Initial\ absorbance - final\ absorbance\ X\ 100}{Initial\ absorbance}$$

Also the hydraulic retention rate of the chamber was determined using the formula as described by Cruz-Salomón et al., 2018:

$$= \text{Hydralic Retention Time (HRT)} = \frac{\text{Volume of anaerobic tank (L)}}{\text{Flowrate (L/day)}}$$


Fig 1: Fabricated Aerobic and Anaerobic Degradation Chambers with Biochar levels higher in the anaerobic chamber and designed for continuous batch fermentation

**3.0 RESULTS**

**3.1 Biochemical Characteristics of Isolates**

The Gram's reaction and biochemical reaction of the isolates revealed that the organisms isolated from rhizosphere soil were three member of the genus pseudomonas, *lysinlibacillus* and *Citrobacter* sp. as show in table 3.1

**Table 3.1: Grams Reaction/ Biochemical characterization of Isolates.**

| Code | Gram Rxn | MR | VP | Oxid-ase | Catal-ase |
|---|---|---|---|---|---|
| **S1** | - Rod | + | - | + | + |
| **S2** | - Rod | + | - | + | + |
| **S3** | +Rod | + | - | + | + |
| **S4** | +Rod | - | + | + | - |
| **S5** | -Rod | - | + | + | - |

| Code | Indole | Mot-ility | Citrate | Gas | $H_2S$ |
|---|---|---|---|---|---|
| **S1** | - | + | - | - | + |
| **S2** | - | - | - | + | - |
| **S3** | - | + | - | - | + |
| **S4** | - | - | - | - | + |
| **S5** | - | + | + | - | + |

| Code | Tentative Organism |
|---|---|
| **S1** | *Pseudomonas* sp. |
| **S2** | *Pseudomaonas* sp. |
| **S3** | *Lynsilibacillus* sp. |
| **S4** | *Pseudomonas sp.* |
| **S5** | *Citorbacter* sp. |

### 3.2 Molecular Characteristics of Isolates

The molecular and phylogentic analysis of the isolates confirmed the isolates to be *Pseudomonas aureginosa* (S1), Pseudomonas aureginosa (S2), *Lynsilibacillus spaericucus* (S3), Pseudomonas Chengduensis (S4) and Citrobacter Freundii (S5). Gel plate and Phylogenetic tree are as shown in Plate 1 and Figure 2 below.

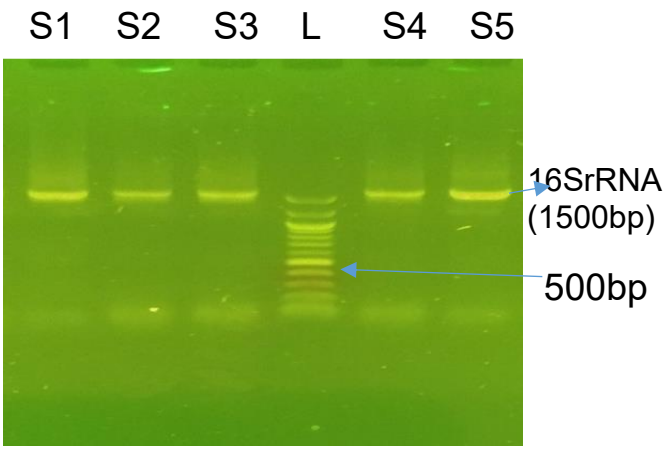


Plate 1: Agarose gel electrophoresis showing the amplified 16S rRNA fragment. Lanes S1-S5 represent the amplified 16SrRNAbands at 1500bp while L represents the 100bp molecular ladder.

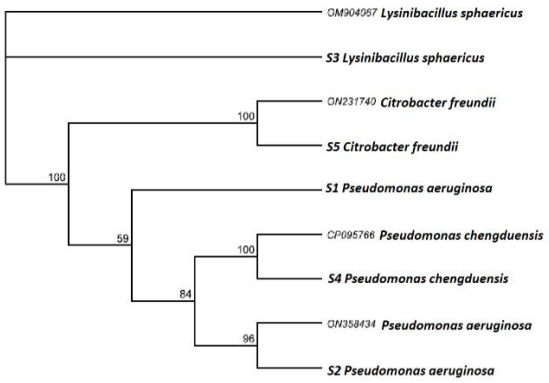


Fig 3.2. Phylogenetic Tree showing eveolutionary relationship between isolates

### 3.3 Decolorization of Disperse Red by Bacterial Consortium under Anaerobic Condition

The percentage degradation of disperse red was highest (56.17%) after 72 hours in the medium with 10% glucose while the medium with 5% glucose had 44.17% decolorization after 72 houre. The least was observed for the 5% medium (11.96%) and 12.85%) for the 10% medium.

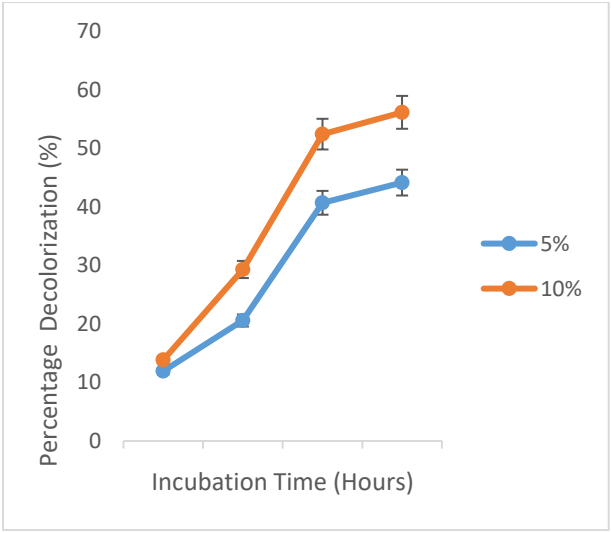


Fig 3.3: Decolourization of Disperse Red by Bacterial Consortium under Anaerobic Condition

### 3.4 Decolorization of Disperse Red by Bacterial Consortium under Aerobic Condition

The percentage degradation of disperse red was highest (71.2%) after 72 hours in the medium with 5% glucose while the medium with 10% glucose had 68.4% decolorization after 72 hours. The least was observed for the 10% medium (2.98%) and 5.2%) for the 5% medium.

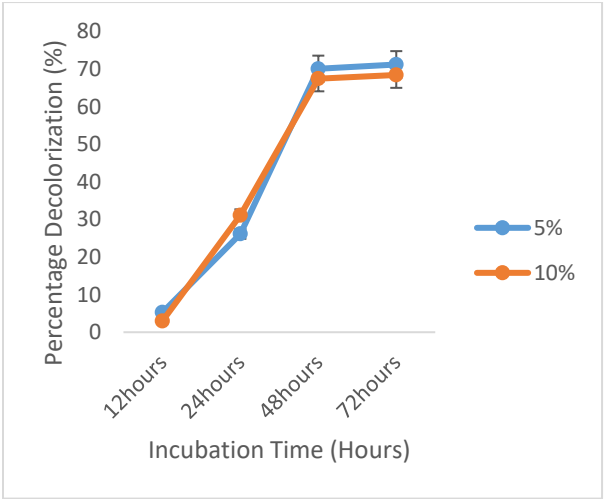


Fig 3.4: Decolourization of Disperse Red by Bacterial Consortium under Aerobic Condition

### 3.5 Decolorization of Disperse Red by Bacterial Consortium in Glass Chambers

The degradation of dye in the double chamber revealed that decolorization during the first anaerobic cycle was maximum after 94hours with 71.95% degradation recorded and minimum 18.06% after 24hours. The first aerobic cycle degraded the disperse red by commenced from the 18.06% inicial and was further degraded up to 90.51%. The 2nd cycle in the anaerobic chamber degraded the dye uptt94.78% while the during the final cycle being the 2nd aerobic cycle, a 98.47% decolorization was recorded. (Fig3.5).

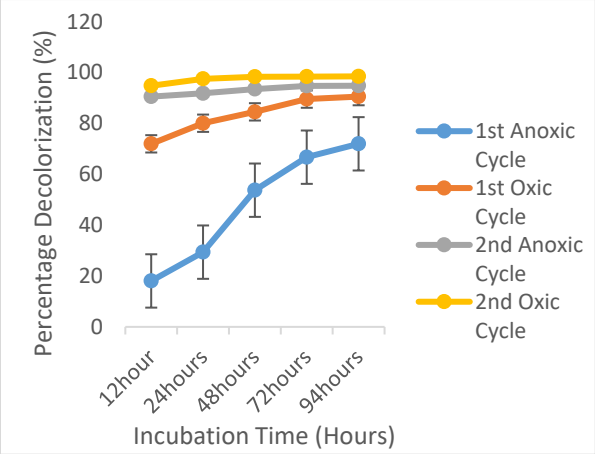


Fig 3.5: Decolourization of Disperse Red by Bacterial Consortium under Aerobic and Anaerobic Conditions in the Fabricated Chambers

### 4.0 Discussions

The study identified the bacterial isolates from rhizosphere soil through Gram's reaction and biochemical tests, revealing the presence of *Pseudomonas*, *Lysinibacillus*, and Citrobacter species. Subsequent molecular and phylogenetic analysis further confirmed the species as *Pseudomonas aeruginosa*(S1,S2), *Lysinibacillus* sphaericus (S3), *Pseudomonas chengduensis* (S4), and *Citrobacter freundii* (S5). Isolation of these organisms from the soil have been previously reported in several other studies (Peng et al., 2020; Guerrero et al., 2023)

The process is crucial as it highlights the diverse metabolic capabilities of these bacteria, which are essential for the degradation of complex pollutants like azo dyes. For instance, Pseudomonas species are known for their versatile metabolic pathways that enable them to degrade a wide range of organic compounds, including dyes (Chen et al., 2018). The study observed that the degradation of Disperse Red was influenced by the glucose concentration in the medium. In a medium with 10% glucose, the highest degradation was 56.17% after 72 hours, while the 5% glucose medium showed 44.17% decolorization. Interestingly, the lowest degradation rates were also observed in these media, indicating that excessive or insufficient glucose may limit the degradation efficiency. These findings align with previous research indicating that the availability of an appropriate carbon source is crucial for the optimal activity of dye-degrading bacteria (Jadhav et al., 2010; Pham et al., 2023).

The higher degradation rate in the medium with 10% glucose can be attributed to the enhanced bacterial growth and metabolic activity due to the abundant carbon source. However, too high glucose concentrations may lead to a shift in bacterial metabolism towards more glucose utilization rather than dye degradation (Kalyani et al., 2009; Chen et al., 2023). Comparing these findings with other studies, the results show both similarities and differences. For instance, Ja Ikram et al. (2022) reported that *Pseudomonas* species could achieve up to 90% decolorization of azo dyes under optimized conditions, which is higher than the 56.17% observed in this study. This discrepancy could be due to differences in experimental conditions, such as the type of dye, initial dye concentration, and the specific bacterial strains used. Moreover, the presence of multiple bacterial species in the consortium could lead to competition for resources, potentially affecting the overall degradation efficiency (Cao et al., 2022; Al-Marri et al., 2023).

The study also examined dye degradation in a double chamber system, involving alternating anaerobic and aerobic cycles. The results showed a maximum decolorization of 71.95% during the first anaerobic cycle after 94 hours, with a significant increase to 90.51% during the subsequent aerobic cycle. This sequential anaerobic-aerobic treatment enhances the degradation efficiency as anaerobic conditions break down the azo bond in the dye, and aerobic conditions further mineralize the intermediate products (Chen et al., 2018; Jayapal et al., 2018). The observed degradation patterns can be explained by the metabolic characteristics of the bacterial species involved. *Pseudomonas aeruginosa*, known for its versatile metabolic pathways, can utilize various carbon sources and degrade complex organic compounds under both aerobic and anaerobic conditions (Rai et al., 2014; Diggle and Whiteley, 2020; Dolan et al., 2020). *Lysinibacillus sphaericus* and *Citrobacter freundii*

also contribute to the degradation process by breaking down dye intermediates formed during the initial degradation steps.

**5.0 Conclusion**

The enormous potential of this microbial community for bioremediation applications was proven by the study on the breakdown of azo dye (Disperse Red) employing a rhizosphere bacterial consortium. The bacterial *isolates Lysinibacillus sphaericus*, *Pseudomonas chengduensis*, and *Citrobacter freundii* were identified by molecular phylogenetic analysis, biochemical testing, and Gram's reaction. The degradation efficiency changed as the concentration of glucose changed. The results of the study also demonstrated the effectiveness of a sequential anaerobic-aerobic conditions, which after the second aerobic cycle may achieve up to 98.47 percent decolorization. These findings highlight how crucial it is to optimize environmental factors, like the concentration of carbon sources and the redox conditions that alternate, in order to promote the biodegradation of azo dyes. The methods and effectiveness of dye breakdown by rhizosphere bacteria are better understood thanks to this research, which also provides a viable treatment for wastewater contaminated with dyes.

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